The effect of high-dose simvastatin on triglyceride-rich lipoprotein metabolism in patients with type 2 diabetes mellitus

William L. Isley,[1,*] John M. Miles,[*] Bruce W. Patterson,[†] and William S. Harris[§]

Division of Endocrinology, Diabetes, Metabolism, and Nutrition,* Mayo Clinic College of Medicine, Rochester, MN; Center for Human Nutrition and Department of Internal Medicine,† Washington University School of Medicine, St. Louis, MO; and St. Luke's Lipid and Diabetes Research Center,§ Mid-America Heart Institute, Kansas City, MO

Abstract Statins decrease triglycerides (TGs) in addition to decreasing low density lipoprotein-cholesterol. Although the mechanism for the latter effect is well understood, it is still unclear how TG decrease is achieved with statin therapy. Because hypertriglyceridemia is common in obese patients with type 2 diabetes mellitus, we studied triglyceride-rich lipoprotein triglyceride (TRL-TG) turnover in 12 such subjects using stable isotopically labeled glycerol. The diabetic subjects were studied after 12 weeks of placebo and after a similar course of therapy with simvastatin (80 mg daily) in a single-blind design. The results were compared with those from six nonobese nondiabetic control subjects. Simvastatin therapy reduced serum TGs by 35% in the diabetic subjects. Compared with the control subjects, TRL-TG secretion was almost 2-fold higher in the diabetic subjects (45.4 ± 4.9 vs. 24.4 ± 1.9 μmol/min; $P < 0.002$) and was unaffected by simvastatin therapy. However, TRL-TG clearance was significantly increased in the diabetic subjects during simvastatin treatment compared with placebo (0.25 ± 0.03 vs. 0.16 ± 0.02 pools/h; $P < 0.002$). This change was accompanied by a 49% increase in preheparin plasma lipase activity ($P < 0.03$) and a 21% increase in postheparin LPL activity ($P < 0.01$). Together, these findings provide strong evidence that the effect of statins on serum TGs is related to an increase in LPL activity, resulting in accelerated delipidation of TRL particles. The effect of high-dose simvastatin on triglyceride-rich lipoprotein metabolism in patients with type 2 diabetes mellitus.—Isley, W. L., J. M. Miles, B. W. Patterson, and W. S. Harris. **The effect of high-dose simvastatin on triglyceride-rich lipoprotein metabolism in patients with type 2 diabetes mellitus. *J. Lipid Res.* 2006.** 47: **193–200.**

Supplementary key words very low density lipoprotein • lipoprotein kinetics • statin

Disordered lipid metabolism is a hallmark of type 2 diabetes mellitus (1). Increased delivery of FFAs to the liver leads to an overproduction of VLDL triglyceride (TG) (2, 3) that is the basis for the characteristic dyslipidemia (high TGs and low high density lipoprotein-cholesterol [HDL-C]) of type 2 diabetes mellitus (4).

HMG-CoA reductase inhibitors, or statins, are widely used in diabetic patients and have been shown to decrease cardiovascular morbidity in individuals with type 2 diabetes (5, 6). The major effect of statins is a reduction in low density lipoprotein-cholesterol (LDL-C) concentrations, primarily mediated by inhibition of the rate-limiting step in cholesterol biosynthesis, resulting in an increase in LDL receptors in the liver (7). In addition, statins can reduce TGs and increase HDL-C (8, 9), all of which lead to a reduced risk for coronary heart disease (CHD) in patients with type 2 diabetes mellitus (5, 6, 10).

The mechanism responsible for the TG-lowering effect of statins is poorly defined. In theory, it could be related to decreased VLDL production (presumably secondary to decreased availability of hepatic free cholesterol for particle assembly), increased clearance of VLDL through the LDL receptor (or other lipoprotein receptors), increased delipidation of VLDL particles via LPL, or a combination of these mechanisms. To address these questions, we determined triglyceride-rich lipoprotein triglyceride (TRL-TG, which is predominantly VLDL) production and clearance in subjects with type 2 diabetes mellitus and hypertriglyceridemia on placebo and high-dose simvastatin therapy (80 mg daily) for 12 weeks and in nondiabetic controls.

RESEARCH DESIGN AND METHODS

Study subjects

The study was approved by the Institutional Review Board of St. Luke's Hospital of Kansas City. Written informed consent

Manuscript received 26 August 2005 and in revised form 11 October 2005 and in re-revised form 27 October 2005.

Published, JLR Papers in Press, October 28, 2005.
DOI 10.1194/jlr.M500387-JLR200

[1] To whom correspondence should be addressed.
e-mail: isley.william@mayo.edu

was obtained from the subjects after the nature of the study was explained.

Six nondiabetic subjects with body mass indices of <30 kg/m^2 (fasting TGs of <150 mg/dl in five subjects, fasting TG of 203 mg/dl in one subject) and 12 obese subjects with type 2 diabetes mellitus and fasting hypertriglyceridemia (200–500 mg/dl) were recruited for these investigations. Individuals with serum creatinine of >1.5 mg/dl, alanine aminotransferase or aspartate aminotransferase of $\geq 2\times$ the upper limit of normal, blood pressure of >170 mmHg systolic or >110 mmHg diastolic, a recent (within 3 months) cardiovascular event, or symptomatic cardiac disease were excluded. The antihyperglycemic therapies of the diabetic subjects were maintained throughout the study and included sulfonylurea (one subject), sulfonylurea plus metformin (three subjects), sulfonylurea plus thiazolidinediones (three subjects), metformin plus thiazolidinediones (one subject), sulfonylurea plus metformin plus thiazolidinediones (two subjects), insulin plus thiazolidinediones (one subject), and insulin plus metformin plus thiazolidinediones (one subject). Diabetes therapy was not altered during the trial.

Only one diabetic subject was taking prescription lipid-lowering medication (atorvastatin, 40 mg twice a day) at the time of study recruitment. The medication was discontinued 4 weeks before beginning the study. Ten of the 12 diabetic subjects were on stable antihypertensive medication throughout the study. Two of the female diabetic subjects were taking oral estrogens, and one was taking an estrogen-progestin combination. One of the female subjects was taking a vaginal estrogen preparation every 2 weeks. Two diabetic subjects were taking thyroid hormone replacement. One of the control subjects took oral estrogen (prescribed for premature menopause) and replacement thyroid hormone throughout the study. Subjects were instructed to consume a low-fat diet.

Experimental protocol

Diabetic subjects received either simvastatin 80 mg daily or matching placebo for two 12 week periods in a single-blind crossover design. Consumption of a low-fat, low-cholesterol diet as recommended by the National Cholesterol Education Program was encouraged. The subjects received placebo first, followed by simvastatin. In the diabetic subjects, TRL-TG turnover studies were performed at the end of each treatment period. Control subjects were studied on one occasion only. Subjects were asked to refrain from alcohol consumption and vigorous exercise for 48 h before the study.

Subjects were admitted to the inpatient Clinical Study Unit at 4:00 PM on the day before the study. At 6:00 PM, a mixed meal (50% carbohydrate, 30% fat, and 20% protein) was given containing calories equal to one-third of energy requirements for weight maintenance, estimated at 1.35 $\times$ basal energy expenditure (Harris-Benedict equation). At 8:00 PM, an infusion cannula was placed in a forearm vein and a retrograde cannula was placed in a contralateral hand vein; the hand was heated for sampling of arterialized venous blood. The three catheters were kept patent by controlled (15 ml/h each) infusions of 0.9% NaCl. Room lights were turned off at 10:00 PM. Care was taken to avoid disturbance of sleep during blood sampling. Subjects remained fasting until completion of the study the next day.

At 2:00 AM, 50 μmol/kg body weight [1,1,2,3,3-$^2H_5$]glycerol (Cambridge Isotope Laboratories, Andover, MA) dissolved in 0.9% saline was injected as a bolus. Blood samples were obtained before the injection of labeled glycerol to determine plasma substrate (glucose, fatty acids, total TGs, and TRL-TG) and background glycerol tracer-to-tracee ratio (TTR) in plasma and TRL-TG. Blood samples were taken at 5, 15, 30, 45, and 60 min, and then every 30 min for 6 h after the labeled glycerol injection, to determine the glycerol TTR in TRL-TG and total plasma TGs and TRL-TG concentrations. Subjects remained in bed for the duration of the study.

Sample analysis

Plasma glucose concentrations were determined on a centrifugal analyzer using a glucose oxidase method. Hemoglobin A_{1c} was determined by high-performance liquid chromatography on a Bio-Rad Variant instrument (Hercules, CA).

For determination of lipids and lipoproteins, blood was drawn into serum separator tubes. Serum total cholesterol, TG, and HDL-C were measured enzymatically on a Cobras Fara II (Roche) using enzymatic reagents and procedures standardized by the Lipid Standardization Program of the Centers for Disease Control and Prevention/National Institutes of Health and Pacific Biometrics Foundation (Seattle, WA) proficiency surveys. HDL-C was determined in the serum supernatant after precipitation of TRL and LDL with dextran sulfate-magnesium chloride as described by Warnick, Benderson, and Albers (11). VLDL-C and LDL-C concentrations were determined by the Friedewald equation (12), or if TGs were >400 mg/dl, by β-quantification. In this procedure, the TRL fraction was isolated from whole serum by ultracentrifugation at 100,000 rpm for 2 h in a Beckman TL-100 using a TLA100.3 rotor. The VLDL layer was removed by aspiration, and the cholesterol content of the infranatant containing HDL and LDL was determined as described above. The LDL-C value was calculated by subtraction of the HDL-C previously measured from the infranatant cholesterol. VLDL-C was calculated as the total cholesterol minus the infranatant cholesterol.

For the TRL kinetic study, blood samples were collected in chilled tubes containing EDTA. Samples were placed in an ice bath, and plasma was separated by centrifugation (2,000 *g* at 4°C for 30 min) within 30 min of collection. Aliquots of plasma (2 ml) were refrigerated at 4°C for subsequent isolation of TRL. The remaining plasma samples were stored at −70°C until the final analyses were performed.

The TRL fraction was isolated by ultracentrifugation. Two milliliters of each plasma sample was transferred into Optiseal tubes (Beckman Instruments, Inc., Palo Alto, CA), covered with a saline solution (d = 1.006 g/ml), and centrifuged in an ultracentrifuge (Beckman Instruments, Inc.) for 16 h at 100,000 *g* and 8°C. The top layer, containing TRL, was removed by tube slicing (Beckman Instruments, Inc.). The exact volume that was recovered (∼1.3 ml) was recorded, and the samples were stored at −70°C until analyzed for TG concentration and the TRL glycerol TTR.

The TTR of glycerol present in TRL-TG was determined by GC-MS, as described previously (13). After the TRL fraction was isolated from plasma, proteins were precipitated with ice-cold acetone, and lipids were extracted with hexane. The lipid extract was dried by Speed-Vac (Savant Instruments, Inc.), and the TG fraction was isolated by thin-layer chromatography and recovered by scraping. TGs were extracted with chloroform-methanol (3:1, v/v) and reacted with acetyl chloride in methanol to form fatty acid methyl esters. The liberated glycerol was derivatized with heptafluorobutyric anhydride and analyzed by GC-MS on an Agilent 5973N MSD equipped with an HP-5MS column, using electron impact ionization and monitoring ions at *m/z* 467 and 472.

LPL assays

Preheparin plasma lipase activity. Endogenous lipolytic activity was measured by incubating plasma with emulsified nonradioactive triolein and then determining the amount of oleic acid

liberated by gas chromatography, as described previously (14). Fasting blood was collected into heparinized tubes, and the plasma was separated and stored at −80°C. Substrate was prepared fresh daily with 200 mg of triolein and 5.68 ml of 90 g/l gum arabic in 50 mM NH_4OH-NH_4Cl buffer (pH 8.5) by sonication (series 4710 semi; Cole-Parmer Instrument Co., Chicago, IL). Then, 1.375 ml of 200 g/l BSA in buffer and 1.375 ml of the internal standard solution were added to the mixture. The internal standard solution was made in advance as follows. Heptadecanoic acid (13.525 mg) was dissolved in 10 ml of methanol and 1 ml of 10 M ammonium hydroxide, then dried under nitrogen. BSA (20 ml of 200 g/l in buffer) was added, and the mixture was sonicated at amplitude 40 for 2 h in an ice bath.

Plasma (100 μl), 880 mmol/l SDS solution (20 μl), buffer (180 μl), and substrate (0.5 ml) were added. Lipase activity was inhibited by the inclusion of 50 mg of NaCl and no SDS in control (blank) samples. The mixtures were vortexed and incubated for 2 h at 28°C. The TG hydrolysis reaction was terminated by adding 5.33 ml of methanol-chloroform-heptane solution (38.4:34.2:27.4%) and 1.5 ml of 0.1 mol/l carbonate-bicarbonate buffer in 1 mol/l NaCl (pH 10.5). After shaking and centrifugation, the supernatant (containing nonesterified fatty acids) was transferred, and 0.5 ml of 0.5 mol/l HCl and 3 ml of hexane were added. The mixture was shaken vigorously and centrifuged at 3,000 rpm for 45 min. The supernatant was transferred and dried under nitrogen. Samples were methylated by adding 1 ml of boron trifluoride and heating at 100°C for 3 min. The methylated fatty acids were extracted by adding 2 ml of distilled water and 2 ml of hexane. The supernatants were dried under nitrogen and analyzed by GC (injection temperature, 200°C; oven temperature, 210°C) with a 30 m SP2330 capillary column (Supelco, Bellefonte, PA). The amount of liberated oleic acid was determined (after subtracting appropriate blanks), and activity was expressed as μmol oleic acid released/h/ml plasma. This assay was found to be linear with time over 4 h with substrate and with plasma (enzyme) concentration. Activity was inhibited by known LPL inhibitors such as NaCl (0.5 M), guanidine HCl (0.5 M), paraoxon (12 μg/ml), and tetrahydrolipstatin (3 μg/ml) (unpublished data) and unaffected by freeze/thawing of plasma.

Postheparin LPL. Postheparin LPL activity was measured in plasma drawn 15 min after the injection of heparin (100 IU/kg body weight). The injection was given in the morning after an overnight fast 3 days after the TRL-TG kinetic studies. The substrate described above was added to 20 μl of a 1:1 mixture of postheparin plasma and buffer, 80 μl of human serum [as a source of apolipoprotein C-II (apoC-II)], and 200 μl of buffer. The blank included 50 mg of NaCl, 20 μl of 880 mmol/l SDS solution, and 180 μl of buffer instead of 200 μl of buffer. For the HL assay, 3.6 mol/l NaCl solution was used instead of buffer. The rest of the procedure was as described for the preheparin assay above. LPL activity was determined to be the difference between total lipase activity and HL activity, both corrected for blank activity.

Calculations

The fractional catabolic rate (FCR) of TRL-TG, which represents the fraction of the TRL-TG pool that disappeared from plasma per hour, was determined as the negative of the monoexponential downslope of TRL-TG glycerol TTR, as described previously (13, 15). There was insufficient information to resolve tracer recycling from TRL-TG turnover using a compartmental model, because only 6 h of data were available (13). Because plasma TRL-TG concentration remained constant throughout the 6 h sampling period, it was assumed that the rate of TRL-TG appearance was equal to the rate of TRL-TG disappearance. The calculations were

$$\text{TRL-TG secretion rate } (\mu\text{mol/min}) = (\text{TRL-TG FCR} \times C_{\text{TRL-TG}} \times \text{PV})/60$$

where $C_{\text{TRL-TG}}$ is the concentration of TRL-TG in plasma and PV is the plasma volume, which was estimated based on each subject's body weight [PV = 0.05 × body weight (kg)]. PV was set equal to the TRL-TG volume of distribution, because TRL is assumed to be restricted to the plasma compartment. TRL-TG clearance is given in pools per hour.

Statistical analyses

Data are presented as means ± SD or SEM when so noted. The statistical significance of mean differences between diabetic subjects and normal control subjects at baseline was determined using Student's *t*-test for independent samples. Differences within the diabetic patients on placebo and simvastatin were determined with a paired *t*-test. Statistics were calculated using the Microsoft Excel 2003 Data Analysis Package.

RESULTS

Baseline characteristics of the study subjects are given in **Table 1**. Although diabetic male subjects were heavier than diabetic female subjects, the body mass index was similar between the two groups. Body weight did not change significantly during the study. Total cholesterol, VLDL-C, and TGs were greater in the diabetic subjects at baseline than in the controls (Table 1; $P < 0.002$). Simvastatin therapy was well tolerated by all diabetic subjects, with no major side effects. There was no significant change in glycemic control (hemoglobin A_{1c} = 8.3 ± 1.4% on placebo and 8.5 ± 1.4% on simvastatin) during the study.

Serum LDL-C was reduced by 49%, whereas VLDL-C and TGs were reduced by 33% and 35%, respectively, by simvastatin compared with placebo ($P < 0.01$). Serum HDL-C increased by 6% compared with placebo ($P = 0.056$).

TABLE 1. Baseline characteristics of 6 (4 men and 2 women) nondiabetic control subjects and 12 (7 men and 5 women) subjects with type 2 diabetes mellitus

Characteristic	Control Subjects	Diabetic Subjects
Age (years)	39 ± 14.9	55.7 ± 7.8[a]
Weight (kg)		
All	85.8 ± 5.9	103.9 ± 19.2[a]
Men		113.5 ± 21.9[b]
Women		89.7 ± 8.4
Body mass index (kg/m^2)		
All	26.7 ± 2.2	35.8 ± 5.7[c]
Men		36.5 ± 7.4
Women		34.9 ± 2.5
Hemoglobin A_{1c} (%)	—	8.3 ± 1.5
Cholesterol (mg/dl)	177 ± 24	242 ± 38[c]
Triglycerides (mg/dl)	129 ± 47	274 ± 58[c]
HDL-cholesterol (mg/dl)	36 ± 12	40 ± 9
VLDL-cholesterol (mg/dl)	26 ± 9	50 ± 18[c]
LDL-cholesterol (mg/dl)	115 ± 19	148 ± 38

Values shown are means ± SD.
[a] $P < 0.04$, control subjects versus diabetic subjects.
[b] $P < 0.003$, male versus female diabetic subjects.
[c] $P < 0.002$, control subjects versus diabetic subjects.

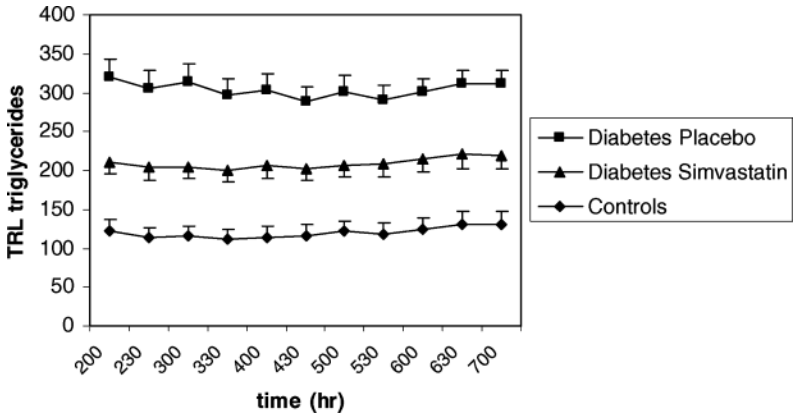

Fig. 1. Triglyceride-rich lipoprotein triglyceride (TRL-TG) concentrations (means ± SEM) overnight in normal subjects and diabetic subjects treated with placebo and simvastatin (80 mg daily). The time scale reflects minutes after administration of the test meal.

There was no statistically significant difference in any of these parameters between male and female diabetic subjects or between diabetic subjects treated with thiazolidinediones and those not treated with these drugs.

As shown in **Fig. 1**, TRL-TG concentrations were in steady state during the study period. Mean TRL-TG was 120 ± 34 mg/dl (mean ± SEM) in controls, compared with 304 ± 10 mg/dl in untreated diabetic subjects ($P < 0.0001$). Mean overnight TRL-TGs were reduced by approximately one-third to 209 ± 7 mg/dl in the diabetic subjects when treated with high-dose simvastatin ($P < 0.0001$).

In the normal subjects, the FCR of TRL-TG was 0.28 ± 0.13 pools/h. It was lower in the diabetic subjects at 0.16 ± 0.07 pools/h, and increased to 0.25 ± 0.10 pools/h with simvastatin treatment (not significantly different from control). **Figure 2** shows the isotopic enrichment curves for each of the study groups over time. The TRL-TG secretion rate was 2-fold greater in diabetic subjects compared with controls (45.4 ± 4.9 μmol/min vs. 24.4 ± 1.9 μmol/min; $P < 0.002$) and unaffected by simvastatin (**Fig. 3**). There was no difference between the male and female diabetic subjects, or between the diabetic subjects treated with thiazolidinediones and those not treated with these drugs (data not shown). However, the TRL-TG disappearance rate, which was lower in the diabetic subjects, was normalized with simvastatin therapy (13.3 ± 1.3 ml/min on placebo, 20.6 ± 1.7 ml/min on simvastatin; $P < 0.003$) (**Fig. 4**). Serum TGs were highly inversely correlated with TRL-TG clearance rate ($P < 0.01$) (**Fig. 5**) but not with TRL-TG secretion.

Preheparin lipase and postheparin LPL activities did not differ significantly between the controls and the patients on placebo (**Table 2**). Treatment of the latter group with simvastatin, however, increased the former by 49% ($P < 0.03$) and the latter by 21% ($P = 0.01$).

DISCUSSION

In this study, postabsorptive TRL-TG turnover rates were measured in untreated nondiabetic subjects and obese persons with type 2 diabetes mellitus on placebo and after treatment with high-dose simvastatin using a bolus injection of [$^2H_5$]glycerol. TRL-TG secretion rates were higher in diabetic subjects compared with controls, and this abnormality was not affected by high-dose simvastatin therapy. TRL-TG clearance was lower in the diabetic subjects and markedly improved with simvastatin therapy. In addition, lipase activity in both preheparin and postheparin plasma increased during treatment with simvastatin. These findings suggest that statins act to decrease serum TGs in diabetic subjects by improving TG clearance and

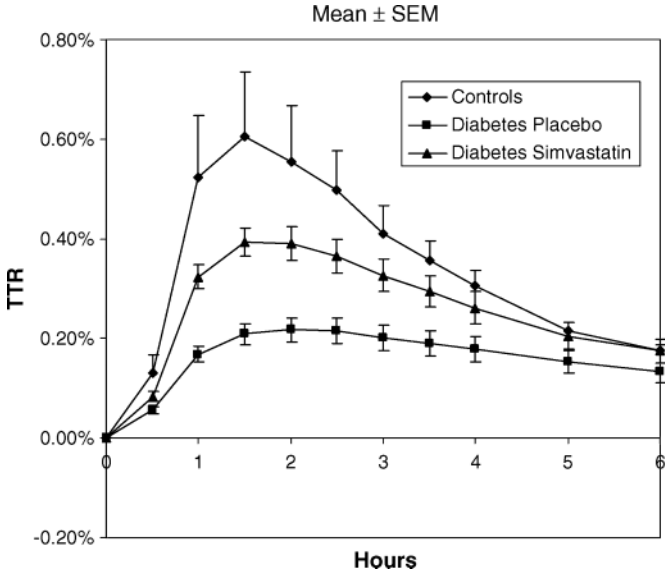

Fig. 2. Isotopic enrichment [tracer-to-tracee ratio (TTR)] over time. Values shown are means ± SEM.

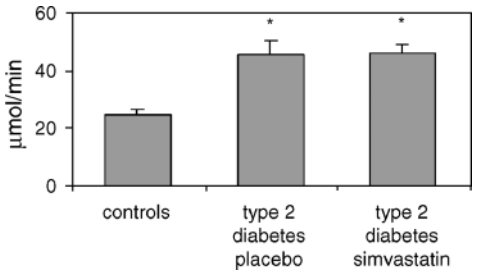

Fig. 3. TRL-TG secretion (means ± SEM). * $P < 0.002$ versus controls.

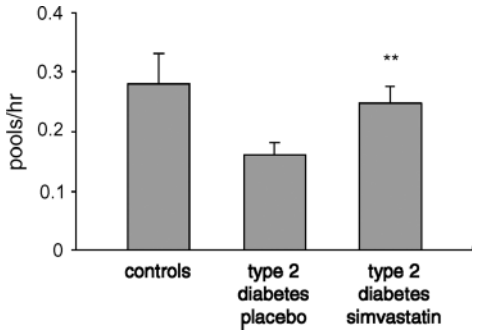

Fig. 4. TRL-TG clearance (means ± SEM). ** $P < 0.002$, simvastatin-treated diabetic subjects versus placebo-treated diabetic subjects.

stimulating intravascular TRL-TG lipolysis while not affecting TRL-TG production.

TGs may be increased in blood by overproduction of or altered clearance of the TGs and TG-rich lipoproteins. In type 2 diabetes, overproduction of TRL occurs (16). Consistent with these findings, we found an increase of TRL-TG secretion in the diabetic subjects.

Previous work in small groups of subjects has suggested that TG lowering by statins could be attributable to either decreased production of VLDL particles, resulting from decreased availability of cholesterol or TGs for particle assembly, or increased catabolism of VLDL, resulting from either enhanced LDL (and perhaps other) lipoprotein receptor uptake or increased delipidation of VLDL particles by LPL (17, 18). Such studies have generally assessed VLDL apoB rather than TRL-TG (17, 18). The concurrent increase in TRL-TG clearance and increase in LPL activity

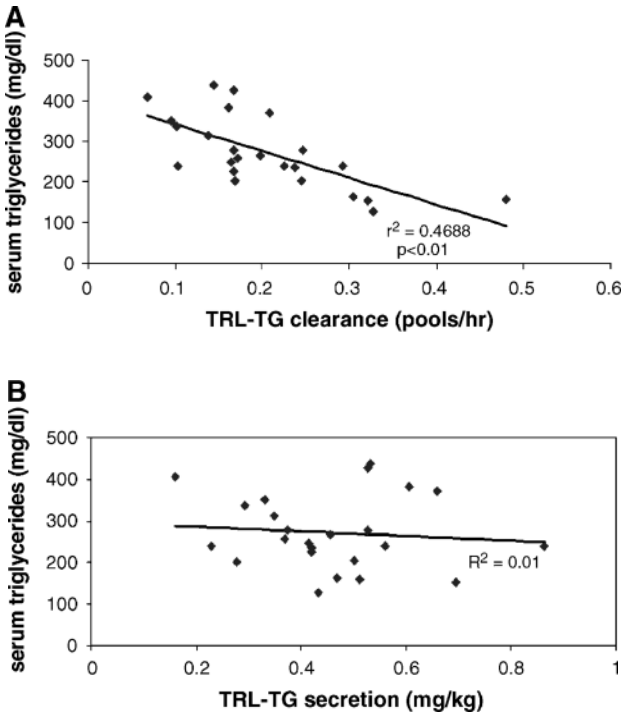

Fig. 5. Serum triglycerides versus TRL-TG clearance ($P < 0.01$) and secretion.

TABLE 2. Effects of simvastatin on lipoprotein lipase activity in subjects with type 2 diabetes mellitus compared with the activity in healthy controls

Lipase	Controls	Type 2 Diabetes on Placebo	Type 2 Diabetes on Simvastatin
Preheparin lipase	0.57 ± 0.50	0.66 ± 0.62	0.98 ± 0.83[a]
Postheparin LPL	5.5 ± 1.8	6.5 ± 0.8	7.9 ± 1.4[a]

Values shown are means ± SD (μmol free fatty acid released/ml plasma/h).

[a] $P < 0.03$ versus subjects on placebo.

observed in this study after treatment with high-dose simvastatin strongly support an increased removal of TGs from TRL as a major mechanism for the TG-lowering effect of high-dose statin therapy.

An alternative explanation of our kinetic studies could be offered if intrahepatic TG production is affected by therapy with high-dose simvastatin and if such newly synthesized TG is labeled and makes a significant contribution to TRL-TG appearance during the observation period. Animal and cell culture studies have shown either no effect (19–21) or an increase (19, 22, 23) in hepatic TG synthesis induced by statin therapy. In previous work validating our techniques, Patterson et al. (13) showed that the monoexponential slope and the model-derived FCR (which accounts for hepatic TG turnover) are essentially equal when the turnover rate is <0.4 pools/h, which was the case in all but one of our studies. Furthermore, the finding of an increase in lipase activity in the diabetic subjects treated with simvastatin provides confirmatory evidence that the effects of the drug on TRL-TG are mediated by increased intravascular lipase-mediated TG clearance.

This major finding of our study is consistent with the recent report by Schneider et al. (24) that atorvastatin treatment (40 mg daily) increased LPL activity in diabetic subjects. Increased postheparin LPL activity has also been seen in subjects with familial combined hyperlipidemia treated with 10–40 mg/day simvastatin (25), in CHD subjects treated with 10 mg/day simvastatin (26), and in high-fat/high-cholesterol-fed rabbits administered atorvastatin or simvastatin at 3 mg/kg/day (21). Our data confirm and extend these observations by showing that statin treatment also increases TRL-TG clearance.

An increase in LPL activity has also been demonstrated with high-dose simvastatin, but not high-dose atorvastatin, in high-fat/high-cholesterol-fed rabbits, in spite of equal lowering of TGs by the two drugs (21). The effect of atorvastatin on TG concentrations was thought to be attributable to an effect on hepatic VLDL synthesis, but a clear explanation for a difference between the two drugs in this rabbit model was not apparent. A similar increase in LPL activity has been seen in rats treated with simvastatin (27). However, neither drug has been shown to affect postheparin LPL activity in normolipidemic rabbits (28).

Other investigators have failed to demonstrate an increase in LPL activity in subjects treated with statins. Kobayashi et al. (29) assessed the effect of atorvastatin 10 mg daily in 21 Japanese hyperlipidemic subjects. Preheparin LPL

activity was not affected by atorvastatin therapy compared with pretreatment values. Similarly, in the Diabetes Atorvastatin Lipid Intervention Study, treatment with 10 or 80 mg atorvastatin daily did not affect postheparin LPL activity (30). Nordoy et al. (31) also failed to find an effect of 10 mg/day atorvastatin on preheparin or postheparin LPL activity in patients with combined hyperlipidemia. Similarly, Castro Cabezas et al. (32) failed to find an effect of atorvastatin at 10–80 mg daily on LPL activity in 18 subjects with familial combined hyperlipidemia. Interestingly, Schneider et al. (24) found that the atorvastatin-induced increase in LPL activity observed in their study accounted for only 37% of TG reduction. Therefore, they assumed that much of the effects of atorvastatin on serum TGs were on VLDL production rather than a decrease in VLDL-TG clearance. Atorvastatin has been shown to decrease VLDL apoB production in some (33) but not all (34, 35) studies in humans. Simvastatin has been shown to reduce VLDL production in normolipidemic subjects (36). The effects of statins on TRL metabolism may be dependent upon the mechanism producing the abnormality (17).

The differences in LPL activity observed between therapy with simvastatin and atorvastatin may reflect methodological differences or differences in study populations. LPL activity has been reported to be low in patients with diabetes (37). However, more recent studies (30, 38) have found normal LPL activity in diabetic patients, similar to the present study. Several potential mechanisms could explain a change in LPL activity during statin therapy. LPL activity can be modulated by alterations in apoC-II (a cofactor for LPL) or apoC-III (an inhibitor of LPL). Both simvastatin and atorvastatin treatment decrease apoC-III (39–41). A decrease in apoC-II levels has been observed in subjects treated with 10–80 mg/day atorvastatin (40). Interestingly, simvastatin has recently been shown to increase LPL activity and mRNA in rat heart and adipose tissue and to decrease apoC-III plasma levels and mRNA expression in the liver (42). Similar results have been seen in cell cultures of 3T3-L1 cells using atorvastatin (42, 43). Furthermore, recent work suggests that statins may activate peroxisome proliferator-activated receptor α, which would be expected to have positive effects on LPL activity (44). Alternatively, statin-mediated depletion of cholesterol may cause a change in sterol regulatory element binding protein, which can bind to the LPL promoter and enhance gene transcription (45).

The lack of effect of simvastatin on TRL-TG secretion is of interest. In a recent study on the kinetics of apoB-100 in nondiabetic subjects with mixed dyslipidemia, baseline hepatic VLDL production was higher and VLDL catabolism was lower relative to controls (18). Specifically, the dyslipidemic subjects produced increased numbers of VLDL particles of normal composition. Treatment with 80 mg/day atorvastatin resulted in decreased VLDL apoB concentrations in some (indicating decreased VLDL production) but not all subjects. Nonresponding subjects had lower apoB production rates at baseline compared with responders. Delipidation of $VLDL_1$ to $VLDL_2$ was increased by atorvastatin treatment by 140%, consistent with the findings of our study. Such a process may make VLDL particles more amenable to receptor-mediated removal.

Recent work by Forster and associates (46) might be viewed as contradictory to our findings. Using trideuterated leucine, the investigators assessed the effect of atorvastatin (40 mg daily) or simvastatin (40 mg daily) on apoB metabolism in nondiabetic subjects with mixed dyslipidemia. The drugs had no effect on VLDL apoB production but increased the clearance of apoB in all its lipoprotein fractions ($VLDL_1$, $VLDL_2$, intermediate density lipoprotein, and LDL). $VLDL_2$ and intermediate density lipoprotein were found to be relatively TG-enriched. The authors postulated that the increased clearance of all of these particles is related to increased receptor-mediated catabolism, although they acknowledged that this hypothesis was not universally consistent with published data (47). Important differences between our findings and those of Forster et al. (46) include our use of diabetic instead of nondiabetic subjects and the assessment of TRL-TG versus VLDL apoB (and other apoB-containing particles) kinetics. Delipidation of TG-rich particles may make them more susceptible to receptor-mediated clearance. It is possible that simvastatin altered the composition of the TRL particles in our study, rendering them a better substrate for LPL, rather than affecting LPL activity directly. Such an effect has been reported for ω-3 fatty acids (48). Studies in which particle number and composition are assessed simultaneously in the same subjects would shed further light on this possibility. Because we studied diabetic subjects, it is possible that apolipoprotein glycosylation has an effect on delipidation or receptor-mediated catabolism that may result in altered TRL metabolism (49, 50).

Our findings support a rationale for combination therapy for patients with mixed dyslipidemia. Statins exert a well-known effect on LDL-C, and our data indicate that in addition they decrease high TG concentrations by increasing TRL-TG clearance. The latter mechanism can be viewed as complementary to agents that reduce TRL-TG secretion, such as niacin (51) and high-dose fish oil (52). Because it is quite possible that cardiovascular risk in patients with mixed dyslipidemia is mediated by both TRL (possibly by altering the size and density of LDL) and LDL itself, therapies that affect both lipoprotein species may provide greater CHD risk reduction than therapies that target either lipoprotein species alone.

Our study has several potential limitations. The diabetic subjects were older and more obese than the controls. It is possible that some of the differences between the two groups can be explained by differences in age and body fat, not diabetes. Ideally, subjects (diabetic and nondiabetic) should be studied while not taking lipid-modulating drugs. Some of our subjects were taking estrogens or thiazolidinediones. Although these agents are known to affect lipid levels, the subjects were on stable doses throughout the study. It is not known whether established estrogen or thiazolidinedione therapy could obscure or modulate the effect of statin treatment on lipid levels. Lastly, we only studied the effect of high-dose simvastatin. The effect seems consistent with complementary studies with moderately

high-dose (40 mg daily) atorvastatin. It is possible that effects on TRL-TG metabolism may be different when using lower doses of statins. Our stable isotope method using monoexponential analysis is well standardized in normalweight and obese normolipidemic subjects but not in hypertriglyceridemic subjects (13, 15, 53–55).

In summary, our study confirms that high-dose simvastatin therapy significantly decreases TRL-TG in obese diabetic subjects and indicates that this reduction appears to be attributable to increased TRL-TG clearance rather than to decreased production. The most likely cause is an increase in intravascular lipolytic activity. This action of high-dose statins may contribute to their ability to reduce CHD risk over and above their effects on LDL-C.

This work was supported in part by a grant from Merck & Co and by National Institutes of Health Grant DK-56341 (Clinical Nutrition Research Unit, B.W.P.). The authors gratefully acknowledge the clinical, laboratory, and organizational support of Sheryl Windsor, Holly Bunyard, Amy Reinert, Crystal Russell-Lopez, Qing Lan Zhang, and Junyoung Kwon and the expert editorial assistance of Michelle Papaconstandinou.

REFERENCES

1. McGarry, J. D. 1992. What if Minkowski had been ageusic? An alternative angle on diabetes. *Science.* **258:** 766–770.
2. Havel, R. J., J. P. Kane, E. O. Balasse, N. Segel, and L. V. Basso. 1970. Splanchnic metabolism of free fatty acids and production of triglycerides of very low density lipoproteins in normotriglyceridemic and hypertriglyceridemic humans. *J. Clin. Invest.* **49:** 2017–2035.
3. 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.
4. Ginsberg, H. N. 1991. Lipoprotein physiology in nondiabetic and diabetic states. Relationship to atherogenesis. *Diabetes Care.* **14:** 839–855.
5. 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.
6. 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, and J. H. Fuller. 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.
7. 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.
8. Schaefer, E. J., J. R. McNamara, T. Tayler, J. A. Daly, J. L. Gleason, L. J. Seman, A. Ferrari, and J. J. Rubenstein. 2004. Comparisons of effects of statins (atorvastatin, fluvastatin, lovastatin, pravastatin, and simvastatin) on fasting and postprandial lipoproteins in patients with coronary heart disease versus control subjects. *Am. J. Cardiol.* **93:** 31–39.
9. Jones, P. H., M. H. Davidson, E. A. Stein, H. E. Bays, J. M. McKenney, E. Miller, V. A. Cain, and J. W. Blasetto. 2003. Comparison of the efficacy and safety of rosuvastatin versus atorvastatin, simvastatin, and pravastatin across doses (STELLAR* Trial). *Am. J. Cardiol.* **92:** 152–160.
10. Collins, R., J. Armitage, S. Parish, P. Sleigh, and R. Peto. 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.
11. Warnick, G. R., J. Benderson, and J. J. Albers. 1982. Dextran sulfate-Mg^{2+} precipitation procedure for quantitation of high-density-lipoprotein cholesterol. *Clin. Chem.* **28:** 1379–1388.
12. Friedewald, W. T., R. I. Levy, and D. S. Fredrickson. 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin. Chem.* **18:** 499–502.
13. Patterson, B. W., B. Mittendorfer, N. Elias, R. Satyanarayana, and S. Klein. 2002. Use of stable isotopically labeled tracers to measure very low density lipoprotein-triglyceride turnover. *J. Lipid Res.* **43:** 223–233.
14. Park, Y., P. G. Jones, and W. S. Harris. 2004. Triacylglycerol-rich lipoprotein margination: a potential surrogate for whole-body lipoprotein lipase activity and effects of eicosapentaenoic and docosahexaenoic acids. *Am. J. Clin. Nutr.* **80:** 45–50.
15. Lemieux, S., B. W. Patterson, A. Carpentier, G. F. Lewis, and G. Steiner. 1999. A stable isotope method using a $[^2H_5]$glycerol bolus to measure very low density lipoprotein triglyceride kinetics in humans. *J. Lipid Res.* **40:** 2111–2117.
16. 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.
17. Aguilar-Salinas, C. A., H. Barrett, and G. Schonfeld. 1998. Metabolic modes of action of the statins in the hyperlipoproteinemias. *Atherosclerosis.* **141:** 203–207.
18. Bilz, S., S. Wagner, M. Schmitz, A. Bedynek, U. Keller, and T. Demant. 2004. Effects of atorvastatin versus fenofibrate on apoB-100 and apoA-I kinetics in mixed hyperlipidemia. *J. Lipid Res.* **45:** 174–185.
19. 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.
20. Funatsu, T., K. Suzuki, M. Goto, Y. Arai, H. Kakuta, H. Tanaka, S. Yasuda, M. Ida, S. Nishijima, and K. Miyata. 2001. Prolonged inhibition of cholesterol synthesis by atorvastatin inhibits apo B-100 and triglyceride secretion from HepG2 cells. *Atherosclerosis.* **157:** 107–115.
21. Verd, J. C., C. Peris, M. Alegret, C. Diaz, G. Hernandez, M. Vazquez, T. Adzet, J. C. Laguna, and R. M. Sanchez. 1999. Different effect of simvastatin and atorvastatin on key enzymes involved in VLDL synthesis and catabolism in high fat/cholesterol fed rabbits. *Br. J. Pharmacol.* **127:** 1479–1485.
22. Scharnagl, H., R. Schinker, H. Gierens, M. Nauck, H. Wieland, and W. Marz. 2001. Effect of atorvastatin, simvastatin, and lovastatin on the metabolism of cholesterol and triacylglycerides in HepG2 cells. *Biochem. Pharmacol.* **62:** 1545–1555.
23. Busch, S. J., R. L. Barnhart, G. A. Martin, M. A. Flanagan, and R. L. Jackson. 1990. Differential regulation of hepatic triglyceride lipase and 3-hydroxy-3-methylglutaryl-CoA reductase gene expression in a human hepatoma cell line, HepG2. *J. Biol. Chem.* **265:** 22474–22479.
24. Schneider, J. G., M. von Eynatten, K. G. Parhofer, J. E. Volkmer, S. Schiekofer, A. Hamann, P. P. Nawroth, and K. A. Dugi. 2004. Atorvastatin improves diabetic dyslipidemia and increases lipoprotein lipase activity in vivo. *Atherosclerosis.* **175:** 325–331.
25. Castro Cabezas, M., T. W. de Bruin, M. Van Linde-Sibenius Trip, L. A. Kock, H. Jansen, and D. W. Erkelens. 1993. Lipoprotein(a) plasma concentrations associated with lipolytic activities in eight kindreds with familial combined hyperlipidemia and normolipidemic subjects. *Metabolism.* **42:** 756–761.
26. Piorunska-Stolzmann, M., A. Piorunska-Mikolajczak, and Z. Mikolajczyk. 2003. Effect of simvastatin on trioleylglycerol hydrolysis and transacylation with cholesterol in serum of outpatients with coronary heart disease. *Drugs Exp. Clin. Res.* **29:** 37–43.
27. Sato, A., K. Watanabe, H. Fukuzumi, K. Hase, F. Ishida, and T. Kamei. 1991. Effect of simvastatin (MK-733) on plasma triacylglycerol levels in rats. *Biochem. Pharmacol.* **41:** 1163–1172.
28. Alegret, M., J. C. Verd, C. Diaz, G. Hernandez, T. Adzet, R. M. Sanchez, and J. C. Laguna. 1998. Effect of hypolipidemic drugs on key enzyme activities related to lipid metabolism in normolipidemic rabbits. *Eur. J. Pharmacol.* **347:** 283–291.
29. Kobayashi, J., T. Maruyama, M. Masuda, and M. Shinomiya. 2001. Effect of atorvastatin treatment on lipoprotein lipase mass in the

pre-heparin plasma in Japanese hyperlipidemic subjects. *Clin. Chim. Acta.* **314:** 261–264.
30. The effect of aggressive versus standard lipid lowering by atorvastatin on diabetic dyslipidemia. The DALI study: a double-blind, randomized, placebo-controlled trial in patients with type 2 diabetes and diabetic dyslipidemia. *Diabetes Care.* 2001. **24:** 1335–1341.
31. Nordoy, A., J. B. Hansen, J. Brox, and B. Svensson. 2001. Effects of atorvastatin and omega-3 fatty acids on LDL subfractions and postprandial hyperlipemia in patients with combined hyperlipemia. *Nutr. Metab. Cardiovasc. Dis.* **11:** 7–16.
32. Castro Cabezas, M., C. Verseyden, S. Meijssen, H. Jansen, and D. W. Erkelens. 2004. Effects of atorvastatin on the clearance of triglyceride-rich lipoproteins in familial combined hyperlipidemia. *J. Clin. Endocrinol. Metab.* **89:** 5972–5980.
33. 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.
34. Watts, G. F., P. H. 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.
35. Chan, D. C., G. F. Watts, P. H. Barrett, T. A. Mori, L. J. Beilin, and T. G. Redgrave. 2002. Mechanism of action of a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor on apolipoprotein B-100 kinetics in visceral obesity. *J. Clin. Endocrinol. Metab.* **87:** 2283–2289.
36. Watts, G. F., R. P. Naoumova, J. M. Kelly, F. M. Riches, K. D. Croft, and G. R. Thompson. 1997. Inhibition of cholesterogenesis decreases hepatic secretion of apoB-100 in normolipidemic subjects. *Am. J. Physiol.* **273:** E462–E470.
37. Taskinen, M. R. 1987. Lipoprotein lipase in diabetes. *Diabetes Metab. Rev.* **3:** 551–570.
38. Rivellese, A. A., C. De Natale, L. Di Marino, L. Patti, C. Iovine, S. Coppola, S. Del Prato, G. Riccardi, and G. Annuzzi. 2004. Exogenous and endogenous postprandial lipid abnormalities in type 2 diabetic patients with optimal blood glucose control and optimal fasting triglyceride levels. *J. Clin. Endocrinol. Metab.* **89:** 2153–2159.
39. Bredie, S. J., H. T. Westerveld, H. C. Knipscheer, T. W. de Bruin, J. J. Kastelein, and A. F. Stalenhoef. 1996. Effects of gemfibrozil or simvastatin on apolipoprotein-B-containing lipoproteins, apolipoprotein-CIII and lipoprotein(a) in familial combined hyperlipidaemia. *Neth. J. Med.* **49:** 59–67.
40. Le, N. A., W. Innis-Whitehouse, X. Li, R. Bakker-Arkema, D. Black, and W. V. Brown. 2000. Lipid and apolipoprotein levels and distribution in patients with hypertriglyceridemia: effect of triglyceride reductions with atorvastatin. *Metabolism.* **49:** 167–177.
41. Lemieux, I., H. Salomon, and J. P. Despres. 2003. Contribution of apo CIII reduction to the greater effect of 12-week micronized fenofibrate than atorvastatin therapy on triglyceride levels and LDL size in dyslipidemic patients. *Ann. Med.* **35:** 442–448.
42. Schoonjans, K., J. Peinado-Onsurbe, J. C. Fruchart, A. Tailleux, C. Fievet, and J. Auwerx. 1999. 3-Hydroxy-3-methylglutaryl CoA reductase inhibitors reduce serum triglyceride levels through modulation of apolipoprotein C-III and lipoprotein lipase. *FEBS Lett.* **452:** 160–164.
43. Bey, L., P. Maigret, H. Laouenan, and M. T. Hamilton. 2002. Induction of lipoprotein lipase gene expression in 3T3-L1 preadipocytes by atorvastatin, a cholesterol- and triglyceride-lowering drug. *Pharmacology.* **66:** 51–56.
44. Landrier, J. F., C. Thomas, J. Grober, H. Duez, F. Percevault, M. Souidi, C. Linard, B. Staels, and P. Besnard. 2004. Statin induction of liver fatty acid-binding protein (L-FABP) gene expression is peroxisome proliferator-activated receptor-alpha-dependent. *J. Biol. Chem.* **279:** 45512–45518.
45. Fajas, L., K. Schoonjans, L. Gelman, J. B. Kim, J. Najib, G. Martin, J. C. Fruchart, M. Briggs, B. M. Spiegelman, and J. Auwerx. 1999. Regulation of peroxisome proliferator-activated receptor gamma expression by adipocyte differentiation and determination factor 1/sterol regulatory element binding protein 1: implications for adipocyte differentiation and metabolism. *Mol. Cell. Biol.* **19:** 5495–5503.
46. 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.
47. Cuchel, M., E. J. Schaefer, J. S. Millar, P. J. 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.
48. Lu, G., S. L. Windsor, and W. S. Harris. 1999. Omega-3 fatty acids alter lipoprotein subfraction distributions and the in vitro conversion of very low density lipoproteins to low density lipoproteins. *J. Nutr. Biochem.* **10:** 151–158.
49. Mamo, J. C., L. Szeto, and G. Steiner. 1990. Glycation of very low density lipoprotein from rat plasma impairs its catabolism. *Diabetologia.* **33:** 339–345.
50. Bucala, R., Z. Makita, G. Vega, S. Grundy, T. Koschinsky, A. Cerami, and H. Vlassara. 1994. Modification of low density lipoprotein by advanced glycation end products contributes to the dyslipidemia of diabetes and renal insufficiency. *Proc. Natl. Acad. Sci. USA.* **91:** 9441–9445.
51. Grundy, S. M., H. Y. Mok, L. Zech, and M. Berman. 1981. Influence of nicotinic acid on metabolism of cholesterol and triglycerides in man. *J. Lipid Res.* **22:** 24–36.
52. Harris, W. S., W. E. Connor, D. R. Illingworth, D. W. Rothrock, and D. M. Foster. 1990. Effects of fish oil on VLDL triglyceride kinetics in humans. *J. Lipid Res.* **31:** 1549–1558.
53. Mittendorfer, B., B. W. Patterson, and S. Klein. 2003. Effect of sex and obesity on basal VLDL-triacylglycerol kinetics. *Am. J. Clin. Nutr.* **77:** 573–579.
54. Mittendorfer, B., B. W. Patterson, S. Klein, and L. S. Sidossis. 2003. VLDL-triglyceride kinetics during hyperglycemia-hyperinsulinemia: effects of sex and obesity. *Am. J. Physiol. Endocrinol. Metab.* **284:** E708–E715.
55. Mittendorfer, B., B. W. Patterson, and S. Klein. 2003. Effect of weight loss on VLDL-triglyceride and apoB-100 kinetics in women with abdominal obesity. *Am. J. Physiol. Endocrinol. Metab.* **284:** E549–E556.