The HMG-CoA Reductase Inhibitor Atorvastatin Increases the Fractional Clearance Rate of Postprandial Triglyceride-Rich Lipoproteins in Miniature Pigs

John R. Burnett, P. Hugh R. Barrett, Paolo Vicini, David B. Miller, Dawn E. Telford, Sandra J. Kleinstiver, Murray W. Huff

Abstract—We have previously shown in vivo that the 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor atorvastatin decreases hepatic apolipoprotein B (apoB) secretion into plasma. To test the hypothesis that atorvastatin modulates exogenous triglyceride-rich lipoprotein (TRL) metabolism in vivo, an oral fat load (2 g fat/kg body wt) containing retinol (50,000 IU) was given to 6 control miniature pigs and to 6 animals after 28 days of treatment with atorvastatin 3 mg \( \cdot \) kg\(^{-1} \cdot \) d\(^{-1} \). A multicompartmental model was developed by use of SAAM II and kinetic analysis performed on the plasma retinyl palmitate (RP) data. Peak TRL (\(d<1.006 \) g/mL; \(S_f>20\)) triglyceride concentrations were decreased 29% by atorvastatin, and the time to achieve this peak was delayed (5.2 versus 2.3 hours; \(P<0.01\)). The TRL triglyceride 0- to 12-hour area under the curve was decreased by 24%. In contrast, atorvastatin treatment had no effect on peak TRL RP concentrations, time to peak, or its rate of appearance into plasma; however, the TRL RP 0- to 12-hour area under the curve was decreased by 20%. Analysis of the RP kinetic parameters revealed that the TRL fractional clearance rate was increased significantly, 1.4-fold (3.093 versus 2.276 pools/h; \(P=0.012\)), with atorvastatin treatment. The percent conversion of TRL RP from the rapid-turnover to the slow-turnover compartment was decreased by 47% with atorvastatin treatment. The TRL RP fractional clearance rate was negatively correlated with very low density lipoprotein apoB production rate measured in the fasting state (\(r=-0.49\)). Thus, although atorvastatin had no effect on intestinal TRL assembly and secretion, plasma TRL clearance was significantly increased, an effect that may relate to a decreased competition for removal processes by hepatic very low density lipoprotein.

Key Words: HMG-CoA reductase inhibitor • atorvastatin • triglyceride-rich lipoproteins • tracer kinetics • compartmental model

Chylomicrons are large, triglyceride-rich, apolipoprotein (apo) B-48–containing lipoproteins that are synthesized and secreted by the enterocyte to transport dietary fat to peripheral tissues. In contrast, VLDLs contain apoB-100 and transport hepatically synthesized triglycerides to their sites of utilization in the peripheral tissues. The assembly and secretion of apoB-containing lipoproteins from the enterocyte and hepatocyte are both complex processes; however, they involve a number of similar steps, as suggested by the absence of both chylomicrons and VLDLs from the plasma of subjects with abetalipoproteinemia.1 In the peripheral circulation, both chylomicrons and VLDLs share a saturable common metabolic removal pathway catalyzed by the hydrolytic enzyme lipoprotein lipase (LPL).2–5 Furthermore, the metabolic end products of the lipolytic process, chylomicron remnants6 and LDLs,7 are cleared from the plasma predominantly by hepatic receptor–mediated processes.

There is increasing evidence from cell culture studies in vitro8,9 and human studies in vivo10–15 that postprandial, triglyceride-rich, apoB-containing lipoproteins are atherogenic. Furthermore, these postprandial lipoproteins may be more closely related to atherogenic risk than those lipoproteins in the fasting state. Consequently, the measurement of postprandial lipids and lipoproteins may be superior to fasting lipid analyses in predicting the existence and/or progression of coronary heart disease.

The most commonly used approach for studying chylomicron metabolism is an indirect one, involving the oral administration of vitamin A (retinol) to endogenously label intestinally derived triglyceride-rich lipoproteins (TRLs).16 The rationale is that retinol is taken up by enterocytes, esterified to retinyl esters [predominantly retinyl palmitate (RP)], incorporated along with other neutral lipids into the core of the chylomicron,17 and secreted into the lymphatics. The assumption that the retinyl esters remain associated with the chylomicron remnant, however, may not be entirely correct. Krasinski et al18 found in humans that during the late postprandial period (\(\geq9\) hours), up to one third of the remaining plasma RP was associated with

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HDL and LDL. Nevertheless, RP data provide a close approximation of plasma chylomicron and chylomicron remnant metabolism during the early postprandial period. The plasma concentrations of retinyl esters with time reflect the balance between (1) the assembly and secretion of chylomicrons into plasma and (2) the formation and clearance of chylomicron remnants. Most studies to date have used the areas under the retinyl ester curves to determine differences in the kinetics of intestinally derived retinyl esters in the postprandial state. However, this approach does not allow one to distinguish the secretion rate from the clearance rate.

3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, an endoplasmic reticulum (ER) protein, catalyzes the rate-determining reductive deacylation of HMG-CoA to mevalonate in cholesterol biosynthesis. A class of lipid-lowering compounds, the HMG-CoA reductase inhibitors, have been shown to interfere with the rate-limiting step in cholesterol biosynthesis. These compounds are potent LDL lowering compounds, the HMG-CoA reductase inhibitors, have been the focus of a recent review by Huff and Burnett.

As reviewed by Cohn, many studies have examined the effect of HMG-CoA reductase inhibitors on postprandial lipoprotein metabolism. Although the HMG-CoA reductase inhibitors as a class tend to decrease postprandial triglyceride concentrations, the effects of treatment are inconsistent but appear to correlate with the degree of fasting plasma triglyceride reduction. No multicompartmental modeling of any retinyl ester kinetic data, however, has been performed in any of these studies.

Atorvastatin is a new synthetic, chiral, tissue-selective inhibitor of HMG-CoA reductase. Clinical trials of atorvastatin in humans have demonstrated marked plasma LDL cholesterol and triglyceride reductions. We have previously shown in vivo that inhibition of HMG-CoA reductase by atorvastatin in miniature pigs significantly decreases hepatic apoB-containing lipoprotein secretion into plasma in the fasting state, with minimal effect on rate of clearance.

We have now developed a multicompartmental model of TRL metabolism and have used kinetic analysis to determine the metabolic parameters of postprandial exogenous TRL in plasma with atorvastatin treatment. These studies were designed to test the hypothesis that the inhibition of cholesterol biosynthesis by atorvastatin modulates exogenous TRL metabolism in vivo.

**Methods**

**Animals and Diets**

Miniature pigs weighing 22 to 25 kg were obtained from a local supplier (Premier Quality Genetics Inc, West Lorne, Ontario). After being acclimated for 1 week, animals were maintained on the experimental diet for 28 days before the postprandial studies. Pigs were studied in pairs, with each pair being same-sex littermates. Each animal received a 590-g ration of diet (B.W.S. Hog Grower, B-W Feed and Seed Ltd) supplemented with lard, butter, and safflower oil (1:0.6:0.2), generating a final polyunsaturated/monounsaturated/saturated fatty acid ratio of 1:1:1. Cholesterol (Fisher Scientific) was added to the diet to a final concentration of 0.1% (0.2 mg/kcal). This diet provided 34% of calories from fat, 49% as carbohydrate, and 17% as protein.

Six animals received the HMG-CoA reductase inhibitor atorvastatin (Parke-Davis) at a dose of ~3 mg · kg body wt · d⁻¹ (80 mg/d) for 28 days before postprandial studies. This dose is equivalent to the maximum therapeutic dose used in humans but is ~2.6-fold greater than that used in humans when body weight is taken into consideration. Atorvastatin is a synthetic inhibitor of HMG-CoA reductase with an IC₅₀ of 7.5 mmol/L for rat liver microsomal HMG-CoA reductase activity. The chemical structure of atorvastatin has been illustrated previously. Atorvastatin was placed in gelatin capsules and, to ensure ingestion, was administered by hand before the daily feeding. The 6 control animals received a placebo capsule. The atorvastatin was given at 9 AM each day after a 24-hour fast.

Two weeks before the postprandial studies, an indwelling silicone elastomer (Silastic) catheter (1.96-mm ID) was surgically implanted in an external jugular vein. Isoflurane USP (Abbott Laboratories Ltd) was used as the anesthetic and ketamine USP (Vetapharm Canada Inc) as the preanesthetic. Catheters that were kept patent by being filled with 7% Dextran 70 allowed blood sampling throughout each postprandial study in unrestrained, unanesthetized animals. The Animal Care Committee of the University of Western Ontario approved the experimental protocol.

**Oral Fat Tolerance Test**

After a 24-hour fast, pigs were fed the diet described above in an amount calculated to provide 2 g fat/kg body wt and either placebo or atorvastatin. This test meal was supplemented with 50 000 IU of retinol (vitamin A capsules USP, Novopharm Ltd) and consumed within 10 minutes. The animals were not fed for the 12 hours of the study but had free access to drinking water. Venous blood samples (20 mL) were drawn at 0 (before the test meal), 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, and 12 hours and collected into tubes containing EDTA-Na₂. Samples were kept on ice before isolation of plasma lipoproteins and protected from light during processing. Plasma was obtained by centrifugation at 1000g for 25 minutes at 4°C. The isolated plasma underwent preparative ultracentrifugation at d = 1.006 g/mL in a Beckman 50.4 Ti rotor at 35 500 rpm at 12°C for 16 hours. TRL fractions (d < 1.006 g/mL; S₃₅ > 20) were isolated by tube slicing, and each plasma sample and TRL fraction was analyzed for cholesterol, triglyceride, and retinyl ester concentrations.

**Retinyl Ester Analysis**

Retinyl ester (RP and retinyl stearate) concentrations were determined in total plasma and in the TRL fraction by a modification of the high-performance liquid chromatography (HPLC) method of Weintraub et al. Extractions and analyses were carried out with HPLC-grade solvents and under subdued light. Retinyl acetate was added to the samples as an internal standard, and retinyl esters were extracted by a mixture of ethanol, hexane, and water (1:5:0.5). The hexane layer was evaporated under nitrogen. The samples were redissolved in ethanol and separated on a 5-μm Hypersil C18 (7.5 × 0.32 cm) column. Pump A consisted of 88% methanol in water, whereas pump B consisted of 75% methanol and 25% isopropanol. Pump A was used at 100% for the first 4 minutes, reduced to 20% pump A to 9 minutes, and further reduced to 0% pump A to 12 minutes, at a flow rate of 0.6 mL/minute. The absorbance of the eluent was measured at 325 nm, and the retinyl ester concentrations were quantified by ratio of peak height to that of the internal standard. Areas under curves (AUCs) for the retinyl ester were calculated. Within-assay and between-assay coefficients of variation for TRL RP were <4.0% and <8.6%, respectively.

**Retinyl Ester Kinetic Analysis**

A compartmental model of TRL metabolism was developed by use of the multicompartmental modeling program SAAM II (SAAM Institute Inc, Seattle, WA) running on a Pentium-based personal computer. The model was developed assuming that the fractional rate constants (k) were time invariant and first order. The model chosen to describe the
data is shown in Figure 1. This model is locally identifiable\cite{44,45} in that it has, a priori, 1 solution for \( k(2,1) \) and \( k(0,3) \) but 2 distinct solutions for \( k(0,4) \) and \( k(4,3) \). One of the 2 solutions for \( k(4,3) \) is consistently negative and therefore must be rejected (all transfer constants must be positive). Thus, the model is uniquely identifiable, with the constraint that all of the transfer rates must be positive. Dietary retinol was used to endogenously label intestinally derived TRL with RP.

Figure 1 shows the compartments of the model and the pathways that connect the compartments. Compartment 1 represents the dosing compartment. On the basis of earlier studies,\cite{43} it was assumed that all of the ingested retinol appeared in chylomicrons as RP. Compartment 2, the delay compartment, contains 5 compartments in series. It was assumed that the residence time of material in compartment 1 and in the delay compartments (compartment 2) were equal. The functions of these compartments were to provide a delay that corresponded to the time required for the synthesis of chylomicrons and their secretion into plasma. From the delay compartment, material enters compartment 3, which is assumed to represent a rapid-turnover chylomicron pool. Chylomicrons in compartment 3 are either removed from plasma or converted to a slower-turnover plasma pool, compartment 4. Chylomicrons and chylomicron remnants in compartment 4 are subsequently removed from plasma. The sum of compartments 3 and 4 represents total plasma TRL RP.

The compartmental model was fit to each individual data set. Simplifying the model to 1 plasma chylomicron pool clearly demonstrated the necessity for the more complex model structure to describe the tail component of the data. We were unable, however, to obtain acceptable coefficients of variation for any of the kinetic parameters reported for animal 3 in the atorvastatin-treated group. The experimental data for this animal did not fit the proposed model, presumably for technical reasons, and this pig represents an outlier relative to the whole population and was thus excluded from subsequent analysis.

**Relationship Between TRL Fractional Clearance Rate (FCR) and Fasting Hepatic VLDL ApoB Synthetic Rates**

To examine the relationship between the kinetics of postprandial retinyl esters and those of fasting hepatic apoB synthesis, 11 pigs (6 control and 5 atorvastatin-treated) participated in both an oral fat tolerance test and the atorvastatin treatment. The TRL RP FCR was increased by 20% (Figure 4; Table 2) and 24%, respectively, in the atorvastatin-treated animals; however, these reductions were not statistically significant. Moreover, the peak TRL triglyceride concentration was decreased 29%, and the time to achieve this peak was delayed (5.2 versus 2.3 hours; \( P<0.01 \)). Consistent with the decrease in peak TRL triglyceride, a decrease in the ratio of TRL triglyceride to protein at the 2-hour time point was observed in all atorvastatin-treated animals. However, this 23% decrease was not statistically significant (\( P=0.081 \); Table 3). The 0- to 12-hour AUC for plasma and TRL RP decreased by 16% (\( P=0.047 \); Figure 4) and 15% (\( P=0.193 \)), respectively. When total plasma retinyl esters were compared, similar differences were observed. No significant differences were seen in either plasma or TRL retinyl esters for any single time point between the control and treatment groups.

A fit of the model to the TRL RP data using the parameters derived from the kinetic analysis for a representative atorvastatin-treated animal is shown in Figure 5. A fit of the model to the mean TRL RP data using the parameters derived from the kinetic analysis is shown in Figure 6. The fractional rate constants, delay times, and FCRs derived from the model analyses are shown in Table 4. The TRL RP \( k(4,3) \) between the rapid-turnover and slow-turnover plasma compartments was decreased by 78% (0.306 versus 1.074 pools/h; \( P=NS \)) with atorvastatin treatment. The TRL RP FCR was increased.
by 1.4-fold in the atorvastatin-treated pigs (3.093 versus 2.276 pools/h; \( P = 0.012 \)). The percent conversion of TRL RP from the rapid-turnover to the slow-turnover compartment decreased markedly, by 47% \( (P = \text{NS}) \), with atorvastatin treatment. The TRL RP FCR was inversely correlated with the change in VLDL apoB production rate \( (r = -0.49) \) for the 11 animals for which fasting apoB kinetic data were also determined (Figure 7). However, the observed relationship was not statistically significant.

Because the chylomicron pool size is changing with respect to time, it is not possible to determine absolute secretion rates. Each pig in the study received the same dose of retinol, and we have made the assumption that the amount of retinol per TRL particle secreted was the same for the control and treatment animals. However, 3 lines of evidence suggest no significant change in the number of intestinal particles assembled and secreted into plasma with atorvastatin treatment. First, the TRL RP curves overlapped during the first 1.5 hours (Figure 4). Second, the TRL RP peak heights and times to peak coincided in both the control and treatment animals. Third, the integral of the mass in compartment 2 provides a time course for the accumulation of the dose in the plasma. We can predict from the model that the time required for 50% of the retinol to appear in the plasma was unaffected by atorvastatin treatment (1.94 versus 1.98 hours; \( P = \text{NS} \)).

**Discussion**

The present experiments were designed to test the hypothesis that inhibition of cholesterol biosynthesis by the potent HMG-CoA reductase inhibitor atorvastatin modulates exogenous TRL metabolism in vivo. These experiments were carried out in miniature pigs fed a fat- and cholesterol-containing diet and either placebo (control) or atorvastatin 3 mg·kg\(^{-1}\)·d\(^{-1}\) (80 mg/d) for 28 days before

### TABLE 2. Postprandial Total Plasma and TRL Lipid, Lipoprotein, and RP Concentrations in Control and Atorvastatin-Treated Miniature Pigs

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Atorvastatin</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak plasma TG, (^*) mmol/L</td>
<td>1.347±0.147</td>
<td>0.984±0.037</td>
<td>NS</td>
</tr>
<tr>
<td>Time to peak plasma TG, h</td>
<td>2.3±0.2</td>
<td>5.2±0.9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>TG AUC 0–12 h, mmol · L(^{-1})·h(^{-1})</td>
<td>7.83±0.75</td>
<td>6.28±0.97</td>
<td>NS</td>
</tr>
<tr>
<td>Peak TRL TG, (^†) mmol/L</td>
<td>1.194±0.140</td>
<td>0.851±0.182</td>
<td>NS</td>
</tr>
<tr>
<td>Time to peak TRL TG, h</td>
<td>2.3±0.2</td>
<td>5.2±0.9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>TRL TG AUC 0–12 h, mmol · L(^{-1})·h(^{-1})</td>
<td>6.63±0.67</td>
<td>5.06±0.82</td>
<td>NS</td>
</tr>
<tr>
<td>Peak plasma RP, ( \mu g/L )</td>
<td>1562±201</td>
<td>1703±250</td>
<td>NS</td>
</tr>
<tr>
<td>Time to peak plasma RP, h</td>
<td>2.3±0.2</td>
<td>2.0±0.4</td>
<td>NS</td>
</tr>
<tr>
<td>RP AUC 0–12 h, ( \mu g · L^{-1}·h^{-1} )</td>
<td>6605±419</td>
<td>5279±710</td>
<td>NS</td>
</tr>
<tr>
<td>Peak TRL RP, ( \mu g/L )</td>
<td>1356±162</td>
<td>1451±240</td>
<td>NS</td>
</tr>
<tr>
<td>Time to peak TRL RP, h</td>
<td>2.3±0.2</td>
<td>2.0±0.3</td>
<td>NS</td>
</tr>
<tr>
<td>TRL RP AUC 0–12 h, ( \mu g · L^{-1}·h^{-1} )</td>
<td>5096±376</td>
<td>4054±720</td>
<td>NS</td>
</tr>
</tbody>
</table>

\(^*\)TG indicates triglyceride; RP, retinyl palmitate. Values represent mean±SEM of determinations from the 6 control and 5 atorvastatin-treated animals.

\(^†\)TRL TG and RP were determined after ultracentrifugation at \( d < 1.006 \) g/mL.
the postprandial studies. These studies used retinol, given with an oral fat load, to endogenously label chylomicrons and their remnants. The results obtained in a multicompartmental model clearly demonstrate that atorvastatin treatment significantly increases the clearance of postprandial TRLs from plasma but has no apparent effect on exogenous TRL synthesis and secretion. The major findings were that atorvastatin treatment (1) significantly decreased fasting cholesterol, triglyceride, and VLDL triglyceride concentrations in plasma; (2) decreased the 0- to 12-hour TRL R P AUC; (3) increased TRL R P FCR; and (4) decreased the percent conversion from the rapid-turnover plasma compartment to the slow-turnover plasma compartment. Furthermore, the TRL R P FCR was negatively correlated with the VLDL apoB production rate.

We have demonstrated previously in miniature pigs that atorvastatin treatment decreases fasting cholesterol, triglyceride, and VLDL triglyceride concentrations in plasma, similar responses have been reported in human subjects. In the present study, decreases were seen in postprandial TRL cholesterol, triglyceride, and RP concentrations. The shapes of the TRL cholesterol and triglyceride curves were similar, with a reduced AUC during the first 4 hours of the study in atorvastatin-treated animals but overlapping curves during the final 8 hours of the study. In contrast, the TRL RP curves were overlapping during the first 1.5 hours after the fat meal, with the atorvastatin-treated pigs having lower values than control animals at the latter time points. The rate at which plasma triglycerides increase after a fat meal is a function of gastric emptying, intestinal absorption, chylomicron assembly and secretion, and LPL-mediated catabolism. However,

**TABLE 3. Postprandial TRL Triglyceride and Protein Concentrations at 2 h in Control and Atorvastatin-Treated Miniature Pigs**

<table>
<thead>
<tr>
<th>TRL Triglyceride, g/L</th>
<th>TRL Protein, g/L</th>
<th>TRL TG/Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.33</td>
<td>0.112</td>
</tr>
<tr>
<td>2</td>
<td>1.23</td>
<td>0.109</td>
</tr>
<tr>
<td>3</td>
<td>1.19</td>
<td>0.105</td>
</tr>
<tr>
<td>4</td>
<td>0.78</td>
<td>0.087</td>
</tr>
<tr>
<td>5</td>
<td>0.30</td>
<td>0.049</td>
</tr>
<tr>
<td>6</td>
<td>0.85</td>
<td>0.082</td>
</tr>
<tr>
<td>Mean</td>
<td>0.95</td>
<td>0.090</td>
</tr>
<tr>
<td>SEM</td>
<td>0.16</td>
<td>0.010</td>
</tr>
<tr>
<td><strong>Atorvastatin</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.49</td>
<td>0.078</td>
</tr>
<tr>
<td>2</td>
<td>0.96</td>
<td>0.090</td>
</tr>
<tr>
<td>4</td>
<td>0.42</td>
<td>0.057</td>
</tr>
<tr>
<td>5</td>
<td>0.25</td>
<td>0.035</td>
</tr>
<tr>
<td>6</td>
<td>0.40</td>
<td>0.055</td>
</tr>
<tr>
<td>Mean</td>
<td>0.50</td>
<td>0.063</td>
</tr>
<tr>
<td>SEM</td>
<td>0.12</td>
<td>0.010</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

*TRL triglyceride and protein were determined after ultracentrifugation at d≤1.006 g/mL.

The increase in postprandial triglycerides may not be entirely of intestinal origin, because recent studies have shown that hepatogenous VLDL triglycerides contribute appreciably to the TRL triglyceride response, particularly after the peak. As discussed below, this may be due to enhanced competition for VLDL triglyceride clearance by postprandial chylomicrons.

Our findings of decreased postprandial RP concentrations, most noticeable at the latter time points, are consistent with

**Figure 4.** Total plasma and TRL RP concentration curves. Results shown are mean±SEM for 6 control and 5 atorvastatin-treated animals. ● indicates atorvastatin; ○, control.

**Figure 5.** TRL RP concentration curve. Data points represent observed data, and the line is the best fit generated by the kinetic model. Results shown are for a representative atorvastatin-treated animal.
those found by Castro Cabezas et al\textsuperscript{25} in familial combined hyperlipidemic subjects treated with simvastatin (mean dose, 24.5 mg/d), Weintraub et al\textsuperscript{23} in type IIb subjects treated with lovastatin (80 mg/d), Gylling et al\textsuperscript{28} in type III (apoE2/2) hyperlipoproteinemic subjects treated with lovastatin (40 mg/d), and Simo et al\textsuperscript{26} in subjects with mild to moderate hypertriglyceridemia (1.75 to 7.5 mmol/L) and low HDL (≤0.9 mmol/L) treated with lovastatin (40 mg/d). These reductions were associated with a mean reduction of 23\% for the plasma RP AUC in these 4 studies. Statistically significant reductions of RP were achieved only in the studies by Weintraub et al\textsuperscript{23} for plasma, $S_f^{1000}$, and $S_f^{1000}$ 0- to 24-hour AUCs and Castro Cabezas et al\textsuperscript{25} for the $S_f^{1000}$ 0- to 24-hour AUC. However, the mechanism for the decreased AUCs could not be determined, because multicompartmental modeling was not carried out.

Although a large number of studies have investigated postprandial lipoprotein metabolism, as reviewed by Cohn,\textsuperscript{22} few have undertaken multicompartmental modeling to analyze chylomicron metabolism.\textsuperscript{43,49–53} To further investigate the apparent enhanced clearance of TRL RP, we developed a multicompartmental model of TRL metabolism, 2 compartments of which represent the total plasma TRL RP. We found that the TRL RP FCR was increased by 1.4-fold with atorvastatin treatment. Furthermore, the percent conversion of RP from the rapid-turnover plasma compartment to the slow-turnover plasma compartment was decreased, by 26\%, in atorvastatin-treated pigs. Although Martins et al.\textsuperscript{54} using lipid emulsions in rats, demonstrated that particle number is

![Figure 6. TRL RP concentration curves. Data points represent observed data, and lines are the best fit generated by the kinetic model. Results shown are for 6 control- and 5 atorvastatin-treated animals. ● indicates atorvastatin; ○, control.](image1)

![Figure 7. Relationship between TRL FCRs and fasting VLDL apoB production rates. Results shown are for 6 control and 5 atorvastatin-treated animals that had both RP and apoB kinetic studies performed. ● indicates atorvastatin; ○, control.](image2)

| Table 4. Calculated Fractional Rate Constants, Delay Times, and FCRs of TRL RP Metabolism in Control and Atorvastatin-Treated Miniature Pigs |
|-----------------|-----------|-----------|-----------|-----------|-----------|-----------|
|                 | $k(2,1)^*$ | $k(0,3)$  | $k(4,3)$  | $k(0,4)$  | FCR       | Conversion |
|                 | Pools/h    | Pools/h   | Pools/h   | Pools/h   | Pools/h   | 3→4, %    |
| Control         |           |           |           |           |           |           |
| 1               | 3.047      | 1.641     | 6.796     | 0.295     | 0.141     | 2.292     | 4.2 |
| 2               | 3.423      | 1.461     | 6.305     | 0.413     | 0.219     | 2.333     | 6.1 |
| 3               | 2.986      | 1.675     | 5.696     | 0.416     | 0.208     | 2.037     | 6.8 |
| 4               | 4.162      | 1.201     | 24.679    | 4.506     | 0.313     | 1.898     | 15.4 |
| 5               | 1.963      | 2.547     | 6.516     | 0.112     | 0.087     | 2.903     | 1.7 |
| 6               | 2.604      | 1.920     | 9.126     | 0.699     | 0.201     | 2.195     | 7.1 |
| Mean            | 3.031      | 1.741     | 9.853     | 1.074     | 0.195     | 2.276     | 6.9 |
| SEM             | 0.303      | 0.189     | 3.004     | 0.691     | 0.031     | 0.142     | 1.9 |
| Atorvastatin    |           |           |           |           |           |           |
| 1               | 1.923      | 2.600     | 7.806     | 0.157     | 0.148     | 3.858     | 2.0 |
| 2               | 3.427      | 1.459     | 4.645     | 0.496     | 0.438     | 2.411     | 9.6 |
| 4               | 2.578      | 1.939     | 6.713     | 0.114     | 0.094     | 3.090     | 1.7 |
| 5               | 4.234      | 1.181     | 10.798    | 0.309     | 0.121     | 3.127     | 2.8 |
| 6               | 4.489      | 1.114     | 5.399     | 0.241     | 0.152     | 2.980     | 2.3 |
| Mean            | 3.330      | 1.659     | 7.072     | 0.241     | 0.191     | 3.093     | 5.1 |
| SEM             | 0.486      | 0.277     | 1.078     | 0.073     | 0.063     | 0.231     | 1.9 |

$k$ indicates fractional rate constant; $dt$, delay time.
an important factor regulating the metabolism of chylomicrons and their remnants, we did not find any evidence for a difference in chylomicron secretion with atorvastatin treatment. The TRL RP curves overlapped during the first 1.5 hours, and TRL RP peak heights and times to peak coincided in both the control and treatment animals.

The mechanism whereby the liver rapidly and specifically removes chylomicron remnants is still unclear. The hepatic uptake of chylomicron remnants has been shown to be mediated by the LDL receptor and LDL receptor–related protein (LRP).55–59 and turnover studies suggest that the liver is the main site of chylomicron metabolism.60 Various possible ligand proteins, including apoE, LPL, and hepatic lipase, have been proposed.61 Heparan sulfate proteoglycans (HSPGs) have been shown to enhance chylomicron clearance.62,63 The VLDL receptor has been implicated in chylomicron remnant removal by nonhepatic tissues.64,65 We have previously shown that inhibition of cholesterol synthesis by atorvastatin decreases both VLDL and LDL apoB production rates in the miniature pig.36 In these studies, neither the VLDL apoB FCR nor LDL apoB FCR was significantly affected by atorvastatin treatment. Furthermore, hepatic or intestinal LDL receptor mRNA abundances, as measured by RNase protection assay, were also unchanged. Our results in this animal model would suggest that upregulation of LDL receptors is not the reason for the enhanced TRL clearance. We could not exclude the possibility of an effect of atorvastatin treatment on LRP or VLDL receptor expression.

Our results differ from those of other postprandial studies using HMG-CoA reductase inhibitors, in that we demonstrated reductions in postprandial TRL triglycerides in the early postprandial period (0 to 4 hours). In contrast to the studies by Simo et al26 and Castro Cabezas et al,25 in which the reduced triglyceride concentrations observed at baseline were maintained throughout the duration of the study, the differences we observed in the postprandial period with atorvastatin treatment were greater than the differences observed at baseline and were confined to the first 4 hours in the postprandial period. This may represent a unique effect of atorvastatin treatment.

The decrease in TRL triglyceride seen in the early (0 to 4 hours) postprandial phase presumably represents a change in lipoprotein composition of postprandial intestinal and/or hepatic lipoproteins with atorvastatin treatment. Consistent with the decrease in peak TRL triglyceride, a trend toward a decrease (23%) in the ratio of TRL triglyceride to protein at the 2-hour time point was observed with atorvastatin treatment (Table 3). It is possible that atorvastatin treatment results in the secretion of a chylomicron particle with a reduced triglyceride content and/or an altered apolipoprotein composition, thereby allowing a more rapid rate of lipolysis and subsequent hepatic clearance of triglyceride-depleted remnants.

Postprandial apolipoproteins, particularly apoC and apoE, and lipid compositional changes in chylomicrons, their remnants, and VLDL, have been demonstrated in humans by immunoaffinity chromatography.66,67 ApoC-III, a component of chylomicrons and VLDL, has been shown to play an important role in TRL metabolism. In vitro, apoC-III can inhibit the hydrolysis of triglyceride68,69 and reduce TRL clearance.70,71 In vivo, transgenic mice overexpressing human apoC-III develop a marked hypertriglyceridemia resulting from impaired clearance of TRL due to apoE insufficiency.72,73 In contrast, homozygous apoC-III knockout mice have hypotriglyceridemia and enhanced TRL clearance.74 ApoE-enriched subfractions of large VLDL from hypertriglyceridemic subjects show enhanced triglyceride hydrolysis by LPL.75 ApoE is important for the hepatic recognition of TRL remnants by the LRP.76,77 The LRP,55–59 and HSPGs.62 HSPGs play a significant role in plasma clearance and hepatic uptake of TRL remnants in mice.53 It is possible that atorvastatin treatment decreases apoC-III and/or increases apoE concentrations of TRL. However, in the present study, TRL apoC-III and apoE concentrations were not determined.

Large TRLs, and in particular chylomicrons, are the favored substrates for LPL. Essentially all TRLs share the same lipolytic pathway, that is, the hydrolysis of the core triglyceride content by LPL. After a fat meal, chylomicrons and endogenous TRLs of hepatic origin (VLDL) are mixed in blood and can compete for LPL. It has been shown that endogenous TRLs accumulate in plasma after oral fat intake11,47,48 because of the failure of these lipoproteins to effectively compete with chylomicrons for lipolysis by LPL.2–5 We have established that in the fasting state, atorvastatin treatment of miniature pigs decreases the production rate of hepatic VLDL apoB.86 Assuming that atorvastatin continued to decrease hepatic VLDL apoB secretion in the postprandial state, the enhanced fractional clearance of intestinally derived TRL observed with atorvastatin treatment would be consistent with decreased competition for removal processes by hepatic VLDL. A negative correlation was observed between TRL (d<1.006 g/mL; Sf 20) RP FCR and VLDL apoB production rate (r = −0.49; P = NS) in the 11 animals in which both protocols had been carried out. Consistent with this observation, Le et al,51 in a group of normolipidemic controls and hypertriglyceridemic subjects, found a positive correlation between the production rate for VLDL apoB in the fasting state and the half-life (the inverse of FCR) of retinyl esters in both Sf 20 to 400 (mostly remnants) and Sf 400 lipoproteins.

In conclusion, we have demonstrated in a multicompartment model of TRL metabolism that the inhibition of HMG-CoA reductase by atorvastatin has no significant effect on intestinal TRL assembly and secretion; however, plasma clearance of TRL was increased, perhaps related to decreased competition for removal processes by hepatic VLDL.

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