Experimental Model of Postprandial Hypertriglyceridemia in Sucrose-Fed Rats and the Effectiveness of Atorvastatin in the Model

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Although postprandial hypertriglyceridemia has drawn attention as an independent risk factor of cardiovascular disease, there is no established animal model that shows a physiological transitory change in lipoprotein metabolism after ingestion of a fatty meal. We developed an animal model of postprandial hypertriglyceridemia using sucrose-fed rats, and used this model to evaluate the effect of atorvastatin on this condition. Compared with normal rats, sucrose-fed rats orally loaded with olive oil showed a high and prolonged increase in plasma triglyceride (TG) concentration accompanied by both an increase in TG secretion and decrease in TG clearance. Atorvastatin (30 mg/kg orally) for 2 weeks reduced not only fasting plasma TG concentration, but also the postprandial TG concentration. Atorvastatin also suppressed rates of TG secretion in both chylomicron (CM)-rich ($d < 0.96$ g/mL) and very–low-density lipoprotein (VLDL) ($d = 0.96$ to1.006 g/mL) fractions after oral fat loading. Further, atorvastatin improved the elimination time of exogenous TG emulsion only in the nonfasted, namely, high plasma TG condition. These results indicate that this animal model satisfactorily replicates the postprandial hypertriglyceridemia observed in humans and may therefore be useful in evaluation of lipid-lowering agents. Furthermore, atorvastatin not only improves fasting but also postprandial lipoprotein metabolism, presumably by reducing TG secretion from the liver or intestine or both, and by secondarily increasing TG-rich lipoprotein clearance by eliminating saturation.

ACCUMULATING EVIDENCE suggests that, in addition to hypercholesterolemia, elevated levels of fasting serum triglyceride (TG) may also be an independent risk factor for cardiovascular disease.[1-4] A recently reported meta-analysis of 17 prospective studies reported that a 1-mmol/L increase in fasting plasma TG level was associated with a 30% increase in the risk of total cardiovascular disease in men and a 75% increase in women.[5] The relative risk of plasma TG level was diminished but remained significant after adjusting for other risk factors such as high-density lipoprotein (HDL)-cholesterol level.

In particular, high levels of plasma TG-rich lipoproteins (TRLs) in type 2 diabetes are considered to be strongly related to atherosclerotic vascular disease.[6-8] In general, not only fasting plasma TG concentration, mainly very–low-density lipoprotein (VLDL),[9] but also postprandial TG concentration, which predominantly occurs in chylomicron (CM) remnants,[10] variably increase in type 2 diabetes patients. Several lines of evidence indicate that these TRLs are atherogenic particles.[11,12] In vitro studies have demonstrated that TRLs derived from patients with hypertriglyceridemia, including VLDL and CM particles, are toxic to endothelial cells and are taken up by macrophages, resulting in foam cell formation.[13] This evidence is supported by reports indicating that postprandial TG level may be more closely related to atherogenic risk than fasting TG level.[14,15]

Although these data have generated interest in postprandial hypertriglyceridemia, the effects of lipid-lowering therapy in preclinical and clinical studies are usually evaluated from fasting lipid concentration. Against this, however, is the fact that postprandial lipoproteins are virtually absent following such a fast. Furthermore, there is no established animal model of postprandial hypertriglyceridemia that shows a physiological transitory change in lipoprotein metabolism after ingestion of a fatty meal. Together, these hinder evaluation of the efficacy of hypotriglyceridemic agents for postprandial hypertriglyceridemia in animal models.

Atorvastatin, a well-established member of the statin class, produces greater decreases than other statins in not only plasma low-density lipoprotein (LDL)-cholesterol levels, but also in plasma TG.[16,17] Recently, Burnett et al demonstrated using miniature pigs that treatment with atorvastatin decreased a transient increase in plasma TG concentration after feeding.[18] Using mathematical compartment models, they also revealed that atorvastatin improved the fractional clearance rate of postprandial TRLs. These findings have been supported by Parhofer et al using atorvastatin in normolipidemic human subjects.[19] However, as yet the effect of atorvastatin on postprandial lipid metabolism in the pathologic condition of postprandial hypertriglyceridemia has not been investigated.

The aim of the present study was to develop an animal model of postprandial hypertriglyceridemia using rats and then use this model to evaluate the effect of atorvastatin on this condition. To further evaluate the mechanism of its improving effect on postprandial lipoprotein metabolism, we examined the effect of atorvastatin on rates of TG secretion in TRL fractions after fat loading, and also evaluated the clearance rate of TG-containing emulsions.

MATERIALS AND METHODS

Materials

Enzymatic lipid assay kits (Cholesterol C-test and triglyceride G-test Wako) and olive oil were purchased from Wako Pure Chemical Industries (Osaka, Japan). Intrafat injection 20% was purchased from Takeda Chemical Industries (Osaka, Japan). Triton WR-1339 (Tyloxapol) was from Sigma-Aldrich Japan (Tokyo, Japan). Atorvastatin was provided

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by Pfizer Pharmaceuticals (Ann Arbor, MI). All other chemicals were of reagent grade.

Animals

Five-week-old male Sprague-Dawley rats (Jcl: SD) were purchased from Clea Japan (Hamamatsu, Japan). The animals were housed in metal cages in a temperature- (23 ± 2°C) and light cycle–controlled colony room (lights on 7:30 AM to 8:30 PM) and had free access to water and standard rat chow (CE-2, Clea Japan, Tokyo, Japan). After matching for body weight, 3 groups of rats were studied: 1 fed standard rat chow (normal group) and the other 2 a synthesized high-sucrose diet during the experimental period (sucrose-induced hypertriglyceridemic groups). The sucrose-enriched diet (Oriental Yeast Co, Tokyo, Japan) contained 18% casein, 68% sucrose, 8% cottonseed oil, 2% beer yeast, and 4% salt, as well as a mix of vitamins, as described previously.[20] Hypertriglyceridemic rats were divided into 2 groups: those in the control group received 0.5% carboxymethyl cellulose alone, while the atorvastatin group was given atorvastatin calcium (3 to 30 mg/kg body weight) suspended in 0.5% carboxymethyl cellulose by daily oral gavage for 2 weeks. Unless specified otherwise, studies were performed after an overnight (12-hour) fast.

Oral Fat Loading Test

One hour after the last administration of drug, rats received the indicated volume of olive oil orally in the fasted state. Blood samples were obtained at 0 (before fat loading), 2, 4, 6, and 8 hours from the tail vein using a heparinized capillary tube (Terumo, Tokyo, Japan). Plasma was isolated immediately by centrifugation at 1,500 × *g* for 10 minutes at room temperature, and stored at 4°C until assay. In separate experiments, to measure TG concentration in TRL fractions, blood samples were obtained from an abdominal vein under anesthesia 2 hours after oral fat loading. VLDL (density [*d*] = 0.96 to 1.006 g/mL) was isolated at a density of 1.006 g/mL at 145,000 × *g* and 16°C for 16 hours after CM-rich fraction ($d < 0.96$ g/mL; representing CM and large VLDL particles) isolation by centrifugation at 36,000 × *g* and 16°C for 30 minutes. TG concentrations in the CM-rich and VLDL fractions were determined by standard enzymatic procedures using commercially available kits. Plasma TG exposure (TG-AUC) were calculated using the trapezoidal rule. Incremental TG-AUC was also calculated by subtracting the fasting value from each postprandial value and calculating the area.

Determination of Exogenous TG Emulsion Clearance Rate

One hour after the last administration of drug, rats were anesthetized with pentobarbital (50 mg/kg intraperitoneally). Intrafat (1.5 mL/kg) was injected into the femoral vein and blood samples were obtained from the tail vein at 5, 25, 45, 65, and 85 minutes after injection using a heparinized capillary tube. Plasma TG concentration was determined as described above. Elimination half-time ($t_{1/2}$) was calculated by the linear least squares method using the logarithmic values of plasma TG concentration in the elimination phase. In this study, for the purposes of investigating the influence of basal plasma TG concentration on the TG clearance rates, these experiments were also evaluated without fasting.

Determination of Rates of TG Secretion in TRL Fractions After Fat Loading

One hour after the last administration of drug in the fasted state, rats were anesthetized with ether and blood samples for evaluation of plasma TG concentration before fat loading were withdrawn from the fundus oculi using capillary tubes (Terumo, Tokyo, Japan). Olive oil or distilled water (2 mL/kg) was loaded orally followed by intravenous injection of Triton WR-1339 (400 mg/kg). Four hours after Triton injection, blood samples were obtained from an abdominal vein under anesthesia. In vivo rates of TG secretion in the CM-rich and VLDL fractions were examined according to previously published methods.[21] Lipoprotein isolation and TG assay were performed as described above.

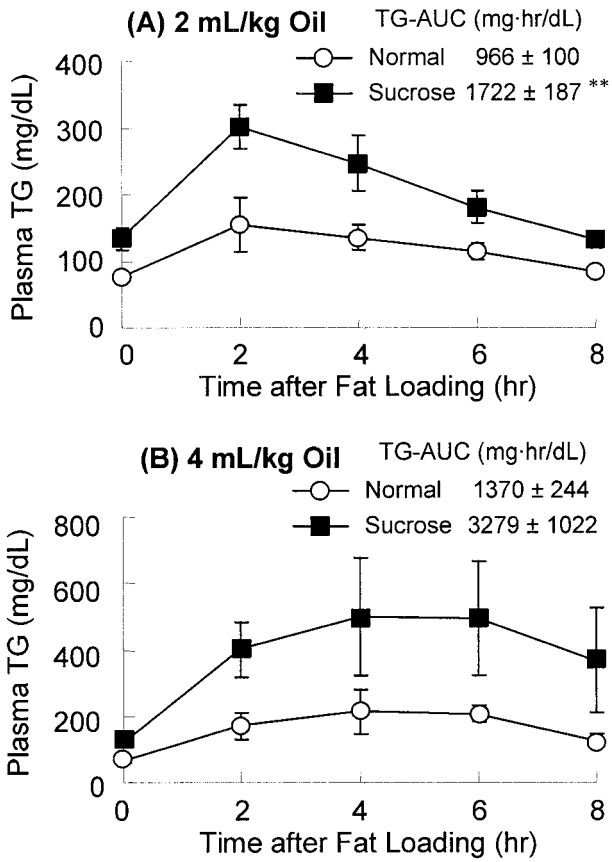

Fig 1. Plasma TG concentration-time profile after olive oil administration in normal and sucrose-fed rats. Rats were maintained for 2 weeks on a normal rat chow diet (Normal) or sucrose-enriched diet (Sucrose). After an overnight fast, olive oil 2 mL/kg (A) or 4 mL/kg (B) was administered. Results are expressed as the mean ± SEM for 6 animals. Plasma TG exposure (TG-AUC) was calculated using the trapezoidal rule. **$P < .01$ v normal group by Student's *t* test.

Statistics

All results were analyzed using Statistical Analysis System version 6.11 (SAS Institute, Cary, NC). The 2-tailed Student's *t*-test was used to compare 2 means, and the Dunnett multiple range test to compare 3 or more groups. Results are presented as the mean ± SEM.

RESULTS

Plasma TG Concentration After Oral Olive Oil Loading

Plasma TG concentration-time profile after olive oil loading was examined prior to evaluation of hypolipidemic drugs (Fig 1). Maximum increases in plasma TG concentration and duration after loading depended on the volume of fat loaded, with a larger volume of fat (4 mL/kg) having too long a duration time and too great a variation in plasma TG to allow evaluation of the compounds for postprandial hypertriglyceridemia. Following loading at 2 mL/kg , the AUC of plasma TG concentration during measurement (0 to 8 hours) in the sucrose-fed rat

Table 1. Effect of Atorvastatin on Plasma TG Concentrations in Sucrose-Fed Rats

Treatment	n	Plasma TG Concentration (mg/dL)	
		Nonfasted	Fasted
Normal	8	197 ± 23	73 ± 5
Control	8	510 ± 44	111 ± 6
		($P < .01$)*	($P < .01$)*
Atorvastatin			
3 mg/kg	8	404 ± 37	118 ± 10
		($P = 0.13$)†	($P = 0.87$)†
10 mg/kg	8	337 ± 34	106 ± 8
		($P < .01$)†	($P = .95$)†
30 mg/kg	8	161 ± 33	83 ± 5
		($P < .01$)†	($P = .04$)†

NOTE. Rats were maintained for 2 weeks on a normal rat chow diet (Normal), or sucrose-enriched diet alone (Control) or with atorvastatin. Blood samples were obtained before (nonfasted) and after an overnight fast. Results are expressed as the mean ± SEM.

Significance was determined by Student's *t* test compared to the respective *normal or †control group.

group was larger than that in the normal chow group (1,722 ± 187 mg · h/dL *v* 966 ± 100 mg · h/dL, $P < .01$). The peak TG concentration in sucrose rats was also higher than in normal rats (301 ± 32 mg/dL *v* 155 ± 40 mg/dL, $P = .02$). Plasma cholesterol concentration was not affected significantly by oral fat loading (data not shown).

Effect of Atorvastatin on Plasma TG Concentrations

To evaluate the hypotriglyceridemic effect of atorvastatin in sucrose-fed rats, plasma TG concentrations were determined. In animals fed sucrose, there were no differences in body weight gain, food consumption, or hepatic amylase concentrations (SGOT and SGPT) during the experiment period between the control and atorvastatin-treated groups (data not shown).

Before an overnight fast, plasma TG concentration in the sucrose control group rose 2.6-fold compared with that in the normal chow group (Table 1). Atorvastatin administration for 2 weeks decreased plasma TG concentration in a dose-dependent manner. At the higher dose (30 mg/kg), atorvastatin lowered plasma TG concentrations by 68% ($P < .01$). After the fast, the high TG concentration in rats induced by the sucrose diet almost disappeared. Nevertheless, plasma TG concentration in the sucrose control group was higher than in the normal chow group by 1.5-fold (Table 1). Although the inhibitory activity of atorvastatin was attenuated in the fasted state compared with that in the fed, it caused a dose-dependent decrease in plasma TG concentration, by 25% at 30 mg/kg ($P = .04$).

Effect of Atorvastatin on Postprandial Hypertriglyceridemia

Postprandial plasma TG concentration after olive oil loading 2 mL/kg at was measured after treatment with atorvastatin. Before the fat load, fasted plasma TG concentration was significantly decreased by 44% ($P < .01$) in the atorvastatin group (Table 2). Atorvastatin also decreased plasma TG concentrations during the 8 hours after fat loading (Fig 2A). Plasma TG exposure (TG-AUC) after fat loading was significantly decreased, by 40% ($P < .01$). However, incremental TG-peak concentration and incremental TG-AUC, obtained by subtracting the fasting TG value from each postprandial TG value after fat loading, tended to decrease but not significantly so (Table 2). Plasma TG concentration in VLDL ($d = 0.96$ to 1.006 g/mL) fraction at 2 hours after fat loading was significantly decreased by 46% ($P < .01$, Fig 2B). Plasma TG concentration in CM-rich ($d < 0.96$ g/mL) fraction tended to decrease to a similar degree, namely, by 39% ($P = .07$).

Effect of Atorvastatin on Clearance Rate of Exogenous TG Emulsion

To determine whether the decrease in postprandial TG concentrations was accompanied by an increase in the clearance rate of TG in plasma, clearance of exogenous TG emulsion was measured (Fig 3). The decay was bi-exponential, with clearance significantly decreased with the sucrose diet as compared to the normal diet group, suggesting a disturbance in plasma TG metabolism in this animal model. In the fasted state, there was no difference in the half time of TG removal from the 5-minute value ($t_{1/2}$) between the atorvastatin-treated and control groups (47 ± 5 minutes and 46 ± 6 minutes, respectively). In the nonfasted state, in contrast, namely, high plasma TG conditions, atorvastatin significantly shortened the $t_{1/2}$ of plasma TG decrease compared with the control group (49 ± 8 minutes and 76 ± 6 minutes, $P = .02$, respectively).

Table 2. Effect of Atorvastatin on Incremental TG-Peak and TG-AUC After Olive Oil 2 mL/kg Loading in Sucrose-Fed Rats

Treatment	n	Plasma TG Concentration (mg/dL)			Incremental TG-AUC (mg·h/dL)
		Fasting	Peak	Incremental Peak	
Normal	10	68 ± 4	163 ± 16	95 ± 17	406 ± 88
Control	10	148 ± 9	360 ± 31	212 ± 27	825 ± 170
		($P < .01$)*	($P < .01$)*	($P < .01$)*	($P = .04$)*
Atorvastatin					
30 mg/kg	10	83 ± 4	232 ± 23	149 ± 25	481 ± 80
		($P < .01$)†	($P < .01$)†	($P = .10$)†	($P = .07$)†

NOTE. Rats were maintained for 2 weeks on a normal rat chow diet (Normal), or sucrose-enriched diet alone (Control) or with atorvastatin. Results are expressed as the mean ± SEM. Plasma TG concentration-time profile after olive oil loading is shown in Fig. 2. Increment TG values were calculated by subtracting the fasting TG value from each postprandial TG value after fat loading.

Significance determined by Student's *t* test compared to the respective *normal or †control groups.

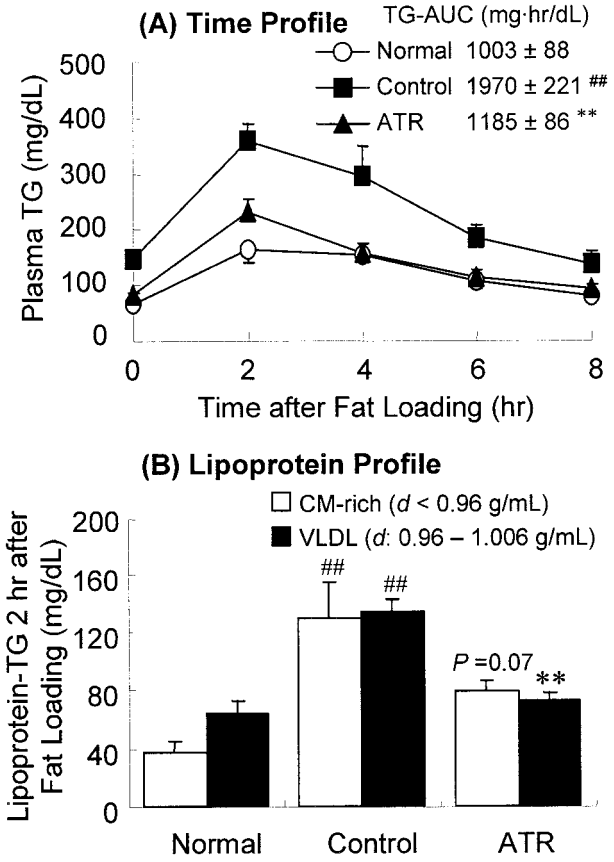

Fig 2. Effect of atorvastatin on plasma TG concentration after olive oil 2 mL/kg loading in sucrose-fed rats. Rats were maintained for 2 weeks on a normal rat chow diet (Normal), sucrose-enriched diet alone (Control), or with atorvastatin 30 mg/kg (ATR). Results are expressed as the mean ± SEM for 10 animals. (A) Time profile of plasma TG concentration. (B) Plasma TG concentration in CM-rich ($d < 0.96$ g/mL) and VLDL ($d = 0.96$ to 1.006 g/mL) fraction at 2 hours after fat loading. ##$P < .01$ *v* normal and **$P < .01$ *v* control by Student's *t* test.

Effect of Atorvastatin on Rates of TG Secretion in TRL Fractions After Fat Loading

To determine whether the decrease in postprandial TG concentration was related to the decrease in rates of TG secretion in CM-rich fraction, VLDL fraction or both, their rates after olive oil loading were measured (Fig 4). The rates of TG secretion in both CM-rich and VLDL fraction after olive oil loading significantly increased with the sucrose diet as compared to the normal diet group. Furthermore, the rate of TG secretion in CM-rich fraction after olive oil loading was 2.3-fold higher than that without loading ($P < .01$), whereas that in VLDL fraction was not affected by loading. Atorvastatin did not affect the rate of TG secretion in CM-rich fraction, but inhibited that in VLDL fraction (29%, $P < .01$) in the fasting condition. However, atorvastatin caused a significant decrease in rates in TG secretion in both fractions after fat loading, by 21% ($P < .01$) and 19% ($P < .01$), respectively.

DISCUSSION

The present experiments showed that sucrose-fed rats orally loaded with olive oil may represent a useful animal model of postprandial hypertriglyceridemia. Recently, Kusunoki et al showed that streptozotocin (STZ)-induced diabetes rats show severe postprandial hyperlipidemia after exogenous fat loading.[22] The sucrose-fed rat has also been reported as a model of diabetes which shows endogenous hypertriglyceridemia accompanied by hyperinsulinemia,[23] impaired glucose tolerance,[24] increased VLDL-TG secretion,[25] and a decrease in both lipoprotein lipase and hepatic TG lipase activities.[26] Our data indicated that a sucrose diet produces not only an increase in the rates of TG secretion in TRLs (Fig 4), but also a decrease in the clearance rate of TG-rich emulsion (Fig 3), reflecting the clinical manifestations of postprandial hyperlipidemia.[10] Sucrose-fed rats orally loaded with 2 mL of olive oil also showed closely similar changes in plasma TG concentration to those observed in postprandial hyperlipidemia patients, whose plasma TG typically increases and remains elevated from 6 to 12 hours after ingestion of a fatty meal.[10] These findings agree well with those of a clinical study in type 2 diabetes patients.[27]

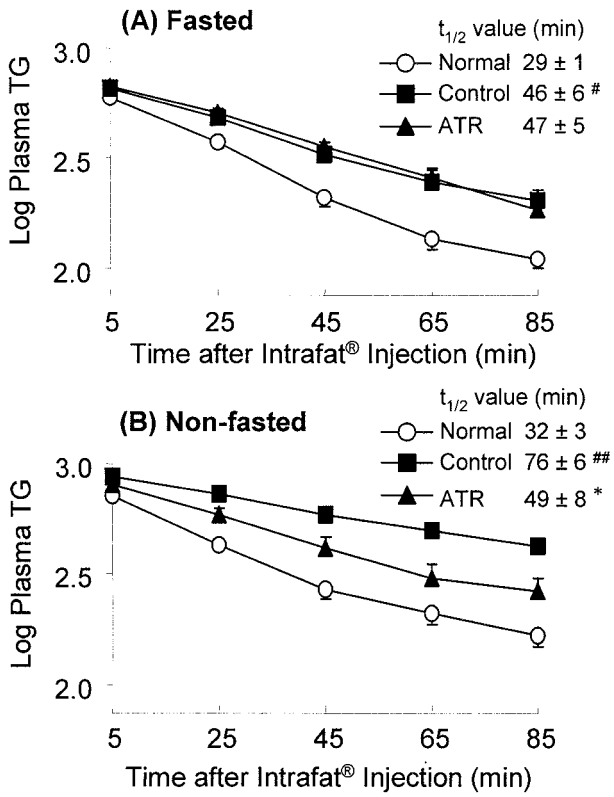

Fig 3. Plasma decay curves after intravenous injection of triglyceride emulsion (Intrafat) in sucrose-fed rats. Rats were maintained for 2 weeks on a normal rat chow diet (Normal), sucrose-enriched diet alone (Control), or with atorvastatin 30 mg/kg (ATR). Intrafat was injected in the (A) fasted or (B) nonfasted (high plasma TG) state at 1 hour after final drug administration. Results are expressed as the mean ± SEM for 7 animals. Elimination half-time ($t_{1/2}$) was calculated by the linear least squares method. #$P < .05$ and ##$P < .01$ *v* normal, and *$P < .05$ *v* control by Student's *t* test.

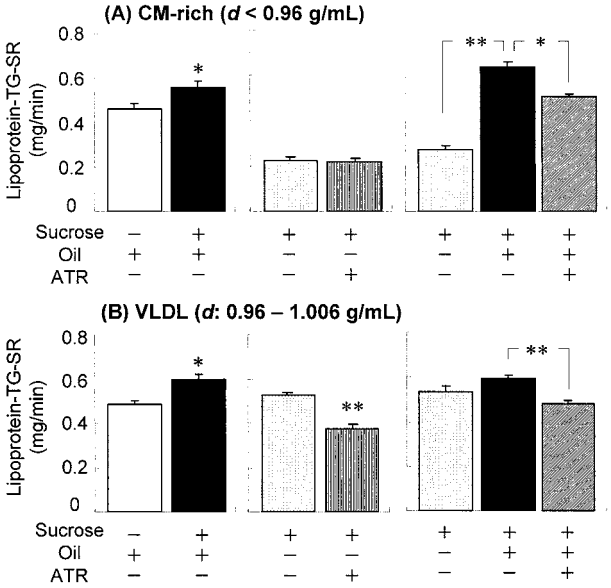

Fig 4. Effect of atorvastatin on rates of TG secretion in CM-rich (d < 0.96 g/mL) and VLDL (d = 0.96 to 1.006 g/mL) fractions in sucrose-fed rats after oral fat loading. Rats were maintained for 2 weeks on a normal chow or a sucrose-enriched diet. Lipoprotein-TG secretion rates (SR) were measured in rats with or without oil loading, and after oil loading in rats treated with or without atorvastatin (ATR). Results are expressed as the mean ± SEM for 7 to 16 animals. *P < .05 and **P < .01 by Student's t test.

Moreover, in our preliminary study, bezafibrate also significantly inhibited postprandial TG concentration after fat loading (data not shown), similar with the previous study in diabetic patients.[28] Taken together, these data indicate that this animal model using sucrose-fed rats may be useful in the investigation of postprandial hypertriglyceridemia.

In the second set of experiments, we evaluated the hypotriglyceridemic effects of atorvastatin in this postprandial hypertriglyceridemic animal model. It has been reported that a high TG concentration in rats induced by a sucrose diet is abolished by overnight fasting.[29] In our study also, plasma TG concentration was markedly decreased from 510 to 111 mg/dL by a 12-hour fast. However, a specific constitutional diathesis to hypertriglyceridemia such as an increase in TG secretion and a decrease in plasma TG clearance rate seems to be still maintained in this condition (Figs 3 and 4).

Our present studies showed that atorvastatin improved not only fasting TG concentration but also the postprandial TG concentration after oral fat loading (Fig 2). This results are consistent with those obtained by Burnett et al, who showed for the first time that atorvastatin improved postprandial lipoprotein metabolism in miniature pigs.[18] Interestingly, the effect of atorvastatin on postprandial hypertriglyceridemia appeared to be arise via the same mechanism as that in lowering basal TG concentration, since adjustment for the fasting plasma concentration of TG (incremental TG-AUC) eliminated the significant treatment effect in the postprandial state (Table 2). However, this result contrasted with that of Burnett et al,[18] who reported a significant reduction in postprandial incremental TG-AUC. Compared with normolipidemic animals, hypertriglyceridemic animals tend to have already increased TG secretion and also various degrees of fasting TG concentration.[25] Therefore, this discrepancy may be accounted for by differences in hypertriglyceridemic state or sample size. Indeed, a study in normolipidemic humans reported that atorvastatin improved incremental TG-AUC,[19] whereas a study with combined hyperlipidemia showed that it lowered total TG-AUC only and not incremental TG-AUC.[30] However, it should be pointed out that total and not incremental TRL level after fat loading is considered a more important clinical parameter, because all lipoproteins present in plasma may interact with the arterial wall.[15]

In our study, clearance rate of exogenous TG emulsion was also evaluated. Although a previous study reported the presence of differences between "true" chylomicrons and artificial TG emulsion such as Intrafat,[31] it is generally assumed that lipid emulsions can be utilized as a tracer of lipoprotein TG because their rate constants for TG clearance are indistinguishable from those for native CM particles.[31,32] Since atorvastatin did not affect the clearance rate of TG emulsion in the fasted state (Fig 3A), it would not seem to act directly on key enzyme activities related plasma TG metabolism, such as lipoprotein lipase and hepatic lipase. This possibility is in accordance with results of Alegret et al obtained in rabbits.[33]

On the other hand, our data indicated that atorvastatin increased the clearance rate of TG emulsion in the nonfasted state, namely, high TG conditions (Fig 3B). In this condition, TG removal seems to be saturated since the $t_{1/2}$ time in sucrose-control rats was prolonged over that in the fasting condition. Given other findings that TRL particles seem to be hydrolyzed by a common process,[34] the increase in clearance rate therefore seems to be a secondary effect related to decreased competition for removal processes among endogenous TG. Considering that the hypotriglyceridemic effect of atorvastatin was mainly through the inhibition of hepatic VLDL-TG secretion in sucrose-fed rats,[21,35] it may be considered that decreased hepatic TG secretion also enhances plasma TG clearance.

The present results also contrast with previous reports that indicated that atorvastatin has no significant effect on intestinal CM-TG secretion.[18,19] In our model, atorvastatin after oral fat loading decreased not only the increased rate of TG secretion in VLDL fraction, but also that in CM-rich fraction (Fig 4). Since our preliminary study indicated that the CM-rich fraction isolated by the ultracentrifugation contained not only CM particles, but also a part of hepatic large VLDL by the high-performance liquid chromatography (HPLC) analysis (data not shown), additional work needs to be done to show that these TRLs are particles of intestinal origin or not. However, recently reported data indicated that CM production may also be regulated by intestinal cholesterol synthesis, and atorvastatin decreased apolipoprotein B_{48} secretion from transformed human intestinal enterocyte ($CaCo_2$ cells) only under stimulatory conditions but not under basal condition.[36] Similarly, atorvastatin significantly decreased TG secretion rates in CM-rich fraction after fat loading but not that in basal condition in our study (Fig 4). Since atorvastatin,[37] as well as other statins,[38] tends to distribute preferentially into the small intestine in addition to accumulating in the liver, the possibility that they improve the

increase in intestinal CM-TG secretion induced by a sucrose diet could not be ruled out. Given our previous findings using HepG2 cells that the inhibitory activity of statins on TG secretion depends on the duration of their inhibition of cholesterol synthesis,[39] atorvastatin may be more effective than other statins due to its longer half-life.[40]

A limitation of this animal model is the species characteristic of rats. Metabolism of plasma cholesterol in rats is known to be significantly different to that in species, such as humans, especially in that HDL particles in rats contain the major portion of plasma cholesterol.[41,42] Therefore, we cannot say whether our results also extend to subjects with abnormalities in plasma cholesterol metabolism in the postprandial state.

In summary, we used sucrose-fed rats to develop an animal model of postprandial hypertriglyceridemia with increased TG secretion rate and depressed TG clearance. We also showed that atorvastatin is able to decrease postprandial plasma TG concentration. The clearance rate of exogenous TG emulsion was increased only in the nonfasted state, namely, the high TG condition, but not in the fasted state, indicating that atorvastatin activates TG elimination by improving the saturation of metabolism. Atorvastatin also lowered not only the rate of TG secretion in VLDL fraction, but also that in CM-rich fraction after oral fat loading. Taken together, these data suggest that the decrease in postprandial plasma TG concentration by atorvastatin is most likely due to decreased secretory activity of hepatic, intestinal or both TG. In contrast, whereas clearance of TG was also enhanced by treatment, this was perhaps a secondary result owing to decreased plasma TG concentration following suppression of TG supplementation into plasma.

REFERENCES

1. MAAS investigators: Effect of simvastatin on coronary atheroma: The Multicentre Anti-Atheroma Study (MAAS). Lancet 344:633-638, 1994

2. Drexel H, Amann FW, Follath F, et al: Plasma triglycerides and three lipoprotein cholesterol fractions are independent predictors of the extent of coronary atherosclerosis. Circulation 90:2230-2235, 1994

3. Hodis HN: Triglyceride-rich lipoprotein remnant particles and risk of atherosclerosis. Circulation 99:2852-2854, 1999

4. Jeppesen J, Hein HO, Gyntelberg F, et al: Triglyceride concentration and ischemic heart disease: An eight-year follow-up in the Copenhagen Male Study. Circulation 97:1029-1036, 1998

5. Hokanson JE, Austin MA: Plasma triglyceride level is a risk factor for cardiovascular disease independent of high-density lipoprotein cholesterol level: A meta-analysis of population-based prospective studies. J Cardiovasc Risk 3:213-219, 1996

6. Pyorala K, Laakso M, Uusitupa M: Diabetes and atherosclerosis: an epidemiologic view. Diabetes Metab Rev 3:463-524, 1987

7. Howard BV: Lipoprotein metabolism in diabetes mellitus. J Lipid Res 28:613-628, 1987

8. Haffner SM, Lehto S, Laakso M, et al: Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. N Engl J Med 339:229-234, 1998

9. Abbasi F, McLaughlin T, Reaven GM, et al: Fasting remnant lipoprotein cholesterol and triglyceride concentrations are elevated in nondiabetic, insulin-resistant, female volunteers. J Clin Endocrinol Metab 84:3903-3906, 1999

10. Burnett JR, Watts GF: Therapeutic considerations for postprandial dyslipidaemia. Diabetes Obes Metab 3:143-156, 2001

11. Nordestgaard BG, Tybjaerg-Hansen A, Lewis B: Influx in vivo of low density, intermediate density, and very low density lipoproteins into aortic intimas of genetically hyperlipidemic rabbits. Roles of plasma concentrations, extent of aortic lesion, and lipoprotein particle size as determinants. Arterioscler Thromb 12:6-18, 1992

12. Mahley RW: Atherogenic lipoproteins and coronary artery disease: Concepts derived from recent advances in cellular and molecular biology. Circulation 72:943-948, 1985

13. Gianturco SH, Bradley WA: Pathophysiology of triglyceride-rich lipoproteins in atherothrombosis: Cellular aspects. Clin Cardiol 22:II7-14, 1999

14. Karpe F, Hellenius ML, Hamsten A: Differences in postprandial concentrations of very-low-density lipoprotein and chylomicron remnants between normotriglyceridemic and hypertriglyceridemic men with and without coronary heart disease. Metabolism 48:301-307, 1999

15. Karpe F, Steiner G, Hamsten A, et al: Postprandial lipoproteins and progression of coronary atherosclerosis. Atherosclerosis 106:83-97, 1994

16. Jones P, Kafonek S, Hunninghake D, et al: Comparative dose efficacy study of atorvastatin versus simvastatin, pravastatin, lovastatin, and fluvastatin in patients with hypercholesterolemia (the CURVES study). Am J Cardiol 81:582-587, 1998

17. Bakker-Arkema RG, Davidson MH, Weiss SR, et al: Efficacy and safety of a new HMG-CoA reductase inhibitor, atorvastatin, in patients with hypertriglyceridemia. JAMA 275:128-133, 1996

18. Burnett JR, Barrett PH, Huff MW, et al: The HMG-CoA reductase inhibitor atorvastatin increases the fractional clearance rate of postprandial triglyceride-rich lipoproteins in miniature pigs. Arterioscler Thromb Vasc Biol 18:1906-1914, 1998

19. Parhofer KG, Barrett PH, Schwandt P: Atorvastatin improves postprandial lipoprotein metabolism in normolipidemlic subjects. J Clin Endocrinol Metab 85:4224-4230, 2000

20. Strobl W, Gorder NL, Patsch W, et al: Effect of sucrose diet on apolipoprotein biosynthesis in rat liver. Increase in apolipoprotein E gene transcription. J Biol Chem 264:1190-1194, 1989

21. Funatsu T, Goto M, Miyata K, et al: Reduction in hepatic non-esterified fatty acid concentration after long-term treatment with atorvastatin lowers hepatic triglyceride synthesis and its secretion in sucrose-fed rats. Biochim Biophys Acta 1580:161-170, 2002

22. Kusunoki J, Aragane K, Sekine Y, et al: Postprandial hyperlipidemia in streptozotocin-induced diabetic rats is due to abnormal increase in intestinal acyl coenzyme A:cholesterol acyltransferase activity. Arterioscler Thromb Vasc Biol 20:171-178, 2000

23. Vrana A, Fabry P, Kazdova L: Liver glycogen synthesis and glucose tolerance in rats adapted to diets with a high proportion of fructose or glucose. Nutr Metab 22:262-268, 1978

24. Gutman RA, Basilico MA, Lombardo YB, et al: Long-term hypertriglyceridemia and glucose intolerance in rats fed chronically an isocaloric sucrose-rich diet. Metabolism 36:1013-1020, 1987

25. Reaven GM, Risser TR, Reaven EP, et al: Characterization of a model of dietary-induced hypertriglyceridemia in young, nonobese rats. J Lipid Res 20:371-378, 1979

26. Basilico MZ, Gutman R, Lombardo YB, et al: Post-heparin plasma hepatic triglyceride lipase and monoglyceride hydrolase activities in hyperlipemia induced by a sucrose rich diet. Biomed Pharmacother 37:36-41, 1983

27. Syvanne M, Vuorinen-Markkola H, Taskinen MR, et al: Gemfibrozil reduces postprandial lipemia in non-insulin-dependent diabetes mellitus. Arterioscler Thromb 13:286-295, 1993

28. Attia N, Durlach V, Girard-Globa A, et al: Post-prandial metabolism of triglyceride-rich lipoproteins in non-insulin-dependent di-

abetic patients before and after bezafibrate treatment. Eur J Clin Invest 27:55-63, 1997

29. Deshaies Y: Plasma lipoprotein cholesterol and triglycerides and lipoprotein lipase activity in epididymal white adipose tissue of rats fed high sucrose or high corn oil diets. Can J Physiol Pharmacol 64:885-891, 1986

30. Boquist S, Karpe F, Hamsten A, et al: Effects of atorvastatin on postprandial plasma lipoproteins in postinfarction patients with combined hyperlipidaemia. Atherosclerosis 162:163-170, 2002

31. Hultin M, Carneheim C, Olivecrona T, et al: Intravenous lipid emulsions: removal mechanisms as compared to chylomicrons. J Lipid Res 36:2174-2184, 1995

32. Bjorkegren J, Packard CJ, Karpe F, et al: Accumulation of large very low density lipoprotein in plasma during intravenous infusion of a chylomicron-like triglyceride emulsion reflects competition for a common lipolytic pathway. J Lipid Res 37:76-86, 1996

33. Alegret M, Verd JC, Laguna JC, et al: Effect of hypolipidemic drugs on key enzyme activities related to lipid metabolism in normolipidemic rabbits. Eur J Pharmacol 347:283-291, 1998

34. Karpe F, Hultin M: Endogenous triglyceride-rich lipoproteins accumulate in rat plasma when competing with a chylomicron-like triglyceride emulsion for a common lipolytic pathway. J Lipid Res 36:1557-1566, 1995

35. Krause BR, Newton RS: Lipid-lowering activity of atorvastatin and lovastatin in rodent species: Triglyceride-lowering in rats correlates with efficacy in LDL animal models. Atherosclerosis 117:237-244, 1995

36. Pal S, Allister E, Mamo JC, et al: Cholesterol esters regulate $apoB_{48}$ secretion in $CaCo_2$ cells. Atherosclerosis 161:55-63, 2002

37. Nemoto H, Ebine H, Oishi S, et al: Pharmacokinetic studies on CI-981 (1); Absorption, distribution and excretion in rats following single oral or intravenous administration. Jpn Pharmacol Ther 26:1133-1145, 1998

38. Duggan DE, Vickers S: Physiological disposition of HMG-CoA-reductase inhibitors. Drug Metab Rev 22:333-362, 1990

39. Funatsu T, Suzuki K, Miyata K, et al: Prolonged inhibition of cholesterol synthesis by atorvastatin inhibits apoB-100 and triglyceride secretion from HepG2 cells. Atherosclerosis 157:107-115, 2001

40. Naoumova RP, Dunn S, Thompson GR, et al: Prolonged inhibition of cholesterol synthesis explains the efficacy of atorvastatin. J Lipid Res 38:1496-1500, 1997

41. Tollefson JH, Liu A, Albers JJ: Regulation of plasma lipid transfer by the high-density lipoproteins. Am J Physiol 255:E894-902, 1988

42. Tall AR: Plasma lipid transfer proteins. J Lipid Res 27:361-367, 1986