Postprandial Hyperlipidemia in Streptozotocin-Induced Diabetic Rats Is Due to Abnormal Increase in Intestinal Acyl Coenzyme A:Cholesterol Acyltransferase Activity

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Abstract—Postprandial hyperlipidemia (PH) is recognized as a significant risk factor for cardiovascular disease. The present study, involving rats with streptozotocin (STZ)-induced diabetes, was performed to establish a PH model and to examine the relation between small intestinal acyl-coenzyme A:cholesterol acyltransferase (ACAT) activity and serum lipid levels in the postprandial state. The small intestinal ACAT activities in normal rats during the experimental period were 4 to 5 pmol/mg protein per minute. In contrast, in the diabetic rats, the ACAT activities were 2 to 3 times higher than activities seen in normal rats from 7 to 21 days after the STZ injection in the absence of a high fat diet and hyperplasia in the gut. In an oral fat-loading test that used diabetic rats that had been injected with STZ (60 mg/kg) intravenously 14 days previously, the postloading changes in the serum concentrations of total cholesterol (TC) and triglyceride (TG) were significantly greater in the diabetic rats than in normal rats. Single oral administration of (1s,2s)-2-[(2,2-dimethylpropyl)-3-nonylureido]cyclohexane-1-yl 3-[(4R)-(2,2,5,5-tetramethyl-1,3-dioxane-4-carbonyl)amino]propionate (F-1394, 3 to 30 mg/kg), a potent ACAT inhibitor, suppressed the post–fat-loading elevation of serum TC levels in the diabetic rats in a dose-dependent manner without affecting serum glucose levels. Furthermore, the small intestinal ACAT activity, serum TG levels, and lymphatic absorption of TC and TG in the rats that were administered F-1394 (30 mg/kg) were reduced by ≈90%, 70%, 30%, and 15%, respectively. This is the first evidence that elevated ACAT activity in the gut, unlike hyperplasia and hyperphagia, induces PH in rats. Our results strongly suggest that F-1394 may be a potential treatment for PH in humans. (Arterioscler Thromb Vasc Biol. 2000;20:171-178.)

Key Words: acyl coenzyme A:cholesterol acyltransferase ■ streptozotocin-induced diabetic rats ■ postprandial hyperlipidemia ■ F-1394 ■ fat-loading test

Coronary heart disease (CHD) resulting from premature atherosclerosis is a major cause of death in insulin-dependent and non–insulin-dependent diabetic patients.1,2 Abnormalities in the metabolism of plasma lipoproteins and lipids in the postprandial state have been reported in diabetic patients.3-9 and now evidence is being accumulated indicating that postprandial increases in triglyceride (TG)-rich lipoprotein,10,11 which is known as one of the atherogenic remnant lipoproteins,12,13 TG,14,15 and cholesterol16 remarkably contribute to the occurrence of CHD in such patients.

A number of studies have reported that rats with streptozotocin (STZ)-induced diabetes (STZ-diabetic rats) shows severe hyperlipidemia after exogenous fat loading.17-19 It has been suggested that the severe hyperlipidemia occurring in STZ-diabetic rats fed a high fat diet could be attributable to a marked increase in fat absorption via the gut, which in turn could be due to an abnormal increase in small intestinal acyl-coenzyme A:cholesterol acyltransferase (ACAT) activity.20 Indeed, repeated administration of an ACAT inhibitor, such as DL-melinamide,21 CL-277082,22,23 or FR 145237,24 markedly decreases both the serum total cholesterol (TC) level and enhanced small intestinal ACAT activity in STZ-diabetic rats fed a high fat diet. However, previous investigators using diabetic models have not been able to find a correlation between the postprandial metabolism of serum lipids and severe hyperlipidemia, although postprandial hyperlipidemia (PH) is found in diabetic patients.3-9

The present study was performed to establish a PH model and to examine the relation between enhanced small intestinal ACAT activity and PH. First, we showed that intestinal ACAT activity was markedly enhanced in STZ-diabetic rats in the absence of both a high fat diet and hyperplasia in the gut. Then we examined the changes in serum lipid levels in the postprandial state in STZ-diabetic rats given a high fat cocktail containing cholesterol and sesame oil by mouth. In addition, we examined the effect of a single administration of (1s,2s)-2-[(2,2-dimethylpropyl)-3-nonylureido]cyclohexane-1-yl 3-[(4R)-(2,2,5,5-tetramethyl-1,3-dioxane-4-carbonyl)amino]propionate (F-1394), a potent ACAT inhibitor,25-28 on serum lipid
levels, lymphatic output of lipids, and small intestinal ACAT activity in STZ-diabetic rats after this oral fat loading.

Methods

Drugs and Chemicals

[1-14C]Oleoyl-coenzyme A (2.0 GBq/mmol) was obtained from New England Nuclear Corp. F-1394 (Fujirebio Inc) was used to produce potent ACAT inhibition.25 STZ was purchased from Wako Pure Chemical Industries Ltd. All other reagents used were standard commercial high-purity materials.

Animals

Male Sprague-Dawley (SD) rats (5 weeks old) were obtained from Charles River Japan (Atsugi, Japan). They were maintained in a temperature- and humidity-regulated room (22±2°C, 55±15%) with controlled lighting (12-hour light/dark cycle). They had free access to tap water and commercial regular chow (F-2, Funabashi Farms) for a week before receiving an STZ injection and throughout the experiments, except where otherwise stated. After an overnight fast, rats were injected with STZ (60 mg/kg) in 50 mmol/L citrate-buffered saline (pH 4.5) via a tail vein to induce diabetes. Control (nondiabetic) rats were injected with vehicle (citrate-buffered saline). The intravenous injections were given under light ether anesthesia. The rats were maintained for various periods, depending on the aim of the experiments, and then euthanized under the anesthesia.

Ex Vivo ACAT Assay

The first 20-cm segment of bowel after the gastric pylorus was removed and discarded. The next 40-cm segment of small intestine was quickly excised, and the lumen was washed out with chilled saline. This segment was opened longitudinally, and the mucosa was scraped off (by using the edge of a microscope slide) and suspended in an ice-cold 0.25 mol/L citrate-buffered saline solution. The suspension was centrifuged at 900g for 10 minutes, and the residue was homogenized in 0.154 mol/L potassium phosphate buffer (pH 6.2). The resulting homogenate was centrifuged at 1000g for 15 minutes, and the supernatant was used for ACAT assays.

ACAT activity was determined by a method involving the incorporation of [14C]Oleoyl-coenzyme A into cholesteryl oleate, as described by Heider et al.26 Except when the ACAT activity was to be determined in the fat-loading test, cholesterol liposomes were exogenously added to the assay system to act as an additional substrate for ACAT and thus to facilitate evaluation of the actual activity of intestinal ACAT. The cholesterol-lysosome suspension was prepared as follows. Cholesterol and lecithin dissolved in ethanol were rapidly added to 0.154 mol/L potassium phosphate buffer (pH 7.4). The resulting suspension containing 1.5 mmol/L cholesterol and 9 mmol/L lecithin was dialyzed against a large amount of the same buffer to remove the ethanol and was then used as a cholesterol-lysosome suspension.

Oral Fat-Loading Test

Rats that had been maintained for 2 weeks after the injection of STZ or its vehicle were fasted overnight and then given by mouth 5 mL high fat cocktail per rat. Blood was collected immediately and at 1, 2, 4, 6, and 8 hours after the oral administration of the cocktail. In each case, blood was collected with the rats under ether anesthesia. The cocktail, which contained 10% (wt/vol) cholesterol, 2% (wt/vol) cholic acid, 25% (wt/vol) sesame oil, 6% (vol/vol) Tween 20, and 0.5% (wt/vol) carboxymethylcellulose sodium (CMC-Na), was prepared by homogenization with a Polytron homogenizer (Kinematica GmbH), F-1394 (3 to 30 mg · 5 mL−1 · kg−1) or its vehicle (0.5% CMC-Na solution) was orally administered to rats immediately before they received the cocktail, and blood was collected 4 hours after administration of the cocktail.

Lymphatic Output of Lipids

Two weeks after the injection of STZ (60 mg/kg IV), the rats were fasted overnight and then were orally given 2.5 mL of saline 1 hour before lymphatic cannulation to visualize a lymph duct. The rats were then anesthetized with sodium pentobarbital (50 mg · mL−1 · kg−1 IP Nembutal, Dainippon Pharmaceuticals) and subjected to cannulation of the thoracic duct as previously described.30–32 A second indwelling catheter was placed in the fore stomach for later administration of the high fat cocktail and/or F-1394. After the surgery, the rats were placed in cages to recover from anesthesia. Each rat was given 3 mL of the cocktail through a gastric tube, and the lymph was collected every 1 hour, starting at 1 hour before the administration of the cocktail to 24 hours after. Also, F-1394 (30 mg · 5 mL−1 · kg−1) or its vehicle (0.5% CMC-Na solution) was given via the gastric tube immediately before the administration of the cocktail. The concentrations of TC and TG in the lymph were determined enzymatically as mentioned above.

Lipoprotein Subfractionation and Measurement of Biochemical Parameters

The concentrations of TC, TG, and glucose in a sample were determined by enzymatic/colorimetric methods with commercial assay kits (Cholesterol E-II HA Test Wako, Triglyceride EII-HA Test Wako, and Glucose II-HA Test Wako, respectively; Wako Pure Chemical Industries). Lipoproteins were isolated by ultracentrifugation, as previously described.33 The following density (d) fractions were determined enzymatically as mentioned above.

Statistical Analysis

The results are expressed as mean±SE. The statistical significance of the differences between groups was determined by means of a Student t test or Aspin-Welch test, and the differences among group in the dose-related experiment were determined by means of a Dunnet, Williams, or Steel test.

Results

Establishment of PH Model in Diabetic Rats

On day 0 (the day of STZ injection), the serum glucose level in the control rats was almost the same as that in the rats injected with STZ. However, the glucose level in the (diabetic) rats injected with STZ was ~400 mg/dL at ≥3 days, whereas in the control (nondiabetic) rats, it remained at <200 mg/dL (Table 1). It was confirmed that diabetes was obviously induced in rats by STZ injection.

In terms of the serum TC and TG levels, there were no significant differences between the nondiabetic and the diabetic rats throughout the experimental period. However, on the 21st day, slight nonsignificant increases in serum TC and TG levels were observed in the diabetic rats (Table 1). Although, in this experiment, the food consumption of regular chow diet was controlled (30 g/d per rat) in both groups to exclude the influence of overeating, nondiabetic rats showed increases in body weight and small intestine weight of 30% and 16%, respectively, whereas the diabetic rats showed little or no change in these parameters throughout the experimental period (Table 1).

The small intestinal ACAT activity in the nondiabetic rats during the experimental period was ~4 to 5 pmol · mg protein−1 · min−1. In contrast, in the diabetic rats, the ACAT activity had reached 11.0±0.7 pmol · mg protein−1 · min−1 at 14 days after the injection and then decreased to 7.4±0.4 pmol · mg protein−1 · min−1 at 21 days after the injection (Figure 1). Therefore, the rats that had received STZ 14 days in advance were used for fat-loading experiments.

The values obtained for the initial concentrations of serum TC in nondiabetic rats and diabetic rats were 71±3 and 58±7.
mg/dL, respectively. In the diabetic rats, the TC level increased rapidly after the oral administration of a high fat cocktail (cholesterol, 0.5 g per rat; sesame oil, 1.25 g per rat); the TC level was 99 ± 8 mg/dL 4 hours after the administration of the cocktail (P < 0.05 versus the nondiabetic rats), and it reached 110 ± 9 mg/dL after 8 hours. In contrast, the TC level in the nondiabetic rats rose more slowly; it reached 77 ± 3 mg/dL 4 hours after the administration of the cocktail and 97 ± 4 mg/dL after 8 hours (Figure 2A). However, the postprandial changes in serum TG were much more drastic than changes in serum TC in the diabetic rats. The values obtained for initial concentrations of TG in the nondiabetic rats and the diabetic rats were 63 ± 4 and 40 ± 6 mg/dL, respectively. The highest level of serum TG in nondiabetic rats was only 167 ± 17 mg/dL (2 hours after the oral administration of the cocktail); thereafter, the level gradually declined to 145 ± 31 mg/dL. In the diabetic rats, the TG level was dramatically increased to 920 ± 282 mg/dL 4 hours after the administration of the cocktail (P < 0.05 versus nondiabetic rats), and it then declined to 503 ± 87 mg/dL after 8 hours (P < 0.01 versus nondiabetic rats) (Figure 2B). Therefore, the time point at 4 hours after the administration of the cocktail was selected to evaluate the effect of an ACAT inhibitor, F-1394, on PH in this model.

Effect of F-1394 on PH in Diabetic Rats

As shown in Table 2, the serum glucose level in the diabetic rats (163 ± 14 mg/dL) was 1.6 times higher than that in the nondiabetic rats (P < 0.01). F-1394 (3 to 30 mg/kg) did not affect the serum glucose levels in the diabetic rats (Table 2, Figure 3A).

Figure 1. Change in small intestinal ACAT activity in rats after STZ injection. Male SD rats were intravenously injected either with STZ at a dose of 60 mg/kg (●) or with its vehicle (○). The rats had free access to tap water, but the daily food ration was controlled (30 g per rat) to ensure equal intake in the 2 groups. ACAT activity was determined after the incorporation of [14C]oleoyl-coenzyme A into the cholesteryl oleate present in a mixture containing a homogenate of small intestinal mucosa and cholesterol liposome (an additional substrate for ACAT). Each symbol represents mean ± SE from 3 rats. §P < 0.001 vs vehicle group (Student’s t test).

Figure 2. Changes in serum TC (A) and TG (B) levels in rats after fat loading. Male SD rats were intravenously injected either with STZ at a dose of 60 mg/kg (●) or with its vehicle (○) and then maintained for 14 days on a diet of regular chow. After fasting overnight, the rats received a high fat cocktail by mouth (at time 0), and blood was collected at the times shown. Each symbol represents mean ± SE from 8 rats. *P < 0.05 and †P < 0.01 vs vehicle group (Student’s t test or Welch’s test).
As shown in Table 2, the serum TC level was significantly higher in the diabetic control rats than that in the nondiabetic rats (P<0.05). Also, as shown in Figure 3B, the serum TC levels reached 115±6 mg/dL 4 hours after the administration of the cocktail in the diabetic control rats. F-1394 (3, 10, or 30 mg/kg PO) reduced the serum TC level in the diabetic rats in a dose-dependent manner (Figure 3B). F-1394 at a dose of 30 mg/kg significantly reduced the serum TC levels to normal (P<0.001; Table 2, Figure 3B). In fact, it was reduced 25%. An analysis of lipoprotein subfractions showed that F-1394 reduced the CM+VLDL (d<1.006) and LDL (1.006<d<1.063) fractions by 54% and 20%, respectively (Table 2).

As shown in Figure 4, the serum TG level was higher in the diabetic control rats than in the nondiabetic rats (P<0.05). Also, as shown in Figure 3C, the serum TG levels reached 1016±298 mg/dL 4 hours after the administration of the cocktail in the diabetic control rats. The serum TG level from the diabetic rats that were given F-1394 at a dose of 3 or 10 mg/kg was reduced by 44% or 24%, respectively; however, there was no statistic difference in serum TG level among the 3 groups. Also, the reduction was not in a dose-dependent manner (Figure 3C), unlike serum TC levels. In contrast, F-1394 at a dose of 30 mg/kg significantly reduced the serum TG level in the diabetic rats (Table 2, Figure 3C).

The ex vivo ACAT activity in small intestinal mucosa from the normal rats and the diabetic rats was measured to ensure the effect of F-1394. As shown in Figure 4, the ACAT activity in normal rats was 0.93±0.39 pmol·mg protein−1·min−1 (P=0.049 versus diabetic control rats), and in diabetic control rats, it was 9 times higher than this (8.65±3.24 pmol·mg protein−1·min−1). In contrast, the activity in the diabetic F-1394–treated rats was significantly less than that in the diabetic control rats (1.10±0.39 pmol·mg protein−1·min−1, P=0.053 versus diabetic control rats).

### Lymphatic Output of Lipids

The lymphatic output of TC or TG and in the presence of F-1394 was followed for 24 hours after administration of the cocktail via the gastric tube. As shown in Figure 5A, the TC output in the diabetic control rats was sharply increased and reached a peak at 5 hours after administration of the cocktail (3.58±0.97 mg/h). Then the change in the TC output was gradually decreased. In contrast to this, the increase in the TC output in the diabetic rats with F-1394 was more moderate than that in the diabetic control rats. The TC output in the diabetic rats with F-1394 reached a plateau at 6 hours after the administration of the cocktail, and then the plateau was maintained until 10 hours after the administration (≈1.4 mg/h). After that, the decline of the TC output behaved as in diabetic control rats.

As shown in Figure 5B, the TG output in the diabetic control rats was drastically increased and also reached a peak.
Discussion

Although many investigators have reported that severe hyperlipidemia can be induced in STZ-diabetic rats by exogenous fat loading and although they have discussed the mechanisms involved, serum lipid metabolism in the postprandial state has not been investigated. In the present study, we performed experiments to determine whether small intestinal ACAT is activated in STZ-diabetic rats in the absence of both high fat diet and hyperplasia in the gut. Hyperglycemia was detectable in rats on the third day after the STZ injection and beyond, and the body weight and small intestine weight remained fairly stable in diabetic rats, whereas they increased progressively in normal rats (Table 1). These results indicated that hyperplasia was not induced in the gut of diabetic rats fed 30 g of regular chow per day per rat, at least up to 21 days after the STZ injection (Figure 1). These results indicate that small intestinal ACAT activity is elevated under diabetic conditions in the absence of both a high fat diet and hyperplasia in the gut, presumably because it is released from its normal control by insulin. In fact, Jiao et al first reported that ACAT activity in the gut was elevated in diabetic rats without a high fat diet and that the enhanced small intestinal ACAT activity can be suppressed by treatment with insulin. Furthermore, several investigators showed that the enhanced ACAT activity in the gut was inhibited by ACAT inhibitors, subsequently resulting in reduced serum TC levels in diabetic rats loaded with a high fat diet. Maechler and colleagues demonstrated that an elevation of intestinal ACAT activity could be observed in diabetic rats only 3 days after STZ injection in the absence of both a high fat diet and hyperplasia in the gut. Our results are almost the same as theirs, despite the differences in study design. Thus, our data suggest that a deficiency of insulin leads to an addition of small intestinal ACAT activity even in the absence of an induction of hyperplasia in the gut and hyperphagia. Jiao et al have reported that insulin inhibited not only fatty acid incorporation into lipids, such as cholesteryl ester (CE), TG, and PL in cultured Caco-2 cells, which are a human intestinal cell line, but also microsomal ACAT activity isolated from the cells; they further suggested that insulin did not affect synthesis of ACAT protein but some modifications of ACAT protein. However, detailed mechanisms of insulin on ACAT activity still remain unclear.

In the past several years, molecular biological approaches have given us new information regarding the ACAT molecule itself and its physiological roles and pathogenicity since Chang et al first cloned the cDNA encoding the human ACAT enzyme, termed ACAT-1. Subsequently, ACAT-1 cDNA has been cloned in hamsters, mice, rabbits, and rats, and ACAT-1 mRNA and/or its product have been found in various tissues, including the small intestine. However, it has been suggested that the ACAT-1 enzyme is not involved in cholesterol absorption via the gut because ACAT-1 gene disruption did not affect cholesterol absorption in mice. Human intestinal ACAT activity was also immuno-depleted by only 20% by the anti-human ACAT-1 antibody DM-10, which inhibited ACAT activities by >80% in the liver, adrenal gland, macrophages, and kidney. Therefore, it has been suggested that there are isoenzymes of ACAT that catalyze cholesterol absorption. Recently, a new ACAT isoenzyme, named ACAT-2, was cloned in mice, monkeys, and humans, and its mRNA was primarily expressed in the liver and small intestine, unlike ACAT-1. As described in the studies mentioned above, the investigators speculated that ACAT-2 is expected to be responsible for CE secretion from the liver and intestine but not for its deposition. Thus, it could be postulated that ACAT-2 is regulated by insulin and that the ACAT-2 enzyme is activated in diabetic conditions. Further investigations are required to distinguish the regulation of ACATs by insulin.

Previous investigators, including Maechler and colleagues, have not been able to show a correlation between the postprandial changes in serum lipids and the severe hyperlipidemia seen in diabetic rats fed a high fat diet. For this reason, we investigated the postprandial levels of serum lipids in diabetic rats (in which the rise in small intestinal ACAT would be expected to be elevated by the use of an oral fat-loading test). Diabetic rats that had been fasted overnight for 14 days after the injection of STZ or its vehicle were used for the oral fat-loading test. The serum TC levels in such
diabetic rats increased rapidly after the oral administration of the high fat cocktail (Figure 2A). Moreover, the increase in serum TG was greatly enhanced in diabetic rats (compared with normal rats) (Figure 2B). From these results, we conclude that PH is induced in STZ-diabetic rats by the feeding of fat. Also, it is suggested that hyperphagia and hyperplasia in the gut are not necessarily the predominant mechanisms for induction of hyperlipidemia in STZ-diabetic rats. These findings support the findings of Maechler and colleagues.22,23 Whereas the TC levels reached steady state at 6 hours after the administration of the cocktail, the TC level should be more increased than that seen in the diabetic rats, as shown in Figure 2A, when the diabetic rats are continuously administered the cocktail or fed a high fat diet. Furthermore, our results may suggest that the severe hyperlipidemia seen in STZ-diabetic rats continually fed a high fat diet occurs as a result of the abnormal increase in fat absorption via the gut that occurs repeatedly in the postprandial state.

We have reported that F-1394 diminished intestinal cholesterol absorption and subsequently reduced serum TC levels in nondiabetic animals fed a high fat diet.26,27 Even in the present oral fat-loading test, a single administration of F-1394, an ACAT inhibitor, to diabetic rats markedly decreased the serum level of TC, including CM + VLDL and LDL fractions (Figure 3B and Table 2), the elevated ACAT activity in the small intestine (Figure 4), and the lymphatic output of cholesterol (Figure 5A). These results strongly suggest that PH is attributable to an abnormal increase in small intestinal ACAT activity and that intestinal ACAT regulates cholesterol absorption via the gut. Moreover, only a high dose of F-1394 (30 mg/kg) unexpectedly decreased TG levels (Figure 3C and Table 2) and the lymphatic output of TG (Figure 5B) in this model, whereas F-1394 decreased TC levels in a dose-dependent manner. Previously, we reported the same observation that a high dose of F-1394 decreased TG levels in the postprandial state in normal beagle dogs fed a high fat diet, whereas F-1394 decreased TC levels in a dose-dependent manner.27 In nondiabetic normal rats fed a high fat diet, F-1394 also decreased serum TG levels in the postprandial state (J.K. et al, unpublished data, 1998). From these findings, it seems that the mechanisms of TG absorption inhibition by F-1394 via the gut are different from that of cholesterol absorption, because the potency of F-1394 in the reduction of TG is not concomitant with that the potency in the reduction of TC (Figure 3). Although the exact relation between TG absorption via the gut and intestinal ACAT activity still remains unclear, it could be that production of CM in the gut is controlled by intestinal ACAT in both the normal and the diabetic rats. Recently, a factor that is predominant in lipoprotein production was found in the liver, namely, microsomal TG transfer protein (MTP).46,47 This protein has also been found in the small intestine, and CE, which is a product of ACAT, and TG have been found to be essential substrates for MTP.46 Thus, the hypotriglyceridemic effect of F-1394 may cause a reduction in CM synthesis in the gut that is secondary to a reduction in MTP activity caused by lack of one of the substrates for MTP (such as CE). Recently, Martins et al49 have shown that an ACAT inhibitor, CL277,082, decreased the lymphatic output of apoB48 and TG, and they have discussed also that synthesis of apoB48 and CM in the intestine may be regulated by the availability of CE. Alternatively, it is suggested that F-1394 may reduce TG absorption as a result of a direct inhibition of MTP activity in the intestine. In fact, a preliminary study in our laboratory has revealed that microsomal lipid transfer activity in rat small intestinal mucosa was affected by high concentrations of F-1394 (>1 mmol/L) in vitro (J.K. et al, unpublished data, 1998), and an incorporation of free fatty acid into TG was not attenuated by F-1394 in cultured Caco-2 cells (J.K. et al, unpublished data, 1994). The distribution study that used radioactive F-1394 has shown that rat small intestine contains a sufficient mass of F-1394 to inhibit such an activity after the oral administration (Fujirebio, unpublished data, 1994). Moreover, a lack of MTP activity is significantly involved in abetalipoproteinemia,57,58 a rare autosomal recessive disease characterized by the absence of VLDL and LDL from plasma, and fat malabsorption.53 Therefore, a partial inhibition of intestinal MTP activity by compounds may cause an inhibition of fat absorption via the gut. Presumably, both mechanisms may be involved in the attenuation of TG absorption. However, at present, these are only speculations, and further studies will be necessary to clarify the actual mechanism.

We have previously demonstrated that F-1394 interferes with cholesterol absorption via the gut as a result of its inhibition of small intestinal ACAT activity in rats fed a high cholesterol diet.26 In the present study, we found that F-1394 decreased serum TC levels, mainly CM + VLDL (Table 2), and that it also inhibited small intestinal ACAT activity ex vivo (Figure 4). In another study in STZ-diabetic rats, no delay in the plasma clearance or liver uptake of CM or CM remnants was observed.52 These data strongly suggest that the hypcholesterolemic action of F-1394 in diabetic rats results mainly from a suppression of cholesterol absorption via the gut. This is in accord with the results of our previous studies26,28 and with those of other studies that used the ACAT inhibitor.21–24,49 Also, F-1394 slightly reduced TC levels in the LDL fraction (Table 2). This result suggests that this compound may affect VLDL secretion from the liver in this model as well. Indeed, the hypcholesterolemic effect by ACAT inhibitors caused by the reduction of VLDL production in the liver that resulted from hepatic ACAT inhibition is expected,53–56 and we have determined that F-1394 prevents VLDL secretion in hyperlipidemic rats induced by Triton WR-1339.57 Another possibility could be that a stimulation of VLDL production by excess plasma concentrations of intestine-derived lipoproteins are diminished by an attenuation of cholesterol absorption by F-1394 in diabetic rats.

Recently, abnormalities of the metabolism of serum lipoproteins,5,3,5,9 TG,4 and TC6–8 in the postprandial state have been reported in diabetic patients. It has been demonstrated that higher cholesterol absorption efficiency has been observed in diabetic patients with CHD but not in patients without CHD.16 That study16 suggests that the abnormal increase of cholesterol absorption in the postprandial state greatly contributes to CHD in diabetic patients. Similarly, increased postprandial triglyceridemia is strongly associated with a constellation of potentially atherogenic and thrombogenic lipoprotein changes. Thus, PH is recognized as a significant risk factor for CHD. The results of the present study suggest that an abnormal increase in intestinal ACAT activity can induce PH in rats. Consequently, a treatment
causing cholesterol malabsorption by F-1394, an ACAT inhibitor, could be beneficial in these patients.

In summary, we established a PH model in STZ-diabetic rats by the single oral administration of a high fat cocktail. The PH was apparently induced by the abnormal increase in intestinal ACAT activity that occurs in STZ-diabetic rats. Indeed, an ACAT inhibitor, F-1394, improved the PH apparently as a result of its inhibition of intestinal ACAT activity.

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