Postprandial Hyperlipidemia in Zucker Diabetic Fatty $fa/fa$ Rats, an Animal Model of Type II Diabetes, and Its Amelioration by Acyl-CoA:Cholesterol Acyltransferase Inhibition

Koji Fujinami*, Kazuhiro Kojima, Katsumi Aragane and Jun Kusunoki
Central Research Laboratories, Fujirebio Inc., 51 Komiya-cho, Hachioji, Tokyo 192-0031, Japan

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ABSTRACT—Postprandial hyperlipidemia (PH) is frequently observed in diabetic patients. We performed an oral fat-loading test in Zucker diabetic fatty (ZDF) $fa/fa$ rats, a model for type II diabetes, to determine whether PH was induced in the rats. Post fat-loading changes in serum cholesterol and triglyceride levels were significantly greater in the $fa/fa$ rats than those seen in their lean littermates and an acyl-CoA:cholesterol acyltransferase (ACAT) inhibitor significantly reduced these levels by 24% and 31%, respectively. Therefore, we confirmed that PH appeared in ZDF $fa/fa$ rats by fat loading and ACAT inhibition may be a potential treatment for PH.

Keywords: Postprandial hyperlipidemia, Zucker diabetic fatty rat, Acyl-CoA:cholesterol acyltransferase

There is accumulating evidence that the postprandial increases in triglyceride-rich lipoproteins, remnant-like particles (RLP), triglyceride and cholesterol make a substantial contribution to the incidence of coronary heart disease (CHD), a major cause of morbidity and mortality in diabetic patients (1, 2). Recently, we have established a postprandial hyperlipidemic (PH) model in the streptozotocin (STZ)-induced diabetic rat, a type I diabetic animal model (3). However, although PH is frequently observed in type II diabetic patients, no animal model for PH in type II diabetic patients has yet been described. To try to remedy this situation, we set out to establish a new PH model using a type II diabetic animal.

In the present study, we used Zucker diabetic fatty (ZDF) $fa/fa$ rats, which have been reported to be a suitable model for human type II diabetes (4), and subjected them to an oral fat-loading test to determine whether they would exhibit PH. Having established that they did exhibit PH, we tested the effect on it of an oral administration of (1S,2S)-2-[3-(2,2-dimethylpropyl)-3-nonylureido]cyclohexane-1-yl 3-[(4R)-N-(2,2,5,5-tetramethyl-1,3-dioxane-4-carbonyl) amino] propionate (F-1394), an acyl-CoA:cholesterol acyltransferase (ACAT) inhibitor which can be expected to reduce lipid absorption via the gut (5).

F-1394 (Fujirebio Inc., Tokyo) was used to induce potent ACAT inhibition in vivo (5). All other reagents and chemicals used were commercial high-purity materials. Male ZDF $fa/fa$ rats and their lean littermates were obtained at 8 weeks of age from Charles River Japan (Atsugi). They were maintained in a temperature- and humidity-regulated room (22 ± 2°C, 55 ± 15%) with controlled lighting (12-h light/dark cycle). They had free access to tap water and commercial regular chow (CRF-1; Oriental Yeast Co., Ltd., Tokyo). All procedures were carried out in accordance with the regulations issued by the Fujirebio Animal Ethics Committee.

To test for an oral fat-loading test, the rats were used between 12 and 15 weeks of age. The high-fat cocktail, which contained 10% cholesterol, 2% cholic acid, 25% sesame oil and 6% Tween 20, was prepared as described previously (3). After an overnight fast, the rats were each given 5 ml of the cocktail by mouth. Blood was collected immediately before and at 1, 2, 4, 6, 8 and 24 h after the oral administration of the cocktail. In each case, blood was collected with the rat under light ether anesthesia.

To test for an effect of ACAT inhibition, the $fa/fa$ rats and their lean littermates were assigned to control and F-1394 groups, respectively, in which serum total cholesterol (TC), triglyceride (TG) and glucose levels were almost the same as the other one. Then, F-1394 (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 h later under ether anesthesia.
The serum concentrations of TC, TG and glucose were measured by enzymatic/colorimetric methods using commercial assay kits (Cholesterol E-HA Test Wako, Triglyceride EII-HA Test Wako and Glucose II-HA Test Wako, respectively; all from Wako Pure Chemical Industries, Osaka).

The results were expressed as the mean ± S.E.M. The statistical difference between groups was assessed by means of a Student’s t- or Aspin-Welch test (SAS software package).

In lean rats, serum TC, TG and glucose levels were, respectively, 80 ± 1, 24 ± 3 and 90 ± 3 mg/dl in the fasting state (t = 0, Fig. 1). In ZDF fa/fa rats, the corresponding values were 1.4, 12.5 and 2.3 times the above values (P < 0.001 vs the lean rats for each parameter). Also, the fa/fa rats were overweight by comparison with their lean littermates (data not shown). Since these findings are consistent with those described in previously (4), the fa/fa rats used here are considered to have suffered from type II diabetes.

In the fa/fa rats, the TC level increased immediately after the oral administration of a high-fat cocktail, and thereafter, it rose progressively throughout the remainder of the experiment to reach 140% of its initial value at t = 24 h. In contrast, in the lean rats the TC level rose more slowly and reached a virtual plateau at t = 6 h (Fig. 1A). As shown in Fig. 1B, the highest serum TG level reached only 79 ± 7 mg/dl (t = 2 h); thereafter, it was cleared rapidly in the lean rats. In contrast, in ZDF fa/fa rats, the TG level increased dramatically to reach 593 ± 22 mg/dl at t = 2 h, and it remained at around this high level for the next 4 h before falling back toward the original level. The area under incremental curve (AUIC) of TC and TG level in the fa/fa rats was significantly greater than that in the lean rats (648 ± 90 and 383 ± 46 mg · dl⁻¹ · h, P = 0.0219; 5343 ± 694 and 633 ± 103 mg · dl⁻¹ · h, P = 0.0053, respectively).

As shown in Fig. 1, the rise in TG in the fa/fa rats was much more dramatic than that in TC, which occurred more gradually. These observations would seem to be consistent with those made in both STZ-diabetic rats (3) and diabetic humans (6). To judge from these findings, in general dietary cholesterol absorption via the gut is a slower and more complex process than TG absorption.

Here we confirmed that PH occurs in ZDF fa/fa rats, an animal model for type II diabetes, as it does in STZ-induced diabetic rats. However, the underlying mechanisms in the fa/fa rats are not clear. The PH in the STZ-induced diabetic rat which lacks insulin apparently induces an excessive absorption of dietary lipids (3), and no delay in the plasma clearance of chylomicrons (CM) or CM remnants has been observed (7). The present study has shown that F-1394 is expected to reduce both TC and TG absorption via ACAT and microsomal TG transfer-protein pathways in the gut (3). On the other hand, according to the existing literature, spontaneous hyperlipidemia in ZDF fa/fa rats occurs primarily as a result of delayed clearance of particular lipoproteins, of which the apolipoproteins show an abnormally altered composition due to a hyperinsulinemia (8). Furthermore, a possible low activity of lipoprotein lipase, which catalyzes TG-rich lipoprotein clearance, may cause such a removal defect in these lipoproteins. Hence, the PH in the fa/ra rats may have been due predominantly to a defective catabolism of exogenous lipoproteins absorbed via the gut. In addition, a significance of baseline hyperlipidemia could
also be one reason for the marked PH in this model.

F-1394 improved the PH seen in fat-loaded ZDF fa/fa rats and significantly reduced serum TC and TG by 24% and 31%, respectively (Table 1). Moreover, ACAT activity in the intestinal mucosa in fa/fa rats was decreased by F-1394 (data not shown). It is suggested that F-1394 interferes with TC and TG absorption via the gut as a result of its inhibition of small intestinal ACAT activity. Thus, an abnormal increase in lipid absorption may be at least partly responsible for the PH seen in this model, which is released from normal control by insulin as well. However, further investigation will be required to clarify the details of the underlying mechanisms.

Recently, it was reported that severe hyperlipidemia could be induced in the db/db mouse, a mouse that develops significant obesity, fasting hyperglycemia and hyperinsulinemia, by feeding it a Western-type diet containing 0.15% cholesterol and 21% fat (9). Although the authors did not describe about postprandial changes in serum lipid levels, it is very likely that PH may have occurred in db/db mice as well as fa/fa rats and STZ-diabetic rats.

Although the cocktail used here contained no sugars, the serum glucose level in both fa/fa and lean littermate increased gradually to reach a peak around 6 to 8 h after fat loading; the AUICs were 1454 ± 168 and 1258 ± 206 mg · dl⁻¹ · h, respectively, \( P = 0.4738 \) (Fig. 1C). In contrast, in fat-loaded fa/fa rats treated with F-1394, the serum glucose level approached the level seen before fat-loading in this strain (Table 1 and Fig. 1C), although there are data indicating that this compound does not reduce sugar absorption or gluconeogenesis (J. Kusunoki et al., unpublished data). It was reported that free fatty acid stimulates glucose production in the liver in diabetes (10). Taken together, these results suggest that absorbed lipids stimulate gluconeogenesis in their liver, and the serum glucose was not elevated in the fat loaded fa/fa rats given F-1394 due to a reduction in exogenous lipid intake by the compound.

In the present study, we established a PH model in ZDF fa/fa rats by giving a single oral administration of a high-fat cocktail. Since the pathological features, such as lipid dismetabolism, of this fa/fa rat are very similar to those of patients with type II diabetes (1), this PH model should be suitable for the exploration of the pathogenesis and potential therapies. Furthermore, PH itself is increasingly being recognised as an important therapeutic target, because it is an independent risk factor for CHD (1, 2).

Hence, F-1394, an orally active ACAT inhibitor, would appear to have potential for use in PH patients, although, as yet, this compound is still not commercially available.

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