Effect of Troglitazone (CS-045) and Bezafibrate on Glucose Tolerance, Liver Glycogen Synthase Activity, and β-Oxidation in Fructose-Fed Rats

I. Inoue, K. Takahashi, S. Katayama, Y. Harada, K. Negishi, A. Itabashi, and J. Ishii

To clarify the relationship between lipid and glucose metabolism abnormalities in fructose-fed rats, we examined whether an improvement of insulin sensitivity by troglitazone (CS-045) or a decrease in plasma lipids by bezafibrate affects the relationship between serum levels of lipid and glucose. In addition, we also examined changes in liver glycogen metabolism and β-oxidation in fructose-fed rats. Troglitazone ameliorated fasting hyperlipidemia, hyperglycemia, and hyperinsulinemia. In addition, it augmented glycogen synthase activity by 53%, and decreased the mitochondrial palmitic acid β-oxidation rate and ketone body production rate by 27% and 55%, respectively. However, hyperglycemia and liver glycogen synthase activity were not improved by bezafibrate treatment despite a marked reduction of serum triglyceride (TG) levels resulting from a 1.76-fold increase in mitochondrial oxidation and a 2.04-fold increase in hepatic ketone body production. These results suggest that abnormalities in glucose and lipid metabolism in fructose-fed rats, which are ameliorated by troglitazone, may be closely linked to reduced glycogen synthase activity in the liver.

ADMINISTRATION of fructose to normal rats leads to hyperinsulinemia, hyperglycemia, hypertriglyceridemia, and elevated blood levels of free fatty acids (FFA), mostly due to the diminished ability of insulin to suppress hepatic glucose output.[1] Fructose-fed rats are widely used as an experimental model of insulin resistance. Although details of the mechanisms responsible for these changes are still unclear, two possibilities can be suggested: (1) the insulin resistance in the liver or peripheral tissues such as muscle and adipose tissue may cause hypertriglyceridemia and elevated blood levels of FFA, which is sometimes observed in patients with non–insulin-dependent diabetes mellitus (NIDDM); or (2) conversely, the hypertriglyceridemia and/or elevated blood levels of FFA may themselves cause insulin resistance in these tissues,[2] a phenomenon known as glucose intolerance in patients with hyperlipemia.[3,4] Moreover, insulin resistance in fructose-fed rats is associated with a reduction of insulin receptor kinase[5] or hepatic glycogen synthase.[6] Recently, Wolf[7] reported that an inhibitor of β-oxidation (inhibitor of carnitine palmitoyltransferase, Etomoxir, BYK Gluden, Germany) increases glucose uptake into the liver and reduces plasma glucose, suggesting that β-oxidation in the liver might be associated with glucose metabolism.

To clarify the relationship between lipid and glucose metabolism abnormalities in fructose-fed rats, we examined whether an improvement of insulin sensitivity by troglitazone or a decrease of plasma lipid levels by bezafibrate affects the relationship between lipid and glucose metabolism. In addition, we also examined changes in liver glycogen synthesis and β-oxidation in fructose-fed rats.

Troglitazone is one of a new class of antidiabetic drugs that decrease plasma glucose, insulin, triglyceride (TG), and FFA in various diabetic animal models and in diabetic patients. Although the exact mechanisms of action of thiazolidinediones such as troglitazone, ciglitazone, pioglitazone, and englitazone are not entirely clear, troglitazone has been reported to improve insulin sensitivity, increase conversion of glucose to glycogen in HepG2 cells from rats,[8] and reduce hepatic gluconeogenic enzyme activity in rats and mice.[9]

On the other hand, bezafibrate is now widely used as an antihyperlipidemic drug that decreases serum TG and FFA levels in diabetic animals and human patients. Moreover, fibrates such as bezafibrate,[4] clofibrate,[3] and gemfibrozil[10] have also been shown to reduce plasma glucose levels in NIDDM patients. It has also been reported that these drugs increase β-oxidation in mitochondria[11] and peroxisomes[12] in the liver, as well as lipoprotein lipase activity in the vascular endothelium. However, details of the relationship between this hypolipidemic effect and glycemic control are still unknown.

The present study was therefore designed to test whether troglitazone or bezafibrate ameliorate hyperinsulinemia, hyperglycemia, hypertriglyceridemia, and elevated blood levels of FFA by augmenting glycogen synthesis and β-oxidation in fructose-fed rats.

MATERIALS AND METHODS

Male Wistar rats were obtained from Charles River, Kanagawa, Japan at the age of 6 weeks. They were housed three per cage and allowed free access to tap water and standard laboratory rat chow from CLEA, Shizuoka, Japan. Two weeks later, blood samples were obtained from a jugular vein to determine plasma glucose, insulin, total cholesterol (TC), TG, phospholipid (PL), and FFA after an overnight fast. The animals were divided into four groups and fed one of the following diets for 5 weeks: (1) normal rat chow (protein 27%, lipid 12%, and carbohydrate 61% of total calories; control group, n = 8); (2) high-fructose diet in which the carbohydrate of the normal rat chow was substituted with fructose, which provided 55% of total calories (fructose group, n = 9); (3) high-fructose diet in combination with troglitazone 70 mg/kg/d (troglitazone group, n = 9); and (4) high-fructose diet in combination with bezafibrate 30 mg/kg/d (bezafibrate group, n = 9). The drugs were dissolved in polyethylene glycol and administered orally by gavage

From the Fourth Department of Medicine, Saitama Medical School, Saitama, Japan.

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Address reprint requests to I. Inoue, MD, Fourth Department of Internal Medicine, Saitama Medical School, 38 Morohongo, Moroyama, Iruma-gun, Saitama 350-04, Japan.

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in the troglitazone group or bezafibrate group. The control group or fructose group received only 2.5 mL/kg vehicle (polyethylene glycol:glycerol:water, 194:12:20 wt/wt). Fasting serum TC, TG, PL, FFA, plasma glucose, and insulin were determined 5 weeks after the start of drug administration. In addition, liver homogenates obtained from each group were used for assay of glycogen synthase according to the method reported by Guinovart *et al*[13] and for assay of β-oxidation by the method reported by Mannaerts *et al.*[14,15] Briefly, glycogen synthase activity was determined as follows. Livers were removed and immersed in ice-cold 0.25 mol/L sucrose–0.005 mol/L EDTA, pH 7.5. All subsequent procedures were performed at 2° to 4°C. The livers were drained, blotted, minced, and homogenized with 3 vol (wt/vol) cold 0.25-mol/L sucrose–0.005-mol/L EDTA, pH 7.5, in a glass homogenizer. Glycogen was determined by the phenol–sulfuric acid method reported by Dubois *et al.*[16] Aliquots of the homogenates were centrifuged at 8,500 × *g* for 10 minutes, and then the supernatants were stored at −35°C until enzyme assay. Glycogen synthase activity was measured using UDP-[^{14}C]-glucose and expressed as micromoles of glucose coverted to glycogen per minute per gram of protein. Total glycogen synthase activity was measured at high glucose-6-phosphate concentration (10 mmol/L) in the presence of 0.2 mmol/L UDP-[^{14}C]-glucose. The active form of glycogen synthase was assayed at low glucose-6-phosphate concentration (0.25 mmol/L) in the presence of 0.2 mmol/L UDP-[^{14}C]-glucose. Percent glycogen synthase activity was calculated as (active form of glycogen synthase activity/total glycogen synthase activity) × 100.

Hepatic β-oxidation was assayed by adding 0.5 mL liver homogenate, 25% (wt/vol) in 0.25 mol/L sucrose containing 0.1% ethanol, to 2 mL buffer containing the following: modified Krebs-Henseleit bicarbonate buffer, pH 7.4, 0.2 mmol/L ^{14}C-labeled palmitic acid (specific activity, 1 Ci/mol) bound to 7.2 mg/mL defatted albumin, 4 mmol/L adenosine triphosphate, 0.5 mmol/L L-carnitine, 0.05 mmol/L coenzyme A, and 2 mmol/L dithiothreitol. The reaction was started by adding an aliquot of homogenate (0.5 mL) and continued at 37°C for 3 minutes with shaking. The reaction was terminated with 12% HCO_4, and labeled CO_2 was trapped in phenethylamine.[17] An aliquot of the neutralized HCO_4 extract was analyzed for ^{14}C content of ketone bodies. Another aliquot was adjusted to pH 4 with 3 mol/L acetate buffer and extracted twice with light petroleum to remove traces of ^{14}C-palmitic acid. The aqueous phase was counted and considered the acid-soluble labeled oxidation product. Palmitic acid oxidation was determined in the presence or absence of 2 mmol/L KCN, and the cyanide-sensitive proportion of total oxidation was regarded as mitochondrial oxidation. Oxidation products were expressed as the total of both labeled CO_2 and acid-soluble labeled oxidation products. The count recovery after centrifugal fractionation was 90% to 95%.

Plasma glucose levels were determined by a glucose oxidase method and insulin levels by a radioimmunoassay using rat insulin as a standard.

All data are expressed as the mean ± SD. The statistics were used with the Kruskal-Wallis test for comparison of data between different groups. Differences were considered significant at P less than .05.

RESULTS

Body weight at the start of medication did not differ significantly between groups. After the study, body weight in the fructose group was significantly lower than in the control group. Treatment of fructose-fed rats with bezafibrate decreased body weight significantly, whereas troglitazone increased body weight (Table 1). Bezafibrate treatment increased liver weight significantly in comparison to the other three groups (Table 1).

TG and FFA concentrations in the troglitazone group or bezafibrate group were significantly lower than in the fructose group after these treatments (Fig 1). Treatment with troglitazone or bezafibrate had no effect on plasma TC concentration (Fig 1).

The fructose group showed significant deterioration of glycemic control. Troglitazone treatment ameliorated fasting hyperglycemia and hyperinsulinemia, but bezafibrate produced no significant change in comparison to fructose-fed rats (Fig 2).

Fructose feeding decreased percent glycogen synthase activity (percent activity) as compared with levels in control rats. Troglitazone treatment increased the percent activity significantly, but did not change glycogen content in the liver (Table 2). Both percent activity and glycogen content in the bezafibrate group showed no significant change in comparison to fructose-fed rats (Table 2).

KCN at 2 mmol/L inhibited generation of total acid-soluble oxidation products by 75% to 85%. Mitochondrial palmitic acid oxidation rate (mitochondrial oxidation) in the fructose group increased slightly but not significantly in comparison to the control group. Although mitochondrial oxidation and ketone body production were significantly decreased in the troglitazone group, they were significantly increased in the bezafibrate group as compared with the fructose group (Table 3).

DISCUSSION

In the present study, long-term administration of fructose to normal rats resulted in the typical characteristics of insulin resistance such as hyperinsulinemia, hyperglycemia, hypertriglyceridemia, and elevated blood levels of FFA, although rats did not become markedly obese. Fructose feeding is known to produce hyperglycemia associated with hyperinsulinemia, which may be due to reduced hepatic glycogen synthase activity.[6] Hypertriglyceridemia in this model may be attributed to elevated hepatic very–low-density lipoprotein TG synthesis and secretion,[18] as well as less efficient elimination of very–low-density lipoprotein TG from plasma.[19,20]

Table 1. Change in Body Weight and Liver Weight (mean ± SD) in Four Rat Groups

Parameter	Control	Fructose	Troglitazone	Bezafibrate
No. of rats	8	9	9	9
Body weight before study (g)	235.8 ± 12.1	236.2 ± 14.0	246.9 ± 5.4	247.1 ± 13.5
Body weight after study (g)	353.1 ± 19.1	311.3 ± 16.6*	344.3 ± 18.1†	309.8 ± 16.7*
Liver weight (g)	11.10 ± 0.93	10.63 ± 0.68	11.70 ± 0.53	16.35 ± 1.46*†

*P < .05 v control group.

†P < .05 v fructose group.

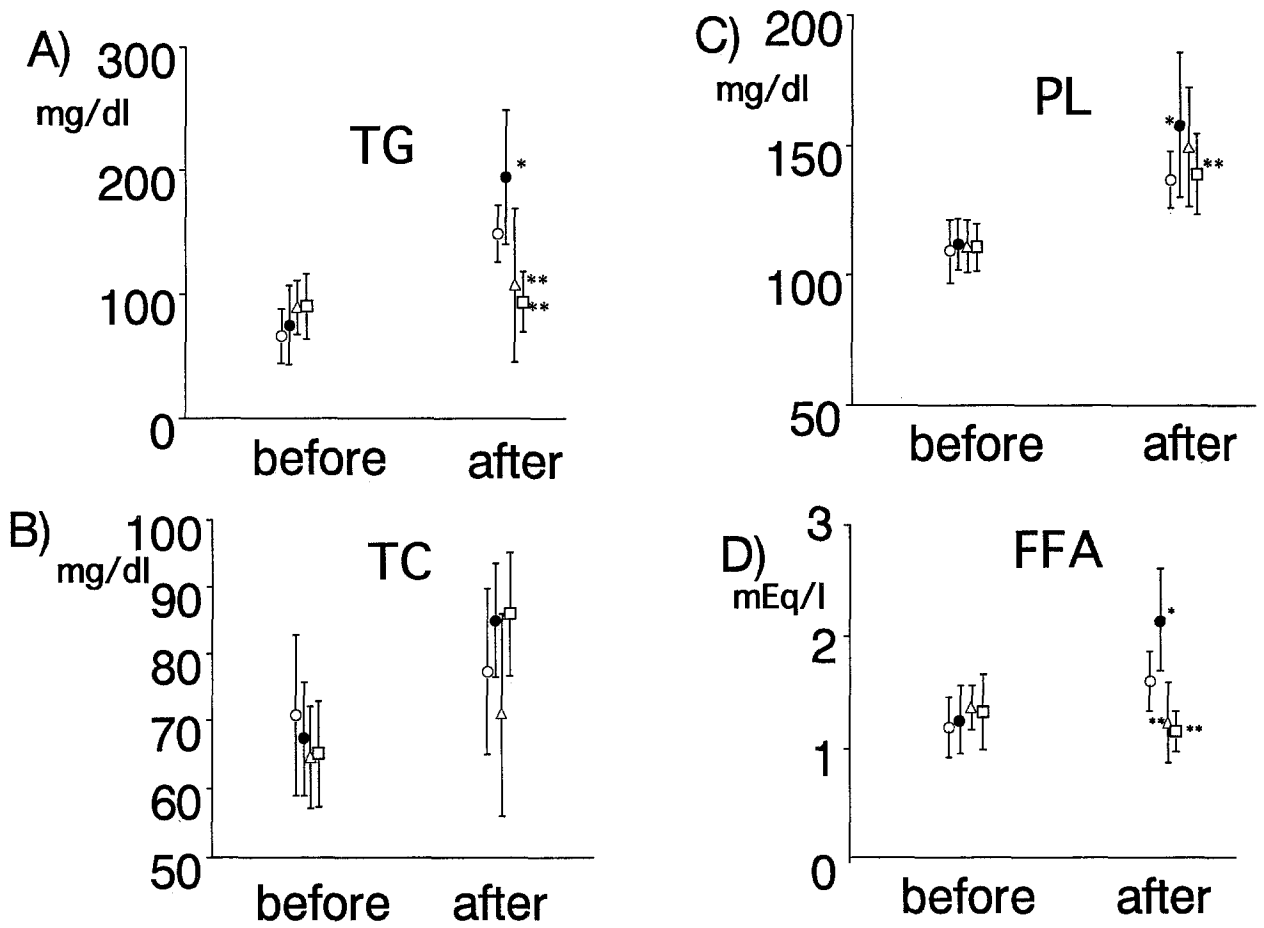

Fig 1. Serum TG (A), serum TC (B), PL (C), and FFA (D) in control rats (n = 8, ○), fructose-fed rats (n = 9, ●), fructose-fed rats treated with bezafibrate 30 mg/kg/d (n = 9, △), and fructose-fed rats treated with troglitazone 70 mg/kg/d (n = 9, □) before and 5 weeks after drug administration. Each point indicates the mean ± SD. *$P < .05$ *v* control rats; **$P < .05$ *v* fructose-fed rats.

Insulin resistance causes hypertriglyceridemia and elevated blood levels of FFA, and, conversely, the latter may lead to the former.[2] In fact, Randle *et al*[2] have demonstrated the inhibitory effect of a high concentration of FFA on the rate of glucose utilization in isolated rat hearts and hemidiaphragms, and this has been termed the glucose–fatty acid cycle. Moreover, the increase in plasma FFA concentration resulting from infusion of TG emulsion has been shown to accelerate endogenous glucose production and cause fasting hyperglycemia in normal humans.[21] In addition, infusion of nicotinic acid, a drug that decreases plasma TG concentration, also decreases plasma glucose and FFA concentrations.[22] Recently, we[4] have reported that bezafibrate is effective in decreasing plasma glucose and serum insulin levels during oral glucose tolerance tests in non-obese patients with hypertriglyceridemia and impaired glucose tolerance. Therefore, TG or FFA themselves may be one of the factors causing deterioration of insulin sensitivity. Thus, reduction of serum TG or FFA may have a beneficial effect in ameliorating insulin resistance.

To address this issue, troglitazone, a so-called insulin sensitizer, or the lipid-lowering agent, bezafibrate, were administered to rats fed a high-fructose diet. Both drugs were found to reduce plasma TG and FFA levels.

However, hyperglycemia was ameliorated only by troglitazone, not by bezafibrate. Of interest is the observation that troglitazone, but not bezafibrate, augmented the diminished hepatic glycogen synthase activity (Table 2). Our data are consistent with the in vitro findings of Ciaraldi *et al*,[8] who reported that troglitazone increased glycogen synthase I activity and reduced gluconeogenesis from lactate in HepG2 cells. Previous studies have shown that glycogen synthase activity can be stimulated by increasing the fraction of the enzyme in the I form. Reduced hepatic glycogen synthase activity has been demonstrated in fructose-fed rats[6] and patients with NIDDM.[23-25] Recently, an association between glycogen synthase gene polymorphism and a strong family history of NIDDM has been reported,[26] suggesting that the glycogen synthase gene may be a candidate gene responsible for insulin resistance.

One further point to be noted is the decreased mitochon-

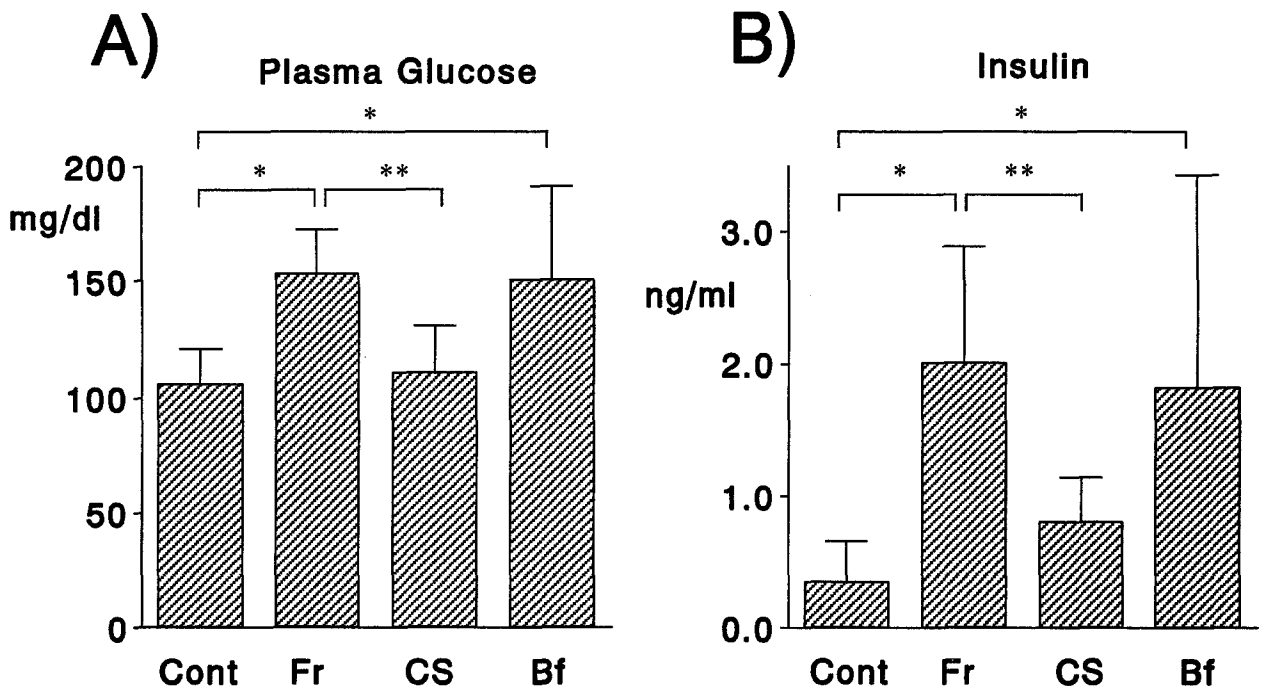

Fig 2. Fasting plasma glucose (A) and serum insulin (B) in control rats (Cont, n = 8), fructose-fed rats (Fr, n = 9), fructose-fed rats treated with troglitazone 70 mg/kg/d (CS, n = 9), and fructose-fed rats treated with bezafibrate 30 mg/kg/d (Bf, n = 9) after 5 weeks of treatment. Each point indicates the mean ± SD. *$P < .05$ v control rats; **$P < .05$ v fructose-fed rats.

drial oxidation of FFA induced by troglitazone and the increase induced by bezafibrate. A previous study by Wolf[7] demonstrated that Etomoxir, a β-oxidation inhibitor, ameliorated fasting hyperglycemia in a dose-dependent manner in diabetic mice, indicating that reduction of FFA oxidation may improve glycemic control. Pioglitazone, another thiazolidinedione, has been reported to increase insulin receptor kinase in muscle,[27] which is known to be reduced by a high-fat, high-sucrose diet.[5] Although it remains to be elucidated whether troglitazone itself has a direct inhibitory effect on β-oxidation or whether the reduction of hepatic very–low-density lipoprotein TG synthesis may occur via improved insulin resistance, troglitazone may be uniquely beneficial for treatment of not only diabetic hyperglycemia but also diabetic dyslipidemia resulting from insulin resistance.

Table 2. Liver Glycogen Synthase Activity (mean ± SD) in Four Rat Groups

Parameter	Control	Fructose	Troglitazone	Bezafibrate
Glycogen content (mg/g wet liver)	3.75 ± 1.10	3.47 ± 0.99	3.35 ± 0.86	3.72 ± 0.42
Total activity (nmol/min/mg protein)	7.83 ± 1.72	7.30 ± 1.65	7.93 ± 1.80	7.35 ± 1.74
Percent activity (%)	40.1 ± 4.1	30.4 ± 3.1*	46.2 ± 4.0†	30.1 ± 3.5*

*$P < .05$ v control group.
†$P < .05$ v fructose group.

On the other hand, administration of bezafibrate to fructose-fed rats increased FFA oxidation (Table 3) together with the decrease of serum TG and FFA levels (Fig 1). However, fasting hyperglycemia was not altered by this maneuver (Fig 2). These data indicate that increased FFA oxidation does not necessarily indicate deteriorated glycemic control. A decrease in the level of FFA may result from increased FFA oxidation or from decreased FFA release from adipose tissue caused by improved insulin resistance. Polyunsaturated fatty acids such as eicosapentanoic acid and docosahexanoic acid have also been reported to increase mitochondrial fatty acid oxidation[28] without deteriorating glycemic control in diabetics.

In conclusion, the present results suggest that abnormalities of glucose and lipid metabolism in fructose-fed rats, which are ameliorated by troglitazone, may be linked to reduced glycogen synthase activity in the liver or to insulin-stimulated improvement of glucose transport utilization. Troglitazone may have unique characteristics in improving the metabolic abnormalities of both glucose and lipids in diabetes mellitus.

Table 3. Palmitic Acid Oxidation (mean ± SD) in Four Rat Groups

Group	Mitochondrial†	Ketone Body†
Control	0.304 ± 0.045	0.215 ± 0.023
Fructose	0.365 ± 0.054	0.224 ± 0.023
Troglitazone	0.265 ± 0.055*	0.123 ± 0.022*
Bezafibrate	0.643 ± 0.031*	0.458 ± 0.039*

*$P < .05$ v fructose group.
†μmol palmitic acid oxidized/min/g liver.

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