The Effect of Pioglitazone on Glucose Metabolism and Insulin Uptake in the Perfused Liver and Hindquarter of High-Fructose–Fed Rats

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To investigate the effect of pioglitazone, a thiazolidinedione oral antidiabetic agent, on the glucose and insulin metabolism in insulin resistance, a perfusion study of the liver and hindquarter was performed in high-fructose–fed rats. Male Wistar albino rats were assigned randomly to one of the following diets for 2 weeks: (1) normal chow (control group), (2) a diet high in fructose (fructose group), or (3) a high-fructose diet plus pioglitazone (pioglitazone intake of ≃ 10 mg/kg body weight; pioglitazone group). The elevated levels of plasma insulin, triglyceride, and free fatty acids (FFA) in the fructose group were normalized by pioglitazone administration. In the perfused liver, the glucagon-induced increment in the glucose output of the fructose (57.1 ± 9.1 μmol/g liver/20 min) and pioglitazone (44.7 ± 10.1 μmol/g liver/20 min) groups was significantly ($P < .01$) higher than that in the control group (27.6 ± 5.7 μmol/g liver/20 min). The level in the pioglitazone group was significantly ($P < .05$) lower than that in the fructose group. In the presence of 100 or 500 μU/mL insulin, the insulin-mediated decrement in the glucagon-induced glucose output of the fructose group (29.8 ± 7.8 or 38.9 ± 9.3 μmol/g liver/20 min) was significantly ($P < .05$) lower than that in the control (45.8 ± 14.2 or 54.5 ± 8.5 μmol/g liver/20 min) and pioglitazone (44.4 ± 9.2 or 56.2 ± 10.8 μmol/g liver/20 min) groups, respectively. In the perfused hindquarter, glucose uptake in the fructose group (8.2 ± 2.0 μmol/g muscle/30 min) was significantly ($P < .05$) lower than that in the control (12.1 ± 2.3 μmol/g muscle/30 min) and pioglitazone (11.8 ± 3.1 μmol/g muscle/30 min) groups. In the presence of 100 or 500 μU/mL insulin, glucose uptake in the fructose group (12.0 ± 5.2 or 17.4 ± 3.0 μmol/g muscle/30 min) was significantly ($P < .05$) lower than that in the control (20.2 ± 2.4 or 23.0 ± 3.1 μmol/g muscle/30 min) and pioglitazone (17.8 ± 2.4 or 20.7 ± 2.0 μmol/g muscle/30 min) groups, respectively. Insulin uptake by the liver and hindquarter was not significantly different in the control, fructose, and pioglitazone groups. These results indicate that pioglitazone improves the increased glucagon-induced hepatic glucose output and decreases insulin-induced muscular glucose uptake without altering insulin uptake in high-fructose–fed insulin-resistant rats.

A NEW CLASS OF PHARMACOLOGIC AGENTS, the thiazolidinediones, has been well known to enhance insulin-stimulated glucose disposal in peripheral tissues in animal models of diabetes and insulin resistance[1-5] and in humans with impaired glucose tolerance or non–insulin-dependent diabetes mellitus.[6-8] In insulin resistance, insulin action is impaired mainly in the liver and skeletal muscles. However, the effect of thiazolidinediones on hepatic and muscular glucose metabolism is unclear. Furthermore, the effect of thiazolidinediones on hepatic and muscular insulin disposal remains to be fully understood, although the biological actions of insulin are elicited by insulin binding to receptors. To elucidate these issues, the effect of pioglitazone, one of thiazolidinedione compounds, on hepatic glucose output and muscular glucose disposal, and on hepatic and muscular insulin uptake, was investigated in the perfused liver and hindquarter of high-fructose–fed insulin-resistant rats.

MATERIALS AND METHODS

Animals

Male Wistar albino rats (4 weeks old) were used in the present study. The animals were assigned randomly to one of the following diets for 2 weeks: (1) normal chow (control group), (2) a diet high in fructose (fructose group), or (3) a high-fructose diet plus pioglitazone (pioglitazone group). The caloric content of the normal chow was distributed as 58% carbohydrate, 12% fat, and 30% protein. The high-fructose diet contained 67% carbohydrate (98% of which was fructose), 13% fat, and 20% protein in calorie. Pioglitazone (Takeda Chemical Industries, Osaka, Japan) was administered in the chow (daily pioglitazone intake of ≃ 10 mg/kg body weight). All animals were allowed free access to water.

Preparation of the Liver

A modified method[9] of Sugano et al[10] was used for isolation and perfusion of the rat liver. The rats were anesthetized with intraperitoneal pentobarbital sodium (30 mg/kg), and the abdomen was opened through a midline incision. The intestines were then placed to the animal's left. The thin strands of connective tissue between the right lobe of the liver and the vena cava were cut and a loose ligature was placed around the inferior vena cava above the right renal vein, superior mesenteric and celiac arteries, and the portal vein. The portal vein was then cannulated and the perfusion pump was started. The ligatures around the portal vein and the arteries were tied. The thorax was then opened and an outflow cannula was inserted through the right atrium into the thoracic vena cava. Finally, the ligature around the abdominal inferior vena cava was tied, thus closing the circuit. The liver was perfused without recirculation with a synthetic medium at a flow rate of 2.5 mL/g liver weight/min.

Preparation of the Hindquarter

A modified method[11,12] of Ruderman et al[13] was used for isolation and perfusion of the rat hindquarter. In brief, after anesthesia with pentobarbital sodium, the abdomen was opened. After injection of heparin (200 U), the abdominal aorta was ligated and then incised between the left renal and iliolumbar vessels. An inflow cannula was inserted and passed to a point midway between the iliolumbar vessels and the aortic bifurcation. The cannula was then fixed in place. Then the perfusion pump was started. All viscera except the urinary bladder, testes, prostate, and seminal vesicle were removed, and some of the major abdominal branches of the great vessels were ligated. Since it was not possible to collect the perfusate quantitatively from the inferior vena

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cava because of anastomotic connections with the vertebral veins, the operated animal was bisected just above the aortic cannulation. The effluent was allowed to drip into the chilled tube. The hindquarter was perfused without recirculation with a synthetic medium at a flow rate of 0.5 mL/g muscle weight/min.

Perfusion Method

Perfusion Medium

The perfusion medium consisted of a Krebs-Ringer bicarbonate buffer containing 3.0% bovine serum albumin and 4.6% Dextran T-70 (Green Cross, Osaka, Japan). To prevent glucose metabolism by erythrocytes and interference for insulin assay by hemolysis, an erythrocyte-free medium was used in the present study. The venous effluent was collected every 5 minutes and stored at −70°C until assay was performed. During perfusion, the medium and the hindquarter were warmed and kept at 37°C and the medium was bubbled with a mixture of 95% O_2 and 5% CO_2. The pH was maintained at 7.4.

Perfusion of Liver

Glucagon-induced glucose output. After a 15-minute perfusion with the medium containing 5.6 mmol/L glucose, 2 mmol/L alanine, and 2 mmol/L lactate, the liver was perfused with the midium containing 200 pg/mL glucagon (Novo Nordisk, Copenhagen, Denmark) for 20 minutes.

Insulin effect on glucagon-induced glucose output. After a 15-minute perfusion with the medium containing 200 pg/mL glucagon, the liver was perfused with the medium containing porcine insulin (100 or 500 μU/mL, crystalline, glucagon-free; Eli Lilly, Indianapolis, IN) for 20 minutes.

Perfusion of Hindquarter

After a 15-minute perfusion with the medium containing 8.3 mmol/L glucose, the hindquarter was perfused with the medium containing insulin (100 or 500 μU/mL) or not containing insulin for 30 minutes.

Calculations

The glucagon-induced increment in glucose output in the liver was calculated by the following formula: (perfusate glucose concentration after glucagon infusion − basal perfusate glucose concentration) × perfusion volume. The insulin-mediated decrement in glucose output in the liver was calculated by the following formula: (basal perfusate glucose concentration − perfusate glucose concentration after insulin infusion) × perfusion volume. Glucose uptake by the hindquarter was calculated by the formula: (basal perfusate glucose concentration − perfusate glucose concentration) × perfusion volume. Insulin uptake was calculated by the formula: (insulin infused during perfusion − effluent insulin during perfusion) × 100(%)/insulin infused during perfusion. The basal perfusate glucose concentration was the mean of the glucose level at 10 minutes and 15 minutes.

Measurements

Glucose concentration was measured by the glucose oxidase method.[14] Plasma triglyceride and FFA concentrations were measured enzymatically using an available commercial kit. Insulin was measured by radioimmunoassay. The least-detectable insulin concentration was 2 μU/mL, and intraassay and interassay coefficients of variation were 4% and 8%.

Statistical Analysis

Data are expressed as the mean ± SD. ANOVA and two-tailed Student's *t* test were used for statistical evaluation.

Table 1. Characteristics of the Rats

Characteristic	Control (n = 6)	Fructose (n = 6)	Pioglitazone (n = 6)
Body weight (g)	177 ± 8	172 ± 9	172 ± 11
Liver weight (g)	8.0 ± 0.5	8.3 ± 0.9	8.1 ± 1.2
Hindquarter weight (g)	15.1 ± 1.0	15.3 ± 0.9	14.6 ± 1.2
Basal blood glucose (mg/dL)	89 ± 3	99 ± 8*	101 ± 11*
Basal plasma insulin (μU/mL)	12 ± 2	27 ± 8†	18 ± 4*‡
FFA (μEq/L)	845 ± 112	1,130 ± 180†	807 ± 143‡
Triglyceride (mg/dL)	105 ± 8	145 ± 20†	101 ± 16‡
Blood pressure (mm Hg)	110 ± 5	109 ± 5	111 ± 6

NOTE. Data are expressed as the mean ± SD.
*$P < .05$, †$P < .01$, significantly different from control.
‡$P < .05$, significantly different from fructose.

RESULTS

Characteristics of the Rat

As shown in Table 1, fasting blood glucose and insulin concentrations in the fructose group (99 ± 8 mg/dL and 27 ± 8 μU/mL) and the pioglitazone group (101 ± 11 mg/dL and 18 ± 4 μU/mL) were significantly ($P < .05$) higher than that in the control group (89 ± 3 mg/dL and 12 ± 2 μU/mL). Fasting plasma insulin concentration in the pioglitazone group was significantly ($P < .05$) lower than that in the fructose group. Fasting plasma triglyceride and free fatty acids (FFA) concentrations in the fructose group (145 ± 20 mg/dL and 1,130 ± 180 μEq/L) were significantly ($P < .01$) higher than that in the control (105 ± 8 mg/dL and 845 ± 112 μEq/L) and pioglitazone (101 ± 16 mg/dL and 807 ± 143 μEq/L) groups, respectively.

Glucose Output by the Perfused Liver

As shown in Fig 1, the glucagon-induced increment in glucose output in the fructose (57.1 ± 9.1 μmol/g liver/30 min)

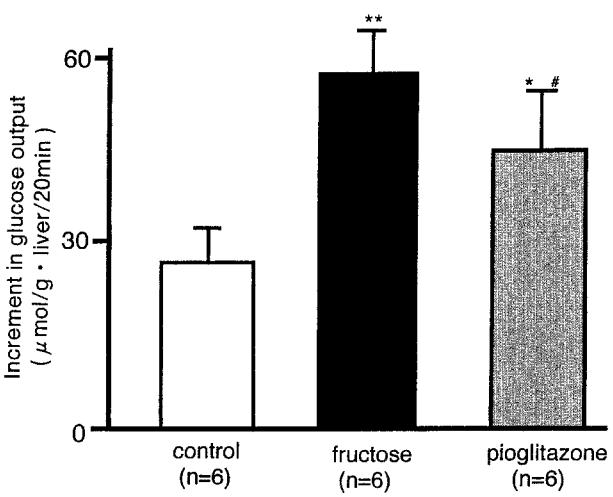

Fig 1. Glucagon-induced increment in glucose output in perfused liver. Bars represent SD. The liver was stimulated by 200 pg/mL glucagon. *$P < .01$, **$P < .001$, significantly different from control. #$P < .05$, significantly different from fructose.

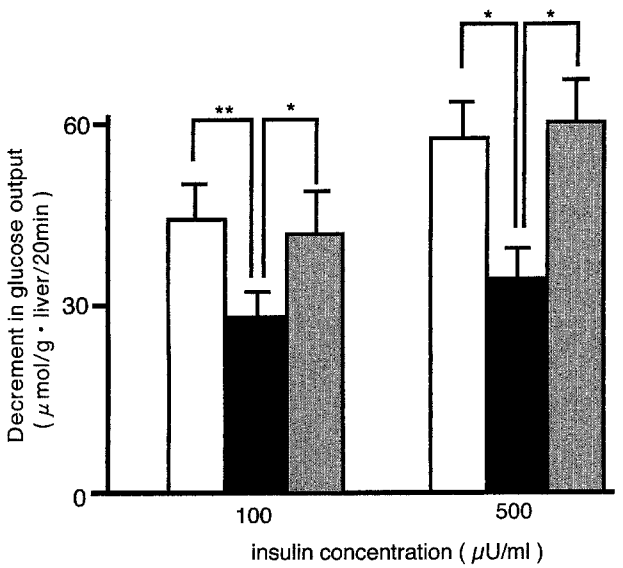

Fig 2. Insulin-mediated decrement in glucagon-induced glucose output in perfused liver. Bars represent SD. (□) Control (n = 6); (■) fructose (n = 6); (▩) pioglitazone (n = 6). *$P < .05$. **$P < .02$.

and pioglitazone (44.7 ± 10.1 μmol/g liver/30 min) groups was significantly ($P < .01$) higher than that in the control group (27.6 ± 5.7 μmol/g liver/30 min). In the pioglitazone group, it was significantly ($P < .05$) lower than that in the fructose group.

As shown in Fig 2, in the presence of 100 or 500 μU/mL insulin, the insulin-mediated decrement in glucagon-induced glucose output in the fructose group (29.8 ± 7.8 or 38.9 ± 9.3 μmol/g liver/30 min) was significantly ($P < .05$) lower than that in the control (45.8 ± 14.2 or 54.5 ± 8.5 μmol/g liver/30 min) and pioglitazone (44.4 ± 9.2 or 56.2 ± 10.8 μmol/g liver/30 min) groups, respectively.

Glucose Uptake by the Perfused Hindquarter

As shown in Fig 3, in the absence of insulin, glucose uptake in the fructose group (8.2 ± 2.0 μmol/g muscle/30 min) was significantly ($P < .05$) lower than that in the control (12.1 ± 2.3 μmol/g muscle/30 min) and pioglitazone (11.8 ± 3.1 μmol/g muscle/30 min) groups.

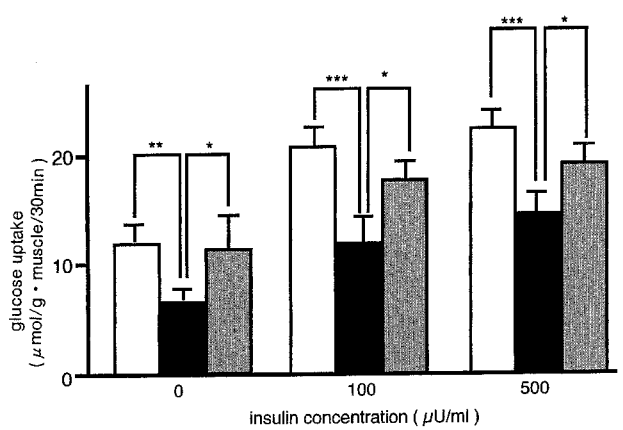

Fig 3. Glucose uptake in perfused hindquarter. Bars represent SD. (□) Control (n = 6); (■) fructose, (n = 6); (▩) pioglitazone (n = 6). *$P < .05$. **$P < .02$. ***$P < .01$.

In the presence of 100 or 500 μU/mL insulin, glucose uptake in the fructose group (12.0 ± 5.2 or 17.4 ± 3.0 μmol/g muscle/30 min) was significantly ($P < .05$) lower than that in the control (20.2 ± 2.4 or 23.0 ± 3.1 μmol/g muscle/30 min) and pioglitazone (17.8 ± 2.4 or 20.7 ± 2.0 μmol/g muscle/30 min) groups, respectively.

Insulin Uptake by the Perfused Liver and Hindquarter

As shown in Table 2, insulin uptake by the liver and hindquarter perfused with 100 or 500 μU/mL insulin was not significantly different in the control (39% ± 3% or 32% ± 4% in the liver, and 22% ± 5% or 27% ± 4% in the hindquarter), fructose (38% ± 5% or 33% ± 6%, and 24% ± 3% or 28% ± 5%), and pioglitazone (40% ± 4% or 35% ± 4%, and 26% ± 3% or 31% ± 3%) groups.

DISCUSSION

Fructose feeding for 2 weeks led to the impairment of insulin ability to suppress hepatic glucose output and to enhance muscular glucose uptake in the present study. In these experimentally induced insulin-resistant rats, pioglitazone treatment ameriolated the elevated levels of plasma insulin, triglyceride, and FFA in vivo and also normalized both the increased glucagon-induced hepatic glucose output and the decreased insulin-mediated muscular glucose uptake, suggesting that pioglitazone improves an environmentally induced, nongenetic form of insulin resistance. The present results were consistent with the report by Lee et al[5] in that troglitazone, a thiazolidinedione compound that is related chemically to pioglitazone, improved hyperinsulinemia and glucose disposal in fructose-induced insulin resistant rats. Tobey et al[15] have reported that the insulin resistance resulting from chronic fructose feeding is due to the diminished ability of insulin to suppress hepatic glucose output, and not to a decrease in insulin-stimulated glucose uptake by the muscles. However, in their study, the rats were fed high fructose for only 7 days. The mechanisms of peripheral insulin resistance caused by this dietary regimen remain incompletely defined. Decreased insulin receptor binding[16] and postreceptor defects[17,18] have previously been reported. Although insulin uptake by the perfused organs is not likely to be a good index of insulin bindings by the organ insulin receptors, insulin uptake by the perfused liver and hindquarter was not significantly different between the fructose group and control group in the present study.

Table 2. Insulin Uptake by Perfused Liver and Hindquarter

Variable	Insulin (100 μU/mL) Uptake (%)	Insulin (500 μU/mL) Uptake (%)
Liver		
Control (n = 6)	39 ± 3	32 ± 4
Fructose (n = 6)	38 ± 5	33 ± 6
Pioglitazone (n = 6)	40 ± 4	35 ± 4
Hindquarter		
Control (n = 6)	22 ± 5	27 ± 4
Fructose (n = 6)	24 ± 3	28 ± 5
Pioglitazone (n = 6)	26 ± 3	31 ± 3

NOTE. Data are expressed as the mean ± SD.

The mechanisms by which pioglitazone normalized the increased glucagon-induced hepatic glucose output and decreased muscular glucose uptake remains to be elucidated. In the present study, pioglitazone treatment did not increase the insulin uptake in the perfused liver and hindquarter, suggesting that the ameriolating effect of pioglitazone on insulin ability may not be due to the increase in insulin uptake.

Inoue et al[19] have reported that troglitazone administration enhanced glycogen synthetase activity in high-fructose–fed rats. In the present study, pioglitazone suppressed the glucagon-induced glucose output from the perfused liver. Hepatic glucose output is assumed to equal hepatic glycogenolysis because of the absence of gluconeogenic precursors in the perfusion medium. These results indicate that thiazolidinediones may improve hepatic insulin resistance by ameriolating insulin's ability to enhance glycogen synthesis and to inhibit glycogenolysis.

Thiazolidinediones are known to be insulin-sensitizing agents. In the present study, pioglitazone administration ameriolated the decreased muscular glucose disposal in vitro in the absence of insulin. These results indicate that pioglitazone influences non–insulin-mediated glucose uptake in the hindquarter. Okuno et al[20] reported that troglitazone did not directly elicit glucose uptake in the perfused hindquarter in the absence of insulin. Pioglitazone probably enhanced non–insulin-mediated glucose uptake through some secondary effect such as a metabolic influence.

Fructose-induced insulin resistance is related to alterations in fat metabolism and increases in FFA, triglycerides, and other fats, and several investigators have shown that elevation of plasma FFA decreases glucose utilization by muscles.[21-23] The decrease in FFA concentrations in vivo may be partly involved in the present results. Pioglitazone has been reported to correct the insulin signaling defects of Wistar fatty rats.[24] Pioglitazone might have ameriolated the insulin signaling in high-fructose–fed rats. The relationship of clinical insulin resistance to fructose-induced insulin resistance is unknown. Further studies are necessary to clarify the mechanisms by which pioglitazone improves insulin resistance in high-fructose–fed rats.

In summary, we conclude that pioglitazone improves increased glucagon-induced hepatic glucose output and decreased insulin-induced muscular glucose uptake without the alteration of insulin uptake in high-fructose–fed insulin-resistant rats.

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