Metabolic Effects of Troglitazone in the Goto-Kakizaki Rat, a Non-obese and Normolipidemic Rodent Model of Non–Insulin-Dependent Diabetes Mellitus

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Troglitazone (TRG) is an orally active antidiabetic agent that increases insulin sensitivity in models of non–insulin-dependent diabetes mellitus (NIDDM), subsequently reducing hyperinsulinemia and hyperglycemia. We examined the effects of TRG on the development and severity of diabetes in the Goto-Kakizaki (GK) rat, a spontaneous, non-obese model of NIDDM. TRG was administered at a dose of 30 mg/kg/d beginning at 4 weeks of age. TRG-treated GK rats were evaluated against Wistar and untreated GK rats at 8, 12, and 16 weeks of age. Untreated GK rats were nonketotic, normolipidemic, hyperglycemic, and had normal fasting insulin levels compared with Wistar rats. TRG treatment decreased glycosylated hemoglobin levels in the GK rat independently of its effects on plasma insulin. In untreated GK rats, intravenous glucose tolerance tests (IVGTTs) showed a hyperglycemic response to glucose loading with severely impaired glucose disposal relative to Wistar controls. TRG treatment was successful in decreasing the glucose area under the curve (AUC) ($P < .03$) but did not improve glucose disposal, suggesting a direct hepatic effect. Ex vivo evaluation of hepatic glucose output (HGO) further supported a direct hepatic action, with 50% reduction in HGO in TRG-treated GK rats ($P < .004$). A euglycemic-hyperinsulinemic clamp performed at 16 weeks of age showed severe insulin resistance in the untreated GK rat, with a glucose infusion rate (GIR) 33% lower than in Wistar rats ($P < .004$). TRG treatment had no effect on this insulin resistance. These results indicate that TRG selectively decreases hepatic glucose production in this unique model of NIDDM independently of its action on peripheral insulin sensitivity or hyperlipidemia.

TROGLITAZONE (TRG) is a new orally active antidiabetic agent of the thiazolidinedione chemical series.[1] This drug has been shown to reverse insulin resistance in patients with non–insulin-dependent diabetes mellitus (NIDDM) and impaired glucose tolerance,[2] and can enhance insulin action in numerous genetic and acquired rodent models of insulin resistance.[3-7] The antihyperglycemic effects of TRG result from its ability to increase insulin-dependent glucose disposal and reduce hepatic glucose production.[2,4] By enhancing insulin action, TRG treatment results in euglycemia at a lower circulating insulin level. In this regard, studies in normal and diabetic rodents[4,7-9] and human clinical trials[2,10,11] have not revealed hypoglycemia as a complication of thiazolidinedione therapy.

Studies in *ob/ob, db/db,* and KK mice and in the Zucker (*fa/fa*) rat have shown that thiazolidinediones potently decrease plasma glucose, insulin, and triglyceride levels.[3-9] Such effects of thiazolidinediones occur not only in genetic models of NIDDM, but also in models in which insulin resistance and hyperlipidemia are induced through dietary manipulation.[12,13] All of these genetic and acquired animal models share the elements of insulin resistance, obesity, and hyperlipidemia, which complicate the interpretation of the mechanism of action of these drugs. In this study, we attempted to assess the metabolic effects of troglitazone in the absence of obesity or elevated lipid levels. We used the Goto-Kakizaki (GK) rat, a spontaneous model of NIDDM that lacks these complicating factors. This model was developed by selective breeding of normal Wistar rats, using glucose intolerance as the selection index.[14,15] GK rats exhibit a moderate but stable fasting hyperglycemia evident at weaning (4 weeks of age), which does not progress to a ketotic state.[16] Although plasma insulin levels may be normal[17,18] or elevated,[19,20] these rats do not show increased insulin secretion in response to glucose. Moreover, euglycemic-hyperinsulinemic clamp studies have also indicated insulin resistance in these animals.[21-23] We report here that TRG can reduce fasting hyperglycemia in GK rats by suppressing hepatic glucose production without increasing glucose disposal.

MATERIALS AND METHODS

Animals

The colony used in these experiments was derived from two male and four female GK rats obtained from Dr Robert Farese (JA Haley Veterans Hospital, Tampa, FL). Rats used for these experiments were from the F_2 through F_6 generation. Nondiabetic Wistar rats (Charles River Laboratories, Wilmington, MA) were used as controls and were age-matched for all procedures. Animals were maintained in an environmentally controlled room with a 12-hour light/dark cycle and had free access to powdered rodent chow (Purina Formulab #5008; St Louis, MO) and water. Animals were divided into three groups: GK control rats, GK rats administered TRG, and Wistar control rats.

Drug Administration

TRG was administered as a powdered food admixture (Purina Formulab #5008). The diet was prepared fresh daily to ensure stability of the drug. Drug administration started at 4 weeks of age and continued through 16 weeks of age. The average daily dose of TRG, 30 mg/kg body weight/d, was calculated from measuring the food intake and body weight of the rats.

Colony Characterization

Fasted serum samples were taken at monthly intervals starting at 4 weeks and continuing through 16 weeks of age. Following a 12-hour overnight fast, rats were anesthetized by intraperitoneal administration of a combination anesthetic consisting of ketamine hydrochloride 60 mg/kg and xylazine hydrochloride 5 mg/kg. Body temperature was maintained during anesthesia through the use of isothermal heating pads. Blood samples were collected from the cranial vena cava, and the following serum analyses were performed: glucose, insulin, triglycerides, cholesterol, ketone bodies, and free fatty acids. Whole blood

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(EDTA) was used for determination of glycosylated hemoglobin. Following sample collection, yohimbine hydrochloride 0.7 mg/kg was administered intramuscularly to reverse the anesthesia.[24]

Glucose Tolerance Tests

Intravenous glucose tolerance tests (IVGTTs) were performed on rats from each group at 8, 12, and 16 weeks of age. The rats were fasted overnight with free access to water. They were anesthetized by intramuscular administration of a combination anesthetic consisting of ketamine hydrochloride 30 mg/kg, xylazine hydrochloride 6 mg/kg, and acepromazine 1 mg/kg. Additional doses of ketamine hydrochloride (10 mg/kg) were administered intramuscularly as needed to maintain anesthesia. Glucose 500 mg/kg was administered as a bolus injection via the penile vein. Blood samples (200 μL) were obtained from the cranial vena cava before and 1, 3, 5, 10, 15, 20, 30, 45, 60, 90, 120, and 180 minutes after the glucose challenge. Blood samples were centrifuged, and serum was taken for glucose analysis. At 16 weeks of age, the remaining serum was frozen and later analyzed for insulin.

Hepatic Glucose Output

Rats were fasted overnight and anesthetized as described for IVGTT. Blood was sampled via cardiac puncture, and then the animals were killed by cervical dislocation. The portal vein was cut for exsanguination, and the liver was flushed with cold, fresh Krebs buffer until blanching was evident and the perfusate was clear. The liver was excised and superficially rinsed in Krebs buffer. In a petri dish, liver lobes were minced into approximately 1-mm^3 pieces with a sharp razor blade. Approximately 100 mg tissue was placed in preweighed scintillation vials filled with 2 mL oxygenated Krebs buffer. No additional exogenous substrate was provided during the period of hepatic glucose output (HGO) measurement. Baseline glucose level was measured immediately on 50-μL aliquots of incubation buffer. Duplicate vials for each time point were then immersed in a 37°C shaking water bath set at 150 oscillations per minute. Aliquots (50 μL) of incubation media were sampled for glucose levels at 15, 30, 45, 60, 90, and 120 minutes from the respective vials. Glucose values were normalized for both tissue weight and supernatant volume.

The HGO assay, as we perform it, measures total HGO. This is a combination of gluconeogenesis and glycogenolysis. Previous measurements in our laboratory using labeled precursors (pyruvate, lactate, and acetate) demonstrate that the majority of total HGO in this assay is due to glycogenolysis.

Euglycemic-Hyperinsulinemic Clamp

Insulin action was assessed under conditions of a euglycemic-hyperinsulinemic clamp. Studies were performed in 16-week-old animals from each test group. Rats were anesthetized as described for the IVGTT. A ventral laparotomy was performed, and the abdominal aorta and vena cava were cannulated. The catheters were tunneled subcutaneously to the back of the neck, exteriorized, and filled with heparinized saline. Upon recovery from anesthesia, rats were individually housed in cages with a harness-and-swivel apparatus. Food and water were available ad libitum.

After a 48- to 72-hour postoperative recovery period, animals were fasted overnight (12 hours) and euglycemic-hyperinsulinemic clamps were performed according to previously reported studies.[25-28] Extension tubing (Intramedic polyethylene tubing #50; Clay Adams, Parsippany, NJ) was attached to the venous catheter by an adapter for simultaneous infusion of glucose and insulin. The arterial catheter was used for blood sampling. Insulin (regular beef-pork insulin; Eli Lilly, Indianapolis, IN) infusion began at time zero at a rate of 12 mU/kg/min and was maintained at a constant rate throughout the study. Blood was sampled at 10-minute intervals and immediately analyzed for glucose levels. Euglycemia was then maintained within a range of 5 to 6 mmol/L by a variable infusion of 20% glucose. Glucose infusion was initiated following a decrease in blood glucose to less than 4.5 mmol/L. The first hour was used to achieve a steady-state blood glucose, and once steady state was achieved it was maintained for 1 hour. Additional blood was obtained before the infusion and at the final sampling time. Serum was separated, frozen, and later analyzed for insulin. The total blood volume sampled was 700 μL, and was replaced with an equal volume of 0.9% sodium chloride.

Analytical Methods

Plasma glucose was determined using a YSI 2700 Glucose Analyzer (YSI, Yellow Springs, OH). A rat insulin radioimmunoassay kit was used to measure serum insulin level, with rat insulin as the standard and an antibody to rat insulin (Linco Research, St Charles, MO). The following quantitative assays were performed in vitro for the estimation of serum levels: triglycerides and free fatty acids (Wako Chemicals USA, Dallas, TX), cholesterol (Boehringer Mannheim Diagnostics, Indianapolis, IN), ketone bodies (Sigma Diagnostics, St Louis, MO), and glycosylated hemoglobin (Isolab, Akron, OH).

Statistics

All data are reported as the mean ± SEM. Statistical significance was calculated using ANOVA. Fisher's protected least significant difference test, Scheffe's test, and Bonferroni/Dunn Correction were used for pairwise comparisons following ANOVA. P less than .05 was considered significant.

RESULTS

Male GK rats were treated with or without TRG starting at 4 weeks of age. Parameters of glucose metabolism in these rats are shown in Table 1. By 4 weeks of age, GK rats exhibited a twofold increase in fasting plasma glucose over the level in Wistar rats ($P < .05$). This hyperglycemia was stable through 16 weeks of age, at which time GK plasma glucose values were 2.3 times those of the Wistar controls ($P < .05$).

Glycosylated hemoglobin values followed a similar progression. In 4-week-old GK rats, glycosylated hemoglobin values were significantly higher than those detected in Wistar controls (10.75% ± 0.71% *v* 6.98% ± 0.34%, $P < .05$). By 16 weeks, glycosylated hemoglobin in GK rats was 2.3 times that in Wistar rats ($P < .05$).

Fasting insulin values in GK rats were not significantly different from those in Wistar control rats at 4, 8, or 16 weeks of age. At 12 weeks, there was a transient but significant hyperinsulinemia noted in the GK animals ($P < .05$). This transitory elevation in insulin levels has not been previously reported, but may be part of the progression of diabetes in this model. At all ages, there were no differences in ketone values.

Treatment with TRG 30 mg/kg/d decreased fasting blood glucose in GK rats at all ages evaluated, but not to statistically significant levels. However, the elevated glycosylated hemoglobin values observed in GK rats were significantly reduced by TRG treatment (9.65% ± 0.55% in treated *v* 16.87% ± 1.48% in untreated GK rats at 16 weeks of age). Additionally, at all ages, fasting insulin levels were decreased with TRG treatment, but this difference was significant ($P < .05$) only at 8 weeks of age.

GK rats were not obese, with a body weight less than that of the control Wistar rats. In contrast to most other models of NIDDM, GK rats were not severely hyperlipidemic (Table 2). Although total plasma triglycerides and cholesterol were moder-

Table 1. Profile of Diabetes in Age-Matched Wistar, GK Control, and GK TRG-Treated Animals

Time (wk)	Wistar Control	GK	
		Control	TRG-Treated
Glucose (mmol/L)			
4 (baseline)	6.67 ± 0.42 (14)	12.64 ± 0.43 (98)*	
8	7.60 ± 0.46 (49)	15.29 ± 0.54 (49)*	14.50 ± 0.62 (16)
12	9.26 ± 0.37 (26)	17.88 ± 0.86 (53)*	16.99 ± 0.92 (10)
16	8.93 ± 0.20 (6)	20.80 ± 1.50 (28)*	17.58 ± 0.79 (17)
Insulin (pmol/L)			
4 (baseline)	74.50 ± 17.77 (8)	52.37 ± 6.17 (25)	
8	65.46 ± 12.56 (16)	67.35 ± 4.55 (33)	42.89 ± 2.29 (9)†
12	60.12 ± 5.75 (19)	113.72 ± 13.62 (31)*	90.28 ± 9.27 (10)
16	62.96 ± 14.48 (7)	51.79 ± 2.91 (41)	38.85 ± 2.73 (6)
Ketone bodies (μmol/L)			
4 (baseline)	172.89 ± 8.64 (28)	182.50 ± 12.49 (28)	
8	166.17 ± 9.61 (44)	170.97 ± 20.17 (23)	193.06 ± 19.21 (24)
12	177.69 ± 12.49 (6)	217.07 ± 38.42 (26)	181.54 ± 22.09 (16)
16	178.65 ± 26.89 (9)	170.01 ± 42.26 (13)	170.00 ± 16.33 (15)
Glycosylated hemoglobin (%)			
4 (baseline)	6.98 ± 0.34 (7)	10.75 ± 0.71 (65)*	
8	8.75 ± 1.24 (7)	7.70 ± 0.25 (22)	8.05 ± 0.26 (13)†
12	8.02 ± 0.43 (18)	10.33 ± 0.43 (20)*	8.80 ± 0.17 (16)†
16	7.41 ± 0.54 (5)	16.87 ± 1.48 (27)*	9.65 ± 0.55 (12)†

NOTE. Values are the mean ± SEM for the number of animals shown in parentheses. Statistical analyses were performed by ANOVA.
*$P < .05$, GK control *v* Wistar.
†$P < .05$, GK control *v* GK + TRG.

ately elevated at 4, 8, and 12 weeks of age, this elevation was well below that in typical insulin-resistant rodents such as the Zucker rat.[29] No differences were noted in plasma free fatty acids. Although statistically significant changes were noted in triglycerides, cholesterol, and free fatty acids at some points following TRG treatment, these changes were inconsistently reliable and of questionable physiologic importance.

The effect of TRG on glucose disposal was evaluated by IVGTT (Fig 1). In all age groups, control Wistar rats showed a normal baseline plasma glucose that was rapidly elevated in response to an intravenous glucose load, and approached baseline within the test period. Control GK rats demonstrated basal hyperglycemia, suggestive of elevated hepatic glucose production. This hyperglycemia was exacerbated by the glucose

Table 2. Blood Lipid Profiles in Age-Matched Wistar, GK Control, and GK TRG-Treated Animals

Time (wk)	Wistar Control	GK	
		Control	TRG-Treated
Triglycerides (mmol/L)			
4 (baseline)	0.37 ± 0.06 (14)	0.81 ± 0.07 (70)*	
8	0.31 ± 0.06 (5)	0.53 ± 0.03 (51)*	0.67 ± 0.11 (16)
12	0.38 ± 0.03 (26)	0.64 ± 0.03 (66)*	0.81 ± 0.14 (9)
16	0.73 ± 0.09 (9)	0.59 ± 0.06 (36)	0.92 ± 0.08 (13)†
Cholesterol (mmol/L)			
4 (baseline)	0.92 ± 0.07 (14)	1.63 ± 0.04 (46)*	
8	1.20 ± 0.06 (24)	2.31 ± 0.18 (32)*	1.86 ± 0.15 (12)
12	1.31 ± 0.05 (6)	1.74 ± 0.06 (42)*	1.29 ± 0.08 (16)†
16	1.87 ± 0.09 (9)	1.86 ± 0.06 (17)	1.81 ± 0.17 (13)
Free fatty acids (g/L)			
4 (baseline)	0.008 ± 0.001 (12)	0.013 ± 0.001 (35)	
8	0.012 ± 0.001 (24)	0.015 ± 0.002 (30)	0.014 ± 0.001 (15)
12	0.011 ± 0.001 (6)	0.011 ± 0.001 (52)	0.018 ± 0.001 (16)†
16	0.009 ± 0.001 (9)	0.012 ± 0.001 (17)	0.017 ± 0.002 (15)†
Body weight (g)			
4 (baseline)	73.00 ± 1.46 (8)	71.40 ± 2.70 (42)	
8	253.20 ± 3.77 (35)	211.25 ± 5.44 (35)*	197.27 ± 5.40 (22)†
12	396.43 ± 8.85 (41)	255.63 ± 3.18 (41)*	288.50 ± 4.78 (10)†
16	466.67 ± 15.04 (32)	300.00 ± 4.08 (32)*	299.62 ± 5.66 (14)

NOTE. Values are the mean ± SEM for the number of animals shown in parentheses. Statistical analyses were performed by ANOVA.
*$P < .05$, GK control *v* Wistar.
†$P < .05$, GK control *v* GK TRG-treated.

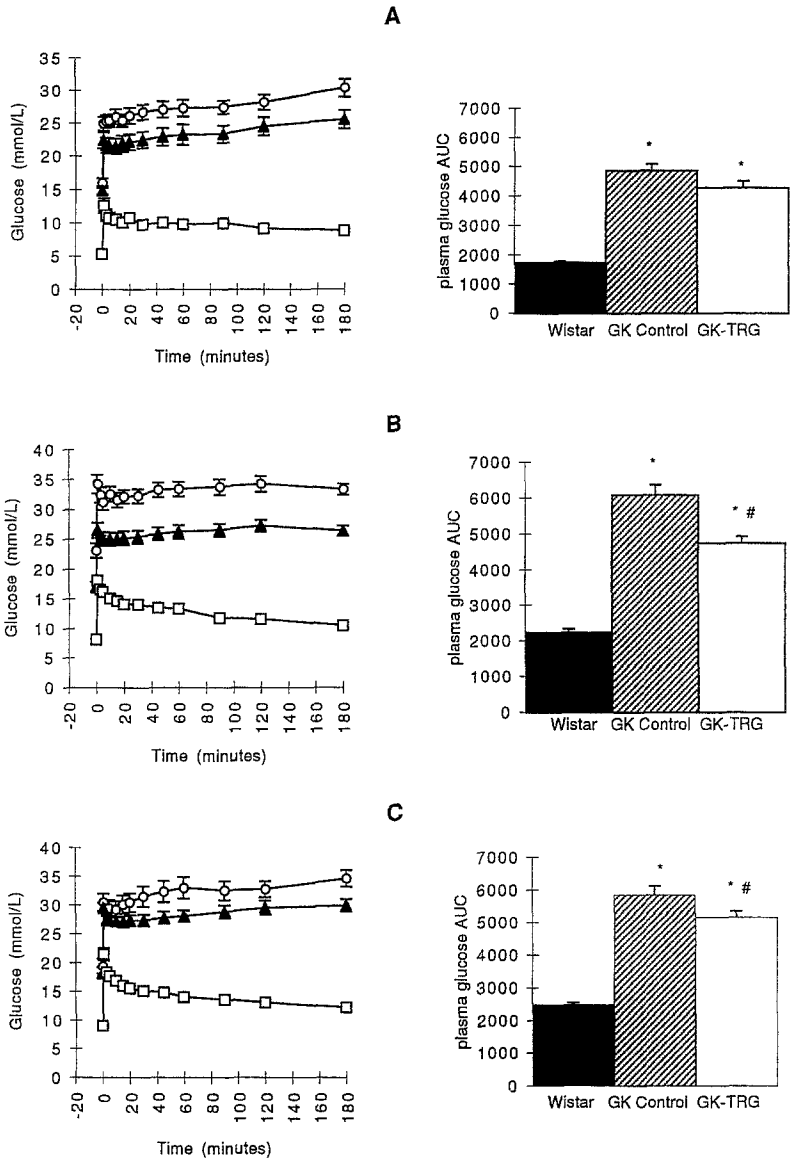

Fig 1. IVGTT glucose values and glucose AUCs for Wistar rats (□), control GK rats (○), and TRG-treated GK rats (▲) at 8 (A), 12 (B), and 16 (C) weeks of age. Following baseline plasma glucose determination, a bolus of glucose (500 mg/kg) was administered intravenously and plasma glucose values were evaluated through 180 minutes postinjection. Each value is the mean ± SE for 8 to 18 animals. *$P < .05$ *v* Wistar. #$P \pm .05$ *v* control GK.

load and did not return to baseline, indicating glucose intolerance and impaired disposal. TRG administration improved overall glucose exposure, but had no effect on the apparent rate of glucose disposal.

The glucose area under the curve (AUC) was calculated to evaluate further the overall glucose exposure (Fig 1). At 8 weeks, control GK rats had an increase in overall glucose exposure that was almost three times that of age-matched Wistar control animals ($P < .001$). This difference in the AUC was stable through 16 weeks of age ($P < .001$). A significant improvement in overall glucose exposure was noted with TRG treatment, such that at 12 weeks of age the AUC for TRG-treated GK rats was 4,728 ± 180, versus 6,073 ± 280 for untreated GK rats ($P < .001$).

Insulin levels were also monitored during the IVGTT (Fig 2). At 16 weeks of age, the insulin response in Wistar rats followed a normal biphasic pattern in response to glucose challenge. GK rats exhibited approximately the same baseline plasma insulin as Wistar animals, but failed to show any response to glucose loading, indicating pancreatic unresponsiveness to glucose. TRG treatment was without effect on this response.

The reduction of basal hyperglycemia and overall glucose exposure without a change in glucose disposal or insulin response noted with TRG suggested a decrease in hepatic glucose production. Therefore, total HGO was examined ex vivo (Fig 3). Glucose production was evaluated using minced liver tissue, and was increased fivefold in 16-week-old control GK tissues relative to Wistar tissues ($P < .001$). This is consistent with previous studies, which have indicated that increased HGO contributes to the development of hyperglycemia in the GK rat.[22,30] TRG treatment decreased this rate by approximately 50%, returning it to the levels found in Wistar control rats ($P < .004$). A similar reduction of total HGO has been seen in vivo following thiazolidinedione treatment in diet-manipulated rats.[30] Although both gluconeogenesis and glycogenolysis contribute to total HGO, the results noted in our ex vivo evaluation reflect primarily glycogenolysis. Previous studies looking specifically at gluconeogenesis have also shown

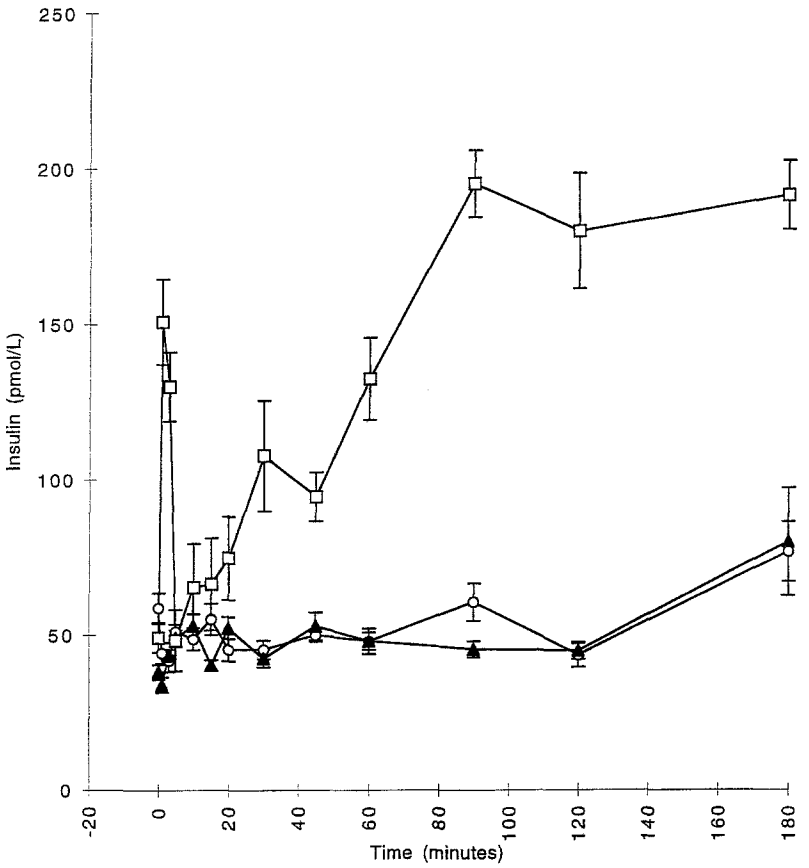

Fig 2. IVGTT insulin values for Wistar rats (□), control GK rats (○), and TRG-treated GK rats (▲) at 16 weeks of age. Following baseline plasma insulin determination, a bolus of glucose (500 mg/kg) was administered intravenously and plasma insulin values were evaluated through 180 minutes postinjection. Each value is the mean ± SE for 7 to 12 animals.

a greater than 50% reduction of glucose production from lactate following TRG treatment of HepG2 cells.[31] Results of these studies further support a direct hepatic action of TRG, affecting both gluconeogenesis and glycogenolysis.

To assess the level of insulin resistance in GK rats, euglycemic-hyperinsulinemic clamp studies were performed at 16 weeks of age (Fig 4). Insulin was infused at a steady rate of 12 mU/kg/min to provide maximal suppression of endogenous glucose production. Final plasma insulin values were greater than 1,000 pmol/L in all groups, with no significant difference between groups, supporting maximal suppression. Blood was sampled at 10-minute intervals following initiation of insulin infusion. Glucose infusion was begun when blood glucose decreased to less than 4.5 mmol/L. Following 1 hour for

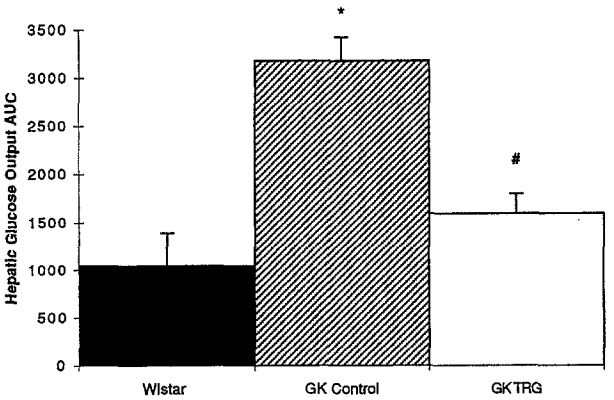

Fig 3. Ex vivo HGO AUC for Wistar rats, control GK rats, and TRG-treated GK rats at 16 weeks of age. Hepatic tissue slices were placed in oxygenated Krebs buffer, and the medium was sampled for glucose levels from baseline through 120 minutes. Each value is the mean ± SE for 6 to 8 animals. *$P < .05$ *v* Wistar. #$P < .05$ *v* control GK.

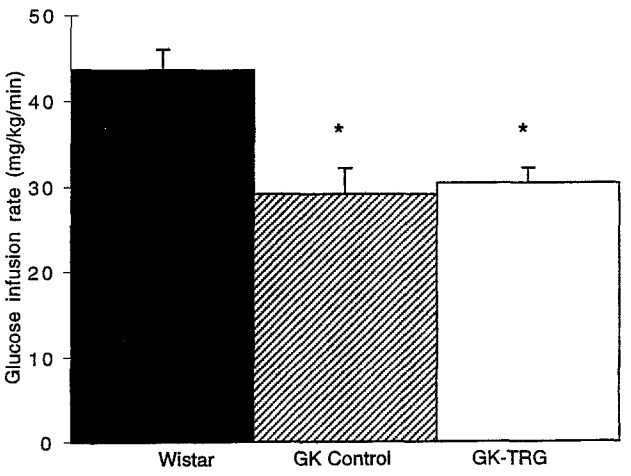

Fig 4. Euglycemic-hyperinsulinemic clamp in awake Wistar rats, control GK rats, and TRG-treated GK rats at 16 weeks of age. Insulin was infused at 12 mU/kg/min to provide maximal suppression of endogenous glucose production. Following 1 hour for stabilization, the GIR necessary to maintain blood glucose at 5 to 6 mmol/L was measured. Values are the mean ± SE for each group (n = 6) over 1 hour. *$P < .05$ *v* Wistar.

stabilization, the glucose infusion rate (GIR) necessary to maintain a steady-state plasma glucose of 5 to 6 mmol/L was measured. The GIR for untreated GK rats was only 67% of that required for control Wistar rats ($P < .004$), indicating severe peripheral insulin resistance (Fig 4). TRG-treated GK rats exhibited no improvement in maximal insulin responsiveness, requiring the same GIR as untreated GK rats.

DISCUSSION

The GK rat is an important new model of NIDDM. Similar to previous studies,[16,18,20] GK rats in our colony were not obese and had lower body weights than the Wistar controls. They exhibited an elevated HGO, fasting hyperglycemia, and impaired glucose disposal in response to a glucose load. An increase in HGO has been shown to contribute to hyperglycemia in this model.[22,30] The failure to dispose of glucose probably results from a combination of diminished insulin secretion and peripheral insulin resistance. The lack of obesity, stability of diabetes, and combination of insulin deficiency and resistance differentiate the GK rat from some of the more commonly used rodent models of NIDDM, including the Zucker (*fa/fa*) rat, KK mouse, *ob/ob* mouse, and *db/db* mouse, all of which are obese and hyperlipidemic.[30,32]

TRG, a new orally active antidiabetic agent, has improved hyperglycemia and insulin resistance in virtually all genetic and acquired obese, hyperlipidemic rodent models of NIDDM. In the KK mouse, *ob/ob* mouse, and Zucker rat, dietary administration of TRG corrected both hyperglycemia and hyperinsulinemia and decreased plasma triglyceride, cholesterol, free fatty acid, and ketone body levels.[4,7-9] In the Zucker rat, thiazolidinediones improved glucose tolerance and decreased insulin secretion following glucose challenge.[4,8,33] On the other hand, administration of these drugs to normal or insulin-deficient diabetic animals failed to alter plasma glucose or insulin or glucose tolerance, although insulin sensitivity was nevertheless increased.[4,8,33]

Of particular interest in this study was the inability of TRG to reverse the insulin resistance observed in the GK rat. Although the drug decreased the total exposure to glucose, there was no effect on the rate of glucose disposal in glucose tolerance tests or on peripheral insulin sensitivity in euglycemic-hyperinsulinemic clamp studies at maximum suppression. The failure of the drug to improve glucose tolerance may result in part from the severe impairment in glucose-induced insulin secretion. Indeed, the effects of TRG and other thiazolidinediones on glucose disposal are thought to result from insulin sensitization, indicating an absolute requirement for insulin. On the other hand, the drug did not improve insulin sensitivity as assessed by the hyperinsulinemic clamp, at least not at this dose level. Dose-dependent effects of thiazolidinediones on plasma insulin and glucose tolerance have been demonstrated in the *ob/ob* mouse and Zucker rat.[3,6] Dose-dependent responses may also occur in the GK rat.

In contrast to its failure to correct insulin resistance, TRG effectively reduced HGO in this normolipidemic and nonketotic model of NIDDM. This was reflected in the significant decrease in glycosylated hemoglobin. Ex vivo evaluation further confirmed this decrease, with a 50% reduction in the total HGO rate in minced liver tissue from TRG-treated GK rats. The reduction in HGO seen with this assay reflected primarily glycogenolysis. Ciaraldi et al[31] have shown that glucose production from lactate measured in HepG2 cells was reduced by 50% following TRG treatment. In both cases, these effects occurred independently of insulin, and TRG treatment did not potentiate insulin action. These results suggest that the effects of TRG on HGO, both in the form of gluconeogenesis and glycogenolysis, are likely to be direct and independent of insulin sensitization.

In vivo, TRG has also resulted in inhibition of gluconeogenesis, acting at the level of fructose bisphosphatase.[7] Given this effect in vivo, the decrease in glycogenolytic activity noted in our HGO assay is not surprising. Inhibiting gluconeogenesis in vivo would result in a decrease in glycogen stores. Following TRG treatment, we presumably begin with a smaller amount of glycogen and therefore show a decrease in total HGO. It is also possible that TRG has a direct effect on the glycogenolytic pathway. The exact biochemical mechanism responsible for this effect is still under investigation. Both in vivo and ex vivo data in the GK rat further support the possibility that the effects of this drug on liver and peripheral tissue may be independent and differ in some respects.[1,7]

An alternative explanation for the data might be related to the signals generated in vivo for the stimulation of insulin secretion. All data generated in these studies have been derived using glucose as the primary secretory challenge. Portha et al[16] have demonstrated an impaired insulin response in the GK pancreas to glucose, whereas the secretory response to arginine was exaggerated. It is possible that in the fed state, other secretagogues are generated that may elicit a nominal insulin secretory response in the GK rat, the metabolic effects of which would be amplified in TRG-treated animals. This may also account for our observation of decreased glycosylated hemoglobin in the absence of significant changes in fasting glucose.

Taken together, the results shown here further confirm the atypical nature of NIDDM in the GK rat. These rats share the insulin and glucose resistance found in other diabetic rodent models, but are unique in the normal lipid profiles and the lack of obesity. Our findings suggest that both glucose and insulin resistance may arise from lesions distinct from those in other models. The insulin resistance observed in these animals is not associated with obesity, hypertriglyceridemia, or increased free fatty acids. Moreover, unlike what is observed in other rodent models of NIDDM, it is not reversed by thiazolidinedione treatment, suggesting a unique lesion. In this regard, recent studies have indicated that selective insulin actions may be impaired in these animals.[21-23] TRG treatment did have a significant effect on HGO in the GK rat independently of its effects on insulin. This model may therefore prove important for further differentiating hepatic and peripheral actions of the thiazolidinediones.

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