Metabolic Effects of Troglitazone on Fat-Induced Insulin Resistance in the Rat

Mousa Khoursheed, Philip D.G. Miles, Ke-Ming Gao, Moon-Kyu Lee, A.R. Moossa, and Jerrold M. Olefsky

Troglitazone is a new orally active hypoglycemic agent that has been shown to ameliorate insulin resistance and hyperinsulinemia in both diabetic animal models and non–insulin-dependent diabetes mellitus (NIDDM) subjects. To determine whether this drug could prevent the development of diet-induced insulin resistance and related abnormalities, we studied its effect on insulin resistance induced by high-fat feeding in rats. Normal male Sprague-Dawley rats were fed a high-fat diet for 3 weeks with and without troglitazone as a food mixture (0.2%) or were fed normal chow. In vivo insulin action was measured using a euglycemic-hyperinsulinemic clamp at two different insulin infusion rates, 4 (submaximal stimulation) and 40 (maximal stimulation) mU/kg/min. Fat feeding markedly reduced the submaximal glucose disposal rate ([GDR], 26.4 ± 1.3 *v* 37.5 ± 1.4 mg/kg/min, $P < .01$) and maximal GDR (55.9 ± 1.3 *v* 64.5 ± 1.3 mg/kg/min, $P < .05$), reduced the suppressibility of submaximal hepatic glucose production ([HGP], 3.2 ± 0.9 *v* 1.5 ± 0.5 mg/kg/min, $P < .05$), and resulted in hyperlipidemia. Troglitazone treatment did not affect any of these parameters. Insulin resistance induced by fat feeding is the first experimental model in which troglitazone failed to correct or partially correct the insulin resistance.

INSULIN RESISTANCE is a central pathophysiological feature of non–insulin-dependent diabetes mellitus (NIDDM). In addition, insulin resistance is a common metabolic abnormality in obesity, hypertension, dyslipidemia, and atherosclerosis,[1] and recently the term syndrome X was coined to characterize these syndromes of insulin resistance–hyperinsulinemia.[2] Environmental, ie, nongenetic, factors such as diet also produce a state of insulin resistance, but do not cause diabetes. Studies in rats have shown that diets high in fructose[3,4] or fat[5,6] result in an impairment of insulin action in both skeletal muscle and the liver.

A variety of orally active hypoglycemic agents are frequently used to help manage the glucose intolerance of NIDDM patients. Thiazolidinediones are a new class of compounds that appear to work by either mimicking or enhancing insulin action without stimulating β-cell insulin secretion, and troglitazone, formerly known as CS-045, is the only compound within this class that is undergoing clinical testing in the United States. Troglitazone proved effective in improving insulin sensitivity and glucose tolerance in NIDDM patients[7,8] and in obese subjects.[9] The agent was also effective in a number of diabetic animal models in decreasing hyperglycemia and hypertriglyceridemia to near-normal levels, and also corrected much of the hyperinsulinemia associated with these animals.[10,11]

We have recently shown that troglitazone restored both peripheral and hepatic insulin sensitivity, plasma lipids, and blood pressure to normal in fructose-induced insulin-resistant rats.[12] However, in terms of a nongenetic etiology of insulin resistance, that induced by a high-fat diet may be clinically more significant considering that the fat content of the North American diet is 40% of total calories,[13,14] with an increasing amount coming from polyunsaturated fat.[15] This study was therefore undertaken to determine whether troglitazone could prevent the development of insulin resistance and related abnormalities induced by a high-fat diet in rats. In vivo insulin action was measured by use of a two-step hyperinsulinemic-euglycemic glucose clamp in chronically catheterized conscious animals.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 75 to 99 g were housed individually under controlled light (12/12) and temperature conditions, and had free access to food and water. After a 2- to 3-day acclimation period, the rats were fed a diet high in fat.

Fat-Fed Rats

The animals were divided into three groups and fed one of the following diets for 3 weeks: (1) normal chow diet, (2) diet high in fat (TD 85418; Harlan Teklad, Madison, WI), and (3) high-fat diet + troglitazone. The high-fat diet was custom-made (E.S. Horton, personal communication, July 1992), whereby 56% of the total calories came from partially hydrogenated vegetable oil (Table 1). Troglitazone was given as a 0.2% admixture, and was freshly made in small amounts every 1 to 3 days and stored at 4°C. Animal weight and food intake were measured daily, and fresh food was given daily.

All procedures were in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health, and were approved by the Animal Subjects Committee of the University of California, San Diego.

Hyperinsulinemic-Euglycemic Clamp

After approximately 14 days on the respective diets, the left jugular vein and the right carotid artery were catheterized (Micro-Renathane MRE-033, 0.033 OD and 0.014 ID; Braintree Scientific, Braintree, MA) under general anesthesia. The anesthetic cocktail consisted of ketamine HCl (50 mg/kg; Ketaset; Aveco, Fort Dodge, IA), acepromazine maleate (1 mg/kg; Butler, Columbus, OH), and xylazine (4.8 mg/kg; AnaSed; Lloyd, Shenandoah, IA) given intramuscularly. Catheters were tunneled subcutaneously and exteriorized at the back of the neck, secured to the skin, and filled with heparinized saline. Catheters were flushed every other day to maintain patency. Ampicillin (1 mg/kg; Aveco) was given prophylactically at the time of surgery. The jugular and carotid catheters

From the Departments of Surgery and Medicine, University of California, San Diego; and the Division of Endocrinology and Metabolism, San Diego Veterans Affairs Medical Center, La Jolla, CA.

Submitted November 21, 1994; accepted February 22, 1995.

Supported in part by grants from the National Institutes of Health (DK 33651), the Veterans Affairs Research Service, Department of Veterans Affairs, Sankyo, Tokyo, Japan, and Parke-Davis, Ann Arbor, MI.

Address reprint requests to Philip D.G. Miles, PhD, Department of Surgery, UCSD Medical Center, 200 W Arbor Dr, San Diego, CA 92103-8400.

0026-0495/95/4411-0020$03.00/0

Table 1. Composition of High-Fat Diet

Ingredient	g/kg
Casein	275.0
Vegetable oil, partially hydrogenated	293.75
Sucrose	153.692
Corn starch	145.0
Cellulose (fiber)	62.5
DL-Methionine	2.5
Mineral mix, AIN-76 (#170915)	43.75
Vitamin mix, Teklad (#40060)	12.5
Calcium carbonate	5.0
Choline bitartrate	6.25
Ethoxyquin (antioxidant)	0.058

were used for infusion and blood sampling, respectively, during the subsequent clamp.

Approximately 7 days after catheter placement, insulin sensitivity was assessed using a two-step hyperinsulinemic-euglycemic glucose clamp. Rats were fasted for 5 to 6 hours before the start of the experimental protocol at approximately 12 noon, and were placed in a restrainer (700R; Braintree Scientific) to which they were accustomed.

Experiments were begun with a priming injection (2.5 μCi/0.5 mL) and constant infusion (0.04 μCi/min) of D-[3-^{3}H]glucose (New England Nuclear, Boston, MA). Tracer glucose was diluted to 5 μCi/mL in saline containing 100 mg/dL unlabeled D-glucose (Malinckrodt, Paris, KY) as carrier and 200 mg/dL sodium benzoate (Malinckrodt) as preservative. Forty minutes after the start of tracer infusion, blood samples (250 μL) were collected in heparinized microtubes at −20, −10, and 0 minutes for determination of plasma glucose concentration and glucose specific activity. At 0 minutes, a submaximal glucose clamp was started. Regular human insulin (Novolin R; Novo Nordisk, Copenhagen, Denmark) was infused at 4 mU/kg/min. The insulin infusate was diluted with saline containing 0.5% human serum albumin (Baxter, Glendale, CA). Blood samples (25 μL) were drawn at 10-minute intervals for immediate determination of plasma glucose (YSI 2300 Glucose/Lactate Analyzer; YSI, Yellow Springs, OH). Based on these values, 20% dextrose (Abbott, Chicago, IL) was variably infused to maintain plasma glucose concentration at approximately 100 mg/dL. Steady state (stable plasma glucose concentration and exogenous glucose infusion rate) was generally achieved within 70 to 90 minutes, at which time three blood samples (250 μL) were collected at 10-minute intervals for determination of glucose specific activity. A maximal glucose clamp was then started. Insulin was infused at 40 mU/kg/min and 50% dextrose (Abbott) was variably infused to maintain plasma glucose concentration at 100 mg/dL. Steady state was achieved within 70 to 90 minutes and maintained for at least 20 minutes. Because of blood volume limitations, tracer infusion was stopped after the submaximal clamp and samples for specific activity were not collected during the maximal glucose clamp. Additional blood samples (250 μL) were collected in microtubes at −10 minutes and at approximately 80 and 170 minutes for determination of plasma insulin concentration.

With the insulin and dextrose infusions continued, the animal was anesthetized (as described earlier) and removed from the restrainer, and 2 mL blood was collected in Lavender Top Vacutainers containing EDTA (Becton Dickinson, Rutherford, NJ) for lipid determination. The animal was then promptly euthanized with pentobarbital.

All blood samples were immediately stored at 4°C. Blood was centrifuged within 20 minutes of collection, and the resultant plasma was stored at −20°C.

Blood Pressure Measurements

A separate group of animals were fed either normal chow, high-fat diet, or high-fat diet + troglitazone. After 3 weeks of feeding, systolic blood pressure in the tail region was measured using an electrosphygmomanometer (PE-300; Narco Bio-Systems, Houston, TX) after prewarming the rat for 15 minutes.[16] All animals had been trained to the apparatus several times before measurement.

Drug Levels

A separate group of animals were fed the fat + troglitazone diet to determine serum concentration of troglitazone and its major metabolite, M-1 (sulfate conjugate of troglitazone). After 2 weeks of feeding, nonfasted animals were anesthetized and 1 mL blood was collected via cardiac puncture. To see if drug levels fluctuated throughout the day, blood was collected at 7 AM, 1 PM, 7 PM, or after a 6-hour fast at 1 PM from another group of rats. Blood samples were allowed to clot and were then centrifuged, and the resultant serum was stored at −20°C.

Assays

Plasma glucose concentration was measured with a YSI glucose analyzer (YSI 23A). Plasma for determination of [3-^{3}H]glucose was deproteinized with perchloric acid and assayed as previously described.[17] Insulin level was measured by radioimmunoassay with a double-antibody immunoprecipitation technique as previously described.[18] Plasma triglyceride, cholesterol, and high-density lipoprotein (HDL) cholesterol levels were measured according to standardized procedures.[19] Troglitazone concentration was determined by Sanyko, Tokyo, Japan.

Calculations

Hepatic glucose production (HGP) and glucose disposal rate (GDR) were calculated for the basal period and the steady-state portion of the submaximal glucose clamp using the Steele equation for steady-state conditions.[20] The use of this form of the equation is possible because dSA/dt = 0 when plasma glucose concentration and rate of exogenous glucose infusion are constant (dSA = derivative of specific activity, dt = derivative of time). During the steady-state portion of the maximal glucose clamp, HGP was assumed to be zero, and therefore GDR was estimated from the rate of exogenous glucose infusion. Values presented are the mean ± SEM. Statistical analysis was performed using two-way ANOVA for unbalanced data (Statistical Analysis System; SAS Institute, Cary, NC) on a microcomputer. Significance was assumed at P less than .05.

RESULTS

Body Weight and Food Intake

The mean daily body weight of the fat-fed group was similar to that of the control chow-fed group (Fig 1A); however, daily food consumption of the fat-fed group was significantly lower than that of the control group (Fig 1B). This was true when the diets were corrected for caloric content (mean over 3 weeks, 65.4 ± 1.4 kcal/d for fat-fed v 80.6 ± 2.0 for chow-fed, $P < .01$). Catheter placement led to a transient decrease in food intake and weight gain, which returned to normal after 3 to 4 days. Plasma insulin concentrations (Table 2) and GDR and HGP (Fig 2) in the basal state were the same among the three groups. However, basal plasma glucose levels were slightly higher in the

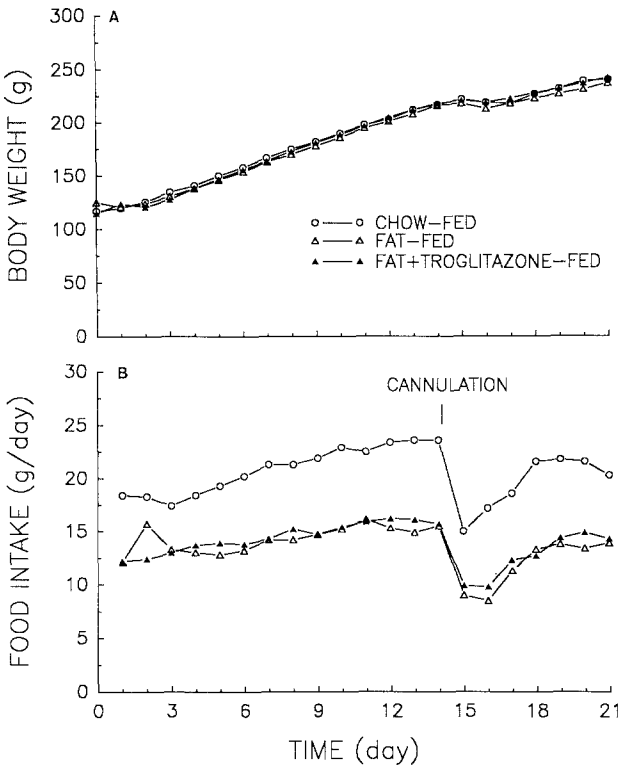

Fig 1. Mean daily body weight (A) and food intake (B) of rats fed chow, fat, or fat + troglitazone. Body weight and food consumption were measured daily, and food intake of the 2 fat-fed groups was significantly lower versus the chow-fed group ($P < .01$).

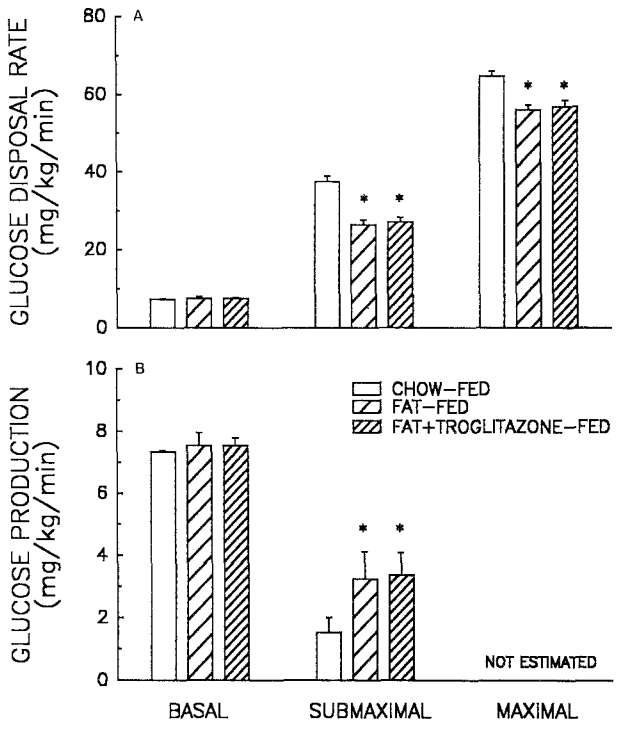

Fig 2. Steady-state GDR (A) and HGP (B) during the basal period, submaximal insulin infusion (4 mU/kg/min), and maximal insulin infusion (40 mU/kg/min) in rats fed chow, fat, or fat + troglitazone. *Significantly different from chow-fed group ($P \leq .05$).

fat-fed group (104 ± 2 mg/dL) versus the chow-fed group (93 ± 2 mg/dL; $P < .05$).

In Vivo Insulin Action

In vivo insulin action was measured using the euglycemic glucose clamp technique in rats at two different insulin infusion rates designed to achieve a submaximal (4 mU/kg/min) and maximal (40 mU/kg/min) stimulation of overall GDR and suppression of HGP. Circulating insulin and glucose concentrations during submaximal and maximal insulin infusions were similar across the three groups (Table 2).

Table 2. Metabolic Characteristics of Rats in Each Group

Characteristic	Chow-Fed	Fat-Fed	Fat-Fed + Troglitazone
No. of animals	11	13	14
Diet duration (d)	22.4 ± 0.2	22.3 ± 0.2	22.1 ± 0.2
Final body weight (g)	246 ± 6	246 ± 6	250 ± 5
Food intake (g/d)	20.1 ± 0.4	13.5 ± 0.3*	13.9 ± 0.3*
Plasma glucose (mg/dL)			
Basal	94 ± 2	104 ± 2*	103 ± 2*
Clamp			
Submaximum	99 ± 3	99 ± 2	105 ± 2
Maximum	101 ± 3	105 ± 2	103 ± 2
Plasma insulin (μU/mL)			
Basal	7 ± 1	11 ± 2	9 ± 1
Clamp			
Submaximum	93 ± 6	101 ± 9	110 ± 8
Maximum	1,668 ± 110	1,674 ± 96	1,729 ± 64
Systolic blood pressure (mm Hg)	109 ± 7	103 ± 5	108 ± 6

NOTE. Values are the mean ± SEM. Systolic blood pressure was measured in a separate group with 6 rats from each diet group.
*Significantly different from basal ($P \leq .05$).

The high-fat diet was effective in inducing both peripheral and hepatic insulin resistance. GDR during submaximal insulin infusion in the fat-fed group was 30% less than that in the chow-fed control group (26.4 ± 1.3 *v* 37.5 ± 1.4 mg/kg/min, $P < .01$), and the maximal GDR was reduced by 14% (55.9 ± 1.3 *v* 64.5 ± 1.3 mg/kg/min, $P < .05$; Fig 2A). During submaximal insulin infusion, the ability of insulin to suppress HGP was impaired (Fig 2B) in that HGP was 112% greater in the high-fat group as compared with the chow group (3.2 ± 0.9 *v* 1.5 ± 0.5 mg/kg/min, $P < .05$). Concomitant feeding of troglitazone with the high-fat diet did not affect the diet-induced development of either peripheral or hepatic insulin resistance (Fig 2A and B). There was no difference between the fat + troglitazone–fed group and the fat-fed group in submaximal GDR (27.1 ± 1.2 *v* 26.4 ± 1.3 mg/kg/min, NS), maximal GDR (56.6 ± 1.7 *v* 55.9 ± 1.3 mg/kg/min, NS), or submaximal HGP (3.4 ± 0.7 *v* 3.2 ± 0.9 mg/kg/min, NS).

Plasma Lipid Levels

Plasma lipid concentrations of the fat-fed group were all significantly greater than those of the chow-fed group (triglyceride, 24 ± 3 *v* 12 ± 1 mg/dL, $P < .01$; cholesterol,

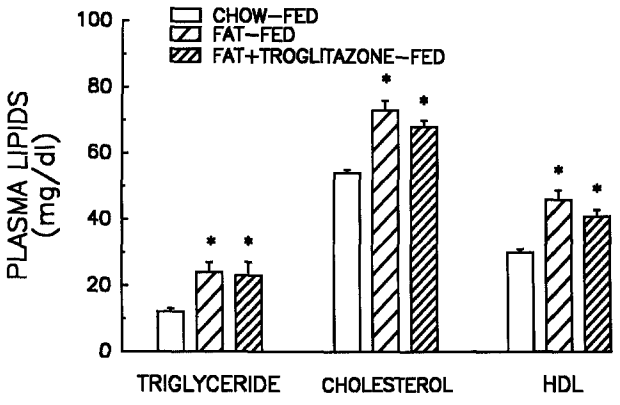

Fig 3. Mean plasma lipid concentrations of rats fed chow, fat, or fat + troglitazone. *Significantly different from chow-fed group ($P \leq .01$).

73 ± 3 *v* 54 ± 2 mg/dL, $P < .01$; HDL, 46 ± 3 *v* 31 ± 1 mg/dL, $P < .01$). Plasma lipid concentrations were unaffected by troglitazone treatment (Fig 3).

Blood Pressure

Three weeks of high-fat feeding did not adversely affect blood pressure. Troglitazone treatment had no effect on blood pressure (Table 2).

Serum Drug Levels

Serum troglitazone and M-1 concentrations of the fat + troglitazone–fed group (n = 8) were 0.25 ± 0.02 and 7.37 ± 0.67 μg/mL, respectively. Because of troglitazone's inability to ameliorate the fat-induced insulin resistance, we examined serum drug levels over a 24-hour period (Table 3). Troglitazone and M-1 levels did not significantly fluctuate over the course of the day. Also, they did not significantly decline following a 6-hour fast. The drug was undetectable in the serum of non–troglitazone-treated groups.

DISCUSSION

In the current report, we have evaluated the impact of troglitazone, a new antidiabetic agent, on the insulin resistance induced by a high-fat diet in normal rats. Troglitazone is a thiazolidinedione, and this class of drug exerts antidiabetic effects by improving insulin action with no effect on insulin secretion. The efficacy of this drug has been reported in various insulin-resistant diabetic animal models and in NIDDM patients. A high-fat diet produces a state of insulin resistance, but does not cause diabetes. Thus, our study provides information on the ability of this drug to influence an environmentally induced, nongenetic form of insulin resistance in a nonhyperglycemic model.

Table 3. Troglitazone and M-1 Plasma Levels Throughout the Day

Parameter	7 AM	1 PM	7 PM	1 PM After 6-Hour Fast
Troglitazone (μg/mL)	0.39 ± 0.05	0.31 ± 0.02	0.37 ± 0.03	0.27 ± 0.02
M-1 (μg/mL)	8.42 ± 0.39	7.41 ± 0.54	7.00 ± 0.53	7.58 ± 0.45

NOTE. Values are the mean ± SEM; n = 8.

This animal model may resemble certain features of the nondiabetic human insulin-resistant states of obesity and impaired glucose tolerance, as recently reviewed.[21] The major finding of this study is that troglitazone failed to prevent the insulin resistance caused by high-fat feeding. This negative report is the first of its kind in which troglitazone was not effective in reversing insulin resistance.

We previously examined the efficacy of troglitazone on another nongenetic form of insulin resistance, ie, that induced by a diet high in fructose.[12] The metabolic abnormalities seen with fructose feeding were virtually identical to those currently seen with fat feeding. Rats fed a diet high in either fat or fructose were insulin resistant in comparison to control animals of similar weight. This was evidenced by both reduced GDR and reduced suppressibility of HGP at an insulin concentration in the midphysiological range, and was consistent with previous studies.[3-6,22] Moreover, the reduced GDR at both submaximal and maximal hyperinsulinemic glucose clamps indicates that the insulin resistance was due to reduced insulin sensitivity and reduced insulin responsiveness. Also, both animal models demonstrated a similar degree of hyperlipidemia. Troglitazone prevented the insulin resistance and hyperlipidemia caused by high-fructose feeding,[12] but had no effect on metabolic abnormalities caused by the high-fat diet.

The differential effects of the drug on these two animal models of insulin resistance indicate that the mechanisms whereby these diets led to insulin resistance are fundamentally different. Furthermore, the site (or sites) of action of troglitazone must be related to the mechanism of fructose-induced insulin resistance and unrelated to the cause of insulin resistance due to high-fat feeding. Although the precise mode of action of troglitazone is unknown and the mechanisms of insulin resistance caused by these two dietary regimens remain incompletely defined, the current results provide some new potential insights. For example, the high-fructose diet led to both peripheral and hepatic insulin resistance, and both were completely prevented by the drug. Since insulin's abilities to stimulate GDR and inhibit HGP represent distinct actions, this indicates that either troglitazone works early in the insulin action pathway at a site common to these two biological events, or that the drug has multiple distal cellular effects resulting in normal insulin action in different target tissues such as liver and skeletal muscle.

The precise mechanism by which a diet high in fat leads to insulin resistance is not clear. However, an increasing body of evidence has demonstrated a link between elevated lipid levels in skeletal muscle and peripheral insulin resistance (for review, see Jenkins et al[23]). The notion that an oversupply of lipids may be important in the etiology of muscle insulin resistance is not new.[24] According to the glucose–fatty acid cycle,[25,26] the elevated intramuscular lipid stores would be used preferentially to glucose and therefore may lead to a decrease in glucose utilization. Indeed, high-fat diets result in increased muscle triglycerides,[27,28] and over a range of dietary fat composition there is a strong negative correlation between triglyceride content

and in vivo insulin-mediated glucose uptake in oxidative muscle types.[29] In addition to dietary intake of lipids, other data indicate that basal hepatic triglyceride output is enhanced in high-fat–fed rats.[30] However, obesity can be thought of as the epitome of an oversupply of lipids. It is characterized by insulin resistance, and many obese subjects are also glucose-intolerant.[9] In contrast to its lack of effect in fat-fed rats, troglitazone treatment has been shown to improve both of these parameters in the obese subject.[9] This discrepancy in efficacy of troglitazone therefore suggests that excessive fat intake and obesity are associated with distinctive forms of insulin resistance. It also suggests that troglitazone is unlikely to influence the glucose–fatty acid cycle.

Troglitazone's ineffectiveness in restoring normal insulin action in fat-fed animals is surprising considering the success of its predecessors. Like troglitazone, ciglitazone[31] and pioglitazone[32,33] belong to the thiazolidinedione class of drugs. In fat-fed rats, these drugs improved insulin sensitivity primarily by increasing insulin-mediated glucose utilization to normal levels.[31,33] Furthermore, ciglitazone has been shown to decrease circulating glucose and insulin concentrations[34,35] and to improve insulin sensitivity[36,37] in several genetically insulin-resistant rodent models. Although ciglitazone is no longer pursued as an antidiabetic drug due to its toxicity, the lack of effect of troglitazone as compared with ciglitazone and pioglitazone is likely due to differing chemical structures.

It is known that high-fructose feeding leads to hypertriglyceridemia, most likely through decreased very–low-density lipoprotein triglyceride catabolism[38] and/or increased very–low-density lipoprotein triglyceride secretion.[39] Similar mechanisms have been proposed to explain the hypertriglyceridemia associated with high-fat feeding.[40] The fact that troglitazone prevented insulin resistance and hypertriglyceridemia in fructose-fed rats but affected neither in fat-fed rats underscores the importance of insulin resistance and hyperinsulinemia in these models, and supports the view that the improvement in insulin action was at least partly responsible for decreasing plasma triglyceride levels in our previous study in fructose-fed animals.

At present, we cannot explain troglitazone's lack of efficacy in fat-fed rats. However, the possibility of insufficient drug dosage can be ruled out. Circulating levels of troglitazone were comparable to levels in our previous study,[12] in which it proved effective in normalizing insulin sensitivity. M-1 concentrations were also similar to previous findings.[12] Moreover, circadian analysis demonstrated that these drug levels remained elevated throughout the day, even after a 6-hour fast (Table 3).

In addition to impaired insulin action, high-fat feeding is also associated with obesity.[41] A surprising finding of our study was the discrepancy between food intake and the high-fat group and that of the control chow-fed group, despite no difference in mean body weight of these groups. Even when corrected for the higher caloric content of the fat diet, fat-fed animals ate an average of 19% fewer calories than the chow-fed group. Although this study did not examine energy balance, the difference in food intake between the two groups is consistent with a high-fat diet–induced decrease in energy expenditure, as reported elsewhere.[6] In that study, it was determined that the thermogenic response of high-fat–fed rats to a carbohydrate meal was substantially decreased as compared with that of weight-matched, high-carbohydrate–fed animals. Moreover, the investigators attributed this difference in thermogenic activity to a marked reduction in brown adipose tissue glucose uptake and greater white adipose tissue mass of the fat-fed group. Similar results of reduced meal-induced thermogenesis have also been shown in humans consuming high-fat diets.[42] This association between insulin resistance and reduced energy expenditure may have important implications for the development of obesity.

In summary, feeding a high-fat diet to normal rats for 3 weeks led to both peripheral and hepatic insulin resistance and hyperlipidemia. These abnormalities were not prevented by concomitant administration of troglitazone. Although troglitazone is effective in the treatment of many forms of insulin resistance, it may not prove useful for those situations in which increased fat intake is a causative factor.

REFERENCES

1. DeFronzo RA, Ferrannini E: Insulin resistance: A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. Diabetes Care 14:173-194, 1991

2. Reaven GM: Role of insulin resistance in human disease. Diabetes 37:1595-1607, 1988

3. Hwang IS, Ho H, Hoffman BB, et al: Fructose-induced insulin resistance and hypertension in rats. Hypertension 10:512-516, 1987

4. Tobey TA, Mondon CE, Azvaroni I, et al: Mechanism of insulin resistance in fructose-fed rats. Metabolism 31:608-612, 1982

5. Kraegen EW, James DE, Storlien LH, et al: In vivo insulin resistance in individual peripheral tissue of the high fat fed rat: Assessment by euglycaemic clamp plus deoxyglucose administration. Diabetologia 29:192-198, 1986

6. Storlien LH, James DE, Burleigh KM, et al: Fat feeding causes widespread in vivo insulin resistance, decreased energy expenditure, and obesity. Am J Physiol 251:E576-E583, 1986

7. Iwamoti Y, Kuzuya T, Matsuda A, et al: Effects of new oral antidiabetic agent CS-045 on glucose tolerance and insulin secretion in patients with NIDDM. Diabetes Care 14:1083-1086, 1991

8. Suter SL, Nolan JJ, Wallace P, et al: Metabolic effects of new oral hypoglycemic agent CS-045 in NIDDM subjects. Diabetes Care 15:193-203, 1992

9. Nolan JJ, Ludvik B, Beerdsen P, et al: Improvement in glucose tolerance and insulin resistance in obese subjects treated with troglitazone. N Engl J Med 331:1188-1193, 1994

10. Fujiwara T, Yoshioka S, Yoshioka T, et al: Characterization of new oral antidiabetic agent CS-045: Studies in KK and ob/ob mice and Zucker fatty rats. Diabetes 37:1549-1558, 1988

11. Fujiwara T, Wada M, Fukuda K, et al: Characterization of CS-045, a new oral antidiabetic agent. II. Effects on glycemic

control and pancreatic islet structure in C57BL/KsJ-db/db mice. Metabolism 40:1213-1218, 1991

12. Lee M-K, Miles PDG, Khoursheed M, et al: Metabolic effects of troglitazone on fructose-induced insulin resistance in the rat. Diabetes 43:1435-1439

13. US Department of Agriculture: Continuing survey of food intakes of individuals: Men 19-50 years, 1 day, 1985. Report No. 85-3, in Nationwide Food Consumption Survey. Hyattsville, MD, Nutrition Monitoring Division, Human Nutrition Information Service, US Department of Agriculture, 1986

14. US Department of Agriculture: Continuing survey of food intakes of individuals: Women and their children 1-5 years, 4 days, 1985. Report No. 85-4, in Nationwide Food Consumption Survey. Hyattsville, MD, Nutrition Monitoring Division, Human Nutrition Information Service, US Department of Agriculture, 1987

15. Rizek RL, Raper NL, Tippett KS: Trends in U.S. fat and oil consumption. J Am Oil Chem Soc 65:722-726, 1988

16. Bunag RD: Validation in awake rats of a tail-cuff method for measuring systolic pressure. J Appl Physiol 34:279-282, 1973

17. Revers RR, Fink R, Griffin J, et al: Influence of hyperglycemia on insulin's in vivo effects in type II diabetes. J Clin Invest 73:664-672, 1984

18. Desbuquois B, Aurbach GD: Use of polyethylene glycol to separate free and antibody-bound peptide hormone in radioimmunoassays. J Clin Endocrinol Metab 33:732-738, 1971

19. Lipid Research Clinics: Manual of Laboratory Operations. Revised Edition. Washington, DC, US Department of Health and Human Services, 1982

20. Steele R: Influence of glucose loading and of injected insulin on hepatic glucose output. Ann NY Acad Sci 82:420-430, 1959

21. Reaven GM: Insulin resistance, hyperinsulinemia, hypertriglyceridemia, and hypertension: Parallels between human disease and rodent models. Diabetes Care 14:195-202, 1991

22. Thorburn AW, Storlien LH, Jenkins AB, et al: Fructose-induced in vivo insulin resistance and elevated plasma triglycerides levels in rats. Am J Clin Nutr 49:1155-1163, 1989

23. Jenkins A, Storlien L, Chisholm DJ, et al: Fatty acid availability and glucose metabolism in skeletal muscle, in Larkins R, Zimmet P, Chisholm D (eds): Diabetes. Amsterdam, The Netherlands, Elsevier, 1989, pp 167-170

24. Olefsky J, Farqufar J, Reaven G: Reappraisal of the role of insulin in hypertriglyceridemia. Am J Med 57:551-560, 1974

25. Randle PJ, Garland PB, Hales CN, et al: The glucose fatty-acid cycle: Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet 1:785-789, 1963

26. Randle PJ, Kerbey A, Espinal J: Mechanisms decreasing glucose oxidation in diabetes and starvation: Role of lipid fuels and hormones. Diabetes Metab Rev 4:623-638, 1988

27. Shafrir E: Intermediary metabolism during the development of obesity and diabetes in the desert rodent *Acomys cahirinus*. Int J Obes 6:9-20, 1982 (suppl 1)

28. Bringholf M, Zaragoza N, Rivier D, et al: Studies on the metabolic effects induced in the rat by a high fat diet. Eur J Biochem 26:360-367, 1972

29. Storlien LH, Jenkins AB, Chisholm DJ, et al: Influence of dietary fat composition on development of insulin resistance in rats. Relationship to muscle triglyceride and w-3 fatty acids in muscle phospholipid. Diabetes 40:280-289, 1991

30. Kraegen EW, Clark PW, Jenkins AB, et al: Early metabolic changes in liver may influence later development of muscle insulin resistance in high fat fed rats. Proc Endocr Soc Aust 32:89, 1989 (abstr)

31. Kraegen EW, James DE, Jenkins AB, et al: A potent in vivo effect of ciglitazone on muscle insulin resistance induced by high-fat feeding of rats. Metabolism 38:1089-1093, 1989

32. Iwanishi M, Kobayashi M: Effect of pioglitazone on insulin receptors of skeletal muscles from high-fat–fed rats. Metabolism 42:1017-1021, 1993

33. Ikeda HS, Taketomi S, Sugiyama Y, et al: Effects of pioglitazone on glocose and lipid metabolism in normal and insulin resistant animals. Arzneimittelforschung 40:156-162, 1990

34. Chang AY, Wyse BM, Gilchrist BJ, et al: Ciglitazone, a new hypoglycemic agent. I. Studies in ob/ob and db/db mice, diabetic Chinese hamsters, and normal and streptozotocin-diabetic rats. Diabetes 32:830-838, 1983

35. Fujita T, Sugiyama Y, Taketomi S, et al: Reduction of insulin resistance in obese and/or diabetic animals by 5-:4-(1-methylcyclohexylmethoxy)benzyl:-thiazolidine-2,4-dione (ADD-3878, U-63,287 ciglitazone), a new antidiabetic agent. Diabetes 32:804-810, 1983

36. Shargill NS, Tatoyan A, Fukushima S, et al: Effect of ciglitazone on glucose uptake and insulin sensitivity in skeletal muscle of obese (ob/ob) mouse: Distinct insulin glucocorticoid effect. Metabolism 35:64-70, 1986

37. Kirsch DM, Bachmann W, Haring HU: Ciglitazone reverses cAMP-induced post-insulin receptor resistance in rat adipocytes in vitro. FEBS Lett 176:49-54, 1984

38. Hirano T, Mamo J, Poapst M, et al: Very-low density lipoprotein triglyceride kinetics in acute and chronic carbohydrate-fed rats. Am J Physiol 255:E236-E241, 1989

39. Zavaroni I, Chen YDI, Reaven GM: Studies of the mechanism of fructose-induced hypertriglyceridemia in the rat. Metabolism 31:1077-1083, 1982

40. Kraegen EW, Clark PW, Jenkins AB, et al: Development of muscle insulin resistance after liver insulin resistance in high-fat–fed rats. Diabetes 40:1397-1403, 1991

41. Danforth E Jr: Diet and obesity. Am J Clin Nutr 41:1132-1145, 1985

42. Acheson KJ, Schutz Y, Bessard T, et al: Nutritional influences on lipogenesis and thermogenesis after a carbohydrate meal. Am J Physiol 246:E62-E70, 1984