Thiazolidinediones Enhance Insulin-Mediated Suppression of Fatty Acid Flux in Type 2 Diabetes Mellitus

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Type 2 diabetes mellitus is characterized by insulin-resistant glucose and lipid metabolism. Thiazolidinediones (TZDs) enhance insulin-mediated glucose disposal, but their effects on lipid kinetics are unknown. We evaluated the effect of the TZD troglitazone on insulin-mediated suppression of fatty acid and glycerol kinetics. Eight obese men and women (body mass index [BMI], 34.1 ± 2.3 kg/m^2) with insulin-requiring type 2 diabetes were studied before and after 12 weeks of troglitazone therapy (400 mg/d). Whole-body and abdominal fat masses were determined by dual-energy x-ray absorptiometry and magnetic resonance imaging, respectively. Palmitate and glycerol rates of appearance (R_a) into plasma were evaluated during a 3-stage hyperinsulinemic euglycemic clamp, which spanned the physiologic range of plasma insulin concentrations that regulate lipolysis. Troglitazone therapy did not alter body composition. Palmitate and glycerol R_a decreased progressively during each stage of hyperinsulinemia ($P < .001$). Suppression of palmitate R_a by insulin was greater after than before troglitazone therapy ($P < .001$), whereas glycerol R_a was unchanged. These results demonstrate that TZDs increase insulin-mediated suppression of fatty acid release into plasma in obese subjects with type 2 diabetes mellitus, which may contribute to their metabolic benefits. However, TZD therapy did not affect whole-body glycerol R_a, possibly because of upregulation of lipoprotein lipase action on plasma triglycerides.

TYPE 2 DIABETES mellitus is characterized by insulin resistance and inadequate compensatory insulin secretion.[1] The consequences of hepatic and peripheral insulin resistance include increased hepatic glucose production, reduced peripheral glucose uptake, increased lipolysis of adipose tissue triglycerides, increased very–low-density lipoprotein (VLDL) production, hyperglycemia, and increased plasma free fatty acid (FFA) concentrations. Many oral therapeutic agents for type 2 diabetes stimulate pancreatic insulin secretion, but do not affect insulin resistance directly. Thiazolidinediones (TZDs), in contrast, represent a novel approach for treating type 2 diabetes by increasing peripheral insulin sensitivity.[2]. Although troglitazone has been voluntarily removed from the marketplace by its manufacturer because of the association of its use with liver disease, several other glitazones continue to be used safely, and new drugs in this class are undergoing clinical testing.

TZDs function as ligands for peroxisome proliferator-activated receptor γ (PPARγ),[3] a member of the nuclear hormone receptor superfamily involved in adipocyte differentiation.[4] TZDs decrease plasma glucose concentration by increasing insulin-mediated peripheral glucose disposal.[5-7] It is likely that TZDs have a direct effect on postinsulin receptor signaling in skeletal muscle,[8] the major site of insulin-mediated glucose uptake. However, it is possible also that the mechanism of TZD's effect on peripheral glucose metabolism involves enhanced insulin sensitivity of adipose tissue. Increased circulating FFA in patients with type 2 diabetes reduces glucose uptake by skeletal muscle,[9] whereas reductions in FFA with TZD therapy are associated with improvements in glycemic control and insulin sensitivity.[10,11] Therefore, an increased antilipolytic effect of insulin could enhance skeletal muscle glucose disposal.

The purpose of the present study was to evaluate the effect of adjunctive TZD treatment on adipose tissue sensitivity to insulin and body composition in patients with type 2 diabetes receiving insulin therapy. We hypothesized that TZDs (troglitazone) would increase the antilipolytic effect of insulin, but would not change body fat mass or body fat distribution.

MATERIALS AND METHODS

Subjects

Eight subjects (4 men, 4 women) with type 2 diabetes mellitus participated in this study, which was approved by the Human Studies Committee and the General Clinical Research Center (GCRC) Scientific Advisory Committee of Washington University School of Medicine. Written informed consent was obtained from all volunteers before their participation. All subjects were being treated with at least 30 U of insulin daily. Subjects were screened with a careful medical examination, including a history, physical examination, resting electrocardiogram, routine blood tests, hemoglobin A_{1C}, oral glucose tolerance test, and a urine pregnancy test for females. Subjects with hemoglobin A_{1C} values between 7% and 11% and peak plasma C-peptide concentrations greater than 1.5 ng/mL during the oral glucose tolerance test were eligible for participation in this study. Individuals with evidence of significant organ system dysfunction or pregnancy were excluded. Subjects were studied on 2 occasions, before and at the end of 12 weeks of TZD therapy.

Body composition assessment. Total body fat and fat-free masses were determined by dual-energy x-ray absorptiometry (Hologic QDR 1,000/w, Waltham, MA).[12] Abdominal (subcutaneous and intra-abdominal) adipose tissue was quantified by magnetic resonance imaging (Siemens, Iselin, NJ).[13] A single slice image at the L_2-L_3 interspace was analyzed for subcutaneous and intra-abdominal adipose tissue content.

Isotope infusion study/hyerpinsulinemic euglycemic clamp. Subjects were admitted to the GCRC at Washington University School of Medicine (day 1). Their habitual insulin regimen was discontinued in the morning, and regular insulin was given subcutaneously with lunch (1:00 PM) and dinner (6:00 PM). Dinner consisted of a standard Amer-

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ican Diabetes Association (ADA) meal containing 12 kcal/kg adjusted body weight (ideal body weight + [(actual body weight - ideal body weight) × 0.25]), which averaged 922 ± 63 kcal and was composed of 55% carbohydrate, 15% protein, and 30% fat. An ADA snack containing 245 ± 39 kcal was served at 9:00 PM. At 8:00 PM, 2 intravenous catheters were inserted; 1 was placed in a dorsal hand or wrist vein for blood glucose monitoring and the second in an antecubital vein for insulin infusion. A constant infusion of insulin was started at an initial rate of 15.16 ± 4.25 mU · m^{-2} · min^{-1} and was adjusted as needed every hour to maintain blood glucose concentration between 80 and 120 mg/dL.

The insulin infusion was discontinued at 5:00 AM on day 2. An isotope infusion protocol was started at 6:00 AM (Fig 1). The catheter placed in the hand or wrist vein was used to obtain arterialized blood samples by placing the subject's hand in a box heated to 60°C. The catheter placed in the antecubital vein was used to infuse hormones and a third intravenous catheter was inserted into the contralateral antecubital vein to infuse stable isotope tracers.

After baseline blood samples were obtained, a primed (1.5 μmol/kg) constant (0.10 μmol · kg^{-1} · min^{-1}) infusion of [1,1,2,2,3-$^2H_5$]glycerol and a constant infusion (0.04 μmol · kg^{-1} · min^{-1}) of [1-^{13}C]palmitate were started and continued for 6 hours. After a baseline period (0 to 90 minutes), a 3-stage hyperinsulinemic, euglycemic clamp was started (Fig 1).[1] Somatostatin (0.12 μg · kg^{-1} · min^{-1}) and growth hormone (0.005 μg · kg^{-1} · min^{-1}) were infused continuously throughout the clamp. Glucagon was not administered, because changes in glucagon concentrations in the physiologic range do not affect lipid kinetics.[14] During each of the three 90-minute stages of the clamp, insulin was infused at rates of 5, 15, and 30 mU · m^{-2} · min^{-1}, respectively. Euglycemia was maintained by a variable rate infusion of 20% dextrose.

Blood samples were obtained before beginning the isotope infusion to determine baseline plasma substrate and hormone concentrations and background isotope enrichments. Blood samples were obtained every 5 minutes during the last 15 minutes of the basal period and each stage of the hyerpinsulinemic euglycemic clamp to determine plasma substrate and hormone concentrations and lipid kinetics. Blood samples also were obtained every 5 minutes throughout the clamp to determine plasma glucose concentrations.

TZD therapy. After the preliminary body composition analyses and isotope infusion study/hyerpinsulinemic euglycemic clamp were completed, each subject began a 12-week course of troglitazone (Rezulin, Parke-Davis, Morris Plains, NJ) therapy (400 mg/d). Blood glucose concentrations were measured and recorded by the participants at least twice daily, and their insulin doses were adjusted as needed to prevent hypoglycemia. After 4 and 8 weeks of troglitazone treatment, blood samples were obtained to assess liver biochemistries, insulin, C-peptide, and hemoglobin A_{1C} concentrations. Upon completion of the 12 weeks of drug therapy, blood tests, body composition analyses, and the isotope infusion study/hyerpinsulinemic euglycemic clamp were repeated.

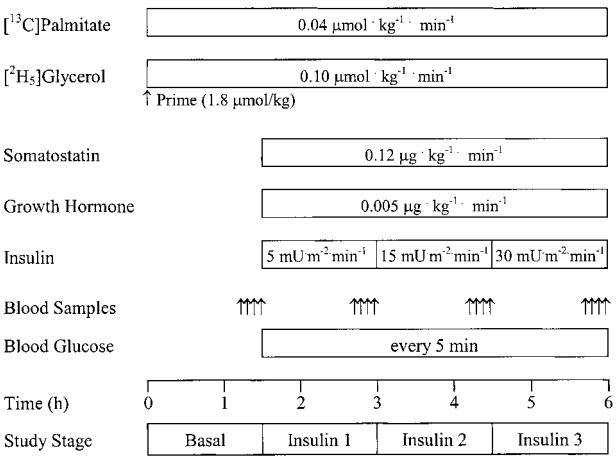

Fig 1. Schematic diagram of isotope infusion study/hyerpinsulinemic euglycemic clamp.

Analyses of Samples

Blood samples. Plasma glucose concentrations were determined enzymatically with an automated analyzer using a glucose oxidase reaction (Glucose AutoAnalyzer; Beckman Instruments, Fullerton, CA). Hemoglobin A_{1C} was determined from whole blood using an automated cation-exchange high-performance liquid chromatography (HPLC) method (Bio-Rad Variant HbA_{1C} program; Bio-Rad Laboratories, Diagnostic Group, Hercules, CA).[15] Plasma insulin[16] and C-peptide[17] concentrations were measured by radioimmunoassay. Plasma total cholesterol, high-density lipoprotein (HDL)-cholesterol, and triglycerides were determined enzymatically (Roche/Hitachi 747 Analyzer; Roche Diagnostics, Indianapolis, IN) using commercially available kits; low-density lipoprotein (LDL)-cholesterol was calculated using the Friedewald equation.[18] Plasma catecholamines were determined by a radioenzymatic method.[19]

Isotopic enrichment of palmitate and glycerol in plasma were determined by gas chromatography-mass spectrometry (GC-MS) using an MSD 5971 system (Hewlett-Packard, Palo Alto, CA) with capillary column. Plasma samples were analyzed for [^{13}C]palmitate enrichment as described previously.[20] FFAs were isolated from plasma and converted to their methyl esters. Ions formed by electron impact ionization and ions at mass-to-charge ratio (*m/e*) 270.2 and 271.2, representing the molecular ions of unlabeled and labeled methyl esters, respectively, were selectively monitored. Plasma samples were prepared for analysis of glycerol isotopic enrichment as described previously.[21] Plasma proteins were precipitated with acetone. A heptafluorobutyric acid derivative of glycerol was formed and ions were produced by electron impact ionization. Glycerol tracer-to-tracee ratios were determined by selectively monitoring ions at *m/e* 253 and 257.

Calculations

Steele's equation for steady-state conditions[22] was used to calculate substrate (palmitate and glycerol) rate of appearance (R_a) in plasma during the last 30 minutes of the basal period and each stage of the hyerpinsulinemic euglycemic clamp. Fatty acid R_a was calculated by dividing palmitate R_a by the percent contribution of palmitate to total FFA concentration.

Statistical Analysis

The effects of TZD treatment on palmitate R_a and glycerol R_a, total body fat mass, and abdominal fat mass were evaluated by analysis of variance (ANOVA) with repeated measures. Significance was accepted at an alpha level of .05. All values are reported as mean ± SE.

RESULTS

Subjects were 52.8 ± 4.5 yr of age, had type 2 diabetes for 10.8 ± 1.6 years, received insulin therapy for 7.1 ± 1.9 years, and were being treated with 71.5 ± 8.8 U of insulin/d upon enrollment into the study. The types of insulin used included Humulin 70/30 (n = 3), NPH (n = 3), and regular + NPH (n = 2). The characteristics of the subjects before and after troglitazone therapy are shown in Table 1. Troglitazone therapy did not cause any changes in body weight or body composition.

Table 1. Subject Characteristics Before and After 12 Weeks of TZD Treatment

	Before	After
Body weight (kg)	101.7 ± 5.1	103.0 ± 5.3
Body mass index (kg/m^2)	34.1 ± 2.3	34.5 ± 2.4
Fat mass (%)	37 ± 4	36 ± 4
Fat mass (kg)	38.8 ± 5.7	38.1 ± 5.4
Fat-free mass (kg)	62.9 ± 3.5	64.8 ± 3.7
Abdominal adipose tissue		
Subcutaneous (cm^2)	345 ± 63	338 ± 66
Intra-abdominal (cm^2)	136 ± 28	143 ± 26
Plasma leptin (ng/mL)	29 ± 9	25 ± 7

NOTE. Values are mean ± SE.

Plasma metabolic factors before and after troglitazone treatment are shown in Table 2. Troglitazone therapy caused a significant decrease in fasting plasma glucose concentration and a trend toward a decrease in plasma HbA_{1C} concentration. There was also a trend toward a decrease in daily insulin requirements (72 ± 9 to 57 ± 10 U/d, $P = .064$). Serum lipid concentrations did not change after 12 weeks of troglitazone therapy (Table 2).

Liver biochemistries were not affected by troglitazone therapy. Mean values before and after 12 weeks of treatment were 17 ± 2 and 13 ± 1 IU/L for alanine amino transferase (SGPT), 21 ± 1 and 22 ± 2 IU/L for aspartate amino transferase (SGOT), 82 ± 5 and 63 ± 4 IU/L for alkaline phosphatase, and 0.6 ± 0.1 and 0.5 ± 0.1 mg/dL for total bilirubin, respectively.

By design, plasma insulin concentration progressively increased with each insulin stage of the hyerpinsulinemic euglycemic clamp and spanned the physiologic range of insulin concentrations known to regulate lipolysis.[23] The plasma insulin concentrations achieved during the clamps performed before troglitazone therapy were reproduced during the clamps performed after therapy (Fig 2).

Plasma glucose concentrations remained stable throughout the 3 stages of the hyperinsulinemic clamp studies, and the concentrations achieved during the pretreatment clamps (129 ± 7, 127 ± 8, 126 ± 8 mg/dL for stages 1, 2, and 3, respectively), were reproduced during the posttreatment clamps (124 ± 4, 124 ± 6, 119 ± 6 mg/dL, P = not significant [NS] between stages and between treatment conditions).

Glucose infusion rate increased with increasing insulin concentrations during the hyperinsulinemic euglycemic clamp procedure, as expected. The rates of glucose disposal, determined during the last 30 minutes of each insulin stage were not different after troglitazone treatment compared with values obtained before treatment (pre: 0.31 ± 12, 0.89 ± 0.20, 1.84 ± 0.56 mg/kg/min; post: 0.51 ± 0.15, 1.02 ± 0.19, 1.88 ± 0.35 mg/kg/min; $P < .05$ between stages, P = NS pre- *v* posttreatment). The short duration (90 minutes) of each stage of the hyperinsulinemic clamp procedure did not permit reaching a steady state in glucose infusion rate.

There was a progressive step-wise decrease in palmitate R_a as plasma insulin concentrations increased during each insulin stage of the hyerpinsulinemic euglycemic clamp (Fig 3). After

Table 2. Plasma Metabolic Factors Before and After 12 Weeks of TZD Therapy

	Before	After	*P* Value
Fasting glucose (mg/dL)	168 ± 12	146 ± 12	.027
HbA_{1c} (%)	8.7 ± 0.5	7.7 ± 0.4	.069
Total cholesterol (mg/dL)	177 ± 11	179 ± 11	
LDL-cholesterol (mg/dL)	109 ± 10	111 ± 7	
HDL-cholesterol (mg/dL)	37 ± 3	38 ± 4	
Triglyceride (mg/dL)	153 ± 30	147 ± 38	

NOTE. Values are mean ± SE.

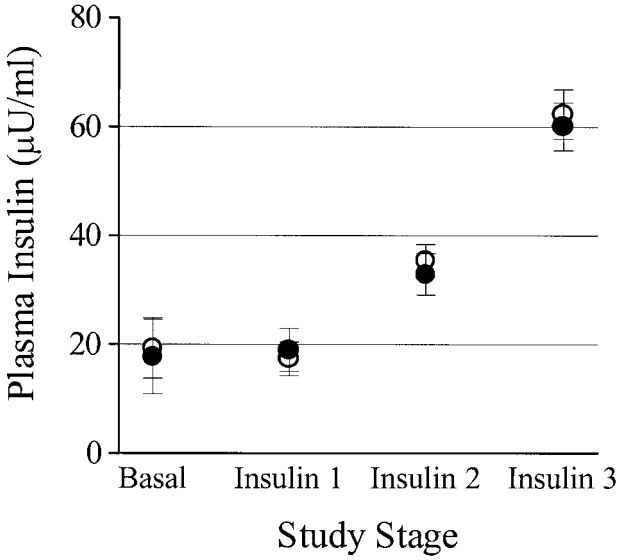

Fig 2. Plasma insulin concentrations during each stage of the isotope infusion/hyerpinsulinemic euglycemic clamp study before (○) and after (●) TZD treatment. Values are mean ± SE.

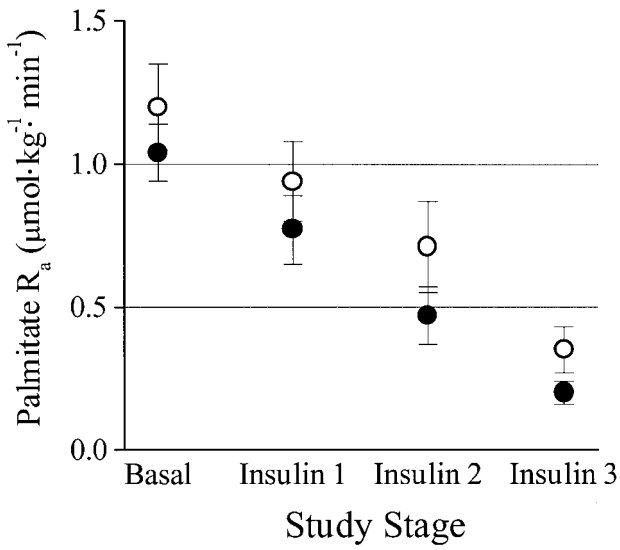

Fig 3. Palmitate R_a during each stage of the isotope infusion/ hyerpinsulinemic euglycemic clamp study before (○) and after (●) TZD treatment. Values are mean ± SE. $P < .05$ after troglitazone therapy relative to before.

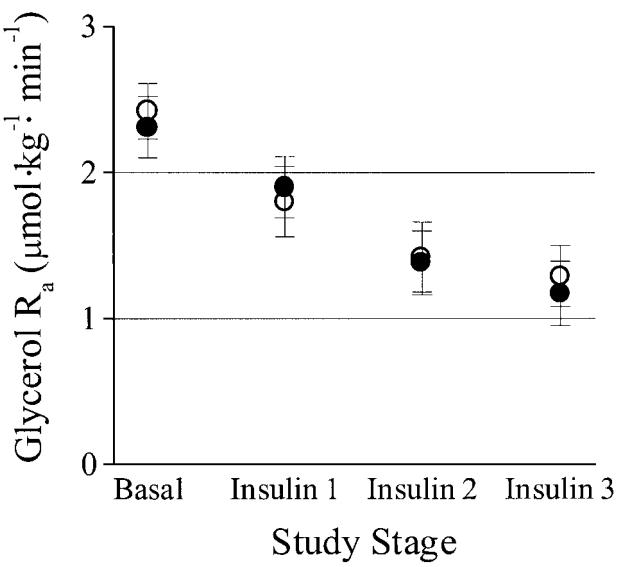

Fig 4. Glycerol R_a during each stage of the isotope infusion/ hyerpinsulinemic euglycemic clamp study before (○) and after (●) TZD treatment. Values are mean ± SE.

12 weeks of troglitazone treatment, basal palmitate R_a was lower, and suppression of palmitate R_a in response to hyperinsulinemia was greater compared with values obtained before TZD treatment ($P < .05$). Glycerol R_a also decreased in a step-wise fashion during each insulin stage of the hyerpinsulinemic euglycemic clamp (Fig 4). However, 12 weeks of troglitazone therapy did not alter basal glycerol R_a or glycerol R_a during hyperinsulinemia.

Basal FFA concentration did not change with treatment (pre: 0.536 ± 0.058; post: 0.458 ± 0.031 μmol/mL $P = .164$). However, there was a trend toward a reduction in total FFA concentrations during hyperinsulinemia after TZD therapy (pre- *v* poststage 1: 0.465 ± 0.083 and 0.376 ± 0.065 μmol/mL, $P = .076$; stage 2: 0.348 ± 0.083 and 0.229 ± 0.066 μmol/mL, $P = .100$; and stage 3: 0.195 ± 0.058 and 0.130 ± 0.059 μmol/mL, $P = .052$.).

DISCUSSION

TZDs are effective agents for treating patients with type 2 diabetes mellitus because they increase insulin-mediated glucose disposal.[6] We hypothesized that TZDs also enhance insulin action in adipose tissue, which contributes to their effect on muscle glucose uptake by decreasing plasma fatty acid availability.[24] Therefore, in the present study, we evaluated the effect of the TZD troglitazone on insulin-mediated suppression of palmitate and glycerol R_a in patients with type 2 diabetes mellitus. Palmitate R_a, which provides an index of fatty acid release from adipose tissue, and glycerol R_a, which provides an index of lipolysis of adipose tissue and plasma triglycerides, were evaluated across a physiologic range of plasma insulin concentrations known to regulate lipolysis. Our results demonstrate that 12 weeks of troglitazone therapy enhance insulin suppression of fatty acid release into plasma, but do not affect glycerol R_a. These data support the notion that TZDs' effect on fatty acid metabolism in adipose tissue contributes to their effect on glucose metabolism in skeletal muscle.

The mechanism(s) responsible for the different effects of troglitazone on palmitate and glycerol kinetics is not clear. It is possible that troglitazone's effect on both insulin and lipoprotein lipase (LPL) action may be responsible. The precise mechanism responsible for troglitazone's effect on adipose tissue lipolytic activity is unknown, but is likely related to the TZDs' function as a ligand for PPARγ.[3] Furthermore, PPARγ may enhance postinsulin receptor signaling.[25,26] The potential importance of TZD activation of PPARγ is underscored by the observation that adipose tissue PPARγ expression is 10- to 30-fold higher than that found in muscle or liver tissue.[27] TZD administration has been shown to increase LPL activity in both adipose tissue[28] and skeletal muscle.[29] In addition, LPL mRNA content was increased when preadipocytes[30] or adipocytes[28] were exposed to a TZD in vitro. An increase in LPL activity in adipose and muscle tissues could increase hydrolysis of circulating triglycerides and increase glycerol, but not fatty acid R_a values. Glycerol released during plasma triglyceride hydrolysis would likely enter the systemic circulation and be detected by our glycerol tracer infusion, whereas released fatty acids would be taken up by local tissue and not be seen by our fatty acid tracer. Therefore, an LPL-mediated increase in plasma glycerol derived from circulating triglycerides may have offset an insulin-mediated decrease in glycerol derived from adipose tissue triglycerides in our subjects after troglitazone therapy.

Alternatively, the greater suppression of palmitate, but not glycerol, R_a in response to insulin after TZD therapy could be explained by effects of troglitazone on PPARγ regulation of fatty acid and glucose transport protein transcription.[3] TZDs cause a marked increase in adipose tissue fatty acid transport protein (FATP) and acyl-CoA synthetase (ACS) mRNA expression in rats.[31] The increase in FATP and ACS presumably is responsible for increased fatty acid uptake by 3T3-L1 preadipocytes incubated with TZDs.[31] In addition, TZDs increase GLUT4 transporter gene expression and glucose uptake in isolated adipocytes,[32,33] which would increase fatty acid reesterification because of glucose conversion to glycerol-3-phosphate required for triglyceride synthesis. Therefore, TZD treatment in our subjects may have enhanced reesterification of fatty acids released during lipolysis because of PPARγ-mediated increases in glucose and fatty acid uptake by adipose tissue. Adipocyte reesterification would prevent fatty acids from entering the systemic circulation, thereby preventing their detection by tracer infusion.

It is likely that TZDs' effect on fatty acid kinetics contributes to the metabolic and clinical benefits observed in patients with type 2 diabetes. Excessive release of FFA into plasma in persons with diabetes may be responsible for (1) alterations in glucose metabolism by impairing insulin's ability to stimulate muscle glucose uptake[24] and suppress hepatic glucose production[24,34,35]; (2) hyperinsulinemia by increasing pancreatic insulin secretion[36] and inhibiting hepatic insulin clearance[37]; and (3) alterations in lipoprotein metabolism by increasing hepatic VLDL production and plasma triglyceride concentrations.[38] We found that 12 weeks of troglitazone therapy decreased mean fasting blood glucose and tended to decrease hemoglobin A_{1c} concentration and daily insulin dose. These results are

consistent with previously published reports involving TZD monotherapy,[6,39] combination therapy with a sulfonylurea,[40] and adjunctive therapy with insulin.[41,42]

Twelve weeks of troglitazone therapy did not affect body weight in our subjects, which is consistent with some,[5,6,40,41,43-47] but not all,[39,42,48,49] previous studies performed in humans. The different effects on body weight between studies may be explained by the duration of treatment and whether troglitazone was used as monotherapy or combination therapy. For example, no changes in body weight were observed after 3[46] or 6 months of troglitazone monotherapy[6] or combination therapy with insulin.[41] However, in the latter study, the same subjects had significant increases in body weight after 74 weeks.[41] In a 52-week trial, body weight increased when troglitazone was used in conjuction with sulfonylurea therapy, whereas troglitazone monotherapy did not cause an increase in body weight.[40] It is possible that longer-term TZD plus insulin therapy in our subjects would have caused an increase in body weight.

Similar to body weight, troglitazone therapy in our subjects did not change total body fat mass or fat distribution. In a previous study, Kelly et al[47] also found that total body fat mass did not change in subjects with type 2 diabetes after 12 weeks of troglitazone therapy. However, in contrast to our results, Kelly et al[47] observed a significant decrease in intra-abdominal fat mass. Other investigators have found that 3 to 6 months of troglitazone monotherapy in patients with type 2 diabetes mellitus tended to decrease intra-abdominal fat and increase subcutaneous fat mass,[49,50] whereas 6 to 12 months of combination troglitazone plus sulfonylurea therapy caused an increase in subcutaneous fat without a change in intra-abdominal fat.[48,49] There are several reasons why we did not observe a change in body fat after TZD plus insulin therapy in our subjects. First, 12 weeks of therapy may not be long enough to cause a detectable change in body composition, and it is possible that continued treatment would have ultimately caused observable changes in body fat and fat distribution. Second, our subjects already were obese at the onset of the study, whereas subjects in previous studies were normal weight[48,50] or overweight[49] at baseline. Third, our subjects may have already experienced considerable weight gain because of long-term insulin therapy before enrollment, whereas most subjects participating in previous studies had been treated with sulfonylureas only. Furthermore, during the course of our study, our subjects received combination TZD and insulin therapy, whereas subjects evaluated in previous studies received TZD monotherapy or combination TZD plus sulfonylurea therapy.[47-50]

We cannot exclude the possibility that TZD treatment increased adipocyte proliferation and cell number. TZD-induced activation of PPARγ increases differentiation of 3T3-L1 preadipocytes to adipocytes[51] and adipose cell number in white adipose tissue of obese Zucker rats.[52] However, the change in adipose tissue morphology of obese Zucker rats was characterized by an increase in the number of small adipocytes and a decrease in the number of large adipocytes, without a change in total body fat mass. Therefore, the data obtained in an obese rodent model are consistent with our observations in obese humans. Troglitazone-induced alterations in fat cell size in obese Zucker rats were associated with a decrease in adipose tissue tumor necrosis factor (TNF)-α and leptin expression[52] and may represent additional mechanisms for the effect of TZDs on insulin sensitivity.

Our study was performed before troglitazone was removed from the marketplace because of its association with liver abnormalities. Careful monitoring of liver biochemistries in our study subjects did not reveal any clinically significant changes during the 12-week trial. Although it is likely that the effects of troglitazone on lipid metabolism observed in the present study are relevant to other TZDs because of their similar mechanism of action, this conclusion should be confirmed by additional studies involving other TZDs.

In summary, the TZD troglitazone increases insulin-mediated suppression of fatty acid release into plasma across a physiologic range of plasma insulin concentrations in patients with type 2 diabetes mellitus. It is likely that this effect on fatty acid kinetics contributes to the metabolic benefits of thiazolidinedione therapy by decreasing FFA delivery to the liver and skeletal muscle, which may decrease VLDL and glucose production, and enhance glucose disposal.

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REFERENCES

1. DeFronzo RA, Tobin JD, Andres R: Glucose clamp technique: A method for quantifying insulin secretion and resistance. Am J Physiol 237:E214-E223, 1979

2. Saltiel AR, Olefsky JM: Thiazolidinediones in the treatment of insulin resistance and type II diabetes. Diabetes 45:1661-1669, 1996

3. Lehmann JM, Moore LB, Smith-Oliver TA, et al: An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor gamma (PPAR gamma). J Biol Chem 270:12953-12956, 1995

4. Wright HM, Clish CB, Mikami R, et al: A synthetic antagonist for the peroxisome proliferator-activated receptor γ inhibits adipocyte differentiation. J Biol Chem 275:1873-1877, 2000

5. Inzucchi SE, Maggs DG, Spollett GR, et al: Efficacy and metabolic effects of metformin and troglitazone in type II diabetes mellitus. N Engl J Med 338:867-872, 1998

6. Maggs DG, Buchanan TA, Burant CF, et al: Metabolic effects of troglitazone monotherapy in type 2 diabetes mellitus. A randomized, double-blind, placebo-controlled trial. Ann Intern Med 128:176-185, 1998

7. Suter SL, Nolan JJ, Wallace P, et al: Metabolic effects of new oral hypoglycemic agent CS-045 in NIDDM subjects. Diabetes Care 15: 193-202, 1992

8. Burant CF, Sreenan S, Hirano K, et al: Troglitazone action is independent of adipose tissue. J Clin Invest 100:2900-2908, 1997

9. Boden G, Chen X, Ruiz J, et al: Mechanisms of fatty acid-induced inhibition of glucose uptake. J Clin Invest 93:2438-2446, 1994

10. Patel J, Anderson RJ, Rappaport EB: Rosiglitazone monotherapy improves glycaemic control in patients with type 2 diabetes: A twelve-week, randomized, placebo-controlled study. Diabetes Obes Metab 1:165-172, 1999

11. Brown KK, Henke BR, Blanchard SG, et al: A novel N-aryl tyrosine activator of peroxisome proliferator-activated receptor-γ re-

verses the diabetic phenotype of the Zucker diabetic fatty rat. Diabetes 48:1415-1424, 1999

12. Kohrt WM: Body composition by DXA: Tried and true? Med Sci Sports Exerc 27:1349-1353, 1995

13. Abate N, Burns D, Peshock RM, et al: Estimation of adipose tissue mass by magnetic resonance imaging: Validation against dissection in human cadavers. J Lipid Res 35:1490-1496, 1994

14. Jensen MD, Heiling VJ, Miles JM: Effects of glucagon on free fatty acid metabolism in humans. J Clin Endocrinol Metab 72:308-315, 1991

15. Halwachs-Baumann G, Katzensteiner S, Schnedl W, et al: Comparative evaluation of three assay systems for automated determination of hemoglobin A1C. Clin Chem 43:511-517, 1997

16. Morgan DR, Lazarow A: Immunoassay of insulin: Two antibody system. Diabetes 12:115-126, 1963

17. Kuzuya H, Blix PM, Horwitz DL, et al: Determination of free and total insulin and C-peptide in insulin treated diabetics. Diabetes 26:22-29, 1977

18. Friedewald WT, Levy RI, Fredrickson DS: Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 18:499-502, 1972

19. Shah SD, Clutter WE, Cryer PE: External and internal standards in the single isotope derivative (radioenzymatic) assay of plasma norepinephrine and epinephrine in normal humans and persons with diabetes mellitus or chronic renal failure. J Lab Clin Med 106:624-629, 1985

20. Patterson BW, Zhao G, Klein S: Improved accuracy and precision of gas chromatography/mass spectrometry measurements for metabolic tracers. Metabolism 47:706-712, 1998

21. Horowitz JF, Coppack SW, Paramore D, et al: Effect of short-term fasting on lipid kinetics in lean and obese women. Am J Physiol 276:E278-E284, 1999

22. Steele R: Influences of glucose loading and of injected insulin on hepatic glucose output. Ann N Y Acad Sci 82:420-430, 1959

23. Campbell PJ, Carlson MG, Hill JO, et al: Regulation of free fatty acid metabolism by insulin in humans: Role of lipolysis and reesterification. Am J Physiol 263:E1063-E1069, 1992

24. Ferrannini E, Barrett EJ, Bevilacqua S, et al: Effect of fatty acids on glucose production and utilization in man. J Clin Invest 72:1737-1747, 1983

25. Bader S, Kiehn R, Haring H: Effect of CS-045 on the activity of insulin receptor kinase in the skeletal muscle of insulin resistant Zucker rats. Diabetes Stoffwechsel 2:56-61, 1993

26. Kellerer M, Kroder G, Tippmer S, et al: Troglitazone prevents glucose-induced insulin resistance of insulin receptor in rat-1 fibroblasts. Diabetes 43:447-453, 1994

27. Spiegelman BM: PPAR-γ: Adipogenic regulator and thiazolidinedione receptor. Diabetes 47:507-514, 1998

28. Lefebvre A-M, Peinado-Onsurbe J, Leitersdorf I, et al: Regulation of lipoprotein metabolism by thiazolidinediones occurs through a distinct but complementary mechanism relative to fibrates. Arterioscler Thromb 17:1756-1764, 1997

29. Sreenan S, Keck S, Fuller T, et al: Effects of troglitazone on substrate storage and utilization in insulin-resistant rats. Am J Physiol 276:E1119-E1129, 1999

30. Schoonjans K, Peinado-Onsurbe J, Lefebvre A-M, et al: PPARα and PPARγ activators direct a distinct tissue-specific transcriptional response via a PPRE in the lipoprotein lipase gene. EMBO J 15:5336-5348, 1996

31. Martin G, Schoonjans K, Lefebvre A-M, et al: Coordinate regulation of the expression of the fatty acid transport protein and acyl-CoA synthetase genes by PPARα and PPARγ activators. J Biol Chem 272:28210-28217, 1997

32. Shimaya A, Kurosaki E, Shioduka K, et al: YM268 increases the glucose uptake, cell differentiation, and mRNA expression of glucose transporter in 3T3-L1 adipocytes. Horm Metab Res 30:543-548, 1998

33. Wu Z, Xie Y, Morrison RF, et al: PPARγ induces the insulin-dependent glucose transporter Glut4 in the absence of C/EBPα during the conversion of 3T3 fibroblasts into adipocytes. J Clin Invest 101: 22-32, 1998

34. Kelley DE, Mokan M, Simoneau J-A, et al: Interaction between glucose and free fatty acid metabolism in human skeletal muscle. J Clin Invest 92:91-98, 1993

35. Mittelman SD, Fu YY, Rebrin K, et al: Indirect effect of insulin to suppress endogenous glucose production is dominant, even with hyperglucagonemia. J Clin Invest 100:3121-3130, 1997

36. Boden G, Chen X, Rosner J, et al: Effects of a 48-h fat infusion on insulin secretion and glucose utilization. Diabetes 44:1239-1242, 1995

37. Peiris AN, Mueller RA, Smith GA, et al: Splanchnic insulin metabolism in obesity. Influence of body fat distribution. J Clin Invest 78:1648-1657, 1986

38. Lewis GF, Uffelman KD, Szeto LW, et al: Interaction between free fatty acids and insulin in the acute control of very low density lipoprotein production in humans. J Clin Invest 95:158-166, 1995

39. Iwamoto Y, Kosaka K, Kuzuya T, et al: Effects of troglitazone: A new hypoglycemic agent in patients with NIDDM poorly controlled by diet therapy. Diabetes Care 19:151-156, 1996

40. Horton ES, Whitehouse F, Ghazzi MN, et al: Troglitazone in combination with sulfonylurea restores glycemic control in patients with type 2 diabetes. The Troglitazone Study Group. Diabetes Care 21:1462-1469, 1998

41. Buse JB, Gumbiner B, Mathias NP, et al: Troglitazone use in insulin-treated type 2 diabetic patients. Diabetes Care 21:1455-1461, 1998

42. Schwartz S, Raskin P, Fonseca V, et al: Effect of troglitazone in insulin-treated patients with type II diabetes mellitus. N Engl J Med 338:861-866, 1998

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

44. 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

45. Antonucci T, Whitcomb R, McLain R, et al: Impaired glucose tolerance in normalized by treatment with the thiazolidinedione troglitazone. Diabetes Care 20:188-193, 1997

46. Kumar S, Boulton AJ, Beck-Nielsen H, et al: Troglitazone, an insulin action enhancer, improves metabolic control in NIDDM patients. Troglitazone Study Group. Diabetologia 69:701-709, 1996

47. Kelly IE, Walsh K, Han TS, et al: Effect of a thiazolidinedione compound on body fat and fat distribution of patients with type 2 diabetes. Diabetes Care 22:228-293, 1999

48. Akazawa S, Sun F, Ito M, et al: Efficacy of troglitazone on body fat distribution in type 2 diabetes. Diabetes Care 23:1067-1071, 2000

49. Mori Y, Murakawa Y, Okada K, et al: Effect of troglitazone on body fat distribution in type 2 diabetic patients. Diabetes Care 22:908-912, 1999

50. Kawai T, Takei I, Oguma Y, et al: Effects of troglitazone on fat distribution in the treatment of male type 2 diabetes. Metabolism 48:1102-1107, 1999

51. Ohsumi J, Sakakibara S, Yamaguchi J, et al: Troglitazone prevents the inhibitory effects of inflammatory cytokines on insulin-induced adipocyte differentiation in 3T3-L1 cells. Endocrinology 136: 1474-1481, 1995

52. Okuno A, Tamemoto H, Tobe K, et al: Troglitazone increases the number of small adipocytes without the change of white adipose tissue mass in obese Zucker rats. J Clin Invest 101:1354-1361, 1998