Acute Effect of Troglitazone on Glucose Metabolism in the Absence or Presence of Insulin in Perfused Rat Hindlimb

Akira Okuno, Kanako Ikeda, Masakazu Shiota, Toshihiko Fujiwara, Shinji Yoshioka, Tsukasa Sugano, and Hiroyoshi Horikoshi

Troglitazone (CS-045) is a new type of antidiabetic agent that decreases plasma glucose by enhancing insulin action in insulin-resistant diabetic animals and non–insulin-dependent diabetes mellitus (NIDDM) patients. To examine the direct effect of troglitazone on glucose metabolism and insulin action in skeletal muscle, we infused troglitazone solution into perfused rat hindlimbs in the presence of 6 mmol/L glucose and in the absence or presence of insulin. In the absence of insulin, even 50 μmol/L troglitazone did not elicit glucose uptake. Troglitazone did increase lactate and pyruvate release at concentrations of 20 μmol/L and higher; however, it decreased the ratio of lactate to pyruvate (L/P ratio) and increased oxygen consumption at concentrations higher than 5 and 20 μmol/L, respectively. In hindlimb muscle, 20 μmol/L troglitazone decreased glycogen content without changing fructose 2,6-bisphosphate (F2,6P2) content in the absence of insulin. Insulin infusion with 250 μU/mL obtained half-maximal effects, causing a 2.8-fold increase in glucose uptake and a 1.5-fold increase in lactate and pyruvate release. When 20 μmol/L troglitazone was infused for 30 minutes together with 250 μU/mL insulin, insulin-induced glucose uptake significantly increased 30 minutes after troglitazone infusion, and this increase was further augmented after withdrawal of troglitazone. In insulin plus troglitazone infusion at 30 minutes after troglitazone removal, glycogen content in hindlimb muscle was significantly decreased compared with that obtained with insulin infusion alone. In summary, in the absence of insulin, troglitazone does not elicit glucose uptake, but causes an increase in glycolysis accompanied by a decrease in muscle glycogen content and L/P ratio and an increase in oxygen consumption. In the presence of insulin, troglitazone increases insulin-induced glucose uptake, and this increase is further augmented after troglitazone removal. Addition of troglitazone to insulin infusion decreased the glycogen content in hindlimb muscle. This decrease in muscle glycogen content may trigger an enhancement of insulin-induced glucose uptake similar to that observed during muscle contraction or epinephrine treatment.

TROGLITAZONE (CS-045) is a new type of antidiabetic agent that enhances insulin action.[1] Various studies have shown that troglitazone decreases plasma glucose in insulin-resistant diabetic animal models[2,3] and patients with non–insulin-dependent diabetes mellitus (NIDDM),[4,5] although it fails to decrease plasma glucose in insulin-deficient diabetic or normal animals.[2] Troglitazone has been shown to decrease plasma glucose by increasing insulin-induced glucose uptake in peripheral tissues in insulin-resistant diabetic animal models and patients with NIDDM. However, it is still unclear whether troglitazone enhances insulin-induced glucose uptake in peripheral tissues through a direct effect or secondary effect such as a metabolic effect. Recently, Lee and Olefsky[6] demonstrated that troglitazone acutely enhanced insulin-induced glucose uptake in a euglycemic clamp study in normal rats. Among peripheral tissues, skeletal muscle is a main target tissue of insulin and plays an essential role in insulin-induced glucose uptake because of its mass in the whole body. Therefore, in this study, we investigated the acute effects of troglitazone on glucose metabolism and insulin action in perfused rat hindlimb.

From the Pharmacology and Molecular Biology Research Laboratories, Sankyo, Tokyo; Department of Veterinary Physiology, College of Agriculture, University of Osaka Prefecture, Osaka, Japan; and Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN.

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Address reprint requests to Toshihiko Fujiwara, PhD, Group Director of Pharmacology and Molecular Biology Research Laboratories, Sankyo Co, Ltd, 2-58, Hiromachi 1-chome, Shinagawa-ku, Tokyo 140, Japan.

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MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats were purchased from Japan SLC (Shizuoka, Japan) and used for experiments at age 6 to 7 weeks (200 to 250 g).

Materials

Troglitazone sodium salt was synthesized by the Medicinal Chemical Laboratories of Sankyo (Tokyo, Japan). Porcine Insulin (I-3505) and fatty acid–free bovine serum albumin ([BSA] A-6007) were purchased from Sigma Chemicals (St Louis, MO). Hexokinase, glucose-6-phosphate dehydrogenase, and lactate dehydrogenase were obtained from Boehringer (Mannheim, Germany). All other reagents and chemicals were of the highest grade commercially available.

Perfusion Medium

Krebs-bicarbonate buffer containing 115 mmol/L NaCl, 5.9 mmol/L KCl, 1.2 mmol/L, $MgCl_2$, 1.2 mmol/L NaH_2PO_4, 1.2 mmol/L Na_2SO_4, 2.5 mmol/L $CaCl_2$, 25 mmol/L $NaHCO_3$, and 6 mmol/L glucose was used after it was gassed with 95% O_2 and 5% CO_2.

Preparation of BSA, Troglitazone, and Insulin Solution

Fatty acid–free BSA was dissolved in perfusion medium (final concentration, 20% wt/vol). The perfusion medium containing BSA was then consecutively filtered by 5.0-, 0.8-, 0.44-, and 0.22-μm filters (Millipore, Bedford, MA) and was used to dissolve troglitazone sodium salt (final concentration, 4 mmol/L). Porcine insulin was dissolved in slightly acidic water (final concentration, 125 mU/mL).

Surgery for Hindlimb Perfusion

Surgery for the hindlimb perfusion was performed according to the methods of Shiota and Sugano[7] and Ruderman et al.[8] In brief, fed rats were first anesthetized with pentobarbital sodium (50 mg/kg intraperitoneally). After all vessels supplying blood to the tissues except for the

left hindlimb were ligated, the aorta was cannulated with a polyethylene tube (16 gauge) to allow inflow of the perfusion medium. Immediately after inflow of the perfusion medium, the vena cava was cut, and the left common iliac veins were cannulated with polyethylene tubing to collect effluents from the left hindlimb.

Perfusion Systems

Rat hindlimbs were perfused at 15 mL/min/leg by flow-through mode. The perfusion medium was gassed with 95% O_2 and 5% CO_2 and kept at 32°C. A solution of BSA alone or in combination with troglitazone was infused throughout the experiment to prevent insulin and troglitazone from attaching to the infusion line. After the perfusion system was stabilized for 30 minutes (preperfusion), we began infusing a 20% wt/vol BSA solution at 0.075 mL/min/leg (final concentration, 0.1%). In the absence of insulin, the BSA solution alone was replaced after 10 minutes with a troglitazone-containing BSA solution for 40 minutes (final concentration, 5, 10, 20, or 50 μmol/L) as shown in the protocol (Fig 1a). To study troglitazone's effects in the presence of insulin, insulin (final concentration, 250 μU/mL) was infused at 40 minutes until the end of the study. Sixty minutes after preparation, the solution of BSA alone was replaced with a troglitazone-containing BSA solution (final concentration, 20 μmol/L) for 30 minutes (Fig 1b). Effluent samples were collected for measuring glucose uptake and lactate and pyruvate release.

Sampling Procedure for Hindlimb Muscle

Just before infusing the 0.1% BSA solution, the right leg muscle was collected to determine initial glycogen and fructose 2,6-bisphosphate (F2,6P2) content. The left leg muscle was collected 30 minutes after troglitazone infusion in the absence of insulin and at the end of insulin infusion (80 minutes after insulin infusion) for insulin infusion alone and insulin plus troglitazone infusion (Fig 1b). Collected muscles were immediately frozen by liquid nitrogen and stored at −80°C until glycogen and F2,6P2 concentrations could be measured. The red portion of the quadriceps muscle (insulin-sensitive red muscle)[9,10] that was isolated from hindlimb muscle and cooled with liquid nitrogen was used for glycogen and F2,6P2 determination.

Analytical Procedure

Glucose concentration was determined by means of a kit using hexokinase and glucose-6-phosphate dehydrogenase (food analysis:D-glucose UV methods; Boehringer, Mannheim, Germany). Lactate and pyruvate concentrations were determined by enzymatic methods using lactate dehydrogenase.[11,12] Oxygen concentration in the effluents was detected with a Polarographic electrode (Iijima Product, Aichi, Japan). Glycogen levels were measured by the method described by Seifter et al.[13] A portion of the muscle sample was powdered by means of a porcelain mortar and pestle cooled with liquid nitrogen, and digested for 20 minutes in a boiling 30% KOH solution. After cooling, the sample was precipitated with 70% EtOH and centrifuged. The precipitate dissolved in water was used for glycogen determination.

F2,6P2 levels were measured according to the method reported by Van Schaftingen et al.[14] A portion of muscle sample added to 50 mmol/L NaOH was powdered by a porcelain mortar and pestle, cooled with liquid nitrogen, and kept at 80°C for 5 minutes. The extracts were cooled and neutralized at 0°C by ice-cold 1 mol/L l-acetic acid in the presence of 20 mmol/L HEPES and then centrifuged for 10 minutes. The supernatants were used for determination of F2,6P2.

Data Analysis

Glucose uptake, lactate and pyruvate release, and oxygen consumption in the hindlimbs were calculated from the differences in concentration between effluents and influents, and the flow rate. Two-sample *t* tests were used in the statistical analysis. *P* less than .05 was considered statistically significant.

RESULTS

Metabolic Effects of Troglitazone in the Absence of Insulin

Glucose uptake. Figure 2 shows the effect of troglitazone infusion for 40 minutes on glucose uptake. Even at the

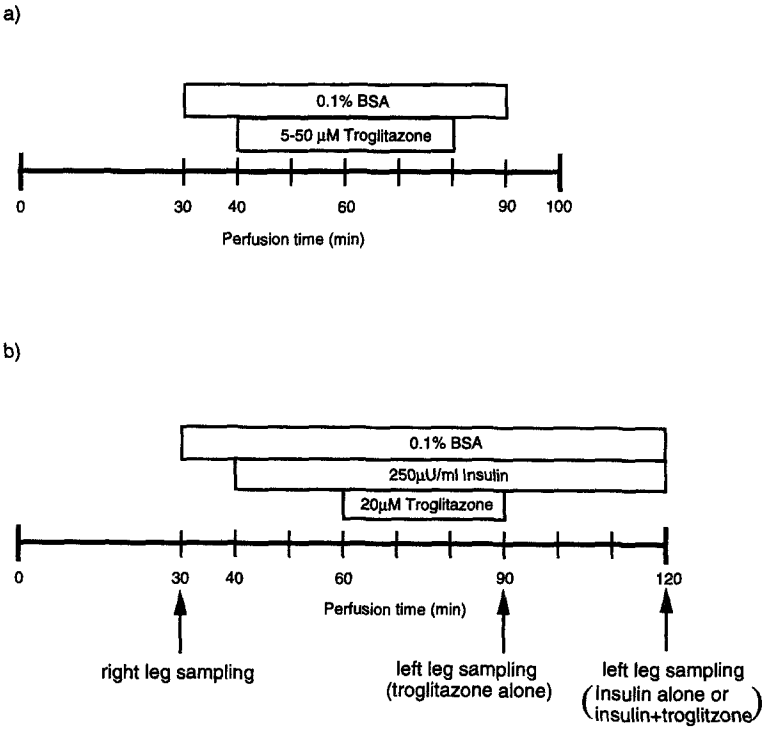

Fig 1. Experimental protocol without (a) or with (b) insulin treatment. Perfusion medium contains 6 mmol/L glucose.

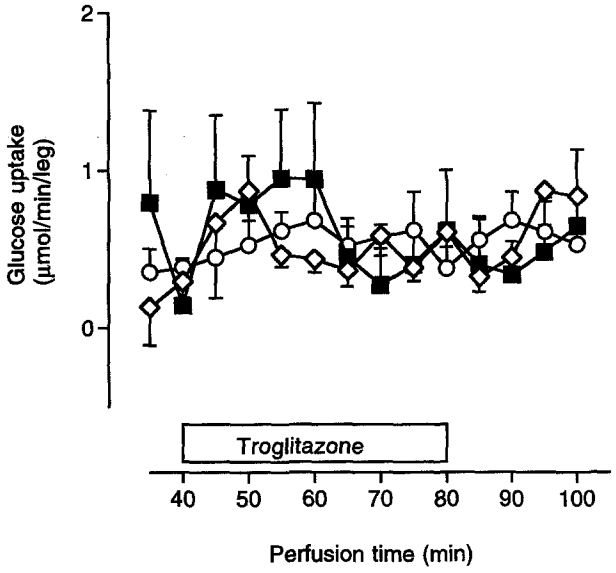

Fig 2. *Effect of troglitazone on glucose uptake in the absence of* insulin. (○) Control group, n = 4; (■) 20 μmol/L, n = 3; (◇) 50 μmol/L, n = 3. Values are the mean ± SEM.

maximum concentration of 50 μmol/L, troglitazone did not elicit glucose uptake in the absence of insulin.

Lactate and Pyruvate Release, Ratio of Lactate to Pyruvate, and Oxygen Consumption

Figures 3 and 4 show the time-course effects of troglitazone on lactate and pyruvate release, the ratio of lactate to pyruvate (L/P ratio), and oxygen consumption. Lactate and pyruvate release gradually increased with troglitazone infusion at 20 and 50 μmol/L, reached a steady state (2.01 ± 0.15 and 3.02 ± 1.21 μmol/min/leg at 20 and 50 μmol/L, respectively) 25 minutes after troglitazone infusion, and remained elevated after troglitazone removal. Compared with lactate and pyruvate release, the L/P ratio immediately decreased with troglitazone infusion as low as 5 μmol/L (troglitazone *v* control, 5.14 ± 0.47 *v* 7.42 ± 0.39, $P < .05$, 20 minutes after troglitazone infusion), with a return to the initial (preinfusion) value promptly after troglitazone removal (Fig 3). With 20 μmol/L troglitazone infusion, oxygen consumption increased gradually, reached a peak value (6.17 ± 0.28 μmol/min/leg) 20 minutes after troglitazone infusion, and returned to the initial value after removal of troglitazone (Fig 4).

Glycogen and F2,6P2 content in perfused hindlimb muscle. Table 1 shows changes in glycogen and F2,6P2 content of red muscle in perfused hindlimb 30 minutes after infusion of troglitazone 20 μmol/L in the absence of insulin. Troglitazone significantly decreased glycogen (−6.01 ± 1.85 mg/g dry weight, $P < .05$) but not F2,6P2 content in hindlimb muscle compared with control muscle.

Effects of Troglitazone on Glucose Metabolism in the Presence of Insulin

Glucose uptake. Figure 5 shows the effect of troglitazone on glucose uptake in the presence of insulin. When insulin was infused at 250 μU/mL for 80 minutes, glucose uptake gradually increased and reached a steady state after 30 minutes. Insulin increased glucose uptake to 2.8 times the basal value (0.35 ± 0.11 μg/min/leg). Troglitazone infusion at 20 μmol/L for 30 minutes during insulin infusion gradually increased insulin-induced glucose uptake, and a significant increase (1.79 ± 0.17 μmol/min/leg) was obtained by the end of 30 minutes. Insulin-induced glucose uptake further increased even

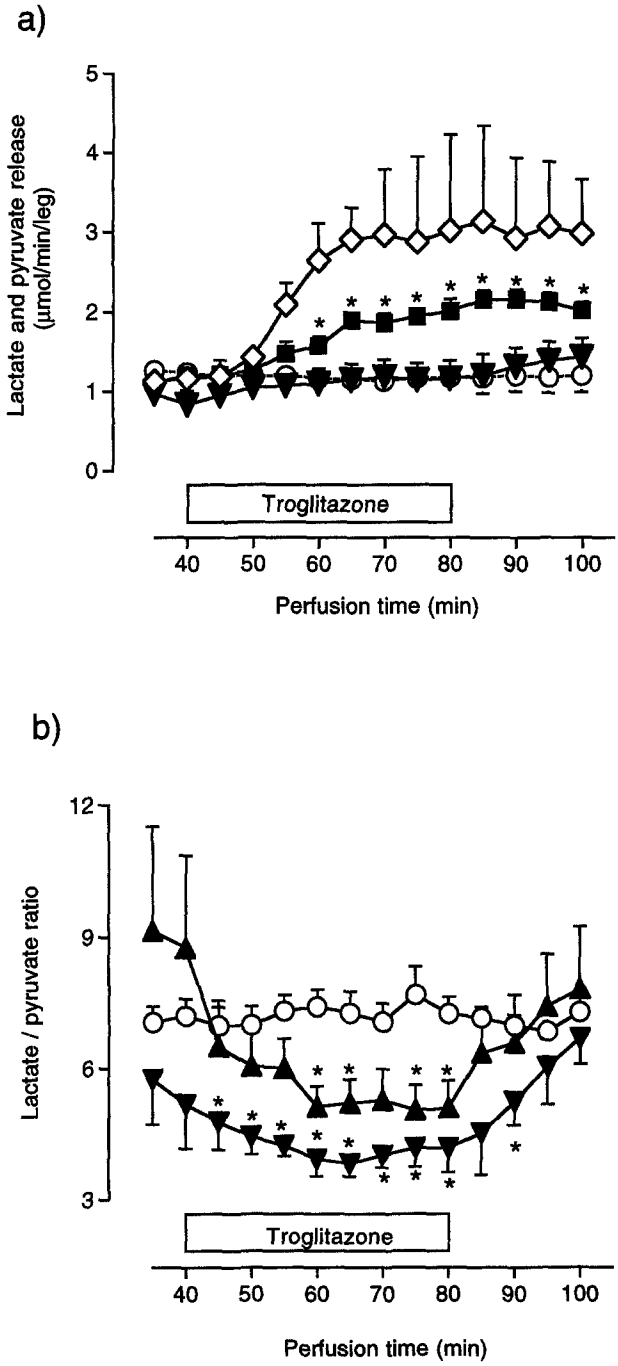

Fig 3. Effects of troglitazone on lactate and pyruvate release (a) and the L/P ratio (b) in the absence of insulin. (○) Control group; (▲) 5 μmol/L; (▼) 10 μmol/L; (■) 20 μmol/L; (◇) 50 μmol/L. Values are the mean ± SEM from 3 to 4 experiments. *$P < .05$ *v* control.

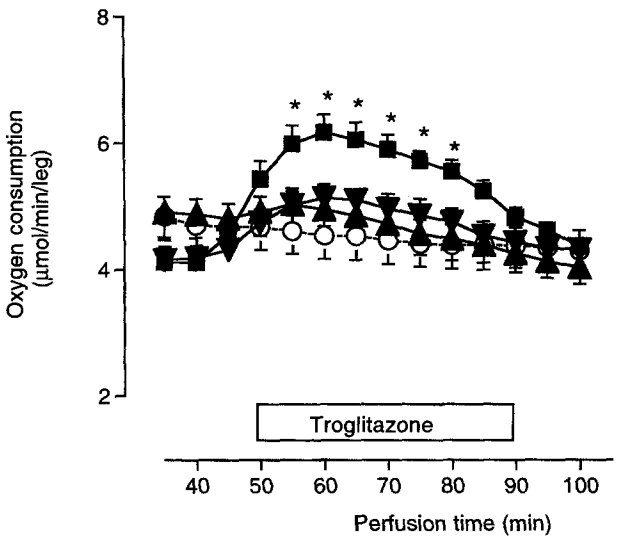

Fig 4. Effect of troglitazone on oxygen consumption in the absence of insulin. (○) Control group; (▲) 5 μmol/L; (▼) 10 μmol/L; (■) 20 μmol/L. Values are the mean ± SEM from 3 to 4 experiments. *$P <$ .05 *v* control.

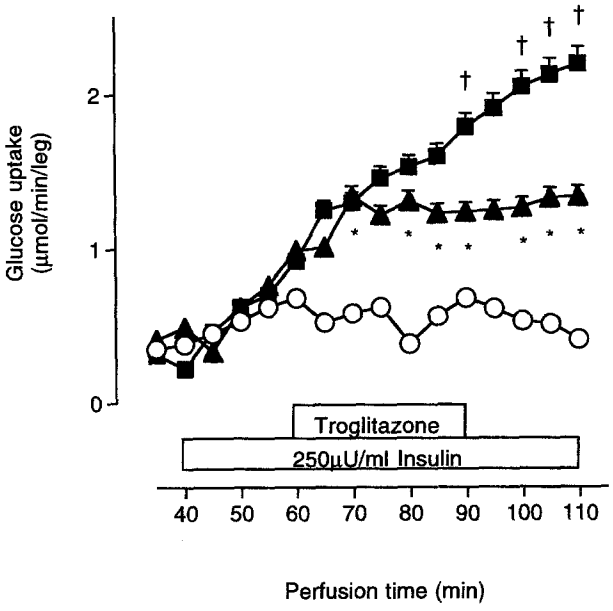

Fig 5. Effect of troglitazone 20 μmol/L on insulin-induced glucose uptake. Values are the mean ± SEM. Each group was from 4 experiments for control (○), 6 for insulin alone (▲), and 7 for insulin plus troglitazone (■). *$P <$ .05, insulin alone *v* control; †$P <$ .05, insulin plus troglitazone *v* insulin alone.

30 minutes after troglitazone withdrawal (2.20 ± 0.18 μmol/min/leg).

Lactate and pyruvate release and the L/P ratio. Figures 6 and 7 show the effects of troglitazone infusion on lactate and pyruvate release, the L/P ratio, and oxygen consumption. Lactate and pyruvate release increased throughout the insulin infusion period (insulin *v* control, 2.33 ± 0.08 *v* 1.22 ± 0.10 μmol/min/leg at the end of insulin infusion). Addition of troglitazone to insulin further increased lactate and pyruvate release, and a significant increase was observed 10 minutes after troglitazone infusion. This augmentation of lactate and pyruvate release was sustained even after cessation of troglitazone infusion (Fig 6a). Insulin infusion tended to decrease the L/P ratio throughout the insulin infusion period. Addition of troglitazone to insulin immediately decreased the L/P ratio, but it returned to the preinfusion level after troglitazone removal (Fig 6b). Insulin infusion did not increase oxygen consumption. Troglitazone infusion with insulin increased oxygen consumption, as it did when infused in the absence of insulin (Fig 7).

Glycogen content in perfused hindlimb muscle. Table 2 shows changes in the glycogen content of red muscle in perfused hindlimb at the end of insulin infusion in the insulin-alone infusion group or insulin plus troglitazone infusion group. Insulin infusion tended to increase glycogen content, but not significantly compared with the control group. In contrast, glycogen content in the insulin plus troglitazone infusion group was significantly decreased (−5.02 ± 3.13 mg/g wet tissue, $P <$ .05) compared with insulin infusion alone (3.11 ± 1.30 mg/g wet tissue).

Table 1. Effect of Troglitazone on Glycogen and F2,6P2 Content in the Red Portion of Quadriceps Femoris Muscle in the Perfused Hindlimb

Group	ΔGlycogen (mg/g dry weight)	F2,6P2 (μg/g dry weight)
Control (n = 5)	−0.21 ± 1.30	0.03 ± 0.44
Troglitazone 20 μmol/L (n = 5)	−6.01 ± 1.85*	−0.26 ± 0.15

NOTE. Troglitazone was infused from 60 to 90 minutes.
*$P <$ .05 *v* control.

DISCUSSION

In the present study, we demonstrated that troglitazone stimulated glucose uptake in the presence of insulin, but not in the absence of insulin, in perfused hindlimbs. These results suggest that troglitazone itself does not have the ability to stimulate glucose uptake, but enhances insulin action in skeletal muscle. The findings are consistent with in vivo findings in which troglitazone decreased plasma glucose in hyperinsulinemic diabetic animals but not in insulin-deficient diabetic animals.[2]

Although troglitazone did not elicit glucose uptake in the absence of insulin, it did increase lactate and pyruvate release and decrease glycogen content in hindlimb muscle, suggesting that troglitazone stimulates glycolysis and glycogenolysis in the absence of insulin in perfused hindlimbs. F 2,6P2 is known to be a stimulator of glycolysis in liver and muscle.[15] Troglitazone did not increase F 2,6P2 in perfused hindlimbs. These results indicate that the increase in glycolysis noted when troglitazone is infused in the absence of insulin is not due to an increase in F 2,6Pc content. Troglitazone decreased the L/P ratio in perfusate at doses as low as 5 μmol/L. Since the L/P ratio in perfusate reflects the cytosolic NAD^+/NADH ratio (lactate + $NAD^+ \leftrightarrow$ pyruvate + NADH),[16] troglitazone seems to increase the cytosolic NAD^+/NADH ratio. In the glycolytic step, NAD^+ in cytosol is used for conversion of glyceraldehyde-3-phosphate (GA-3P) to 1,3-bisphosphoglycerate (1,3-PGA) (GA-3P + NAD^+ + $P_i \leftrightarrow$ 1,3-PGA + NADH). Therefore, troglitazone may stimulate glycolysis between GA-3P and 1,3-

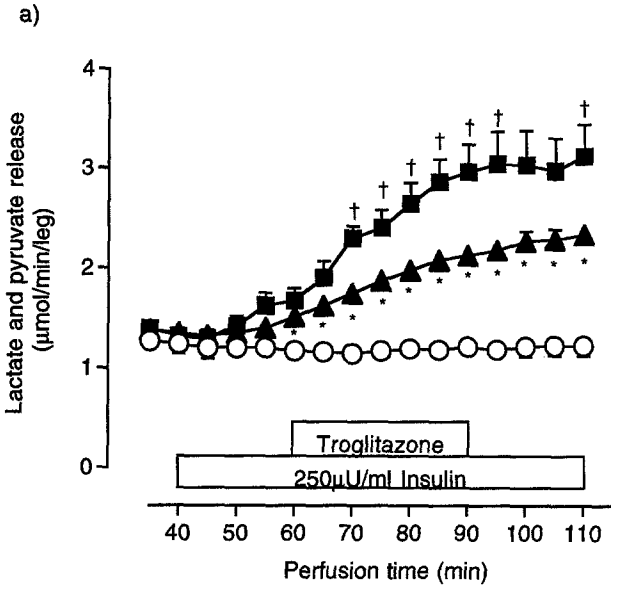

Fig 6. Effects of troglitazone on lactate and pyruvate release (a) and the L/P ratio (b) in the presence of insulin. Values are the mean ± SEM. Each group was from 4 experiments for control (○), 6 for insulin alone (▲), and 7 for insulin plus troglitazone (■). *$P < .05$, insulin alone *v* control; †$P < .05$, insulin plus troglitazone *v* insulin alone.

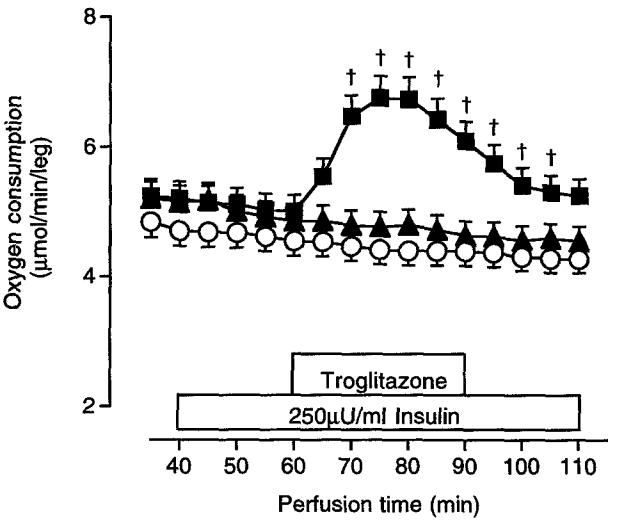

Fig 7. Effect of troglitazone on oxygen consumption in the presence of insulin. Values are the mean ± SEM. Each group was from 4 experiments for control (○), 6 for insulin alone (▲), and 7 for insulin plus troglitazone (■). †$P < .05$, insulin plus troglitazone *v* insulin alone.

PGA by increasing the cytosolic NAD^+/NADH ratio. In addition, it is possible that troglitazone increases glycolytic flux secondary to stimulating glycogenolysis.

In the absence of insulin, troglitazone increases oxygen consumption in perfused hindlimbs. One can postulate that oxygen is consumed by glucose oxidation and/or lipid oxidation (using intracellular lipid). However, it is unlikely that troglitazone stimulates lipid oxidation, because the perfusion medium contained no free fatty acids and an increase in lipid oxidation would suppress glycolysis by accumulating intracellular acetyl coenzyme A (Randle cycle[17,18]). Thus, troglitazone may stimulate glucose oxidation, which results in enhanced glycolysis in the absence of insulin.

Insulin infusion at 250 μU/mL caused a 2.8-fold increase in glucose uptake and a 1.5-fold increase in lactate and pyruvate release by the end of infusion compared with basal values. These data indicate that some portion of the glucose was stored as glycogen (Table 1) or converted to CO_2.

Troglitazone infusion with insulin significantly increased insulin-induced glucose uptake and lactate and pyruvate release. Surprisingly, in the presence of insulin, glucose uptake further increased after troglitazone removal. Glycogen content in perfused hindlimb 20 minutes after troglitazone removal was significantly decreased during infusion of insulin plus troglitazone compared with insulin infusion alone (Table 1). This result suggests that troglitazone inhibits insulin-induced glycogen synthesis and that the glycogenolytic effect of troglitazone is predominant compared with the glycogenic effect of insulin.

Table 2. Effect of Insulin and Insulin Plus Troglitazone on Glycogen Content in the Red Portion of Quadriceps Femoris Muscle in the Perfused Hindlimb

Group	ΔGlycogen (mg/g dry weight)
Control (n = 5)	0.30 ± 1.21
Insulin 250 μU/mL (n = 7)	3.11 ± 1.30
Insulin 250 μU/mL + troglitazone 20 μmol/L (n = 7)	−5.02 ± 3.13*

NOTE. Insulin and troglitazone were infused from 40 to 120 minutes and from 60 to 90 minutes, respectively.

*$P < .05$ *v* insulin.

The potentiation of insulin-induced glucose uptake after troglitazone removal in perfused hindlimb is similar to the enhancement of insulin-induced glucose uptake in muscle after muscle contraction[19,20] and epinephrine treatment[21] in which muscle glycogen content is decreased. After both muscle contraction and epinephrine treatment, it is hypothesized that glycogen depletion enhances insulin-stimulated glucose uptake.[20,21] Therefore, in the phase after troglitazone removal, glycogen depletion may potentiate insulin-induced glucose uptake.

In conclusion, (1) in the absence of insulin, troglitazone did not elicit glucose uptake, but increased glycolysis and decreased glycogen content in hindlimb muscle, and these changes may be induced by a decrease in the cytosolic $NAD^+/NADH$ ratio or an increase in oxygen consumption; (2) in the presence of insulin, troglitazone increased insulin-induced glucose uptake and increased glycolysis, and this increase was further enhanced after troglitazone removal. Insulin infusion increased glycogen content in hindlimb muscle, but addition of troglitazone to insulin decreased the glycogen content in hindlimb muscle. A decrease of glycogen content may trigger augmentation of insulin-induced glucose uptake in hindlimb muscle in the presence of insulin, just as muscle contraction does.

REFERENCES

1. Hofmann CA, Colca JR: New oral thiazolidinedione antidiabetic agents act as insulin sensitizer. Diabetes Care 15:1075-1078, 1992
2. Fujiwara T, Yoshioka S, Yoshioka T, et al: Characterization of a new antidiabetic agent CS-045. Studies in KK and ob/ob mice and Zucker fatty rats. Diabetes 37:1549-1558, 1988
3. 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 at a late stage of the diabetic syndrome in C57BL/KsJ-db/db mice. Metabolism 40:1212-1218, 1992
4. 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
5. 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
6. Lee MK, Olefsky JM: Acute effect of troglitazone on insulin action in normal rats. Metabolism 44:1166-1169, 1995
7. Shiota M, Sugano T: Characteristics of rat hindlimbs perfused with erythrocyte- and albumin-free medium. Am J Physiol 251:C78-C84, 1986
8. Ruderman NB, Houghton CRS, Hems R: Evaluation of the isolated perfused rat hindquarter for the study of muscle metabolism. Biochem J 124:639-651, 1971
9. James DE, Jenkins AR, Kraegen EW: Heterogeneity of insulin action in individual muscle in vivo: Euglycemic clamp studies in rats. Am J Physiol 248:E567-E574, 1985
10. James DE, Burleigh KM, Kraegen EW: In vivo glucose metabolism in individual tissues of the rat. J Biol Chem 261:6366-6374, 1986
11. Gutmann I, Wahlefeld AW: L -(+)-Lactate: Determination with lactate dehydrogenase and NAD, in Bergmeyer HU (ed): Methods of Enzymatic Analysis, vol 3 (ed 2). New York, NY, Academic, 1974, pp 1492-1495
12. Passonneau JW, Lowry OH: Pyruvate: Fluorimetric assay, in Bergmeyer HU (ed): Methods of Enzymatic Analysis, vol 3 (ed 2). New York, NY, Academic, 1974, pp 1452-1456
13. Seifter S, Dayton S, Novic B, et al: The estimation of glycogen with the anthrone reagent. Arch Biochem Biophys 25:191-200, 1950
14. Van Schaftingen E, Leder B, Bartrons R, et al: A kinetic study of pyrophosphate fructose-6-phosphate phosphotransferase from potato tubers. Application to a microassay of fructose-2,6-bisphosphate. Eur J Biochem 129:191-195, 1982
15. Hue L, Rinder MH: Role of fructose 2,6-bisphosphate in the control of glycolysis in mammalian tissues. Biochem J 245:313-324, 1987
16. Williamson DH, Lund P, Krebs HA: The redox state of free nicotinamide-adenine dinucleotide in cytoplasm and mitochondria of rat liver. Biochem J 103:514-527, 1967
17. 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
18. 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
19. Garetto LP, Richter EA, Goodman MN, et al: Enhanced muscle glucose metabolism after exercise in the rat: The two phases. Am J Physiol 246:E471-E475, 1984
20. Cartee GD, Young DA, Sleeper MD, et al: Prolonged increase in insulin-stimulated glucose transport in muscle after exercise. Am J Physiol 256:E494-E499, 1989
21. Nolte LA, Gulve EA, Holloszy JO: Epinephrine-induced in vivo muscle glycogen depletion enhances insulin sensitivity of glucose transport. J Appl Physiol 76:2054-2058, 1994