

Exercise and Thiazolidinedione Therapy Normalize Insulin Action in the Obese Zucker Fatty Rat

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Thiazolidinediones and exercise are both known to improve insulin action independently. Therefore, we determined whether combined therapy could normalize insulin action in the Zucker fatty (ZF) rat. Rats were fed troglitazone as a 0.2% food admixture over a 3-week exercise training period (treadmill running 5 days/week, 20 m/min, 0% grade, 60 min/day). Subsequent to drug and/or exercise therapy, animals were chronically cannulated in the carotid artery (sampling) and jugular vein (infusion). After a 4-day recovery from surgery, animals were exposed to a hyperinsulinemic ($40 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) euglycemic clamp ($8.5 \pm 0.12 \text{ mmol/l}$; $P = 0.45$ between groups). Independently, exercise ($n = 7$) and troglitazone ($n = 7$) improved the glucose disposal rate 20% ($P = 0.04$) and 76% ($P = 0.001$), respectively, when compared with untreated ZF controls ($n = 11$). In combination, exercise and troglitazone therapy ($n = 6$) produced significant increments in the following: tracer-determined glucose disposal rate (combined therapy, $52.4 \pm 2.9 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, vs. untreated ZF, $25.8 \pm 0.8 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$; $P = 0.0001$), total GLUT4 protein (twofold increase; $P = 0.001$), insulin receptor substrate (IRS)-1 protein (fourfold increase; $P = 0.0001$), and Akt phosphorylation (2.9-fold increase; $P = 0.002$). In conclusion, 1) exercise and troglitazone therapy each improved insulin action in the ZF rat, whereas the combination of the two led to complete normalization of insulin sensitivity, and 2) combination treatment also resulted in normalization of GLUT4 total protein, IRS-1 protein, and Akt phosphorylation compared with lean littermates. *Diabetes* 49:2154–2159, 2000

Insulin resistance is a central pathophysiological feature of obesity and type 2 diabetes (1,2). The genetically obese Zucker fatty (ZF) rat (*fa/fa*) is a well-characterized model of insulin resistance, exhibiting a range of metabolic abnormalities including severe hyperinsulinemia, dyslipidemia, and adipocyte hypertrophy (3–5). Attenuated insulin-stimulated glucose disposal resulting from decreased transport is a major manifestation of insulin resistance in ZF rats; however, the precise mechanism responsible for this

defect remains unknown. This defect in glucose transport appears to be specific to insulin action because glucose uptake in response to exercise, muscle contraction, and hypoxia appear normal in these animals (6–8).

Interestingly, whereas significant reductions in glucose transport capacity have been observed, there is no apparent reduction in GLUT4 transporter protein expression in the insulin-resistant obese ZF rat when compared with insulin-sensitive lean Zucker littermates (7,9–12). Thus, the defect in glucose uptake is thought to be constrained to one or more loci within the insulin-signaling cascade (13–15).

Chronic exercise improves insulin sensitivity and glucose tolerance (16–18). In insulin-resistant subjects, 6 weeks of exercise training was shown to increase whole-body maximal insulin-stimulated glucose disposal 2.7-fold (19). Whereas this increase in insulin sensitivity, as defined by increments in glucose disposal, has not previously been thought to be attributable to elevations in protein or phosphorylation states of key insulin-signaling molecules, increases in GLUT4 mRNA and total protein have consistently been demonstrated (10,20,21). Recently, however, a twofold increase in maximal insulin-stimulated phosphatidylinositol (PI) 3-kinase activity was shown in exercise-trained individuals (22). This improvement in PI 3-kinase activation led to a 30% increase in the glucose disposal rate, yielding a significant relationship between PI 3-kinase activation and glucose disposal rate ($r = 0.60$, $P < 0.02$) (23). The precise physiological adaptations that occur as a result of chronic exercise that produce improved insulin sensitivity remain to be elucidated.

Troglitazone is a member of the thiazolidinedione (TZD) class of insulin sensitizers that activate peroxisome proliferator-activator receptor (PPAR)- γ . When ligands stimulate the PPAR- γ nuclear receptor, a variety of response genes are stimulated or repressed (24). Although the exact target genes for insulin sensitization remain unknown, it is felt that induction or repression of key glucoregulatory proteins underlies the therapeutic effect of these compounds. Indeed, it has been demonstrated in vitro that TZD treatment can lead to increased GLUT4 expression (25).

Because exercise and troglitazone bring about improvements in skeletal muscle insulin sensitivity via divergent cellular pathways, we sought to ascertain whether exercise and troglitazone in combination could have additive beneficial effects on insulin action in ZF rats.

RESEARCH DESIGN AND METHODS

Animals. Female FZ (*fa/fa*) and lean Zucker (*fa/??*) rats (Charles River, Wilmington, MA) were received at 8 weeks of age and housed individually under controlled light (12:12 light:dark) and temperature conditions. Animals had access to food and water ad libitum.

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FFA, free fatty acid; GDR, glucose disposal rate; HGO, hepatic glucose output; ID, internal diameter; IRS, insulin receptor substrate; PI, phosphatidylinositol; PKB, protein kinase B; PPAR, peroxisome proliferator-activator receptor; TZD, thiazolidinedione.

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

Study design. Obese ZF rats were randomly divided into four experimental groups: sedentary untreated ZF controls ($n = 11$), exercise ($n = 7$), troglitazone ($n = 9$), and exercise plus troglitazone ($n = 9$). Lean littermates ($n = 11$) were also received at 8 weeks of age, remained sedentary, and were fed standard rat diet ad libitum. Animals participating in the exercise protocols were acclimated to treadmill running (15 m/min, 30 min/day at a 0% grade for 1 week). Subsequent to treadmill acclimation, the workload and duration were increased to 20 m/min, 60 min/day at a 0% grade for 3 weeks. Before and after each training bout, rats ran an additional 30 min at 15 m/min, which served as a warm-up and cooldown. The training protocol was extended through the cannulation recovery period (week 12), with the final acute bout of exercise performed 18–20 h before the glucose clamp. Animals participating in the troglitazone groups were fed the drug as a 0.2% food admixture (~200 mg/day) beginning at week 9 and extending through week 12. The troglitazone-enriched diet was prepared in small amounts every 2 days and stored at 4°C. Animal weight and food intake were measured daily. Plasma troglitazone concentration was assayed from plasma taken after the clamp. Troglitazone concentration for both treated groups averaged $2.8 \pm 0.4 \mu\text{g/ml}$ but was undetectable in nontreated animals (Table 1).

Surgery and clamp procedure. One week before the experiment, animals (11 weeks of age) were chronically cannulated under single-dose anesthesia (42 mg/kg ketamine HCl, 5 mg/kg xylazine, 0.75 mg/kg acepromazine maleate; administered intramuscularly) in the jugular vein for infusion of glucose, tracer, and insulin (dual cannula internal diameter [ID] 0.03 cm; Dow Corning Silastic, Midland, MI) and in the carotid artery (Intramedic polyethylene tubing PE-50; Clay Adams, Becton Dickinson, Sparks, MD) for sampling. All cannulae were tunneled subcutaneously, exteriorized at the back of the neck, and encased in silastic tubing (0.2-cm ID) sutured to the skin. Animals were allowed a 4-day recovery from surgery to regain body weight.

Six hours before the euglycemic-hyperinsulinemic clamp, food was withdrawn from the cage. All animals were exposed to the same general glucose clamp protocol. Ninety minutes before the clamp, animals were weighed and placed into a modified metabolic chamber. Basal samples were drawn at -60 and 0 min. A priming dose of 5 μCi of D-[3-³H]glucose (New England Nuclear, Boston MA) was administered, and a tracer constant infusion (0.167 $\mu\text{Ci}/\text{min}$) was initiated at -60 min. After 60 min of tracer equilibration and basal sampling at time 0, glucose (variable infusion, 50% dextrose; Abbott Labs, Chicago) and tracer plus insulin (40 mU \cdot kg⁻¹ \cdot min⁻¹; Novolin R; Novo Nordisk, Copenhagen, Denmark) infusions were started simultaneously.

Small blood samples (70 μl) were drawn at 10-min intervals and immediately analyzed for glucose (YSI 2300 Glucose Analyzer; Yellow Springs Instruments, Yellow Springs, OH) to maintain the integrity of the glucose clamp throughout the duration of the experiment. Larger blood samples (250 μl) were taken at basal, -60 min, and 0 min and at 100 and 120 min for determination of tracer specific activity, insulin, free fatty acid (FFA), and glucose. All blood samples were immediately centrifuged, and plasma was stored at -80°C for subsequent analysis. After terminal blood sampling at 120 min (500 μl), animals were euthanized with a dose of Nembutal (100 mg/kg i.v.) and tissues were excised, immediately quick frozen, and stored at -80°C for subsequent metabolic analyses.

Analytical procedures. Plasma determination of glucose was assayed by the glucose oxidase method (Yellow Springs Instruments). Plasma insulin was measured via a radioimmunoassay kit (Linco Research, St. Charles, MO). Plasma glucose specific activity was measured in duplicate after zinc sulfate and barium hydroxide deproteinization. Plasma FFA levels were measured enzymatically with a commercial kit (NEFA C; Wako Chemicals, Richmond, VA).

Red quadriceps muscle (30–100 mg) was homogenized in liquid nitrogen and treated with lysis buffer containing phosphatase and protease inhibitors. After a 10-min incubation, the lysates were clarified by centrifugation (10,000g at 4°C). Before Western blot analysis, muscle lysates were analyzed for total protein. Samples were separated by SDS-PAGE on 5, 7.5, or 10% polyacrylamide gels. Proteins were transferred to polyvinylidene difluoride membranes (Immobilon-p; Millipore, Bedford, MA) using an SD Transblot apparatus (Bio-Rad, Hercules, CA) and blotted with PY20 (Transduction Laboratories, Lexington, KY), insulin receptor (Santa Cruz Biotechnology, Santa Cruz, CA), insulin receptor substrate (IRS)-1 (anti-rat carboxy-terminal IRS-1; Upstate Biotechnology, Lake Placid, NY), GLUT4 (Chemicon International, Temecula, CA), phospho-Akt (New England Biolabs, Beverly, MA), and Akt antibodies according to the manufacturer instructions. After incubation with horseradish peroxidase-conjugated secondary antibodies, proteins were visualized by enhanced chemiluminescence. Band intensities were quantified by den-

sitometry on a Hewlett-Packard ScanJet II using NIH-Image 1.6 software. No group differences for total muscle protein as reflected by total α -actin content ($P = 0.32$) were observed.

Calculations. Hepatic glucose output (HGO) and glucose disposal rate (GDR) were calculated using Steele's equation (26). Values presented are expressed as means \pm SE. Statistical analyses were performed using analysis of variance with Tukey's post hoc comparison for identification of significance within and between groups (SPSS graduate pack; SPSS, Chicago). Significance was set a priori at $P < 0.05$.

RESULTS

Baseline values. Initial body weights were the same for ZF rats received at 8 weeks of age (mean weight 243 ± 10 g). Four weeks of exercise led to a decrease in the amount of weight gain compared with troglitazone-treated animals (331 ± 9 vs. 363 ± 7 g, respectively; $P = 0.02$). As anticipated, lean Zucker littermates weighed significantly less (180 ± 5 g; $P = 0.00001$) than all four ZF groups. Although untreated ZF animals were not overtly hyperglycemic (9.21 ± 0.33 mmol/l), troglitazone and exercise plus troglitazone treatment yielded a significant reduction (22%) in glycemia (Table 1). Exercise alone led to a slight but nonsignificant decrease in basal plasma glucose (Table 1).

Untreated ZF rats were substantially hyperinsulinemic (Table 1). Exercise produced a 30% reduction in basal insulin levels, whereas troglitazone treatment led to a 41% reduction in basal insulin concentration. Combination therapy did not further reduce insulinemia, and insulin levels for lean controls were significantly lower than those of all three treated ZF groups.

Compared with untreated ZF rats, exercise, troglitazone treatment, and combination treatment all led to reduced plasma FFA levels to the range seen in lean controls (Table 1). **Glucose clamp studies.** As seen in Table 1, steady-state plasma glucose and insulin levels were comparable in all groups during the clamp. As expected, the insulin-stimulated GDR was lower in untreated ZF rats compared with lean littermates (Fig. 1A). Both chronic exercise and troglitazone treatment led to an improvement in insulin sensitivity (as seen by an increase in GDR); however, the effect of troglitazone was greater than that for exercise. When used in combination, the effects of exercise and troglitazone were additive and led to a complete normalization of GDR in the ZF rat. Thus, independently, exercise and troglitazone treatment moderately improved GDR, whereas the combination of the two led to complete normalization of this aspect of insulin action.

As seen in Table 1, basal HGO rates were the same in all groups. Whereas the ability of insulin to inhibit HGO was the same in lean and untreated ZF rats, the exercise and the combined therapy led to a marked increase in the ability of insulin to suppress HGO.

As seen in Fig. 1B, total GLUT4 protein content was decreased by 37% in skeletal muscle from untreated ZF rats compared with lean littermates ($P = 0.09$, two-tailed test; $P < 0.045$, one-tailed test). Interestingly, exercise, troglitazone treatment, and combination therapy led to normalization of GLUT4 protein content, and these effects were roughly comparable to the magnitude of improvement in insulin-stimulated GDR, as seen in Fig. 1A.

As seen in Fig. 2, a statistically significant (30–35%) decrease in insulin receptor protein content was observed for untreated ZF rats compared with lean controls. This statistically significant decrease remained constant across all treat-

TABLE 1

Effect of exercise and troglitazone on body weight, arterial glycemia, insulin concentration, FFA concentration, HGO, and plasma troglitazone in the ZF rat

	Untreated ZF	Exercise	TZD	Exercise + TZD	Lean
<i>n</i>	11	7	7	6	11
Body weight (g)	341 ± 12	331 ± 9	363 ± 7	347 ± 16	180 ± 5 [†]
Arterial glucose concentration (mmol/l)					
Basal	9.2 ± 0.33	8.1 ± 0.4	7.5 ± 0.2	7.2 ± 0.3	8.6 ± 0.2
Steady-state clamp	8.5 ± 0.04	8.6 ± 0.2	8.6 ± 0.12	8.5 ± 0.18	8.4 ± 0.07
Insulin concentration (nmol/l)					
Basal	0.73 ± 0.04*	0.52 ± 0.08* [†]	0.43 ± 0.03* [†]	0.36 ± 0.03* [†]	0.16 ± 0.01 [†]
Steady-state clamp	18.4 ± 2.4	18 ± 3.2	18.6 ± 2.6	14.7 ± 3.0	13.7 ± 1.6
FFA concentration (mmol/l)					
Basal	1.5 ± 0.27	0.72 ± 0.06 [†]	0.89 ± 0.1	0.92 ± 0.14	0.74 ± 0.06 [†]
Steady-state clamp	1.0 ± 0.2	0.53 ± 0.06 ^{†‡}	0.30 ± 0.07 ^{†‡}	0.29 ± 0.04 ^{†‡}	0.28 ± 0.05 ^{†‡}
HGO (mg · kg ⁻¹ · min ⁻¹)					
Basal	11.4 ± 0.75	11 ± 0.78	10 ± 0.67	9 ± 0.66	10.4 ± 1.0
Steady-state clamp	7.8 ± 0.2 [‡]	3.5 ± 0.5* ^{†‡}	6 ± 0.8 [‡]	4.4 ± 0.90 ^{†‡}	6.0 ± 0.85* [‡]
Troglitazone (µg/ml)	0.00	0.00	2.9 ± 0.45* [†]	2.7 ± 0.34* [†]	0.00

Data are means ± SE. *Significance vs. lean Zucker controls (*P* < 0.05); [†]significance vs. untreated ZF rats (*P* < 0.05); [‡]significance vs. corresponding basal value (*P* < 0.05).

ment groups. In contrast (Fig. 2B), insulin receptor tyrosine phosphorylation was markedly decreased only in untreated ZF rats compared with controls. This phosphorylation defect was ameliorated after chronic exercise and troglitazone therapy but was completely normalized in combined therapy animals, although the difference between the combined therapy group and single treatment groups did not reach statistical significance.

Qualitatively, results observed for skeletal muscle IRS-1 protein content (Fig. 3) were similar to GDR. In the muscle samples obtained at the termination of the euglycemic clamp, there was a striking decrease (70%) in IRS-1 protein in the untreated ZF rats. This defect was increased toward normal in a stepwise fashion in the exercise-treated, troglitazone-treated, and combination therapy groups, such that protein levels were restored to normal after chronic exercise with TZD therapy.

Akt total protein was comparable across all study groups; thus, no significant difference was observed (Fig. 4A and B). In contrast, there was a marked decrease in insulin-stimulated Akt serine phosphorylation in the untreated ZF rats compared with lean controls (Fig. 4C and D). Whereas chronic exercise had no significant effect on insulin-stimulated Akt phosphorylation, the striking defect in phosphorylation in untreated ZF rats was essentially normalized by combination therapy. These findings demonstrate that the insulin-signaling pathway leading to Akt phosphorylation, markedly blunted in untreated ZF rats, was restored to functional normality when animals were chronically exercised and treated with troglitazone.

DISCUSSION

Insulin resistance is a characteristic feature of type 2 diabetes and other pathophysiological states in humans; therefore, the treatment of insulin resistance is an important therapeutic

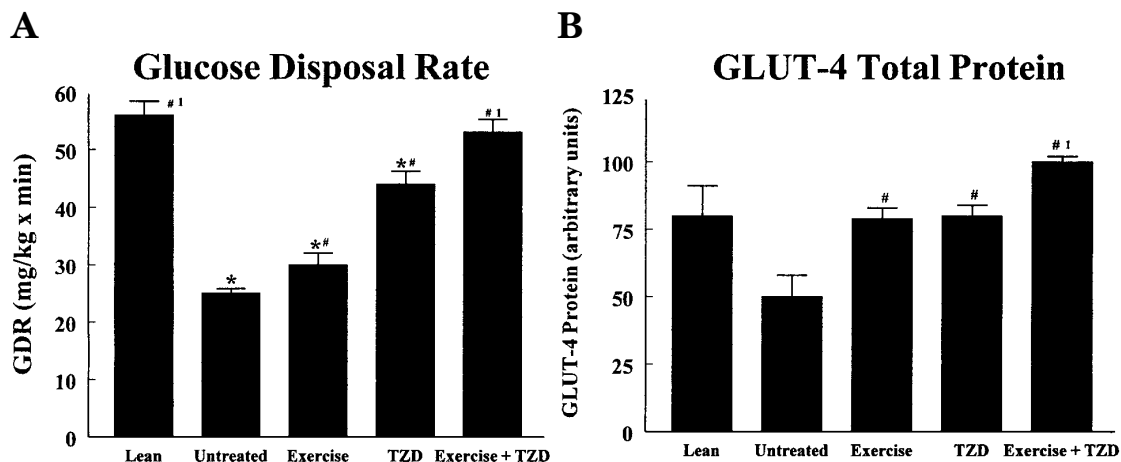


FIG. 1. Whole-body insulin sensitivity assessed by the euglycemic-hyperinsulinemic clamp technique in lean Zucker and obese ZF rats after exercise and troglitazone therapy. Data are expressed as means ± SE for (A) steady-state GDR and (B) GLUT4 total protein. The five experimental groups include the following: lean, untreated ZF, exercise ZF (4), TZD (troglitazone-fed ZF), and exercise plus TZD. *Significance vs. lean control (*P* < 0.05); #significance vs. obese untreated control (*P* < 0.05); #1significant elevation above single treatment groups (*P* < 0.05).

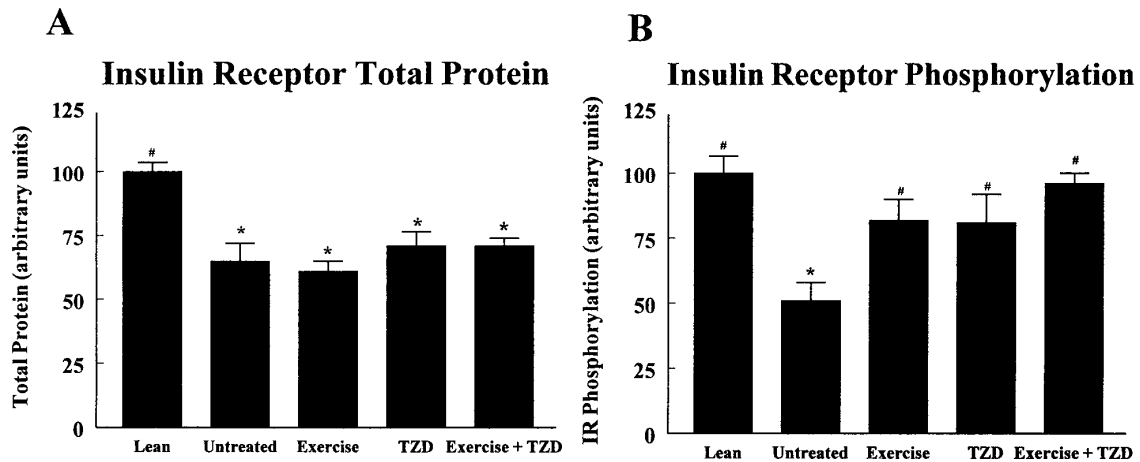


FIG. 2. The effect of exercise and troglitazone on muscle (red quadriceps) insulin receptor (IR) total protein (A) and insulin receptor phosphorylation (B) after a euglycemic-hyperinsulinemic clamp for the five experimental groups: lean, untreated ZF, exercise ZF, TZD (troglitazone-fed ZF), and exercise plus TZD. Data are expressed as means \pm SE. *Significance vs. lean control ($P < 0.05$); #significance vs. obese untreated control ($P < 0.05$).

goal. Chronic exercise as well as treatment with an insulin-sensitizing TZD agent are two well-known methods for improving insulin sensitivity. It has been shown that these two therapeutic modalities exert their beneficial effects through distinct and independent mechanisms (27,28). Based on this fact, we hypothesized that chronic exercise training as well as TZD treatment could result in additive insulin-sensitizing effects, which if applicable to humans, would be of therapeutic importance. To test this idea, we treated insulin-resistant ZF rats with chronic exercise, a TZD, or a combination of these two therapies. Troglitazone was used as a representative well-characterized example of the TZD class of compounds.

The major findings of this study are that both exercise training and troglitazone treatment partially ameliorate the insulin resistance of the ZF rat but when used in combination, the effects are additive, leading to essential normalization of insulin-stimulated glucose disposal. More specifically, compared with lean controls, ZF rats displayed a 55% decrease in insulin-stimulated GDR. Exercise training alone led to a 20% improvement, and troglitazone treatment alone led to a 76% improvement, but the combination therapy resulted in a 95% amelioration of insulin resistance.

If similar results could be reproduced in humans, then these findings have significant therapeutic implications. TZDs are commonly used to treat insulin resistance, and

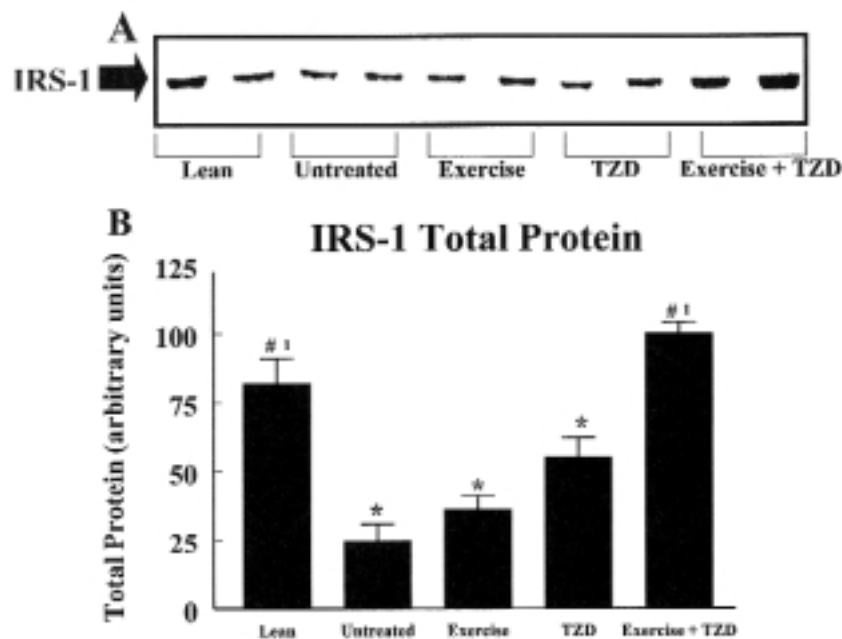


FIG. 3. The effect of exercise and troglitazone on IRS-1 total protein after a euglycemic-hyperinsulinemic clamp for the five experimental groups: lean, untreated ZF, exercise ZF, TZD (troglitazone-fed ZF), and exercise plus TZD. A: Representative blot from red quadriceps muscle for two animals per group. B: Blots were quantified via densitometry and expressed as means \pm SE. *Significance vs. lean control ($P < 0.05$); #significance vs. obese untreated control ($P < 0.05$); #significance vs. single treatment groups ($P < 0.05$).

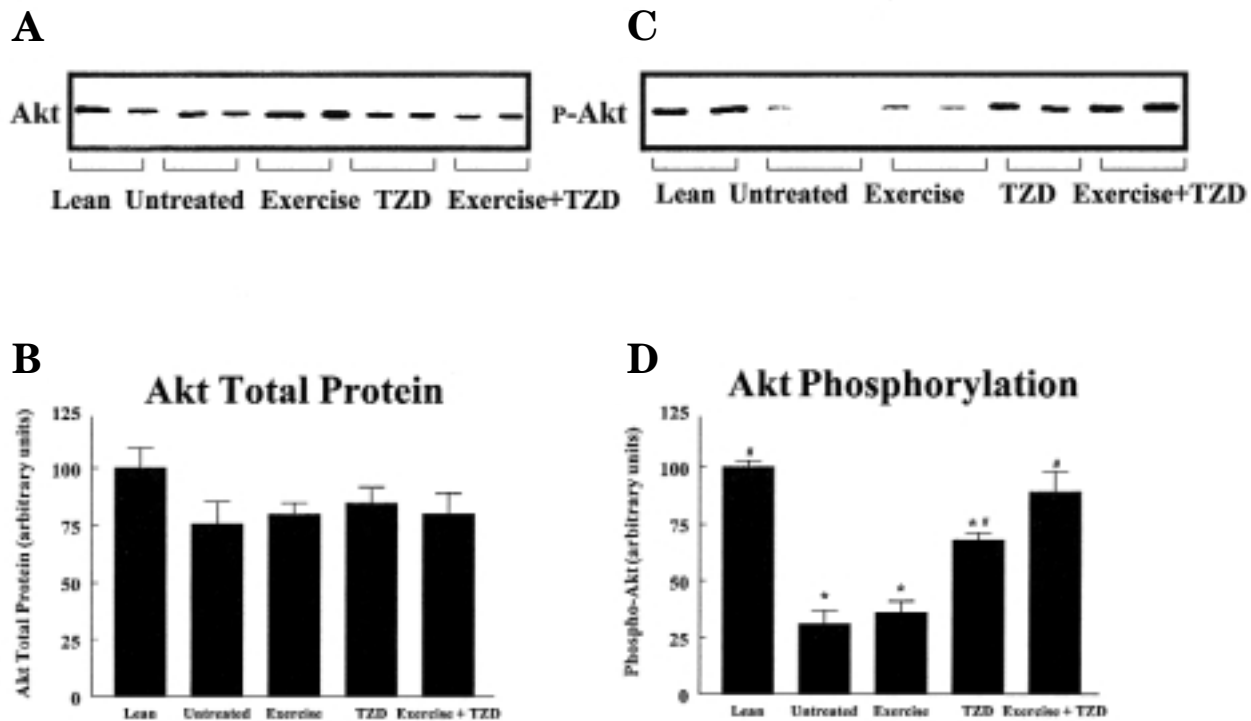


FIG. 4. The effect of exercise and troglitazone on muscle Akt total protein (A and B) and Akt phosphorylation (C and D) after a euglycemic-hyperinsulinemic clamp for the five experimental groups: lean, untreated ZF, exercise ZF, TZD (troglitazone-fed ZF), and exercise plus TZD. A and C: Representative blots of Akt and phospho-Akt (p-Akt), respectively, from red quadriceps muscle for two animals per group. B and D: Blots were quantified via densitometry and expressed as means \pm SE. *Significance vs. lean control ($P < 0.05$); #significance vs. obese untreated control ($P < 0.05$).

their beneficial effects in a variety of insulin-resistant states in humans have been well described. Exercise training has also been shown to improve insulin sensitivity, although long-term exercise programs have been difficult to maintain in large numbers of subjects. The additive effects that we have observed in combination therapy raise the possibility that the implementation of a moderate chronic exercise program in patients treated with TZDs could have substantial therapeutic effects to normalize insulin sensitivity. This should lead to glycemic reductions in patients with type 2 diabetes and could also ameliorate other sequelae of insulin resistance, e.g., dyslipidemia and hypertension.

Our studies also shed some light on the potential mechanisms underlying amelioration of insulin resistance. Total GLUT4 content measured in skeletal muscle of ZF rats has yielded variable results, with some studies reporting a decrease in specific muscle types and others finding no change (7,10–12). In the current investigation, we found a 37% decrement in total skeletal muscle GLUT4 protein content in ZF rats compared with lean rats ($P = 0.09$, two-tailed test; $P = 0.045$, one-tailed test). Although we did not measure plasma membrane GLUT4 content during insulin stimulation (reflecting GLUT4 translocation), these results are consistent with the possibility that a deficiency of GLUT4 protein contributes to decreased insulin-stimulated GDR in ZF rats. Consistent with this idea, exercise, troglitazone, and combination therapy led to a stepwise increase in skeletal muscle GLUT4 content that was highly correlated ($r = 0.60$, $P < 0.001$) with the stepwise increase in insulin-stimulated GDR. It has been previously reported that chronic exercise training and TZD treatment augment GLUT4 levels, possibly through different

mechanisms (23,25). The additive effects of these two therapies on GLUT4 levels shown in the current investigation are fully consistent with the additive effects of these two therapies to improve overall insulin sensitization.

It is unlikely that changes in GLUT4 content entirely explain the insulin-sensitizing effects of exercise and troglitazone treatment because various defects in the intracellular insulin-signaling pathway in ZF rats have been previously reported (14,29,30). Along these lines, Akt/protein kinase B (PKB) is a serine/threonine kinase that is thought to be a possible component of the insulin-stimulated GLUT4 translocation pathway. Akt/PKB undergoes serine/threonine phosphorylation upon insulin stimulation. These events are downstream of and dependent on PI 3-kinase and PI-dependent kinase 1 (PDK1) (31,32). In current studies, we find a striking (69%) decrease in Akt/PKB phosphorylation in untreated ZF rats despite no reduction in total skeletal muscle Akt protein. Insulin-stimulated Akt phosphorylation was unaffected by chronic exercise. However, troglitazone treatment led to a substantial increase, whereas the combination of exercise and troglitazone treatment led to normalization of this phosphorylation event. Insulin-stimulated Akt phosphorylation cannot be explained by differences in total protein because Akt protein was identical in the groups. These results suggest that exercise and troglitazone treatment led to functional normalization of insulin signaling—at least with respect to the steps leading to Akt/PKB phosphorylation.

Obviously, the insulin receptor is upstream of Akt/PKB phosphorylation, and our results show a significant (30%) decrease in insulin receptor total protein in all ZF rats—

untreated and treated alike. Perhaps more importantly, there is an even greater (50%) decrease in insulin-stimulated receptor tyrosine phosphorylation, which was improved to normal or near-normal levels after exercise and troglitazone treatment. Clearly, enhanced insulin-stimulated receptor phosphorylation would be expected to improve insulin signaling, with a subsequent increase in insulin-stimulated Akt/PKB phosphorylation.

IRS-1 is a well-described substrate of the insulin receptor that, after tyrosine phosphorylation, associates with and activates PI 3-kinase (33). Our findings show a marked deficiency in IRS-1 protein content in untreated ZF rats, with a stepwise improvement back to normal levels after exercise, troglitazone, and combination therapy. Taken together, these results indicate that the treatment-induced increases in GLUT4 protein, coupled with the improvements in insulin signaling, provide a reasonable explanation for the effects of exercise, troglitazone, and combination therapy to improve insulin-stimulated GDR in these animals.

In conclusion, our findings show a marked state of insulin resistance in ZF rats, which is associated with various defects in the insulin-signaling–glucose transport pathway. Troglitazone treatment caused a marked improvement in overall insulin sensitivity, which was associated with beneficial changes in various components of the insulin-signaling pathway. Chronic exercise also produced insulin sensitization; however, the combination of these two therapies led to essential normalization of insulin-stimulated GDR in ZF rats despite marked obesity. Furthermore, insulin receptor phosphorylation, IRS-1 protein content, and Akt/PKB phosphorylation were also normalized by the combined therapy. Thus, despite persistence of the obese state in these animals, the combination of chronic exercise and troglitazone treatment led to an apparent normal state of insulin sensitivity. If similar results are obtained in humans, then concerted efforts to apply these methods of combination therapy would be warranted.

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