Peroxisome Proliferator–Activated Receptor-α Agonist Treatment in a Transgenic Model of Type 2 Diabetes Reverses the Lipotoxic State and Improves Glucose Homeostasis

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Abnormalities in insulin action are the characteristics of type 2 diabetes. Dominant-negative muscle-specific IGF-I receptor (MKR) mice exhibit elevated lipid levels at an early age and eventually develop type 2 diabetes. To evaluate the role of elevated lipids in the progression of the diabetic state, MKR mice were treated with WY14,643, a peroxisome proliferator–activated receptor (PPAR)-α agonist. WY14,643 treatment markedly reduced serum fatty acid and triglyceride levels within a few days, as well as muscle triglyceride levels, and subsequently normalized glucose and insulin levels in MKR mice. Hyperinsulinemic-euglycemic clamp analysis showed that WY14,643 treatment enhanced muscle and adipose tissue glucose uptake by improving whole-body insulin sensitivity. Insulin suppression of endogenous glucose production by the liver of MKR mice was also improved. The expression of genes involved in fatty acid oxidation was increased in liver and skeletal muscle, whereas gene expression levels of hepatic gluconeogenic enzymes were decreased in WY14,643-treated MKR mice. WY14,643 treatment also improved the pattern of glucose-stimulated insulin secretion from the perfused pancreata of MKR mice and reduced the β-cell mass. Taken together, these findings suggest that the reduction in circulating or intracellular lipids by activation of PPAR-α improved insulin sensitivity and the diabetic condition of MKR mice. *Diabetes* 52:1770–1778, 2003

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ACO, acyl-CoA oxidase; BrdU, 5-bromo-2′deoxyuridine; CPT-1, carnitine palmitoyl transferase 1; FA, fatty acid; IGF-IR, IGF-I receptor; PPAR, peroxisome proliferator–activated receptor.

Type 2 diabetes results from both a loss of insulin sensitivity and defects in β-cell function (1). Insulin insensitivity reflects an impaired insulin-stimulated glucose uptake in skeletal muscle and adipocytes and a reduced insulin-induced inhibition of hepatic glucose production. Early β-cell dysfunction is seen as a loss of first-phase insulin secretion in response to a glucose challenge. Because most obese individuals have insulin resistance but only diabetic patients fail to show β-cell compensation, type 2 diabetes in humans is distinguishable by the presence of β-cell dysfunction (2).

Intensive investigation has suggested that excess lipids (circulating or intracellular) in obesity and type 2 diabetes are important causative factors for insulin resistance in liver and skeletal muscle (3–14). Elevated triglycerides and fatty acids (FAs) in pancreatic β-cells also interfere with insulin production and glucose-stimulated insulin secretion (15–18). Recent data demonstrated that increased protein kinase C activity by accumulation of intracellular FA metabolites such as long-chain acyl-CoAs and diacylglycerol results in enhanced serine phosphorylation of insulin substrate receptor 1. This results in an interference with tyrosine phosphorylation, leading to inhibition of the signaling cascade of events that normally culminates in insulin-stimulated glucose uptake (19–21).

We have recently created a transgenic mouse model of severe insulin resistance by overexpressing a dominant-negative IGF-I receptor (IGF-IR) in skeletal muscle (MKR mice) (22). The hybrid formation of mutated IGF-IR and the endogenous IGF-IR and insulin receptors caused impairment of both insulin and IGF-I signaling pathways in skeletal muscle. This defect in skeletal muscle led to insulin resistance in fat and liver and rapidly progressed to β-cell dysfunction and type 2 diabetes. The above changes were associated with significant elevations in serum FA and triglyceride levels and increased triglyceride deposits in liver and muscle in MKR mice, suggesting that increased circulating and accumulated lipids in tissue may be causative factors for the progression from severe muscle insulin resistance to type 2 diabetes.

Peroxisome proliferator–activated receptor (PPAR)-α, a member of the nuclear hormone receptor superfamily,

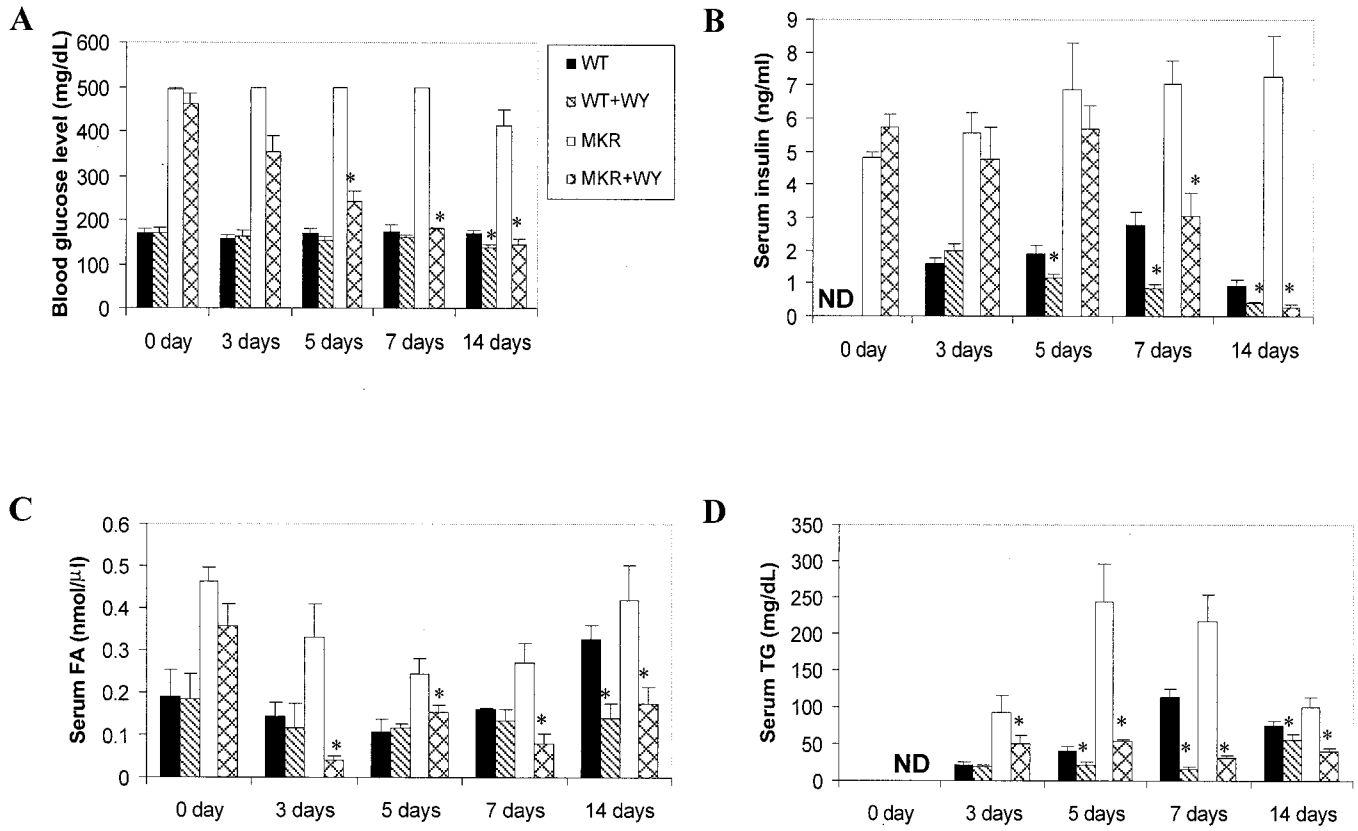

FIG. 1. Effect of WY14,643 treatment on blood glucose (*A*), serum insulin (*B*), FA (*C*), and triglyceride (*D*) levels in WT and MKR mice. Six-week-old MKR and WT mice were fed diets supplemented either without or with WY14,643 for 0–14 days. In the nonfasting state, blood or serum was collected before treatment and after 3, 5, 7, and 14 days of treatment, as indicated. Results are expressed as means ± SE (n = 5–6 in each group); *differences between treated and untreated were significant at $P < 0.05$. ND, not determined; TG, triglycerides.

regulates the expression of genes encoding various enzymes involved in lipid metabolism (23). A PPAR-α agonist, WY14,643, has been shown to induce FA β-oxidation in muscle and liver and to reduce triglyceride stores in these tissues (24–26). Treatment with a PPAR-α agonist improved insulin resistance by lowering lipid levels in both rodents and humans (13,25,27–29). Furthermore, recent studies have shown that expression of PPAR-α was decreased in islets of Zucker diabetic fatty rats (30) as well as by longer exposure to FAs (18). Acute activation of PPAR-α by WY14,643 treatment improved insulin secretion at low glucose concentrations in isolated rat pancreatic islets (31), suggesting that lipid metabolism is involved in β-cell function.

In the present study, we treated MKR mice with WY14,643 to determine whether alteration in lipid levels can mediate the progression from severe insulin resistance to full-blown type 2 diabetes. Our findings demonstrate that PPAR-α agonist treatment reduces circulating and stored lipids and improves the diabetic state in MKR mice, suggesting that glucolipotoxicity is associated with the progressive nature of this condition.

RESEARCH DESIGN AND METHODS

Animals. Mice were maintained on a 12-h light/dark cycle and were fed NIH-07 rodent food diets (Zeigler Brothers, Gardners, PA), with water available ad libitum. Generation and characterization of MKR mice have been described previously (22,32). Homozygous MKR male mice (FVB/N background) and age- and sex-matched wild-type (WT) mice (at 6–8 weeks of age) were used in this study. Genotyping of MKR mice was performed by Southern blot analysis as described previously (22). Mice were fed diets supplemented with or without WY14,643 (ChemSyn Laboratories, Lenexa, KS) as 0.05% of a powder-type diet (AIN-93 G [Dyets, Bethlehem, PA]). The WY14,643 was mixed with the powder-type diet using a coffee grinder. Blood was obtained from the tail vein and glucose levels were measured using a Glucometer (One Touch II; LifeScan) under nonfasting conditions. At the end point of the experiment, mice were killed after anesthetization using 2.5% Avertin in 15–17 μl/g body wt in the nonfasting state between 10:00 A.M. and noon. Tissues were collected and frozen in liquid nitrogen for RNA extraction and to assay tissue triglyceride levels. Livers were fixed in 4% paraformaldehyde and processed by American Histolabs (Gaithersburg, MD) for histological study. All experiments were performed in accordance with National Institutes of Health guidelines and with the approval of the Animal Care and Use Committee of the National Institute of Diabetes and Digestive and Kidney Diseases.

Serum assays. Serum was collected from the tail vein between 10:00 A.M. and noon under nonfasting conditions. Insulin levels were measured using a radioimmunoassay kit (Linco Research, St. Charles, MO). FA and triglyceride levels were determined using an FA kit (Roche, Indianapolis, IN) and GPO-Trinder kit (Sigma, St. Louis, MO), respectively.

Tissue triglyceride determination. Liver and muscle triglyceride levels were measured as previously described (33). Briefly, after tissue extraction with chloroform:methanol, measurement of triglyceride levels was performed by ethanolic KOH hydrolysis, and then a radiometric assay was used for glycerol.

Hyperinsulinemic-euglycemic clamp. Mice were treated with or without WY14,643 for 1 week, as described above. After an overnight fast, hyperinsulinemic-euglycemic clamp studies were conducted as described previously (34).

RNA analysis. Total RNA was isolated using the TRIzol reagent (Life Technologies, Rockville, MD), and Northern blot analysis was performed as described previously (35).

Perfused pancreas. Studies were performed on nonfasted mice anesthetized with 20 mg/kg xylazine and 100 mg/kg ketamine. The procedure for pancreas isolation was based on that of Grodsky et al. (36). The pancreas was isolated from the stomach, spleen, and duodenum in vivo via ligation. Auxiliary arteries and the aorta above the celiac axis were ligated. The aorta below the celiac axis and the hepatic portal vein were cannulated using PE50 tubing

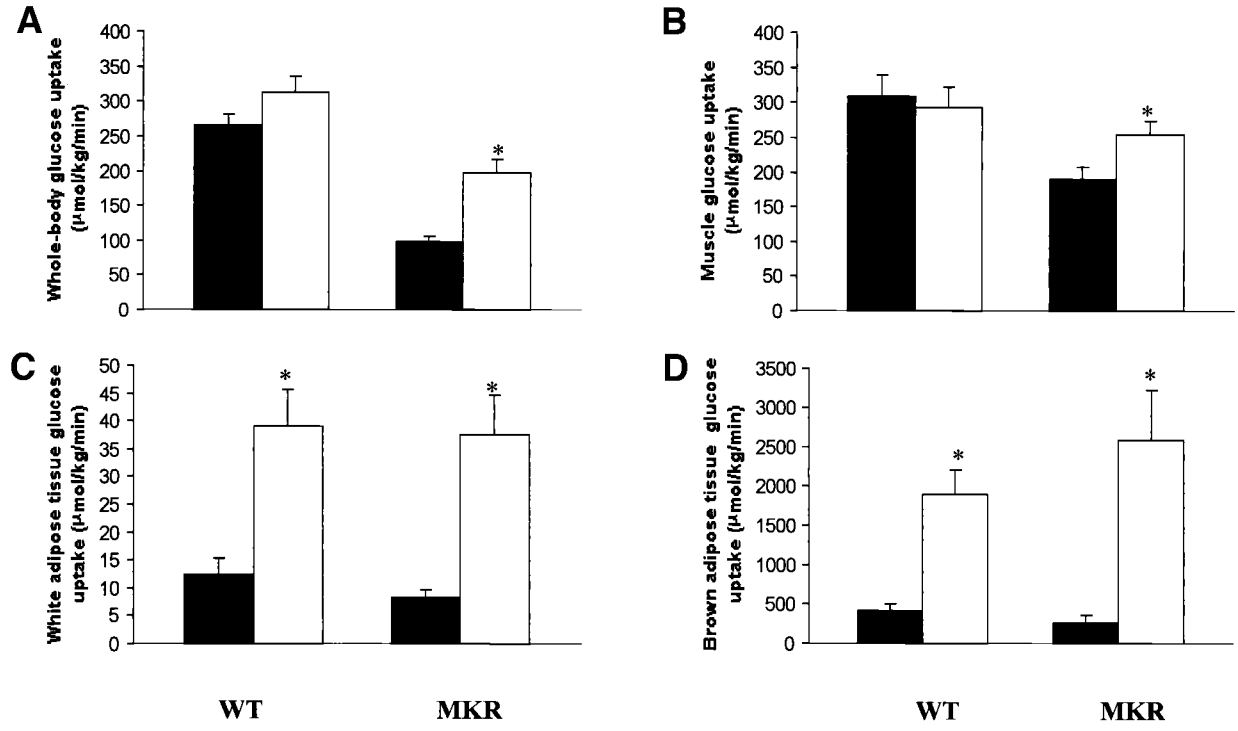

FIG. 2. Effect of WY14,643 on whole-body, skeletal muscle, and adipose tissue glucose uptake during hyperinsulinemic-euglycemic clamp analysis. Six-week-old male WT and MKR mice were fed diets supplemented either without (■) or with (□) WY14,643 for 2 weeks. After 1 week of treatment, various parameters were determined using hyperinsulinemic-euglycemic clamp analysis. *A*: Whole-body glucose uptake. *B*: Muscle glucose uptake. *C*: White adipose tissue glucose uptake. *D*: Brown adipose tissue glucose uptake. *Significance at $P < 0.05$. Data are expressed as means ± SE ($n = 6$ in each group).

(Intramedic, Parsippany, NJ). The pancreas was perfused with a Krebs-Ringer buffer solution containing 2% BSA, glucose, and 3% dextran via the arterial cannula. The perfusate was maintained at 37°C, and gassed with a mixture of 95% O_2/5% CO_2 to achieve a pH of 7.4. After 20 min of preperfusion with Krebs-Ringer buffer solution containing 1.4 mmol/l glucose, the pancreas was perfused with 1.4 mmol/l glucose solution for 4 min, 16.7 mmol/l glucose for 20 min, 1.4 mmol/l glucose for 15 min, and finally 20 mmol/l L-arginine plus 16.7 mmol/l glucose for 10 min. Fractions were collected via the portal vein and assayed for insulin as previously described by Joseph et al. (37).

Pancreas harvesting and tissue processing. Eight-week-old MKR mice were injected intraperitoneally with 5-bromo-2′deoxyuridine (BrdU) (Sigma) 5 h before pancreas removal. Their pancreata were then excised, weighed, and fixed in 4% paraformaldehyde, and sections were processed by American Histolabs (Gaithersburg, MD) for immunostaining study.

Immunostaining. Sections (~4 μm) from MKR and WT mice were costained for insulin and BrdU. A rabbit anti–guinea pig primary antibody (Dako, Mississauga, ON) and a biotinylated goat anti-rabbit secondary antibody (Vector Laboratories, Burlington, ON) were used for insulin detection. The sections were then treated with Ultra Streptavidin Horseradish Peroxidase from the USA Level 2 Detection System (Signet Laboratories, Dedham, MA) and stained with 3,3′-diaminobenzidine (Dako) for visualization. For BrdU detection, a mouse anti-BrdU primary antibody (Clone IU4; Caltag Laboratories, Burlingame, CA) and a biotinylated horse anti-mouse secondary antibody (Vector Laboratories) were used, followed by treatment with the same USA Level 2 Detection System used for insulin and nickel 3,3′-diaminobenzidine (Vector Laboratories) staining for visualization. Sections were counterstained with hematoxylin. Images of each section were acquired using an Olympus B×60 microscope connected to a Photometrics CoolSNAP color camera (Roper Scientific, Trenton, NJ). Each slide was covered systematically, and 25–65 fields at a final magnification of 100× were analyzed for each pancreas.

Quantification of β-cell mass and proliferation. Pancreatic β-cell mass and proliferation were determined as previously described (37) with minor modifications. Briefly, insulin-stained pancreatic area (i.e., β-cell area) of each section was measured using Image-Pro Plus software (Media Cybernetics, Silver Spring, MD). β-Cell area was then multiplied by the pancreatic weight to obtain the β-cell mass. Proliferation was quantified as the percentage of β-cells positive for BrdU staining.

Statistical analysis. The data are expressed as means ± SE. Statistically significant differences within genotypes were determined using a one-factor ANOVA followed by a *t* test. Integrated insulin release was determined by calculating area under curve using Microcal Origin 6.0.

RESULTS

WY14,643 treatment improves hyperglycemia and reduces serum triglycerides and free FA levels in MKR mice. Six-week-old MKR mice were treated with WY14,643 as a dietary supplement for 2 weeks. There was no difference in food intake with or without WY14,643 treatment between genotypes (data not shown). Within 1 week, WY14,643 treatment dramatically decreased blood glucose levels in MKR mice from 461 ± 23.5 to 181 ± 3.4 mg/dl (levels similar to that seen in WT mice). The normal blood glucose levels were maintained in MKR mice over the following week (Fig. 1*A*). WY14,643 treatment for 2 weeks also induced a modest but significant decrease in blood glucose levels in WT mice from 170 ± 7.8 to 138 ± 6.4 mg/dl (Fig. 1*A*). Following WY14,643 treatment, serum insulin levels fell from the (initial) hyperinsulinemic range of 5.7 ± 0.4 to 3.0 ± 0.7 ng/ml after 1 week of treatment (Fig. 1*B*) and to 0.3 ± 0.05 ng/ml at 2 weeks treatment (Fig. 1*B*). In MKR mice, serum FAs fell by ~50% from 0.4 ± 0.08 to 0.2 ± 0.04 nmol/μl after 2 weeks of WY14,643 treatment of the MKR mice reduced serum triglyceride and FA levels after the third day, prior to the reduction in blood glucose and insulin levels, which did not occur until day 5 or 7, respectively (Fig. 1). This temporal difference suggests that the fall in FAs and triglycerides may play a role in the subsequent decrease of blood glucose and serum insulin to normal levels.

Hyperinsulinemic-euglycemic clamp demonstrates that WY14,643 treatment improves insulin sensitiv-

TABLE 1
Effects of WY14,643 treatment on various metabolic parameters measured during basal and hyperinsulinemic-euglycemic clamp

	Basal period					Clamp period			
	Mice (n)	Body weight (g)	Blood glucose (mg/dl)	Insulin (ng/ml)	Endogenous glucose production (μmol · kg^{-1} BW · min^{-1})	Blood glucose (mg/dl)	Glucose infusion (μmol · kg^{-1} BW · min^{-1})	Insulin (ng/ml)	Endogenous glucose production (μmol · kg^{-1} BW · min^{-1})
WT	6	24.8 ± 0.7	142 ± 9	0.3 ± 0.0	116 ± 12	116 ± 4	227 ± 14	1.8 ± 0.3	39.2 ± 10.3
WT + WY	6	20.7 ± 0.5#	130 ± 9	0.5 ± 0.2	142 ± 7	115 ± 5	321 ± 24**	1.7 ± 0.2	−9.2 ± 5.3**
MKR	6	17.6 ± 0.5	243 ± 24	0.7 ± 0.1	164 ± 18	184 ± 18	0.5 ± 1	1.8 ± 0.1	97.5 ± 7.4
MKR + WY	6	16.3 ± 0.5*	159 ± 15**	0.3 ± 0.1**	177 ± 30	111 ± 2**	183 ± 10#	1.5 ± 0.1*	14.2 ± 14.5#

Data are means ± SE. Six-week-old WT and MKR mice were treated either with or without WY14,643 for 1 week. Mice were then fasted for 12–14 h and subjected to a hyperinsulinemic-euglycemic clamp, as described in RESEARCH DESIGN AND METHODS. *$P < 0.05$; **$P < 0.01$; #$P < 0.001$ within genotypes. BW, body weight.

ity. To determine whether the improvement in blood glucose levels in response to WY14,643 treatment in MKR mice was associated with improved insulin sensitivity, mice were subjected to hyperinsulinemic-euglycemic clamp analysis after 1 week of WY14,643 treatment. The glucose infusion rate required to maintain euglycemia was significantly higher after WY14,643 treatment in both MKR and WT mice (Table 1). In MKR mice treated with WY14,643, the glucose infusion rate was dramatically increased to 183 ± 10 μmol · kg body wt^{-1} · min^{-1} compared with 0.5 ± 1 μmol · kg body wt^{-1} · min^{-1} in the untreated group. Basal endogenous glucose production was significantly higher in MKR mice than in WT mice (Table 1) and was slightly increased in response to WY14,643 treatment in both WT and MKR mice. This may be due to an increased liver size in the WY14,643-treated mice (Table 2). During the clamp, endogenous glucose production levels in WY14,643-treated MKR mice were significantly reduced from 97.5 ± 7.4 to 14.2 ± 14.5 μmol · kg body wt^{-1} · min^{-1} (Table 1), suggesting that liver insulin sensitivity is enhanced in response to WY14,643 treatment.

Whole-body glucose uptake was significantly increased (~100%) after WY14,643 treatment in MKR mice (Fig. 2*A*). Muscle glucose uptake was also significantly increased (by 20%) in MKR mice in response to WY14,643 treatment (Fig. 2*B*). In addition, glucose uptake in brown and white adipose tissues was dramatically increased in MKR mice after WY14,643 treatment (Fig. 2*C* and *D*). In WT mice, glucose uptake in muscle did not change in response to WY14,643 treatment, whereas glucose uptake in white and brown adipose tissue was increased to a similar degree as in WY14,643-treated MKR mice (Fig. 2*B–D*). These findings suggest that WY14,643 treatment improves whole-body glucose uptake in the MKR mice mediated, at least in part, by increasing glucose uptake in muscle and fat.

Liver and muscle triglycerides content are reduced by WY14,643 treatment. Previous studies have shown that fibrates reduce body weight and improve insulin sensitivity in rodents (13,25,28). In MKR and WT mice, body weight gain was decreased by 1.6 and 2.9 g, respectively, over the 2-week WY14,643 treatment period (Table 2). Liver weight increased after WY14,643 treatment in both MKR and WT mice (Table 2). In contrast to liver, the weight of adipose and muscle tissues in both MKR and WT mice was decreased in response to WY14,643 treatment by 75 and 20%, respectively (Table 2). Therefore, the decrease in overall body weight in response to WY14,643 treatment may be primarily due to a reduction in fat content of the various peripheral tissues.

Liver and muscle triglyceride content were measured to determine whether reduced tissue triglyceride content is associated with improvement of insulin sensitivity in liver and muscle (13,25,28). Liver triglyceride levels in WY14,643-treated MKR and WT mice were significantly reduced by ~30% (from 22.1 ± 2.5 to 15.4 ± 1.2 μmol/g and from 14 ± 2.3 to 8.7 ± 0.9 μmol/g, respectively) (Fig. 3*A*). Muscle triglyceride levels were significantly lowered by ~20% in WY14,643-treated MKR mice (from 15 ± 0.3 to 12.3 ± 0.7 μmol/g) but were not changed in WT mice (Fig. 3*B*). Thus, improved insulin sensitivity in muscle and liver in response to WY14,643 treatment may be due to the reduced triglyceride levels observed in these tissues.

WY14,643 treatment regulates gene expression of enzymes involved in fatty oxidation and gluconeogenesis in both liver and muscle. The effect of the PPAR-α agonist on increased FA oxidation (24–26) is a

TABLE 2
Effect of WY14,643 on whole-body, liver, adipose tissue, and muscle weight in WT and MKR mice

	WT	WT + WY	MKR	MKR + WY
Body weight gain (g)	3.14 ± 0.64	0.15 ± 0.67*	2.05 ± 0.30	0.41 ± 0.38*
Liver weight (g)	1.32 ± 0.04	2.75 ± 0.19*	1.72 ± 0.12	2.52 ± 0.11*
Epididymal adipose tissue weight (g)	0.87 ± 0.09	0.17 ± 0.02*	0.40 ± 0.03	0.10 ± 0.01*
Inguinal adipose tissue weight (g)	0.67 ± 0.08	0.18 ± 0.01*	0.18 ± 0.01	0.11 ± 0.01*
Quadriceps muscle weight (g)	0.34 ± 0.02	0.29 ± 0.01*	0.19 ± 0.01	0.14 ± 0.01*
Combined gastrocnemius & soleus muscle weight (g)	0.34 ± 0.01	0.26 ± 0.01*	0.21 ± 0.01	0.16 ± 0.01*

Data are means ± SE (n = 6–7 in each group). After 2 weeks of WY14,643 treatment, mice were killed and different tissue weight was measured. *Significance at $P < 0.05$, within genotypes.

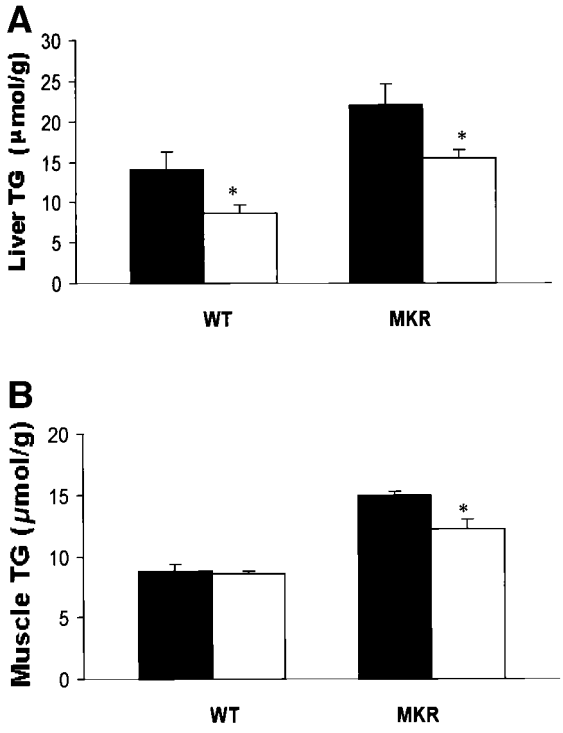

FIG. 3. Treatment with WY14,643 decreases triglyceride (TG) content in liver (*A*) and quadriceps (*B*) muscle of MKR mice. Six-week-old male WT and MKR mice received diets supplemented either with or without WY14,643 for 2 weeks. Data from untreated (■) and treated (□) mice are shown. Data are expressed as average ± SE (n = 4–6 in each group). *Significance at $P < 0.05$.

possible mechanism involved in the reduction of liver and muscle triglyceride content, and also the serum FA and triglyceride levels, in response to WY14,643 treatment. Previous studies have shown that increased FA oxidation by the PPAR-α agonist was mediated by induction of the expression of peroxisomal and mitochondrial β-oxidation genes including acyl-CoA oxidase (ACO) and carnitine palmitoyl transferase 1 (CPT-1) (38). Consistent with previous studies, WY14,643 treatment significantly increased the steady-state levels of ACO and CPT-1 mRNA levels in the liver and muscle of both WT and MKR mice (data not shown). The level of CD36, an FA uptake protein, was also significantly increased in liver of both groups after WY14,643 treatment, whereas it was not changed in muscle of either WT or MKR mice (Fig. 4). The mRNA level of uncoupling protein 3, a mitochondrial membrane transporter possibly involved in mitochondrial FA transport in skeletal muscle (39–42), was increased after WY14,643 treatment in MKR mice, but unchanged in WT mice (Fig. 4). These results indicate that WY14,643 treatment is likely to increase the flux of free FAs from peripheral tissues to liver and to enhance hepatic and muscle lipid catabolism. Levels of mRNA encoding PEPCK and glucose-6-phosphatase, enzymes that regulate gluconeogenesis, were significantly reduced in MKR mice in response to WY14,643 treatment but were unchanged in WT mice (Fig. 4), suggesting that there is a different response between diabetic and normal mice after WY14,643 treatment.

WY14,643 treatment improves the pattern of glucose-stimulated insulin secretion in MKR mice. Perfusion studies were performed on the pancreas isolated in situ to determine whether the WY14,643-induced reduction in lipid and glucose levels was able to improve the impaired β-cell function of MKR mice (22). Basal insulin secretion at 1.4 mmol/l glucose was significantly higher in MKR mice than in WT mice (Fig. 5*A* and *B*). Glucose-stimulated insulin secretion at 16.7 mmol/l glucose elicited a biphasic secretory pattern in WT mice, whereas MKR mice displayed a monophasic and exaggerated response (Fig. 5*A*). After WY14,643 treatment for 3 weeks, MKR mice exhibited significantly reduced basal insulin secretion (Figs. 5*A* and *B*). A trend toward the restoration of the first phase of insulin secretion was seen (Fig. 5*A*); however, insulin secretion at 16.7 mmol/l glucose in WY14,643-treated MKR mice was not significantly different from untreated MKR mice (Fig. 5*C*). Relative secretory capacity of the β-cell, evaluated by the insulin response to 20 mmol/l arginine in the presence of 16.7 mmol/l glucose, demonstrated that MKR mice had a significantly greater insulin response than WT mice (Fig. 5*D*). WY14,643 treatment for 3 weeks resulted in an arginine response in MKR mice similar to that in WT mice. Collectively, these results demonstrate the restoration of normal basal insulin secretion, with some improvement in glucose-stimulated insulin secretion in the MKR mice after WY14,643 treatment.

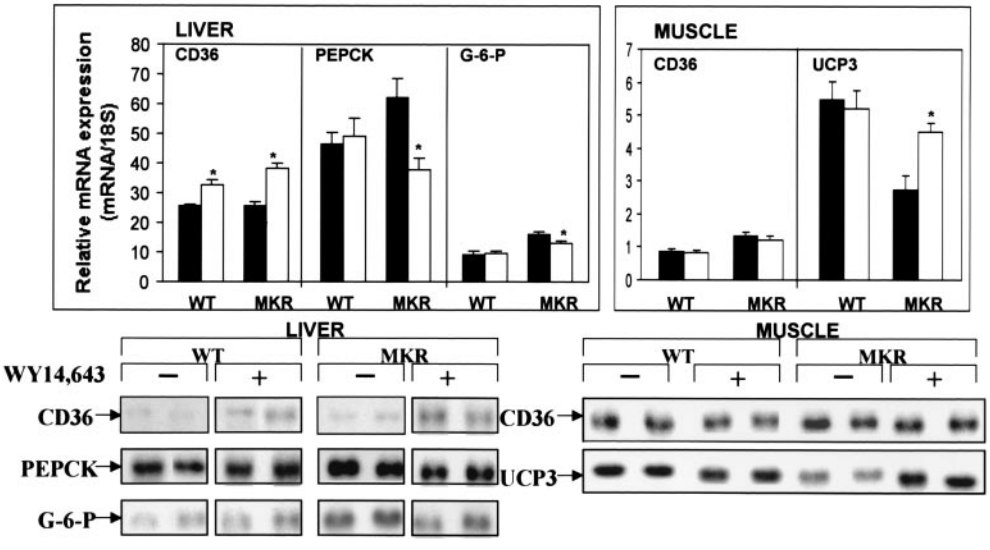

FIG. 4. Effect of WY14,643 on liver and muscle mRNA expression of genes related to gluconeogenesis and lipid metabolism. Livers and muscles were collected from mice fed diets either without (■) or with (□) WY14,643 for 2 weeks. Lower panels show representative Northern blots and upper panels show average mRNA expression levels, normalized to 18S RNA. CD36, FA uptake protein; G-6-P, glucose-6-phosphatase; UCP3, uncoupling protein 3. *Significance at $P < 0.05$. Data are expressed as average ± SE (n = 6–7 in each group).

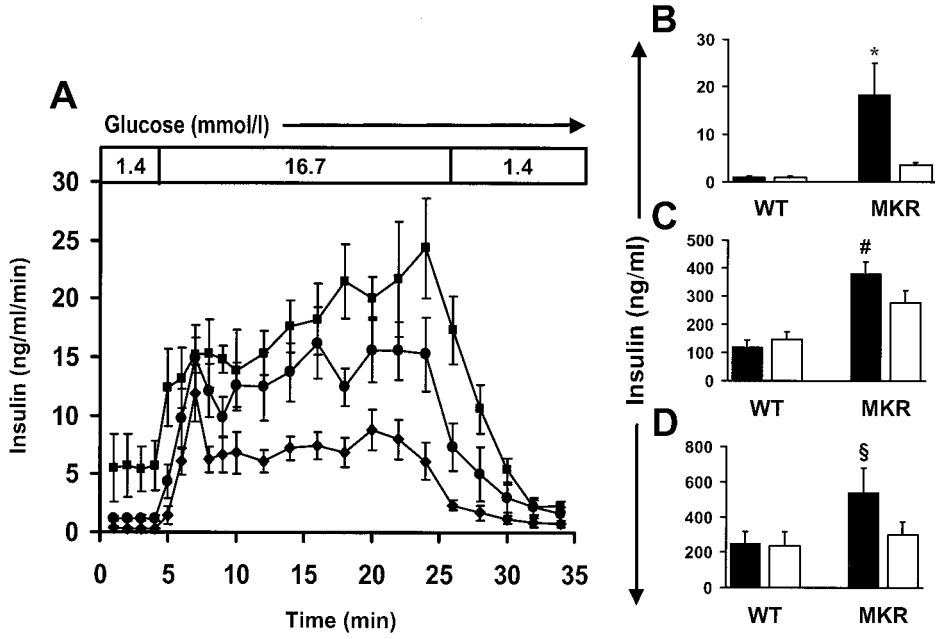

FIG. 5. Effects of WY14,643 treatment on insulin secretion from the perfused pancreas. Data from WY14,643-treated (□) and -untreated (■) mice are shown. *A*: The pattern of insulin secretion during pancreas perfusion in MKR (■), WY14,643-treated MKR (●), and WT (◆) mice. *B*: Integrated insulin release under 1.4 mmol/l (basal) glucose. *$P < 0.01$ vs. all other groups. *C*: Integrated insulin release under 16.7 mmol/l (high) glucose. #$P < 0.001$ vs. WT and WY14,643-treated WT. *D*: Integrated insulin release under a combination of 20 mmol/l arginine and 16.7 mmol/l glucose. §$P < 0.05$ vs. MKR. All data are expressed as the mean ± SE (n = 5–11 in each group).

To examine the possible mechanisms underlying the exaggerated glucose- and glucose/arginine-stimulated insulin response in MKR mice, we examined β-cell mass from the pancreata of these animals. β-Cell mass was significantly increased in MKR mice compared with age-matched WT controls (Fig. 6*A*; MKR vs. WT: 8.20 ± 0.56 vs. 2.10 ± 0.14 mg, $P < 0.001$, $n = 4$); however, this increase was attenuated in MKR mice after 13 days of WY14,643 treatment, such that the β-cell mass was not significantly different from WT controls (Fig. 6*A*). An increase in β-cell mass could be mediated by increased β-cell proliferation (replication), neogenesis (differentiation of β-cells from precursor cells), β-cell hypertrophy, or a combination of these mechanisms. Assessment of proliferation through BrdU incorporation demonstrated a significant increase in $BrdU^+$ β-cells in MKR mice (Fig. 6*B*; MKR vs. WT: 3.20 ± 0.56 vs. 1.15 ± 0.34% of β-cells, $P < 0.05$, $n = 4$). This result suggested that an increased β-cell mass in these mice was at least in part mediated by an increased number of proliferating β-cells. However, in WY14,643-treated MKR mice, no increase in the incidence of proliferation was seen; in fact, proliferation in these mice was less than that for WT controls (Fig. 6*B*; MKR+WY14,643 vs. WT: 0.15 ± 0.07 vs. 1.15 ± 0.34% of β-cells, $P < 0.001$, $n = 4$–5), implicating a mechanism through which WY14,643 treatment attenuates the increase in β-cell mass in MKR mice.

DISCUSSION

The present study demonstrates that within a few days of the onset of treatment with the highly selective PPAR-α agonist WY14,643 (43), MKR mice showed a marked reduction in serum FAs and triglycerides. This result was followed by a decrease in blood glucose and serum insulin levels to normal (WT) ranges. Using the hyperinsulinemic-euglycemic clamp, we demonstrated that normalization of glucose homeostasis was associated with an improvement in whole-body insulin sensitivity, increased insulin-stimulated glucose uptake in muscle and fat, and improved insulin responsiveness in liver. Because muscle glucose uptake was increased to a much smaller degree when compared with whole-body glucose uptake, we propose that significant contributions may have come from improved insulin-induced glucose uptake in fat, liver, and heart (44).

Closer examination of the effects of WY14,643 on FA metabolism showed that there was an increase in the expression of mRNA for two enzymes involved in FA oxidation in the liver (CPT-1 and ACO). Along with this presumed increase in FA metabolism, there were concomitant marked reductions in triglyceride levels and in the expression of the gluconeogenic enzymes PEPCK and glucose-6-phosphatase in liver. Furthermore, enhanced insulin sensitivity was observed, as indicated by the suppression of hepatic glucose production under hyperinsulinemic-euglycemic clamp conditions. Therefore, these results suggest that the accumulation of triglycerides in liver causes this tissue to become more insulin resistant. Interestingly, reduction of muscle triglyceride levels was associated with a significant increase in insulin-stimulated glucose uptake in muscle of MKR mice after treatment. Previously, it has been shown that in vitro insulin-stimulated glucose uptake into muscle is reduced by 80–90% in MKR mice compared with WT mice (22). Therefore, the partial recovery of this function, as demonstrated in the present study, may reflect a functional defect in otherwise normal insulin receptors. This result raises the possibility that the original findings of insulin resistance in muscle may have been only partly due to the formation of hybrids between dominant-negative IGF-IRs and endogenous insulin receptors. In this regard, increased accumulation of triglycerides in muscle may inhibit insulin signaling in MKR mice. Even though the precise mechanisms by which decreased lipid content in liver or muscle reduces insulin resistance were not determined in this study, treating MKR mice with WY14,643, which reduces muscle triglyceride content, can partially reverse the defect in insulin signaling.

Evidence from other studies suggests an important role

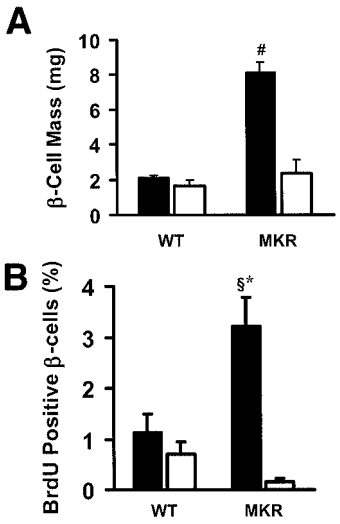

FIG. 6. Effects of WY14,643 treatment on β-cell mass and proliferation. Data from WY14,643-treated (□) and -untreated (■) mice are shown. *A*: β-Cell mass. #$P < 0.001$ vs. all other groups. *B*: β-Cell proliferation as assessed by BrdU incorporation over a 5-h period. *$P < 0.05$ vs. WT; §$P < 0.001$ vs. WY14,643-treated MKR. All data are expressed as the mean ± SE (n = 4-5 in each group).

for tissue lipid levels in insulin action (8,12). Despite a similar effect of WY14,643 treatment on lowering serum lipid levels in both genotypes, insulin-stimulated glucose uptake in skeletal muscle was not increased in WT mice after treatment, whereas it was associated with a 20% increase in WY14,643-treated MKR mice. This modest effect may reflect the modest reduction in muscle triglyceride content in MKR mice after WY14,643 treatment. WY14,643 treatment in WT mice did not change muscle triglyceride levels. Furthermore, we observed a reduction in liver triglyceride levels with improved hepatic responsiveness to insulin in both WT and MKR mice after WY14,643 treatment. These findings could indicate that tissue lipid levels are more closely associated with improvement in insulin sensitivity than circulating lipid levels.

Diet-induced diabetes is associated with insulin resistance and impaired β-cell function (45). Using models of diet-induced diabetes, we have shown that the most profound islet feature is exaggerated basal insulin secretion, followed by reduced glucose-stimulated insulin secretion (37). Glucolipotoxicity has been proposed to account for β-cell dysfunction leading to overt diabetes (45,46). Supporting this concept, the present study demonstrated that reductions in serum FAs and triglyceride levels with a PPAR-α agonist is associated with improved β-cell function (primarily basal insulin secretion). Normalized insulin secretion in MKR mice may result from several factors, including improved glucose sensing, the reduction of intracellular fat accumulation, and reduced β-cell mass and/or insulin content. β-Cell mass is a major determinant of the amount of insulin that can be secreted, and it is dynamic, increasing, or decreasing to meet changing insulin demands to maintain euglycemia (47). In our previous study (22), we showed that the islets of MKR mice are significantly enlarged, suggesting that β-cell mass is increased and that insulin secretory capacity is therefore heightened. These suppositions are borne out by our present study, which demonstrates exaggerated basal and glucose-stimulated insulin secretion (Fig. 5) and a significantly increased β-cell mass in MKR mice (Fig. 6). The increase in β-cell mass is likely a major cause of the exaggerated increased secretion. The increase appears to be mediated by the increased incidence of β-cell proliferation in MKR mice, demonstrated in this study. However, proliferation may not be the sole contributor given that β-cell mass is regulated by other processes, including apoptosis, neogenesis, and hypertrophy (48). Since increased β-cell mass is seen with insulin resistance, we propose that the increased β-cell mass of MKR mice is a compensatory response to the insulin resistance in these mice, mediated by increased β-cell proliferation. Furthermore, WY14,643-induced PPAR-α activation attenuates the increased proliferation, leading to a normalized β-cell mass. This change in β-cell mass is likely responsible, at least in part, for the trend toward normalization of insulin output under both basal and stimulatory conditions seen in these mice after WY14,643 treatment (Fig. 5). In support of improved glucose sensing, pancreatic β-cells express the PPAR-α, -β, and -γ isoforms, and activation of PPAR-γ in the Zucker diabetic fatty (*fa/fa*) rat model restored glucose-stimulated insulin secretion by reduction of intracellular fat accumulation, as well as by increased expression of the glucose transporter GLUT2 in β-cells (49). In this study, we did not directly measure the lipid levels in pancreatic β-cells, but after extrapolation from the changes in lipid levels in serum and tissues (muscle and liver), we propose that decreased intracellular lipid accumulation in β-cells may also play a role in recovery of β-cell function in MKR mice. Our observation that reduction in lipid levels preceded the improvement of the pattern of glucose-stimulated insulin secretion in β-cells may reflect the causative effect of lipids on the pathogenesis of type 2 diabetes in MKR mice.

The Randle cycle hypothesis (50) was generally accepted to explain muscle insulin resistance induced by increased FA oxidation. This hypothesis suggests that increased levels of FAs in muscle produce more acetyl-CoA and citrate, which inhibit the activity of pyruvate dehydrogenase and phosphofructokinase, the main enzymes for glucose oxidation. This process results in a decrease in glucose oxidation and eventually impairment of glucose uptake. However, recent studies suggest that lipid storage is more closely related to insulin resistance in skeletal muscle than to increased FA oxidation (12). Our findings further support these recent data by showing that WY14,643 treatment-induced FA oxidation followed by reduction of lipid accumulation reduces insulin resistance and enhances insulin sensitivity in muscle as well as in other tissues and is associated with a recovery in pancreatic β-cell responsiveness to glucose.

In summary, induction of FA catabolism by WY14,643 treatment, which activates PPAR-α, leads to a reduction in circulating lipid levels and accumulation of triglycerides in tissues (liver and skeletal muscle), induced as a result of a defect in IGF-I signaling and insulin signaling in muscle. This hypolipidemic effect of WY14,643 treatment appears to improve insulin sensitivity and β-cell function. Thus, we suggest that lipotoxicity plays a pivotal role in the development of type 2 diabetes in the MKR mouse model.

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