Phloridzin Improves Hyperglycemia But Not Hepatic Insulin Resistance in a Transgenic Mouse Model of Type 2 Diabetes

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The chronic hyperglycemia that occurs in type 2 diabetes may cause deterioration of β-cell function and insulin resistance in peripheral tissues. Mice that express a dominant-negative IGF-1 receptor, specifically in skeletal muscle (MKR mice), exhibit severe insulin resistance, hyperinsulinemia, dyslipidemia, and hyperglycemia. To determine the role of hyperglycemia in the worsening of the diabetes state in these animals, MKR mice were treated with phloridzin (PHZ), which inhibits intestinal glucose uptake and renal glucose reabsorption. Blood glucose levels were decreased and urine glucose levels were increased in response to PHZ treatment in MKR mice. PHZ treatment also increased food intake in MKR mice; however, the fat mass was decreased and lean body mass did not change. Serum insulin, fatty acid, and triglyceride levels were not affected by PHZ treatment in MKR mice. Hyperinsulinemic-euglycemic clamp analysis demonstrated that glucose uptake in white adipose tissue was significantly increased in response to PHZ treatment. Despite the reduction in blood glucose following PHZ treatment, there was no improvement in insulin-stimulated whole-body glucose uptake in MKR mice and neither was there suppression of endogenous glucose production by insulin. These results suggest that glucotoxicity plays little or no role in the worsening of insulin resistance that occurs in the MKR mouse model of type 2 diabetes. Diabetes 53:2901–2909, 2004

Type 2 diabetes is a polygenic disorder characterized by defects in insulin secretion and insulin sensitivity, which lead to dysregulation of glucose and lipid metabolism (1). Progression of the disease can be exacerbated by consumption of high-calorie foods, lack of exercise, age, medication, obesity, and body fat distribution (2,3). Hyperglycemia (glucotoxicity) and dyslipidemia (lipotoxicity) impair β-cell function and increase insulin resistance in peripheral tissues, such as muscle, liver, and adipose tissue (4,5).

In a previous study (6,7), we developed a transgenic mouse that expresses a dominant-negative IGF-1 receptor (IGF-1R) specifically in skeletal muscle (the MKR mouse model). The formation of hybrid receptors between the mutant and endogenous IGF-1 and insulin hemireceptors markedly inhibited both IGF-1R and insulin receptor activity. This led to severe insulin resistance in muscle, which then caused secondary insulin resistance to develop in liver and adipose tissue. MKR mice exhibit β-cell dysfunction, hyperinsulinemia, dyslipidemia, and hyperglycemia. Thus, the MKR mouse is an excellent model for studying the molecular mechanisms underlying the development of type 2 diabetes (6).

To determine whether lipotoxicity played a role in the characteristics of type 2 diabetes exhibited by MKR mice, the mice were treated with WY14,643, a peroxisome-proliferator–activated receptor-α agonist that induces fatty acid β-oxidation in muscle and liver and decreases triglyceride stores in these tissues (8). Treatment with WY14,643 markedly decreased serum fatty acid and triglyceride levels, decreased triglyceride stores in muscle and liver, and subsequently normalized glucose and insulin levels. Hyperinsulinemic-euglycemic clamp analysis showed that WY14,643 treatment improved insulin sensitivity in liver and adipose tissue and insulin secretion by pancreatic β-cells. These findings suggested that improving dyslipidemia abrogates lipotoxicity and improves insulin sensitivity in MKR mice (9).

Phloridzin (PHZ) is an antidiabetic agent that is found primarily in apple peels (10). PHZ inhibits intestinal glucose uptake via the sodium D-glucose cotransporter and similarly inhibits renal glucose reabsorption (11,12). Correction of hyperglycemia with PHZ has been shown to normalize the effects of insulin on glucose metabolism in the liver and other peripheral tissues such as muscle and adipose tissue in diabetic rat models (13). β-Cell abnormalities were also completely corrected when diabetic rats were treated with PHZ to normalize plasma glucose levels (14). Muscle-specific GLUT4 knockout mice showed severe insulin resistance and glucose intolerance from 8 weeks of age (15). When these mice were treated with PHZ, insulin-stimulated glucose uptake in adipose tissue and insulin-induced suppression of hepatic glucose production were normalized, whereas insulin-stimulated whole-body and skeletal muscle glucose uptake remained decreased and urine glucose levels were increased in response to PHZ treatment.
PHLORIDZIN FAILS TO IMPROVE INSULIN RESISTANCE

FIG. 1. Effect of PHZ treatment on blood glucose (A), urine glucose (B), serum triglycerides (C), serum free fatty acids (D), and serum insulin levels (E) in 8-week-old male WT and MKR mice. Mice were treated with either PHZ (■) or vehicle (□) for 2 weeks, as described in RESEARCH DESIGN and METHODS. Blood and urine samples were collected in the nonfasting state 3 h after PHZ injection. Data are expressed as means ± SE, with n = 8–12 mice in each group. *P < 0.05 for PHZ vs. vehicle group; #P < 0.05 for WT vs. MKR from the same treatment group.

decreased (16). It was proposed by the authors that a primary defect in muscle glucose uptake could lead to secondary defects in insulin action in adipose tissue and liver due to glucotoxicity, which contributed to the whole-body insulin resistance and to the development of diabetes in these muscle GLUT4 knockout mice.

The present study was designed to determine the role of glucotoxicity in the development of secondary insulin resistance and subsequent type 2 diabetes in MKR mice. The mice were treated with PHZ for 2 weeks, which decreased serum blood glucose levels and increased glucose uptake in white adipose tissue. However, PHZ treatment had no effect on the level of insulin resistance in the whole body or in liver. These data suggest that glucotoxicity only partially contributes to secondary insulin resistance. Lipotoxicity appears to play a greater role in the exacerbation of the insulin resistance in the MKR model of type 2 diabetes.

RESEARCH DESIGN AND METHODS

Mice were maintained on a 12:12 h light/dark cycle and were fed the NIH-07 diet (Zeigler Brothers, Gardners, PA) ad libitum. Male wild-type (WT) and MKR mice (FVB/N background) were studied at 6–8 weeks of age. PHZ (Sigma, St. Louis, MO) was dissolved in a solution containing 10% ethanol, 15% DMSO, and 75% saline and was injected subcutaneously at a dose of 0.4 g/kg twice daily for 2 weeks. Control mice (male WT and MKR littermates) were injected with the same volume of vehicle solution. Body weight was monitored daily. All procedures and studies were conducted in accordance with National Institutes of Health guidelines as approved by the Animal Care and Use Committee of the National Institute of Diabetes and Digestive and Kidney Diseases.

Food intake. Mice were caged individually and treated with either PHZ or vehicle, as described above. The amount of food in the feeding container was measured at days 0 and 10 of treatment. Food intake was normalized to the body weight of each mouse and was expressed as g · g body wt⁻¹ · day⁻¹ (17,18).

Blood and urine assays. Blood samples were obtained from the tail vein, and serum glucose levels were determined using a glucometer (OneTouch; Lifescan, Milpitas, CA). The same glucometer was used to measure glucose levels in various dilutions of urine, from 1× to 0.05×. For urine collection, the mice were put in clean cages for several seconds, and the urine was collected immediately. Pressure was applied to the bladder only when necessary. Blood samples were collected under nonfasting conditions 3 h after PHZ injection at 8:00 A.M. to determine insulin, fatty acid, triglyceride, leptin, adiponectin, and resistin levels. Serum insulin, leptin, adiponectin, and resistin levels were measured using a commercial radioimmunoassay kit (Linco Research, St. Charles, MO). Fatty acid and triglyceride levels were analyzed using a commercial fatty acid analysis kit (Roche, Indianapolis, IN) and the GPO-Trinder kit (Sigma), respectively.

Insulin tolerance test. Mice were treated with either PHZ or vehicle for 2 weeks, as described above. Insulin tolerance tests were then performed on animals that had been fasted for 6 h starting at 8:00 A.M. The evening before the test, mice received the final injection of PHZ or vehicle at 6:00 p.m. Recombinant human insulin (Sigma) was injected intraperitoneally (0.75 IU/kg). Blood glucose levels were measured 0, 15, 30, 60, and 120 min after the injection, as described above.

Glucone tolerance test. After the last PHZ or vehicle treatment at 6:00 p.m., animals were fasted overnight. Glucose was injected intraperitoneally (2 g/kg) at 8:00 A.M. the next morning. Blood glucose levels were measured 0, 15, 30, 60, and 120 min after the injection, as described above.

Determination of the body composition in live mice. Mice were treated with either PHZ or vehicle for 2 weeks, as described above. Body composition was measured in nonanesthetized mice using the Bruker minispec NMR analyzer mq10 (Bruker Optics, Woodlands, TX).

Histology. The left gonadal fat pad was removed following the killing of the mouse and fixed overnight in 4% paraformaldehyde in PBS. The tissues were then transferred to 70% ethanol and embedded in paraffin. Samples were cut into 5-μm sections, and hematoxylin and eosin staining was performed. Between 200 and 300 adipocytes were quantified from two to three sections of each sample using MacBas V2.52 software (Fuji PhotoFilm). From this, the
average adipocyte area was calculated by dividing the individual-for-cell pixel by pixels of a 100-μm standard (a pixel represents a 1-μm² area).

**Hypermisulinemic-euglycemic clamp.** The clamp studies were performed exactly as developed by Kim and colleagues (16,19–21). The clamp technique was validated in a set of control experiments (J.K. Kim, personal communication). The study involved a primed insulin infusion (19). Mice were treated with either PHZ or vehicle for 2 weeks, as described above. On day 10 of treatment (4 days before the clamp experiment), mice were anesthetized with 100 mg/kg ketamine and 10 mg/kg xylazine. A catheter was inserted into a lateral incision on the right side of the neck and advanced into the superior vena cava via the right internal jugular vein. The catheter was then sutured into place, according to the protocol of MacLeod and Shapiro (22). The evening before the clamp analysis, mice received the final injection of PHZ or vehicle at 6:00 P.M. and were then fasted overnight. To conduct experiments in awake animals with minimal stress, a tail-restrain method was used during experiments (16,20,21). The stressfulness of the procedure was assessed by measuring epinephrine levels (J.K. Kim, personal communication).

The basal rates of glucose turnover were measured by continuous infusion of [3-3H]glucose (0.02 μCi/min) for 120 min, which followed a bolus of 2.5 μCi, starting at 9:00 a.m. Blood samples (20 μl) were taken at 90 and 115 min of the basal period for the determination of plasma [3H]glucose concentration. A 120-min hyperinsulinemic-euglycemic clamp was started at 11:00 a.m. Insulin was infused as a bolus of 300 mU/kg over a period of 3 min, followed by continuous insulin infusion at the rate of 2.5 mU·kg⁻¹·min⁻¹ (Humulin R; Eli Lilly, Indianapolis, IN) to raise the plasma insulin concentration to 4 nmol/l. During the clamp study, mice were restrained, and blood samples (20 μl) were collected via a small nick in the tail vein at 15-min intervals for the immediate measurement of plasma glucose concentration, and 20% glucose was infused at variable rates to maintain plasma glucose at ~200 mg/dl in MKR mice (because of severe insulin resistance, this is the lowest glucose level we can reach) (23–25). Insulin-stimulated whole-body glucose flux was estimated using a prime continuous infusion of high-pressure liquid chromatography–purified [3-3H]glucose (10 μCi bolus, 0.1 μCi/min; NEN Life Science Products, Boston, MA) throughout the clamps (16,20,21). To estimate insulin-stimulated glucose transport activity and metabolism in skeletal muscle, 2-deoxy-D-[1-14C]glucose (NEN Life Science Products) was administered as a bolus (10 μCi) at 45 min before the end of clamps. Blood samples (20 μl) were taken at 80, 85, 90, 100, 110, and 120 min after the start of clamps for the determination of plasma [3H]glucose, 2-deoxy-D-[1-14C]glucose and [3H]2O concentrations. Additional blood samples (10 μl) were collected before the start and at the end of clamp studies for measurements of plasma insulin concentration. All infusions were performed using microdialysis pumps (CMA/Microdialysis, Acton, MA). At the end of the clamp period, animals were anesthetized with ketamine and xylazine injection. Within 5 min, gastrocnemius muscle from hindlimbs, epididymal and brown adipose tissue, and liver were removed. Each tissue, once exposed, was dissected within 2 s, frozen immediately using liquid N2-cooled aluminum blocks, and stored at −70°C for later analysis.

**Statistical analysis.** Results are expressed as means ± SE. Statistically significant differences were determined using a one-factor ANOVA followed by a t test.

**RESULTS**

**PHZ treatment improves hyperglycemia without affecting dyslipidemia or hyperinsulinemia in MKR mice.** At the age of 6–8 weeks, male MKR mice had higher blood and urine glucose levels, higher serum triglycerides, and hyperinsulinemia compared with WT mice (Fig. 1). Two weeks of PHZ treatment significantly decreased blood glucose levels in MKR mice (from 345 ± 27.7 to 212 ± 17.0 mg/dl). However, PHZ had no effect on blood glucose levels in the WT mice (Fig. 1A). PHZ treatment significantly increased urine glucose levels in both MKR and WT mice, as compared with vehicle-treated littersmates, which confirmed a functional effect of PHZ (Fig. 1B). However, serum triglycerides and insulin levels were not changed in response to PHZ treatment. Free fatty acid level was significantly reduced in PHZ-treated WT mice; however, it was not changed in PHZ-treated MKR mice (Fig. 1C–E).

**PHZ treatment decreases fat mass in MKR mice.** Compared with WT controls, vehicle-treated MKR mice had slightly lower average body weights (Fig. 2A). Body composition analysis showed that MKR mice had 30% higher levels of fat mass and 20% lower levels of lean mass than WT mice (Fig. 2B–C). Whole-body fat mass in MKR mice was significantly reduced in response to PHZ treatment (1.40 ± 0.18 vs. 2.29 ± 0.16 g in vehicle-treated MKR mice).
mice). In contrast, PHZ treatment had no effect on fat mass in WT mice. PHZ treatment had no effect on lean mass in either MKR or WT mice.

To better understand the mechanisms underlying the fat loss exhibited by the PHZ-treated MKR mice, we measured food intake. Daily food intake normalized to body weight was higher in MKR mice than that in WT mice, regardless of whether the mice received PHZ or vehicle treatment (Table 1). PHZ treatment had no effect on food intake in WT mice; however, it caused a further 7.8% increase in food intake in MKR mice ($P = 0.02$). Thus, the loss of adipose mass in PHZ-treated mice was not due to a decrease in food intake.

To confirm the body composition data obtained by nuclear magnetic resonance, we measured organ weights in an independent set of mice. Mice were treated with either PHZ or vehicle for 2 weeks, as described. The weight of white adipose tissue in MKR mice was decreased in response to PHZ treatment (Fig. 2D–E). When expressed as a percentage of body weight, gonadal and inguinal fat pad weights decreased by 27 and 30%, respectively. In contrast, PHZ treatment had no effect on white adipose tissue weight in WT mice. Neither WT nor MKR mice exhibited any changes in brown adipose tissue weight in response to PHZ treatment ($P < 0.05$ for WT vs. MKR from the same treatment group; $P < 0.05$ for PHZ vs. vehicle group). Samples were collected from nonfasting mice between 9:00 A.M. and 12:00 P.M.

**TABLE 1**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>WT + vehicle</th>
<th>WT + PHZ</th>
<th>MKR + vehicle</th>
<th>MKR + PHZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>30.2 ± 0.7</td>
<td>30.4 ± 0.5</td>
<td>24.6 ± 0.7*</td>
<td>24.1 ± 0.4*</td>
</tr>
<tr>
<td>Food intake (g · g body wt$^{-0.75}$ · day$^{-1}$)</td>
<td>0.29 ± 0.01</td>
<td>0.29 ± 0.01</td>
<td>0.32 ± 0.01*</td>
<td>0.35 ± 0.01†</td>
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<tr>
<td>Liver weight (g)</td>
<td>1.63 ± 0.06</td>
<td>1.82 ± 0.05†</td>
<td>1.78 ± 0.08*</td>
<td>1.75 ± 0.07</td>
</tr>
<tr>
<td>Muscle weight (g)</td>
<td>1.85 ± 0.04</td>
<td>1.65 ± 0.05†</td>
<td>1.00 ± 0.03*</td>
<td>0.92 ± 0.03*</td>
</tr>
<tr>
<td>Pancreas weight (g)</td>
<td>0.23 ± 0.01</td>
<td>0.22 ± 0.01</td>
<td>0.25 ± 0.01</td>
<td>0.22 ± 0.02</td>
</tr>
<tr>
<td>Serum leptin (ng/ml)</td>
<td>12.7 ± 1.2</td>
<td>7.1 ± 2.0†</td>
<td>6.7 ± 0.7*</td>
<td>4.5 ± 0.4†</td>
</tr>
<tr>
<td>Serum adiponectin (μg/ml)</td>
<td>2.9 ± 0.3</td>
<td>4.2 ± 0.3</td>
<td>5.5 ± 0.6*</td>
<td>4.2 ± 0.3†</td>
</tr>
<tr>
<td>Serum resistin (ng/ml)</td>
<td>4.3 ± 0.1</td>
<td>4.2 ± 0.3</td>
<td>4.4 ± 0.3</td>
<td>5.5 ± 0.7</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE, with $n = 7–12$ mice in each group. *$P < 0.05$ for WT vs. MKR from the same treatment group; †$P < 0.05$ for PHZ vs. vehicle group.

Improvement in hyperglycemia is incapable of overcoming whole-body insulin resistance in MKR mice. MKR mice displayed hyperinsulinemia and hyperlipidemia as early as 2 weeks of age (data not shown). In contrast, postprandial glucose levels did not increase in MKR mice until ~3–4 weeks of age (6). To determine whether glucotoxicity plays an important role in the development of secondary insulin resistance and type 2 diabetes in MKR mice, insulin tolerance tests were performed. As shown in Fig. 4A, PHZ treatment did not improve insulin sensitivity in MKR mice. PHZ treatment also had no effect on glucose intolerance in MKR mice, despite the PHZ-induced reduction in ambient circulating blood glucose levels (Fig. 4B). As shown in Fig. 4E, serum insulin levels were not significantly decreased in response to PHZ treatment in MKR mice. These data suggest that PHZ treatment did not improve whole-body insulin sensitivity, despite the improvement in circulating glucose levels.

**PHZ treatment increases glucose uptake in white adipose tissue in MKR mice.** To examine whether the PHZ-induced improvement in blood glucose levels in MKR mice was associated with improved insulin sensitivity in liver and adipose tissue, MKR mice were subjected to hyperinsulinemic-euglycemic clamp studies after 2 weeks of PHZ treatment. Basal glucose and insulin levels were similar in vehicle-treated and PHZ-treated MKR mice (Fig. 5A and Table 2). During the clamp, insulin was infused at a rate of 2.5 mU · kg$^{-1}$ · min$^{-1}$, after a bolus of 300 mU/kg, and glucose was infused at a variable rate in an attempt to maintain plasma glucose levels within the normal range in WT mice (140–160 mg/dl). However, blood glucose levels did not fall to <201 mg/dl in MKR mice because these animals had severe insulin resistance.

PHZ treatment did not alter glucose infusion rate (GIR) and endogenous glucose production in MKR mice compared with vehicle-treated controls (Fig. 5B–C). Whole-body glucose and muscle glucose uptake were also unchanged in response to PHZ treatment in MKR mice (Fig. 5D–E). Similarly, PHZ treatment did not affect the rates of glycogen synthesis in skeletal muscle or liver in MKR mice (Table 2).

Interestingly, glucose uptake into white adipose tissue was increased by 52% in MKR mice treated with PHZ ($16 ± 0.3$...
2.3 μmol · g⁻¹ · min⁻¹) compared with MKR mice treated with vehicle (10.5 ± 1.2 μmol · g⁻¹ · min⁻¹) (Fig. 5F). Glucose uptake into brown adipose tissue also increased in response to PHZ treatment in MKR mice (313 ± 76 μmol · g⁻¹ · min⁻¹), as compared with vehicle treatment (184 ± 36 μmol · g⁻¹ · min⁻¹) (Fig. 5G). Thus, PHZ improved glucose uptake into adipose tissue, but it did not affect whole-body glucose disposal and neither did it improve insulin sensitivity in muscle or liver of the MKR mice.

DISCUSSION
MKR mice overexpress a dominant-negative IGF-1R specifically in skeletal muscle. As a result, both the IGF-1R
and insulin receptor signaling systems are inhibited in the skeletal muscle of these animals. MKR mice were shown to develop insulin resistance, hyperinsulinemia, dyslipidemia, and glucose intolerance as early as 2–3 weeks of age (6,7). Thus, MKR mice provide an excellent model to study molecular mechanisms underlying the onset and progression of type 2 diabetes.

When MKR mice were treated with the peroxisome-proliferator-activated receptor-α agonist, WY14,643, dyslipidemia was improved, followed by a gain in insulin sensitivity (9). However, the role of hyperglycemia in the development of secondary insulin resistance and eventually type 2 diabetes in MKR mice is not clear because these mice already show glucose intolerance at the age of 3 weeks, without full-blown diabetes (6). In the present study, we demonstrated that treatment of MKR mice with PHZ improved hyperglycemia, but not dyslipidemia. PHZ treatment significantly increased glucose uptake in white adipose tissue in MKR mice during a hyperinsulinemic-euglycemic clamp. However, sensitivity to insulin with respect to whole-body glucose uptake, glucose uptake in muscle, and endogenous glucose production in liver did not improve in MKR mice in response to PHZ treatment.

Genetic defects together with environmental influences can induce insulin resistance and type 2 diabetes. As a result, glucotoxicity may lead to β-cell dysfunction, as manifested by reduced insulin content and secretion as well as impaired insulin action in peripheral target tissues (26). Kim et al. (16) demonstrated that glucotoxicity that occurs in response to muscle-specific inactivation of the glucose transporter GLUT4 (muscle GLUT4 knockout) can lead to secondary defects in insulin action in adipose tissue and liver. These secondary defects were found to contribute to higher levels of insulin resistance and to the eventual development of diabetes. PHZ treatment prevented these acquired abnormalities in insulin action in liver and adipose tissue in muscle GLUT4 KO mice. In the present study, improvement in glucose levels increased insulin-stimulated glucose uptake only in white adipose tissue. This is in striking contrast to the findings in muscle GLUT4 KO mice (16). The basis for this discrepancy may be due to differences in the state of lipid metabolism between the two types of mice. It has been shown (15) that cholesterol and free fatty acid levels remained normal in muscle GLUT4 KO mice up to the age of 6 months and that triglyceride levels tended to be lower in muscle GLUT4 KO mice. However, MKR diabetic mice exhibited dyslipidemia from the age of 2–3 weeks. Our study therefore suggests that dyslipidemia played a more important role than glucotoxicity in the onset of diabetes.

It has been suggested (27,28) that either hyperglycemia, dyslipidemia, or both play an important role in insulin resistance in adipose tissue. In this study, improvement of hyperglycemia was associated with increased insulin sensitivity in white adipose tissue. However, whole-body glucose uptake, muscle glucose uptake, and endogenous glucose production in liver did not improve. A previous study (29) showed that normalization of blood glucose in type 1 diabetic rats reversed insulin-resistant glucose transport in adipose cells, which is consistent with our current findings. In a separate study, we have shown that treating MKR mice with thiazolidinediones was associated with improvements in dyslipidemia and insulin sensitivity in adipocytes, but not in whole-body or liver insulin sensitivity or in the diabetic state (H.K., M. Haluzik, O.G., S.Y., J.P., H.S., U.B. Pajvani, P.E. Scherer, D.L., unpublished observations). This suggests that, in and of itself, improved insulin sensitivity in adipose tissue does not affect whole-body glucose homeostasis. Furthermore, these findings indicate that hepatic function plays a major role in the MKR diabetic mouse model.

Increased deposition of triglycerides and/or other lipid metabolites in the liver often results in insulin resistance within the liver (30). Triglyceride levels were twofold higher in the livers of MKR mice compared with those in WT mice. Previously, we showed (9) that treating MKR mice with WY14,643 improves dyslipidemia and hyperglycemia, which led to improved insulin resistance in the liver. In contrast, treating MKR mice with PHZ did not improve dyslipidemia, despite the observed improvement in hyperglycemia. Several previous studies (13,14,31,32) have reported that PHZ treatment reverses hyperglycemia and normalizes insulin sensitivity in liver. The differences between those studies and our current study are probably due to the dissimilarity in the animal models. Most studies were performed on animals with type 1 diabetes that was

FIG. 4. Effect of PHZ treatment on insulin and glucose tolerance in 8-week-old male WT and MKR mice. Mice were treated with either PHZ or vehicle (V) for 2 weeks, as described in Research Design and Methods. (A) Animals were fasted for 6 h, injected intraperitoneally with insulin (0.75 IU/kg), and blood glucose was measured at the indicated time points. (B) Mice were fasted overnight, injected intraperitoneally with glucose (2 g/kg), and blood glucose was monitored for 2 h. Data are expressed as means ± SE, with n = 6 mice in each group.
induced by streptozotocin treatment or by pancreatec-
tomy. Those animal models exhibit little obvious dysli-
pidemia or insulin resistance. Thus, dyslipidemia may have
contributed in a major way to insulin resistance in the liver
of MKR mice. Improvement of hyperglycemia failed to
overcome insulin resistance in the liver, and no subse-
cquent improvements in whole-body glucose uptake were
observed.

FIG. 5. Effect of PHZ treatment on whole-body, skeletal muscle, and adipose tissue glucose uptake during hyperinsulinemic-euglycemic clamp
studies in MKR mice. Mice were treated with either PHZ (●) or vehicle (○, V) for 2 weeks, as described in RESEARCH DESIGN AND METHODS. Mice were
fasted overnight (after the last injection of vehicle or PHZ) before performing a hyperinsulinemic-euglycemic clamp analysis, as described in
RESEARCH DESIGN AND METHODS. A: Time course of plasma glucose levels before and during the clamp studies. B: GIR. C: Clamp endogenous glucose
tissue (BAT) glucose uptake. Data are expressed as means ± SE, with n = 7–12 mice in each group. *P < 0.05 for PHZ vs. vehicle group.
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TABLE 2
Effect of PHZ treatment on various metabolic parameters during the basal (12-h fast) period and hyperinsulinemic-euglycemic clamp in MKR mice

<table>
<thead>
<tr>
<th></th>
<th>MKR + vehicle</th>
<th>MKR + PHZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>21.5 ± 0.8</td>
<td>20.7 ± 0.7</td>
</tr>
<tr>
<td>Basal plasma glucose (mg/dl)</td>
<td>310 ± 41</td>
<td>320 ± 25</td>
</tr>
<tr>
<td>Clamp plasma glucose (mg/dl)</td>
<td>201 ± 21.0</td>
<td>227 ± 25</td>
</tr>
<tr>
<td>Basal plasma insulin (mg/ml)</td>
<td>2.2 ± 0.7</td>
<td>2.2 ± 0.4</td>
</tr>
<tr>
<td>Clamp plasma insulin (mg/ml)</td>
<td>3.7 ± 0.8</td>
<td>3.6 ± 0.5</td>
</tr>
<tr>
<td>Basal EGP (μmol·kg⁻¹·min⁻¹)</td>
<td>170.2 ± 24.1</td>
<td>157.6 ± 21.0</td>
</tr>
<tr>
<td>Clamp gas glycogen synthesis (μmol·kg⁻¹·min⁻¹)</td>
<td>1.76 ± 0.6</td>
<td>1.1 ± 0.4</td>
</tr>
<tr>
<td>Clamp quad glycogen synthesis (μmol·kg⁻¹·min⁻¹)</td>
<td>2.2 ± 0.5</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td>Clamp liver glycogen synthesis (μmol·kg⁻¹·min⁻¹)</td>
<td>4.0 ± 1.4</td>
<td>3.0 ± 1.0</td>
</tr>
</tbody>
</table>

Data are means ± SE. n = 7–12 mice per group. EGP, endogenous glucose production; gas, gastrocnemius; quad, quadriceps.

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REFERENCES

It has been well established that obesity is an important determinant of insulin sensitivity (2). Adipose tissue is now accepted as an endocrine organ, which secretes various adipokines, including leptin, adiponectin, and resistin, to regulate insulin sensitivity and other physiological processes (33,34). The levels of adipokines released from fat mass are altered in insulin-resistant states and in diabetes (35). Treatment of MKR mice with PHZ decreased fat mass and improved insulin sensitivity in white adipose tissue. Adipocytes from PHZ-treated MKR mice were smaller on average than that from vehicle-treated MKR mice. It is possible that the marked excretion of glucose via the urine and inability of muscle to utilize the glucose resulted in increased lipolysis, causing a reduction in adipocyte size. Serum adiponectin levels were higher in MKR mice than that in WT mice. While we have no explanation for this consistent finding, we speculate that it may be due to compensation for the severe insulin resistance seen in MKR mice. Serum adiponectin levels were decreased in PHZ-treated MKR mice following the fat mass loss, which may at least partially explain the failure to observe improvements in insulin sensitivity in liver. It has been shown (36–39) that circulating leptin levels are closely correlated with body fat content and adipocyte size. Serum leptin levels were decreased in PHZ-treated WT mice. PHZ-treated WT mice had reduced amount of gonadal fat (Fig. 2D) and muscle (Table 1). Thus, PHZ-treated WT mice appear to be in an energy-deficient state, which may explain the lower leptin levels. Another possible explanation of lower leptin levels in PHZ treated mice is increased clearance of leptin via kidney (33,40).

In conclusion, MKR mice, which exhibit severe insulin resistance and diabetes, exhibit improved circulating glucose levels in response to treatment with PHZ. However, this effect on circulating blood glucose levels was not associated with any improvements in whole-body insulin sensitivity or glucose homeostasis. These data suggest that lipotoxicity, but not gluotoxicity, plays a major role in the development and progression of type 2 diabetes in this animal model.


