A Nonspecific Phosphotyrosine Phosphatase Inhibitor, Bis(maltolato)oxovanadium(IV), Improves Glucose Tolerance and Prevents Diabetes in Zucker Diabetic Fatty Rats

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The molecular basis of insulin resistance, a major risk factor for development of Type II diabetes, involves defective insulin signaling. Insulin-mediated signal transduction is negatively regulated by the phosphotyrosine phosphatase, PTP1B, and numerous studies have demonstrated that organo-vanadium compounds, which are nonselective phosphotyrosine phosphatase inhibitors, have insulin-mimetic properties. However, whether or not vanadium compounds can prevent the transition from insulin resistance to overt diabetes is unknown. We compared the ability of bis(maltolato)oxovanadium(IV) (BMOV), an orally bioavailable organo-vanadium compound, and rosiglitazone maleate (RSG), a known insulin sensitizer, to prevent development of diabetes in Zucker diabetic fatty (ZDF) rats. Treatment began at 6 weeks of age when animals are insulin resistant and hyperinsulinemic, but not yet hyperglycemic, and ended at 12 weeks of age, which is 4 weeks after ZDF rats typically develop overt diabetes. BMOV-treated ZDF rats did not develop hyperglycemia, showed significant improvement in insulin sensitivity, and retained normal pancreatic islet morphology and endocrine cell distribution, similar to RSG-treated animals. BMOV and RSG treatment also prevented the hyperphagia and polydipsia present in untreated ZDF rats; however, BMOV-treated ZDF rats gained much less weight than did RSG-treated animals. Circulating levels of adiponectin decreased in untreated ZDF rats compared to lean controls, but these levels remained normal in BMOV-treated ZDF rats. In contrast, in RSG-treated ZDF rats, plasma adiponectin levels were nearly 4-fold higher than in lean control rats, primarily as a result of a large increase in the amount of low–molecular weight forms of adiponectin in circulation. These data demonstrate that phosphatase inhibition offers a new approach to diabetes prevention, one that may have advantages over current approaches. Exp Biol Med 230:207–216, 2005

Key words: protein tyrosine phosphatase; BMOV; insulin sensitivity; adiponectin; ZDF rat

Introduction

Type II diabetes is characterized by insulin resistance in muscle, liver, and fat and by defects in insulin secretion from pancreatic β cells (1–4). The molecular basis of insulin resistance, a major risk factor for the development of diabetes, involves a defect in the insulin signaling pathway. Insulin exerts its effects by binding to the insulin receptor (IR), which activates an intrinsic insulin receptor tyrosine kinase (IRK), resulting in the phosphorylation of key tyrosine residues (autophosphorylation) in the IR and proximal signaling molecules such as insulin receptor substrate-1 (IRS-1). These signaling events result in a series of responses that occur within minutes (e.g., glucose uptake) or many hours (e.g., mitogenesis) (5, 6). The underlying defect in Type II diabetes is an attenuation of the insulin signaling cascade (7). The IRK activity has been shown to be negatively regulated by phosphotyrosine phosphatases such as PTP1B (8–12). Recently it was reported that PTP1B null mice have increased insulin sensitivity and decreased susceptibility to diet-induced obesity, but otherwise appear normal (13). In addition, enhanced PTP activity or PTP1B mRNA stability has been shown in patients with insulin resistance and Type II diabetes and was reduced following improved insulin sensitivity associated with weight loss (14–16). Treatment of diabetic o*b*/*ob* mice with antisense PTP1B leads to normalization of glucose and insulin levels (17). Thus, inhibition of PTP1B activity has emerged as a novel approach for the treatment of insulin resistance (18).

The Zucker diabetic fatty (ZDF) rat exhibits a number

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of characteristics that simulate the development of Type II diabetes. The ZDF rat was originally derived from the Zucker fatty rat, which carries a spontaneous mutation in the leptin receptor (*fa* gene) (19) that causes obesity. Although Zucker fatty rats are obese and insulin resistant, they maintain normal blood glucose levels through increased insulin production and secretion. However, ZDF rats become slightly hyperglycemic beginning at about 7 weeks of age, and their serum glucose levels reach greater than 300 mg/dl by 9–10 weeks of age. Their insulin levels are high (hyperinsulinemic) from 5 to 10 weeks of age and drop to control levels thereafter. At this stage, the ZDF animals are clearly insulin resistant and go on to develop overt diabetes by 12 weeks of age, since the β cells cannot adequately compensate for the insulin resistance by increasing insulin production and secretion (20). The evolution of diabetes in the ZDF rat is associated with disruption of the islet cell architecture, β-cell degranulation, and increased β-cell death. It is not clear whether these changes precede or develop as a result of the increase in plasma glucose (21); however, early intervention with thiazolidinediones (rosiglitazone) prevented the onset of hyperglycemia and disruption of the β-cell architecture (22, 23).

Numerous studies both *in vivo* and *in vitro* have demonstrated the insulin-like properties of vanadium compounds (24–28). We and others have shown that the orally bioavailable organo-vanadium compound bis(maltolato)oxovanadium(IV) (BMOV) inhibits multiple tyrosine phosphatases, including PTP1B *in vitro*, and BMOV treatment decreased plasma insulin levels in the Zucker fatty rat (29, 30). In addition, long-term (10-week) treatment with BMOV in ZDF rats beginning at 15 weeks of age, in the presence of hyperglycemia and depleted pancreatic insulin stores, greatly reduced plasma glucose levels and effectively improved β-cell function (31). However, whether or not phosphatase inhibition with BMOV can prevent the development of diabetes and preserve islet cell architecture in this model is not known. In this study, we sought to examine the effect of treatment with BMOV in the ZDF rat before the onset of hyperglycemia as well as the results of such a treatment on the functional response of the pancreas.

Materials and Methods

Materials. BMOV was purchased from Organica Feinchemie GmbH, Wolfen, Germany (catalog No. 14920).

Animals. Five-week-old ZDF rats and lean ZDF littermates were obtained from Charles River Laboratories (Indianapolis, IN). The animals were allowed *ad libitum* access to water and ground food (Purina 5008; PMI Nutrition International, St. Louis, MO). The rats were housed individually in a plastic cage with contact bedding and were allowed 5 days to acclimate to their new environment before the onset of the study. The use and care of rats in these studies were in accord with Procter & Gamble Pharmaceuticals laboratory animal research guidelines. The fatty rats were randomly divided into treated (BMOV or rosiglitazone maleate [RSG]) and untreated groups. Lean ZDF littermates served as an untreated, nondiabetic control group. A total of five rats were studied in each treatment group. Baseline measurements were taken for fed blood glucose, body weight, and food and water intake. Body weight and food and fluid intake were assessed three times a week throughout the study period. BMOV was administered in the drinking water at an average daily dose of 44.5 mg/kg/day, and RSG was administered in the food at 4 mg/kg/day.

Blood Sampling and Assays. Blood samples were obtained weekly from the saphenous veins of rats eating *ad libitum*, and the fed blood glucose levels were determined using the One Touch Ultra glucometer (Life Scan, Milpitas, CA). Plasma insulin levels were measured using the ultrasensitive rat insulin enzyme-linked immunosorbent assay kit from Crystal Chem, Inc. (Downers Grove, IL), and plasma adiponectin levels were assayed by radioimmunoassay using a kit from Linco Research, Inc. (St. Charles, MO). Hemoglobin A1c levels were measured using a DCA 2000+ analyzer from Bayer Diagnostics (Tarrytown, NY).

Glucose Tolerance Test. At the end of the 6-week treatment period, a jugular vein catheter was surgically implanted, and the rats were allowed to recover for 24 hrs. Following a 16-hr fast, an oral glucose tolerance test was performed. Glucose was administered via oral gavage at a dose of 2 g/kg of body weight. Blood was sampled at 0, 30, 60, 90, and 120 min using a Culex automated blood sampler from Bioanalytical Systems (West Lafayette, IN). The blood was collected in sodium heparin tubes, centrifuged, and the plasma was analyzed for glucose levels using a Yellow Springs, Inc. (YSI) 2700 (Yellow Springs, OH) blood glucose analyzer. The remaining plasma was frozen at –80°C for insulin analysis.

Insulin Sensitivity Index. The insulin sensitivity index is defined as $ISI_{0,120} = MCR/\log_{10} MPI$ (32). Metabolic clearance rate (MCR) = m/MPG, where m = milligrams of glucose administered + (0-min plasma glucose – 120-min plasma glucose) × 0.19 × body weight (BW)/120 min; BW is body weight in kilograms; mean plasma glucose (MPG) is the mean of the 0- and 120-min plasma glucose levels in milligrams per liter; and mean plasma insulin (MPI) is the mean of the 0- and 120-min plasma insulin concentrations in milliunits per liter.

Tissue Sampling. Animals were sacrificed via exsanguination while under a deep surgical plane of isoflurane general anesthesia. Skeletal muscle was immediately frozen in liquid nitrogen and was then stored at –80°C until use. The pancreas was immediately placed in a solution of 4% paraformaldehyde in 0.1 *M* phosphate buffer, pH 7.4, allowed to fix for 24 hrs, then placed in cold phosphate-buffered saline (PBS), pH 7.4, and stored at 4°C until shipped to Pathology Associates, Inc. (West Chester, OH) for paraffin embedding and sectioning.

Histology. Hematoxylin and eosin (hematoxylin-eosin) sections were processed and stained by Pathology Associates, Inc.

Immunohistochemistry. Guinea pig anti-porcine insulin and rabbit anti-human glucagon antisera were both obtained from Dako Corporation (Carpinteria, CA) and were used at 10 μg/ml and 30 μg/ml, respectively. Fluorescein-labeled donkey anti–guinea pig and Cy3-labeled donkey anti-rabbit antisera were obtained from Jackson Immunoresearch Laboratories, Inc. (West Grove, PA) and were used at 5 μg/ml and 2 μg/ml, respectively. Paraffin-embedded sections were hydrated and permeabilized for 30 mins in 0.5% Triton X-100 in PBS, then incubated for 1–2 hrs in 5% normal donkey serum to block nonspecific antibody binding. Sections were incubated overnight at 4°C in a primary antibody solution containing guinea pig anti-porcine insulin and rabbit anti-human glucagon in PBS containing 0.2% bovine serum albumin (BSA). After primary antibody incubation, sections were rinsed for 30 mins in three changes of PBS and then incubated for 1 hr in secondary antibody solution containing fluorescein-labeled donkey anti–guinea pig, Cy3-labeled donkey anti-rabbit antiserum and the fluorescent nuclear stain DAPI. Following secondary antibody incubation, sections were washed twice in PBS and once in 0.1 *M* Tris-HCl, pH 8.2, before being mounted on microscope slides in *p*-phenylenediamine (PPD) glycerol and cover slipped.

***In Vivo* Insulin Receptor Activation.** To assess the effect of BMOV on insulin receptor activation *in vivo*, fasted male Sprague-Dawley rats (250–300 g) were infused with saline or BMOV (15 μmoles) over 5 mins, followed immediately by a 10-min infusion with saline or insulin (1.5 μg/100 g BW) via a carotid artery catheter. Animals were sacrificed and skeletal muscle tissues were removed. Frozen tissue (250 mg) was homogenized in 50 m*M* Tris-HCl (pH 7.5), 150 m*M* NaCl, 1 m*M* EDTA, 1 m*M* sodium orthovanadate, 1 m*M* NaF, and one complete protease inhibitor tablet (Roche Diagnostics, Indianapolis, IN) per 50 ml buffer. Homogenates were centrifuged at 5000 *g* for 10 mins at 4°C, and the supernatant was centrifuged at 100,000 *g* for 60 mins. The cell membrane pellets were solubilized in homogenization buffer containing 1% NP-40 and 0.25% sodium dodecyl sulfate (SDS), and protein concentrations were determined by the BCA assay (Pierce Biotechnology, Rockford, IL). The insulin receptor was immunoprecipitated from 1 mg total protein using 10 μg of an anti-insulin receptor-β antibody (C-19; Santa Cruz Biotechnology, Santa Cruz, CA) at 4°C overnight. The complex was captured using protein A/G-Plus agarose beads (Santa Cruz Biotechnology) for 1 hr at 4°C. The beads were sedimented at 10,000 *g* for 1 min, washed once in cold RIPA buffer, and bound proteins were eluted by boiling for 5 mins in 30 μl of 1× sample buffer (62 m*M* Tris-HCl, pH 6.8; 10% glycerol; 2% SDS; 0.1 *M* DTT; and 0.0025% bromphenol blue). Samples were run on an 8% SDS-polyacrylamide gel electrophoresis (PAGE) gel and transferred to a polyvinylidene fluoride (PVDF) membrane. Western blotting was performed using an anti-phosphotyrosine antibody (PY99; Santa Cruz Biotechnology) diluted 1:1000 in 2.5% BSA in 50 m*M* Tris (pH 7.5), 150 m*M* NaCl–0.1% Tween-20 (TTBS) for 2 hrs at room temperature. The membrane was washed five times in TTBS and then incubated with a horseradish peroxidase–labeled goat anti-mouse antibody (Pierce Biotechnology) in TTBS for 1 hr at room temperature. The membrane was washed in TTBS, and the signal was detected using ECL Western blotting reagents (Amersham Biosciences, Piscataway, NJ). The blot was stripped and reprobed with the anti-insulin receptor-β antibody (C-19) diluted 1:1000 to measure the total amount of insulin receptor. Films were scanned and quantitated using Quantity One software (Bio-Rad Laboratories, Hercules, CA).

Analysis of Circulating Forms of Adiponectin. Plasma samples (1 μl from each animal) were added to 10 μl of 1× sample buffer without DTT and heated for 10 mins at 95°C (33). Samples were run on a 4%–20% Tris-Glycine SDS-PAGE gel and transferred to a PVDF membrane. Western blotting was performed using an anti-globular domain antibody of adiponectin (MAB 3608; Chemicon International, Temecula, CA), diluted 1:10,000, and a horseradish peroxidase–labeled goat anti-mouse antibody, as described above.

Statistics. The results for blood glucose levels, plasma insulin and adiponectin concentrations, ISI, body weight, and food and water intake are expressed as mean ± SD and were analyzed using one-way analysis of variance. A $P < 0.05$ value was considered as the level of statistical significance, and group differences were determined using the Tukey-Kramer test.

Results

BMOV Enhances Activation of the IRK *In Vivo*. Skeletal muscle membrane extracts from BMOV- and/or insulin-treated rats were analyzed for activation of the insulin receptor tyrosine kinase (Fig. 1). BMOV alone and insulin alone increased IRK activation by ~1.6-fold and ~4-fold, respectively. However, the combination of BMOV and insulin treatment augmented IRK autophosphorylation by ~10-fold. These results indicate that BMOV increases the insulin response by enhancing IRK activation and demonstrate the potential insulin-sensitizing effects of BMOV.

BMOV Prevents the Development of Overt Diabetes in ZDF Rats. Two weeks into the treatment period, when the rats were 8 weeks of age, fed blood glucose concentrations were significantly elevated in untreated ZDF rats (~2-fold) compared to the lean control animals that do not develop hyperglycemia (Fig. 2). Blood glucose levels were markedly elevated (~4-fold) by the third week of treatment (9 weeks of age) and were even higher (~5-fold) by the end of the study in ZDF rats

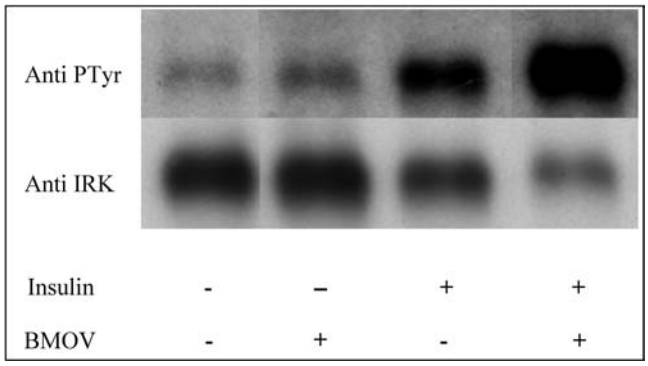

Figure 1. Western blots of activated and total insulin receptor in skeletal muscle membrane preparations from rats injected with vehicle, bis(maltolato)oxovanadium(IV) (BMOV) alone, insulin alone, or insulin + BMOV. The extracts were immunoprecipitated with insulin receptor antibody and probed with anti-phosphotyrosine antibody (AntiPTyr, top row) and insulin receptor antibody (AntiIRK, bottom row). The various treatments are indicated below.

compared to lean controls. In contrast, there was no increase in fed blood glucose concentrations in either the BMOV- or RSG-treated fatty rats compared to the lean control group at any of the weekly time points (Fig. 2). Similarly, hemoglobin A1c levels at the end of the 6-week treatment period in BMOV- or RSG-treated rats were not significantly different than those observed in lean control rats (lean controls, 3.5% ± 0.3%; ZDF controls, 9.2% ± 0.6%; ZDF-RSG–treated, 3.3% ± 0.1%; and ZDF-BMOV–treated, 4.0% ± 0.2%). Thus, BMOV treatment prevented the development of hyperglycemia in ZDF rats, as did RSG treatment.

BMOV Improves Glucose Tolerance and Enhances Insulin Sensitivity in ZDF Rats. The response to a glucose tolerance test at the termination of 6 weeks of treatment is shown in Figure 3. Plasma glucose levels (Fig. 3a) were significantly elevated in the fatty control group compared to the lean control rats at all time points tested. In the untreated ZDF rats, plasma glucose concentrations increased from fasting levels of 300 mg/dl to nearly 600 mg/dl by 30 mins and were still greatly increased over baseline levels 2 hrs after the oral glucose challenge. The RSG- and

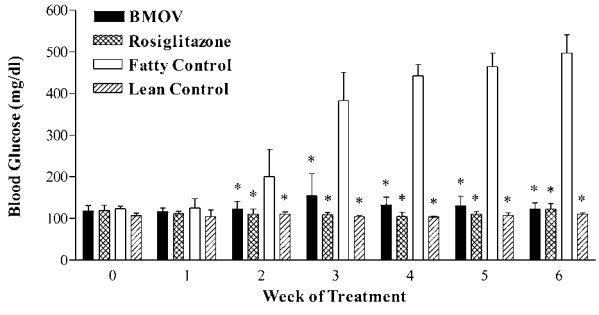

Figure 2. Weekly fed blood glucose levels (mean ± SD) in age-matched lean and fatty control rats and fatty rats treated with bis(maltolato)oxovanadium(IV) (BMOV) or rosiglitazone from 0 to 6 weeks of treatment. * $P < 0.01$ versus fatty control rats.

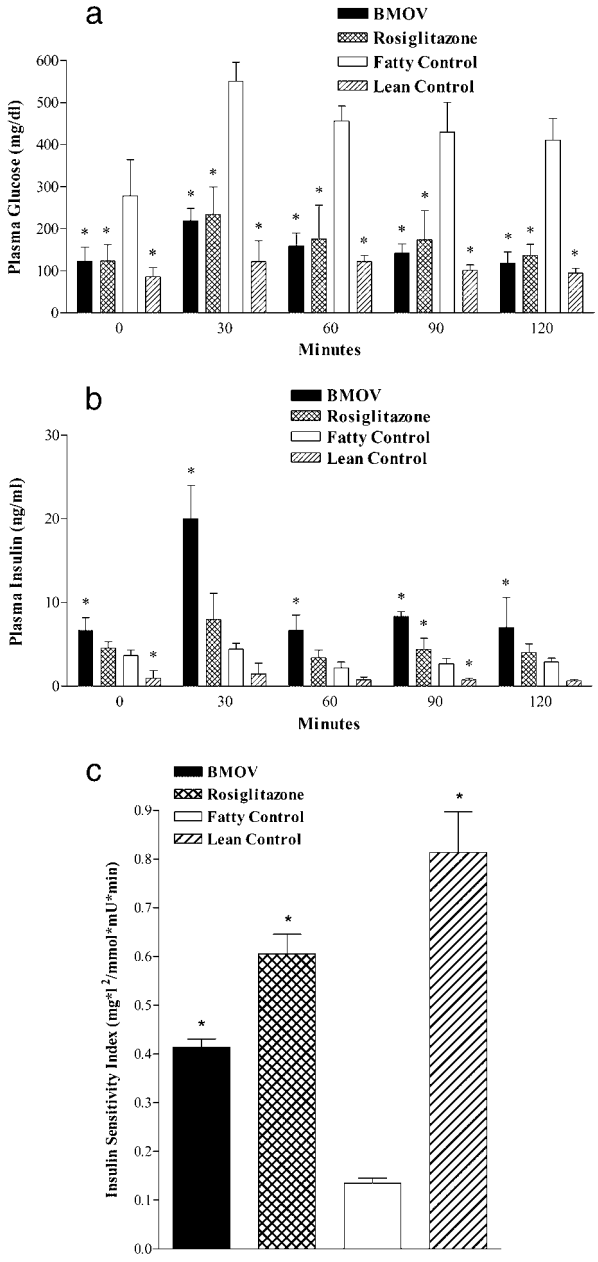

Figure 3. Glucose tolerance test and insulin sensitivity index. Following a 16-hr fast, an oral glucose tolerance test was administered to lean and fatty control rats and fatty rats treated with bis(maltolato)oxovanadium(IV) (BMOV) or rosiglitazone after 6 weeks of treatment: (a) plasma glucose levels, (b) plasma insulin levels, and (c) insulin sensitivity index ($ISI_{0,120}$). All values are mean ± SD. * $P < 0.01$ versus fatty control rats.

BMOV-treated animals showed a significant elevation in plasma glucose concentrations at 30 mins but returned to basal levels by 60 mins after glucose administration. The plasma glucose concentrations in the lean control rats did not change significantly throughout the glucose tolerance test (GTT).

Fasting plasma insulin concentrations (Fig. 3b) were significantly greater in all groups compared to the lean control rats and remained higher at all time points throughout the GTT study period. In addition, BMOV-

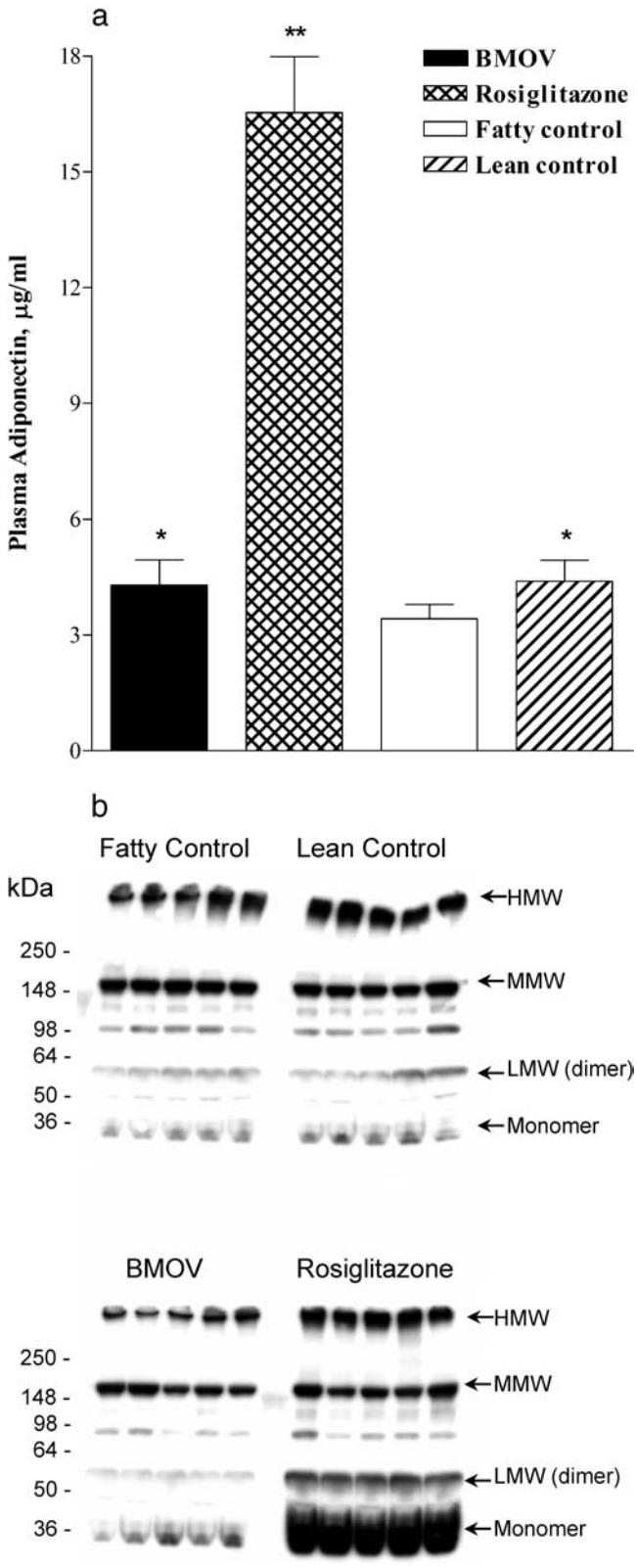

Figure 4. Circulating adiponectin in age-matched lean and fatty control rats and fatty rats treated with bis(maltolato)oxovanadium(IV) (BMOV) or rosiglitazone after 6 weeks of treatment: (a) plasma adiponectin concentrations (mean ± SD), and (b) size distribution of various forms of adiponectin in plasma by Western blot. HMW, high molecular weight; MMW, medium molecular weight; and LMW, low molecular weight (dimer). * $P < 0.05$ or ** $P < 0.01$ versus fatty control rats.

treated rats showed higher fasting insulin levels than the other groups. Both BMOV- and RSG-treated rats exhibited increased plasma insulin concentrations 30 mins after glucose administration but returned to their respective fasting levels by 60 mins. Our results from the GTT experiment demonstrate an enhancement in insulin secretion in those animals treated with BMOV.

The euglycemic hyperinsulinemic clamp test has been the gold standard for determining insulin sensitivity (34). In our study, we used the plasma glucose and plasma insulin levels obtained during the glucose tolerance test to measure insulin sensitivity according to the method reported by Gutt *et al*. (32). These data have been shown to correlate well with the euglycemic hyperinsulinemic clamp homeostasis model assessment and other methods of insulin sensitivity measurement. The ISI results (Fig. 3c) indicate that there is a significant increase in insulin sensitivity in the BMOV- and RSG-treated fatty animals when compared to untreated ZDF rats.

BMOV Stabilizes Plasma Adiponectin Levels in ZDF Rats. At the start of the treatment period (6 weeks of age), plasma adiponectin levels in the fatty rats were higher than in lean controls (8.4 ± 0.3 vs. 5.7 ± 0.1 µg/ml, $P < 0.001$). In the lean rats, circulating adiponectin remained at 4–5µg/ml at the end of the study. However, adiponectin levels in untreated fatty rats fell and showed a 23% decrease compared to lean rats at the end of the 6-week treatment period (Fig. 4a). In BMOV-treated animals, plasma adiponectin remained at the same level as in the lean control group, while in RSG-treated fatty rats, plasma adiponectin rose nearly 4-fold over the lean controls after 6 weeks of treatment (Fig. 4a). Thus, BMOV treatment stabilized plasma adiponectin concentrations, indicating an improvement in insulin sensitivity.

Next, we analyzed the different forms of adiponectin present in circulation using nonreducing SDS-PAGE, as previously described by Waki *et al*. (33). This method has been used to successfully separate and identify the various molecular-weight forms of adiponectin. The results indicated that the greatest difference among the groups was the predominance of the ~30-kDa monomeric form of adiponectin, as well as a higher level of the low–molecular weight ~60-kDa dimer in circulation in RSG-treated animals, compared to the other groups (Fig. 4b). High–molecular weight (>250 kDa), medium–molecular weight (>150 kDa), and possibly other molecular-weight species were also observed in all groups.

BMOV Does Not Affect Food or Fluid Intake or Weight Gain in ZDF Rats. Food and fluid intake was assessed 72 hrs before the beginning of administration of test substances, then three times a week thereafter until the end of the study. BMOV-treated and lean control rats did not alter their total food or water intake significantly throughout the treatment period (Table 1a). RSG-treated and untreated fatty rats increased their total food and water intake steadily through the end of the study (Table 1a), but

Table 1. Food and Water Intake (mean ± SD) in Age-Matched Lean and Fatty Control Rats and Fatty Rats Treated with Bis(maltolato)oxovanadium(IV) (BMOV) or Rosiglitazone for 6 Weeks: (a) Total Food and Water Intake and (b) Food and Water Intake Normalized to Body Weight (BW)

		BMOV	Rosiglitazone	Fatty control	Lean control
(a) Total food and water intake					
Food intake (g/24 hrs)	Week 0 (pretreatment)	24.80 ± 14.29	23.99 ± 1.46	28.87 ± 10.56	21.18 ± 8.07
	Week 6 of treatment	23.37 ± 1.94*	41.13 ± 1.84	44.84 ± 4.95	20.11 ± 1.04*
Water intake (ml/24 hrs)	Week 0 (pretreatment)	26.19 ± 8.80	34.30 ± 8.15	29.74 ± 4.16	24.87 ± 2.73
	Week 6 of treatment	25.14 ± 2.73*	60.49 ± 3.89*	161.83 ± 31.43	29.20 ± 1.76*
(b) Food and water intake/g body weight					
		BMOV	Rosiglitazone	Fatty control	Lean control
Food intake (g/24 hrs/g BW)	Week 0 (pretreatment)	0.146 ± 0.072	0.139 ± 0.006	0.166 ± 0.065	0.160 ± 0.074
	Week 6 of treatment	0.061 ± 0.003*	0.075 ± 0.002*	0.120 ± 0.014	0.062 ± 0.003*
Water intake (ml/24 hrs/g BW)	Week 0 (pretreatment)	0.157 ± 0.046	0.200 ± 0.052	0.169 ± 0.018	0.184 ± 0.018
	Week 6 of treatment	0.066 ± 0.006*	0.110 ± 0.007*	0.431 ± 0.080	0.090 ± 0.005*

* $P < 0.01$ versus fatty control rats.

when normalized to body weight, the food and water intake for the RSG-treated rats also remained at the same level as in the lean control animals (Table 1b). Thus, BMOV and RSG treatment prevented the hyperphagia and polydipsia present in untreated ZDF rats and maintained food and water intake at levels not significantly different from those associated with the lean control group. Consistent with food intake, BMOV-treated rats gained an amount of weight similar to that of the control fatty rats (Fig. 5). In contrast, RSG-treated animals showed a significant increase in body weight compared to fatty control rats by the third week of treatment, despite eating the same amount of food, when normalized to body weight. The average body weights at the end of the treatment period were as follows: lean controls, 325 g; ZDF controls, 375 g; ZDF-RSG–treated, 549 g; and ZDF-BMOV–treated, 381 g. These results indicate that phosphatase inhibition could have advantages over PPARγ agonists for insulin sensitization.

BMOV Preserves Islet Morphology in ZDF Rats. Morphological and ultrastructural examination of pancreatic tissue at the end of the study revealed that the untreated ZDF rats displayed disrupted islet architecture compared to lean control rats, as indicated by pancreatic islet hyperplasia (Fig. 6) and derangement of α-cell distribution within the islet (Fig. 7). In lean control rats, glucagon-staining α cells are present as a mantle of cells around the insulin producing β cells, but in islets from untreated fatty rats, the α cells are more randomly distributed throughout the pancreatic islets (Fig. 7). In contrast, these changes were ameliorated in the RSG-treated group, as reported previously (22, 23). Similarly, the BMOV-treated group demonstrated preserved islet morphology and endocrine marker distribution. These data demonstrate that the beneficial effects of BMOV treatment on islet pathology may be a direct consequence of improving insulin sensitivity and preventing hyperglycemia.

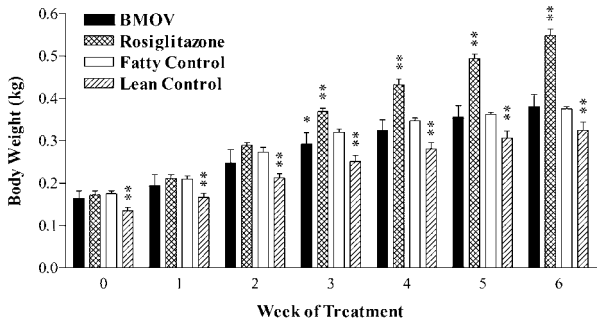

Figure 5. Body weight (mean ± SD) in age-matched lean and fatty control rats and fatty rats treated with bis(maltolato)oxovanadium(IV) (BMOV) or rosiglitazone from 0 to 6 weeks of treatment. * $P < 0.05$ or ** $P < 0.01$ versus fatty control rats.

Discussion

The pathogenesis of Type II diabetes is complex and in most instances involves a defect in both β-cell function and insulin sensitivity (35). The ZDF rat progresses through several stages in the development of diabetes in a relatively predictable age-dependent fashion (36). It has been recently demonstrated by Griffen *et al*. (37) that the ZDF rat also has a genetic defect in the β cells that results in decreased insulin gene transcription and is independently inherited from their leptin receptor (*fa* gene) mutation. The lean ZDF control rats used in this study are heterozygous for the leptin receptor mutation (*fa*/+), do not develop obesity, and are able to maintain normoglycemia despite the defect in β-cell insulin expression. However, the homozygous (*fa*/*fa*) ZDF rats become obese and eventually also develop β-cell failure and overt diabetes, possibly because the independently inherited β-cell defect impairs their ability to tolerate the metabolic alterations caused by the insulin-resistant state.

Several different types of therapeutic agents are currently used for the treatment of Type II diabetes.

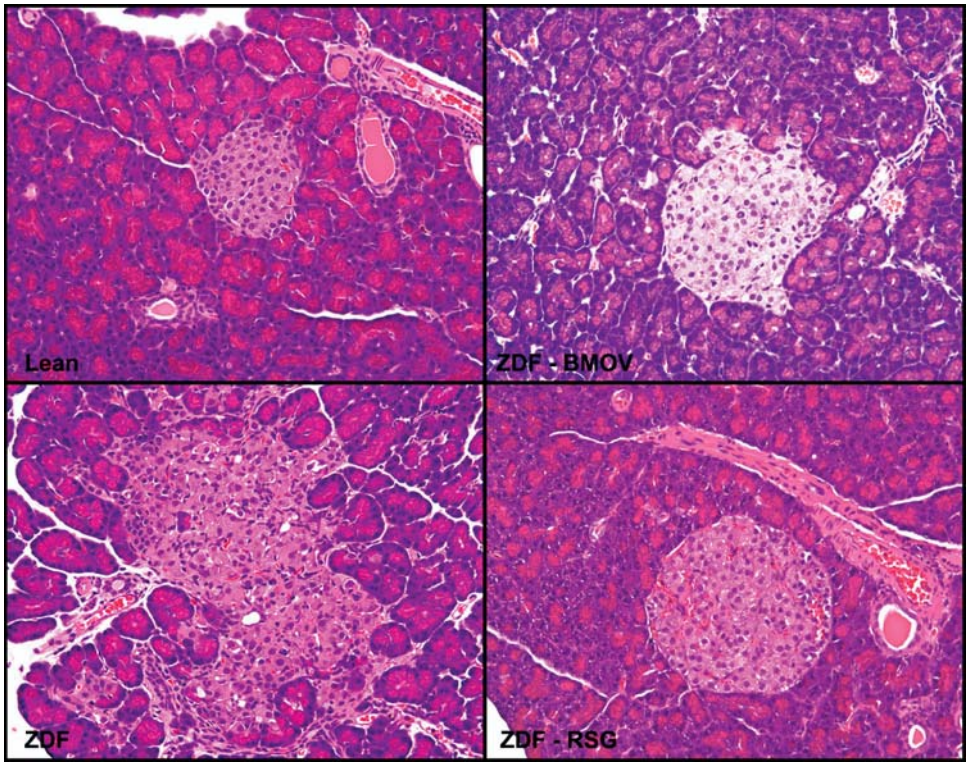

Figure 6. Hematoxylin and eosin staining of pancreatic islets from age-matched lean (upper left) and fatty (lower left) control rats and fatty rats treated with bis(maltolato)oxovanadium(IV) (BMOV) (upper right) or rosiglitazone (RSG) (lower right) after 6 weeks of treatment. Magnification: ×200.

Thiazolidinediones, agonists of the nuclear receptor, PPARγ, enhance insulin sensitivity and improve metabolic control in patients with Type II diabetes (38). Drugs such as α-glucosidase and metformin have also been shown to enhance insulin sensitivity through different mechanisms (39, 40). Recently, protein tyrosine phosphatases, PTP1B in particular, have been shown to negatively regulate the activation of the insulin receptor, indicating that phosphatase inhibition could represent a novel approach to insulin sensitization (8–12). Consistent with this idea, vanadium compounds have been shown to inhibit multiple phosphotyrosine phosphatases, including PTP1B, and to enhance insulin sensitivity in rodent models of diabetes and in diabetic patients (24–31). In the present study, we demonstrate that long-term treatment of ZDF rats with BMOV beginning at 6 weeks of age, before the onset of hyperglycemia but at a point at which the animals are insulin resistant and hyperinsulinemic, prevented the development of overt diabetes, as observed by weekly fed blood glucose levels, which remained in the normal range throughout the treatment period. Plasma glucose levels also rapidly returned to normoglycemic levels in response to oral administration of glucose (GTT) in BMOV-treated animals, when compared to untreated fatty controls, indicating improved glucose tolerance. ZDF rats treated with BMOV, however, had higher fasting insulin concentrations and secreted more insulin in response to glucose. Similar results have also been reported by other investigators after long-term (10-week) treatment with BMOV in ZDF rats beginning at 15 weeks of age, in which both plasma insulin levels and pancreatic insulin content increased compared to that of untreated ZDF rats (31). These effects are likely due to the effect of BMOV treatment on improving insulin sensitivity and maintaining normal glycemia, which prevents the hyperinsulinemia and subsequent depletion of pancreatic insulin stores and the development of diabetes that occurs in untreated ZDF rats.

The BMOV-treated group also exhibited reduced food and water intake when compared to untreated ZDF fatty rats, as was the case in previously reported results for vanadium compounds in this and other diabetic rat models (41–43). Diminished food intake has been related to improvement in insulin action in both ZDF rats and humans, possibly through downregulation of neuropeptide Y (44, 45). BMOV-treated rats gained weight at the same rate as the untreated fatty rats despite eating and drinking significantly less food and water. In contrast, RSG-treated rats gained more body weight than the untreated fatty rats despite eating the same amount of food as the lean control rats, when normalized to body weight. The main difference between the BMOV and RSG groups was in the amount of fat accumulation. Excessive subcutaneous and visceral fat was noted in the abdominal and thoracic regions of the RSG-treated group.

Recently, RSG has been shown to inhibit a futile cycle

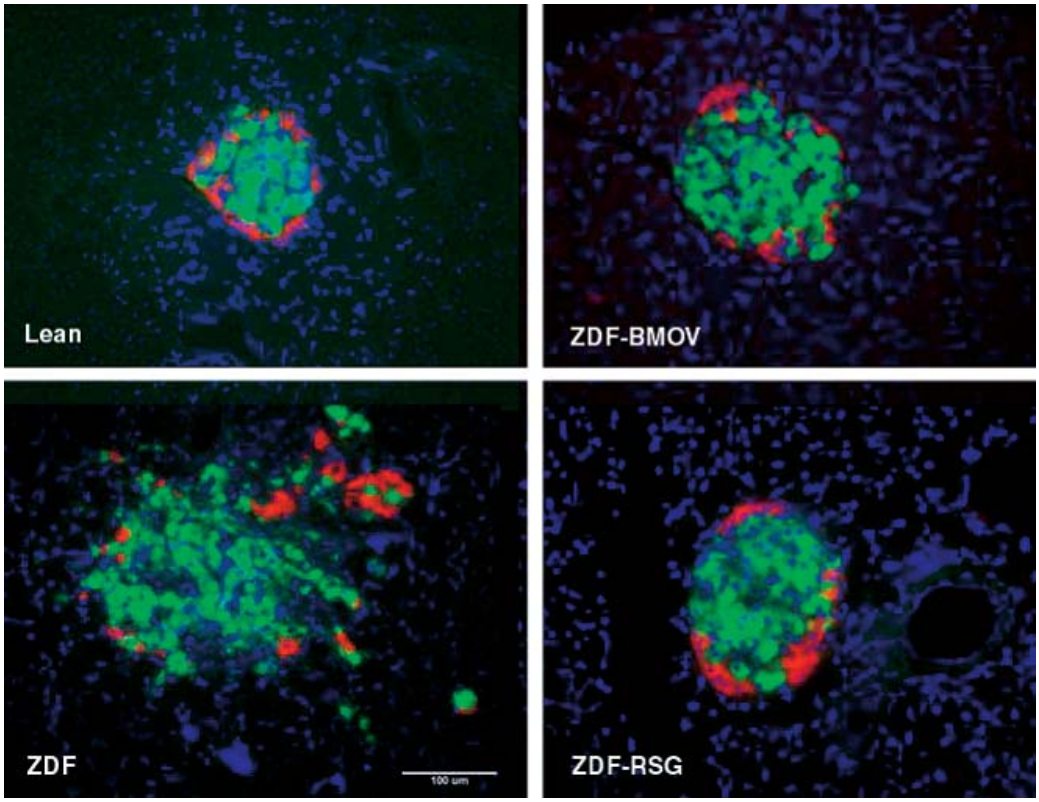

Figure 7. Immunostaining of pancreatic islets from age-matched lean (upper left) and fatty (lower left) control rats and fatty rats treated with bis(maltolato)oxovanadium(IV) (BMOV) (upper right) or rosiglitazone (RSG) (lower right) after 6 weeks of treatment. Sections were stained for glucagon (red) and insulin (green). Magnification: ×200.

of fat synthesis/storage in adipocytes by the activation of glycerol kinase mRNA and enzyme activity (46). Therefore, the increase in body weight observed in RSG-treated animals could be explained by the action of RSG to enhance glycerol kinase activity and mRNA levels. Thus, studies with BMOV provide proof of concept for PTPase inhibition for treatment of Type II diabetes and indicate benefits above and beyond those offered by the current therapy with PPARγ agonists.

Adiponectin, also known as ACRP30, is a hormone secreted by mature adipocytes and may play a role in the development of insulin resistance. It has been demonstrated that plasma adiponectin levels are decreased in the instances of obesity (47) and Type II diabetes (48, 49), whereas conversely, weight loss and pharmacological improvement of insulin sensitivity by TZD treatment are associated with increased adiponectin levels (50–52). In the present study, we also found that circulating adiponectin levels were decreased in untreated fatty diabetic control rats. BMOV-treated rats maintained normal adiponectin levels like those in the lean controls, indicating an improvement in insulin sensitivity. RSG treatment, however, caused a large increase (4-fold) in plasma adiponectin compared to lean rats, which could be due to a direct effect of PPARγ activation on adipogenesis by RSG.

Waki *et al.* (33) have recently demonstrated the importance of the adiponectin multimer distribution, in addition to the total adiponectin concentration, in both healthy and diseased states. Therefore, we analyzed the plasma samples for both the total (quantitative) amount of adiponectin and the distribution of various molecular-weight forms. Interestingly, we observed a large increase in the low–molecular weight forms of adiponectin (monomer and dimer), and we noted that the monomer was the predominant form of adiponectin in the RSG-treated animals when compared to BMOV-treated and control animals, possibly as a result of a direct effect of RSG on adipogenesis.

The development of diabetes is also associated with histopathological changes in the pancreatic islets and decline of insulin content in the ZDF rat (21, 53). At 6 weeks of age, the pancreatic islets of the ZDF rats were round or oval with a mantle of peripheral glucagon-producing α cells around the core of the islets. The lean control rats maintained this architecture throughout the study, while the islet architecture in untreated ZDF rats became larger and more elongated and showed greater irregularity at the boundaries than did that of lean control rats. The dense immunostaining for insulin-producing β cells exhibited by the lean control rats was also reduced in the fatty control rats. This evidence of pancreatic degeneration and disordered architecture (scattered α cells) was markedly reduced in ZDF rats treated with BMOV or RSG,

indicating improved pancreatic structure preservation and function in these animals. An increase in insulin sensitivity would remove the demand for further adaptation of the β cells in these leptin receptor-deficient rats and could possibly have a direct effect on maintaining islet integrity.

The present study clearly demonstrates that phosphatase inhibition with BMOV treatment, like RSG treatment, prevented the development of diabetes in ZDF rats. Moreover, BMOV treatment also resulted in improved β-cell architecture and function, as did RSG treatment. Thus, treatment of insulin resistance before the onset of diabetes, regardless of the mechanism, appears to be important in delaying the onset of diabetes. Whether or not phosphatase inhibition will have similar beneficial effects in patients in the early stages of glucose intolerance will be dependent upon the development of potent, selective, and orally bioavailable phosphatase inhibitors targeting PTP1b, or perhaps other phosphatases that are critical regulators of insulin action.

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