MBX-102/JNJ39659100, a Novel Peroxisome Proliferator-Activated Receptor-Ligand with Weak Transactivation Activity Retains Antidiabetic Properties in the Absence of Weight Gain and Edema

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MBX-102/JNJ39659100 (MBX-102) is in clinical development as an oral glucose-lowering agent for the treatment of type 2 diabetes. MBX-102 is a nonthiazolidinedione (TZD) selective partial agonist of peroxisome proliferator-activated receptor (PPAR)-γ that is differentiated from the TZDs structurally, mechanistically, preclinically and clinically. In diabetic rodent models, MBX-102 has insulin-sensitizing and glucose-lowering properties comparable to TZDs without dose-dependent increases in body weight. In vitro, in contrast with full PPAR-γ agonist treatment, MBX-102 fails to drive human and murine adipocyte differentiation and selectively modulates the expression of a subset of PPAR-γ target genes in mature adipocytes. Moreover, MBX-102 does not inhibit osteoblastogenesis of murine mesenchymal cells. Compared with full PPAR-γ agonists, MBX-102 displays differential interactions with the PPAR-γ ligand binding domain and possesses reduced ability to recruit coactivators. Interestingly, in primary mouse macrophages, MBX-102 displays enhanced antiinflammatory properties compared with other PPAR-γ or α/γ agonists, suggesting that MBX-102 has more potent transrepression activity. In summary, MBX-102 is a selective PPAR-γ modulator with weak transactivation but robust transrepression activity. MBX-102 exhibits full therapeutic activity without the classical PPAR-γ side effects and may represent the next generation insulin sensitizer. (Molecular Endocrinology 23: 975–988, 2009)

Peroxisome proliferator-activated receptor (PPAR)-γ belongs to the nuclear receptor superfamily of transcription factors and is a member of the NR1C subgroup that includes PPAR-α and PPAR-δ. PPARs are ligand-activated nuclear receptors that display a wide range of biological effects. Although PPAR-γ is most abundantly expressed in adipose tissue and is a known activator of fat cell formation, PPAR-γ also negatively regulates osteoblastogenesis (1) and has recently emerged as a key modulator of inflammatory and immune responses (2). PPAR-γ ligands include a surprisingly diverse array of natural and synthetic molecules among which the thiazolidinediones (TZDs) are the best characterized (3, 4).

PPAR-γ activates transcription in a ligand-dependent manner by binding directly to specific PPAR-response elements in target genes as a heterodimer with the retinoic X receptor. Agonist binding within the PPAR-γ ligand-binding domain (LBD) causes conformational changes leading to the exchange of corepressor for coactivator proteins and a switch from gene repression to activation (5). Synthetic PPAR-γ ligands described as full agonists appear to share a common binding mode, in which the binding domain is differentiated from the LBD structure. Partial PPAR-γ agonists appear to interact with a unique, secondary site at the LBD and in addition to acting as agonists may also negatively influence activation (5). Non-TZD partial PPAR-γ agonists have been identified as selective PPAR-γ ligands by screening libraries for compounds with reduced transactivation activity but robust transrepression activity. MBX-102/JNJ39659100 (MBX-102) is in clinical development as an oral glucose-lowering agent for the treatment of type 2 diabetes. MBX-102 is a non-TZD selective partial PPAR-γ agonist binding within the PPAR-γ ligand-binding domain and possesses reduced transactivation but robust transrepression activity. MBX-102 exhibits full therapeutic activity without the classical PPAR-γ side effects and may represent the next generation insulin sensitizer. (Molecular Endocrinology 23: 975–988, 2009)

Abbreviations: ATRA, All-trans-retinoic acid; CBP, CREB-binding protein; ChIP, chromatin immunoprecipitation; DMSO, dimethylsulfoxide; ET-1, endothelin-1; FABP4, fatty acid-binding protein 4; FBS, fetal bovine serum; FCB, fat cell buffer; FRET, fluorescence resonance energy transfer; GyK, glycerol kinase; HRP, horseradish peroxidase; LBD, ligand-binding domain; LPS, lipopolysaccharide; MCP-1, monocyte chemoattractant protein-1; NCoR, nuclear receptor corepressor; PEPCCK, phosphoenolpyruvate carboxykinase; PGC1α, PPAR-γ coactivator 1α; PPAR, peroxisome proliferator-activated receptor; pRNA, small interfering RNA; SMRT, silencing mediator for retinoid and thyroid-hormone receptor; SP1, selective partial PPAR-γ agonist 1; SP2, selective partial PPAR-γ agonist 2; SREC1, steroid receptor coactivator 1; TIF2, transcriptional intermediary factor 2; TR, time resolved; TRAP220, thyroid hormone receptor-associated protein 220; TZD, thiazolidinedione; WT, wild type; ZDF, Zucker diabetic fatty; ZF, Zucker fatty; ZL, Zucker lean.
acidic head groups interact with three key amino acid residues (H323, H449, and Y473) within the ligand-binding pocket. These interactions configure the C-terminal activation function 2 domain to form a charge clamp in which a conserved glutamate in the activation function 2 helix and a conserved lysine in the LBD grip the LXXLL-containing helical motifs present in most nuclear receptor coactivators (5–7). Interestingly, synthetic ligands that induce partial PPAR-γ transactivation in cell-based assays bind the LBD in a different manner and do not directly interact with the AF-2 helix. These partial agonists exhibit reduced ability to recruit coactivators, diminished adipogenic capacity, and attenuated gene signatures in cultured adipocytes (8–10). Benefits of PPAR-γ activation are demonstrated by the current clinical use of PPAR-γ modulating agents as antidiabetic drugs. Actos (pioglitazone) and Avandia (rosiglitazone), two potent PPAR-γ agonists of the TZD class, induce remarkable insulin sensitization and improved glycemic control in patients with type 2 diabetes (11, 12). However, despite their proven efficacy, these drugs possess a number of deleterious side effects, including significant weight gain, peripheral edema, increased risks of congestive heart failure, and decreased fasting plasma glucose were observed in type 2 diabetics (29, 30). The insulin sensitization properties of halofenate involve PPAR-γ activation because halofenate was recently identified as a non-TZD, selective partial PPAR-γ agonist (31). Although the antidiabetic activities of halofenate were comparable to those of rosiglitazone, halofenate was clearly differentiated from the full agonist by its inability to promote adipogenesis in vitro or weight gain in vivo (31).

We selected MBX-102, the (−) enantiomer of halofenate for further clinical development, and it is currently in phase II clinical development as an oral glucose-lowering agent for the treatment of type 2 diabetes. In this report, we demonstrate that MBX-102 is a PPAR-γ agonist selective for the PPAR-γ receptor. Like halofenate, MBX-102 is a weak transactivator of PPAR-γ and possesses equivalent antidiabetic properties without displaying the classical PPAR-γ-linked side effects observed with full agonists. Importantly, we show that MBX-102 potently transrepresses inflammatory genes. These findings suggest that MBX-102 preferentially activates PPAR-γ transrepression rather than coactivator recruitment and transactivation and potentially explain its antidiabetic efficacy in the absence of side effects.

Results

MBX-102 acid is a selective partial PPAR-γ agonist that is distinct from rosiglitazone

MBX-102 (Fig. 1A) is the (−) enantiomer of halofenate, a drug previously described as a partial PPAR-γ agonist (31). MBX-102 is a pro-drug ester (Fig. 1A), that is rapidly and completely modified in vivo by nonspecific serum esterases to the mature free acid form MBX-102 acid (Fig. 1B), which is the circulating form of the drug. For these reasons, MBX-102 was used for in vivo studies whereas the acid form was used for all in vitro studies.

To investigate whether MBX-102 acid behaves as a PPAR-γ agonist, its ability to bind and activate PPAR-γ in vitro was determined. As shown in Fig. 1C, MBX-102 acid completely displaced a fluorescent PPAR-γ ligand from the ligand-binding domain (LBD) of human PPAR-γ in a dose-dependent manner. In this assay, MBX-102 acid and rosiglitazone displayed EC50s of approximately 35 and about 0.1 μM, respectively. Dose-dependent activation of human GAL4-PPAR-γ was also observed in response to MBX-102 acid and rosiglitazone, with EC50s of about 12 μM for MBX-102 acid and approximately 1 μM for rosiglitazone (Fig. 1D). The maximal transactivation activity of MBX-102 acid was about 10% of that observed with rosiglitazone (Fig. 1D), suggesting MBX-102 acid functions as a partial PPAR-γ agonist in this context. This weak degree of transactivation of PPAR-γ was similar in three different cell lines (HEK, CHO, and CV1 cells; data not shown). MBX-102 did not trans-
activate human GAL4-PPAR-α or -δ (supplemental Fig. 1 published as supplemental data on The Endocrine Society’s Journals Online web site at http://mend.endojournals.org), indicating that MBX-102 acid is highly selective for human PPAR-γ. A very similar PPAR-activation profile of MBX-102 acid was observed for mouse and rat orthologs, including the absolute selectivity for PPAR-γ, the degree of partial agonism, and EC₅₀ values for PPAR-γ activation (supplemental Fig. 1 and supplemental Table 1). MBX-102 acid also had the ability to antagonize rosiglitazone-dependent PPAR-γ activation (supplemental Fig. 2), suggesting that the site of binding for MBX-102 acid overlaps with that of rosiglitazone and supporting the characterization of MBX-102 acid as a partial agonist. Interaction with Tyr473 in helix 12 of the LBD of PPAR-γ is required for the functional activity of full agonists, and mutation of Tyr473 to alanine (Y473A) abolishes the ability of full agonists to activate PPAR-γ (24, 32). The requirement of Tyr473 for the activity of MBX-102 acid was assessed using GALA-human PPAR-γ LBD containing the Y473A mutation. As expected, the Y473A mutation significantly compromised the potency of rosiglitazone and right shifted its transactivation curve with a 36-fold increase in EC₅₀ (WT, ~1.9 μM; Y473A, ~69 μM) (Fig. 1E). In contrast, the Y473A mutation had no effect on the ability of MBX-102 acid to activate PPAR-γ (EC₅₀: WT, ~28 μM; Y473A ~27 μM) (Fig. 1F). These data indicate that MBX-102 binds to the PPAR-γ LBD in a manner that is distinct from but overlapping with rosiglitazone. These results were not unexpected given that partial and full PPAR-γ agonists bind in the same ligand-binding pocket but may bind in a different manner (10).

**MBX-102 acid increases insulin sensitivity of 3T3-L1 adipocytes, and the insulin sensitization effect is PPAR-γ dependent**

PPAR-γ plays a central role in glucose metabolism. TZDs sensitize adipocytes to insulin as shown by increased glucose uptake at submaximal insulin concentrations. The ability of MBX-102 acid to modulate the insulin sensitivity of 3T3-L1 adipocytes was investigated. The PPAR-γ dependency of the insulin sensitization was simultaneously assessed by using small interfering RNA (siRNA)-mediated PPAR-γ gene silencing.

Differentiated 3T3-L1 adipocytes were treated with dimethyl sulfoxide (DMSO), rosiglitazone, or MBX-102 acid in the presence of a submaximal concentration of insulin (0.05 nM), or with endothelin-1 (ET-1, a PPAR-γ-independent stimulator of glucose transport) alone.

In PPAR-γ-containing adipocytes, both insulin and ET-1 increased glucose transport. Insulin-stimulated glucose transport was further enhanced by treatment with either rosiglitazone or MBX-102 acid (Fig. 2A). In PPAR-γ-deficient adipocytes, the enhancement of insulin-stimulated glucose uptake by both PPAR-γ ligands was abolished, confirming the PPAR-γ dependence of the insulin sensitization effect. PPAR-γ silencing was confirmed both at the protein (Fig. 2B) and mRNA (Fig. 2C) levels and was found to be greater than 90%. Near identical results were obtained using a siRNA targeting a different sequence in PPAR-γ (supplemental Fig. 3). ET-1-stimulated glucose uptake was unaffected by PPAR-γ silencing, confirming that the glucose transport system in the PPAR-γ-silenced adipocytes remained intact (Fig. 2A).

**MBX-102 displays robust antidiabetic and insulinsensitizing properties in rodent diabetic models**

Antidiabetic properties including glucose lowering in preclinical models is a hallmark of full PPAR-γ agonists and has also been reported for partial agonists (27). The antidiabetic properties of MBX-102 were evaluated by assessing its glucose-lowering activity in several rodent models of type 2 diabetes, including ob/ob, db/db mice, and Zucker fatty diabetic (ZDF) rats. In all models tested, short-term treatment with MBX-102 led to robust, significant glucose lowering (Table 1).

The ability of MBX-102 to cause insulin sensitization was also examined in vivo. ZDF rats were treated for 4–7 d with either vehicle or MBX-102 (100 mg/kg) after which their insulin sensitivity was determined using a hyperinsulinemic-euglycemic clamp. A significant reduction in plasma insulin was observed after MBX-102 treatment (Table 2). When compared with the control group, MBX-102-treated rats showed significant increases in the glucose infusion rate (Fig. 3A) and in the rate of glucose disposal (Rd Clamp values) (Fig. 3B), suggesting that MBX-102 increases insulin sensitivity and glucose utilization in peripheral tissues. In addition, compared with the control rats, MBX-102-treated rats also showed significantly decreased hepatic glucose output in the clamped state (Fig. 3C), suggesting that MBX-102 may also positively affect liver insulin sensitivity in ZDF rats.
A pronounced, dose-dependent lowering of triglyceride was observed with all doses of both compounds (Fig. 4C). Moreover, treatment with 30 mg/kg of either MBX-102 or rosiglitazone led to maximal increases in plasma adiponectin levels (Fig. 4D). These results demonstrate that MBX-102 has physiological efficacy that is equivalent to the full agonist rosiglitazone.

In contrast, MBX-102 and rosiglitazone displayed very different side effect profiles (Fig. 4, E–H). Body weight was elevated after rosiglitazone treatment but not by MBX-102 (Fig. 4E). Rosiglitazone caused a gradual, dose-dependent, significant increase in cumulative body weight gain at doses as low as 1 mg/kg whereas no significant increase in body weight gain was observed with MBX-102 at any dose (supplemental Fig. 5A). Differential modulation of food intake was also recorded between both drugs (supplemental Fig. 5B). Heart weight was unaffected by MBX-102 treatment, but a significant increase was observed at the highest dose of rosiglitazone tested (30 mg/kg; Fig. 4F). Increased heart weight has been observed in preclinical studies with other PPAR-γ agonists, is related to increases in plasma volume, and may be predictive of edema (33). Rosiglitazone markedly increased the mass of the epididymal white adipose (EWAT, Fig. 4G) and of the intrascapular brown adipose (IBAT, Fig. 4H) in a dose-dependent manner, whereas marginal increases were detected only with the highest dose of MBX-102 (Fig. 4, G and H). These observations confirm that, at equivalent efficacious doses, MBX-102 displays a more desirable side effect profile compared with rosiglitazone.

**TABLE 1.** MBX-102 lowers fasting plasma glucose levels in diabetic rodent models

<table>
<thead>
<tr>
<th>Animal model</th>
<th>Treatment duration (days)</th>
<th>Mean fasting plasma glucose ± SEM (mg/dl)</th>
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<tr>
<td>db/db</td>
<td>12</td>
<td>426.1 ± 34.7 (n = 12)</td>
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<tr>
<td></td>
<td></td>
<td>224.5 ± 17.0* (n = 12)</td>
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<tr>
<td></td>
<td></td>
<td>190.4 ± 29.3* (n = 12)</td>
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<tr>
<td></td>
<td>9</td>
<td>409.9 ± 40.1 (n = 12)</td>
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<tr>
<td></td>
<td></td>
<td>177.9 ± 8.4a (n = 12)</td>
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<tr>
<td></td>
<td></td>
<td>221.9 ± 9.8a (n = 8)</td>
</tr>
<tr>
<td>ZDF</td>
<td>11</td>
<td>397.0 ± 57.5 (n = 6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>144.7 ± 10.9 (n = 6)</td>
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<tr>
<td></td>
<td></td>
<td>184.8 ± 17.2 (n = 6)</td>
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*P < 0.001 vs. vehicle group; **P < 0.01; one-way ANOVA and Tukey’s multiple comparison test.

**FIG. 2.** MBX-102 acid enhances insulin sensitivity in vitro in 3T3-L1 adipocytes. Rosiglitazone and MBX-102 acid enhanced glucose uptake activity at submaximal concentration of insulin (0.05 nM) in 3T3-L1 adipocytes (A, Scramble). Silencing of PPAR-γ with P17 siRNA duplexes, as assessed at the protein (B) and message levels (C), fully abolished enhancement by both PPAR-γ agonists (A, PPAR-γ P17). In contrast, enhancement of basal glucose transport by ET-1 was not abolished in PPAR-γ-deficient adipocytes (A, PPAR-γ P17), indicating the cells were able to properly respond to non-PPAR-γ stimuli. Values represent mean ± SEM (n = 4; *P < 0.001 vs. DMSO-treated adipocytes, two-way ANOVA and Bonferroni multiple comparison test). FC, Fold change.

**Long-term treatment with MBX-102 led to comparable efficacy compared with rosiglitazone while lacking the typical PPAR-γ side effects**

Full PPAR-γ agonists cause unwanted side effects such as body weight gain, edema, and increased risk of congestive heart failure (4). To examine MBX-102 therapeutic efficacy and safety profile, a long-term study using the Zucker Fatty (ZF) insulin-resistant rat model was performed (Fig. 4). Multiple doses of MBX-102 and rosiglitazone were administered for 50 d to male ZF rats. Doses of 3, 10, 30, 60, and 100 mg/kg of MBX-102 and 0.3, 1, 3, 10, and 30 mg/kg of rosiglitazone were used. As shown in Fig. 4A, treatment with both compounds led to a dramatic decrease in plasma insulin levels. Maximal insulin lowering was detected with 10 mg/kg of rosiglitazone and 60 mg/kg of MBX-102. Both drug treatments also resulted in a dose-dependent, significant decrease in the insulin resistance index (Fig. 4B), which was assessed after 43 d of treatment by performing an oral glucose tolerance test (supplemental Fig. 4). The magnitude of the decrease in insulin resistance was comparable between the two compounds at high doses (10 and 30 mg/kg of rosiglitazone; 60 and 100 mg/kg of MBX-102; Fig. 4B). A
acid also displayed markedly decreased ability to stimulate differentiation of murine 3T3-L1 preadipocytes (supplemental Fig. 6). When compared with rosiglitazone, MBX-102 acid was also found to differentially regulate adipogenic and lipogenic genes in human differentiated primary adipocytes (Fig. 5C). As anticipated, rosiglitazone significantly induced the expression of phosphoenolpyruvate carboxykinase (PEPCK), fatty acid-binding protein 4 (FABP4), and CD36, whereas MBX-102 acid showed attenuated induction. In 3T3-L1 adipocytes, MBX-102 acid modulated some, but not all, of the PPAR-γ responsive genes evaluated. Specifically, MBX-102 acid and rosiglitazone similarly decreased the mRNA levels of 11β-hydroxysteroid dehydrogenase type 1 (HSD11B1) and increased the mRNA levels of pyruvate dehydrogenase kinase 4 and ANGPTL4 (Fig. 5D). As observed in human adipocytes, rosiglitazone induced the expression of key lipogenic genes including glycerol kinase (GyK), PEPCK, FABP4 and CD36, whereas MBX-102 acid showed little (GyK, PEPCK) to no induction (FABP4, CD36) of these same genes (Fig. 5E).

MBX-102 acid displays differential recruitment of coactivators compared with rosiglitazone

Ligand-dependent nuclear hormone receptor activation regulates gene expression through the dissociation of corepressors and subsequent recruitment of coactivators (34). The differential binding of MBX-102 acid to the PPAR-γ LBD may lead to an alteration in the ability to dissociate corepressors and/or to recruit coactivators, possibly explaining the differential gene regulation observed in mature adipocytes treated with MBX-102 acid.

### TABLE 2. General characteristics of the experimental ZDF rats used for the clamp study

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>MBX-102 (100 mg/kg)</th>
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<tbody>
<tr>
<td>Posttreatment body weight (g)</td>
<td>353.4 ± 5.4</td>
<td>351.2 ± 6.0*</td>
</tr>
<tr>
<td>Basal glucose concentration (mg/dl)</td>
<td>183.1 ± 20.5</td>
<td>134.2 ± 8.1*</td>
</tr>
<tr>
<td>Clamped glucose concentration (mg/dl)</td>
<td>152.2 ± 2.2</td>
<td>159.1 ± 1.1a</td>
</tr>
<tr>
<td>Basal insulin concentration (ng/ml)</td>
<td>11.8 ± 1.1</td>
<td>4.6 ± 1.1b</td>
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Values represent mean ± SEM (n = 7 for vehicle-treated group and n = 6 for MBX-102-treated group. * P > 0.05; ** P < 0.001 vs. vehicle-treated, unpaired two-tailed t test).

As observed in human adipocytes, rosiglitazone induced the expression of key lipogenic genes including glycerol kinase (GyK), PEPCK, FABP4 and CD36, whereas MBX-102 acid showed little (GyK, PEPCK) to no induction (FABP4, CD36) of these same genes (Fig. 5E).
and rosiglitazone. We compared the ability of MBX-102 acid and rosiglitazone to modulate the interaction of corepressors and coactivators with PPAR-γ using a time resolved (TR)-fluorescence resonance energy transfer (FRET) assay. MBX-102 acid was able to efficiently displace the corepressors NCoR and silencing mediator for retinoid and thyroid-hormone receptors (SMRT) in a dose-dependent manner and to a similar degree as rosiglitazone (Fig. 6A). In contrast, MBX-102 acid displayed a greatly reduced ability to recruit coactivators compared with rosiglitazone. The relative magnitude of thyroid hormone receptor associated protein 220 (TRAP220), PPAR-102 acid modulated some PPAR-γ-responsive genes similarly (D) and some differentially (E) compared with rosiglitazone. Values represent mean ± SEM and are representative of at least two to three independent experiments (***, P < 0.001; one-way ANOVA and Tukey’s multiple comparison test). FC, Fold change.

**Effect of MBX-102 acid on osteoblast/adipocyte differentiation pathways in mesenchymal cells**

Osteoblasts and adipocytes are derived from a common cell lineage of progenitor cells present in the bone marrow. Terminal differentiation of these progenitor cells to either osteoblasts or adipocytes depends on the temporal expression of transcription factors and external effectors. Full PPAR-γ agonists promote adipogenesis and reciprocally inhibit osteoblastogenesis. The murine mesenchymal cell line C3H10 T1/2 was used to address the effect of MBX-102 on osteoblast and adipocyte differentiation markers. As shown in Fig. 7A, rosiglitazone treatment of C3H10 T1/2 cells inhibited retinoic-acid [all-trans-retinoic acid ATRA]-stimulated alkaline phosphatase expression, an early marker of bone cell phenotype, whereas treatment with MBX-102 acid did not inhibit the expression of this bone cell marker. Moreover, a high dose of MBX-102 acid was able to partly antagonize the adipogenic capacity of rosiglitazone (Fig. 7B).

**MBX-102 displays potent antiinflammatory properties**

TZDs have beneficial antiinflammatory effects, and the ability of the PPAR-γ agonist rosiglitazone to repress inflammatory responses in macrophages has recently been demonstrated (for review, see Ref. 2). The antiinflammatory properties of MBX-102 acid were investigated using mouse primary peritoneal macrophages. Before lipopolysaccharide (LPS) treatment, mouse macrophages were treated with either rosiglitazone or MBX-102 acid. Changes in secreted cytokines were evaluated 24 h after LPS treatment. As shown in Fig. 8A, MBX-102 acid decreased the levels of LPS-stimulated proinflammatory cytokines to a greater [monocyte chemoattractant protein-1 (MCP-1), IL-1β, and IL-6] or slightly less (IL-12p40) extent than rosiglitazone. Similar changes were observed at the gene expression level (supplemental Fig. 7). To assess whether the MBX-102 antiinflammatory effects are PPAR-γ dependent, PPAR-γ deficient [knock-out (KO)] primary mouse macrophages were used. As shown in Fig. 8B, PPAR-γ protein levels were dramatically reduced. In these cells, MBX-102 was unable to repress MCP-1, indicating this effect was mediated through PPAR-γ. Similar results were obtained for IL-12p40. In contrast, upon MBX-102 treatment repression of IL-6 was observed both in wild-type (WT) and KO macrophages, suggesting the suppression of IL-6 secretion in promoter whereas robust recruitment of coactivators was observed after treatment with rosiglitazone (Fig. 6B).
these cells is PPAR-γ independent. Interestingly, rosiglitazone retained some ability to transrepress these cytokines in the KO cells. MBX-102 also displayed impressive antiinflammatory properties in vivo. Decreased macrophage content and MCP-1 gene expression were found in epididymal adipose tissue derived from both db/db mice (data not shown) and ZF rats (supplemental Fig. 8) chronically treated with MBX-102.

MBX-102 displays a unique transactivation/transrepression profile compared with glitazones, glitazars, or other selective partial PPAR-γ agonists

Although MBX-102 acid is a weak transactivator of PPAR-γ, it is a potent transrepressor of proinflammatory genes in vitro and in vivo. Based on the role of macrophage PPAR-γ and transrepression in the insulin-sensitizing efficacy of PPAR-γ agonists, we compared the transrepression profile of several selective γ (MBX-102, selective partial PPAR-γ agonist 1 (SP1), selective partial PPAR-γ agonist 2 (SP2), troglitazone, rosiglitazone, and pioglitazone) or dual α/γ (muraglitazar and tesaglitazar) PPAR agonists. Compounds were initially selected based on their potential for transactivating FABP4 in 3T3-L1 preadipocytes. As shown in Fig. 9A, the compounds tested fell in three categories including weak partial activation (MBX-102 acid and SP1), partial activation (SP2, pioglitazone), or full activation (rosiglitazone, troglitazone, muraglitazar, and tesaglitazar) of FABP4 gene expression. MCP-1 secretion from LPS-stimulated primary mouse macrophages was used to profile the transrepression potential of each compound. Although all compounds significantly decreased MCP-1 secretion, the transrepression potential differed between compounds and was not correlated to their transactivation activity (Fig. 9B). As summarized in Fig. 9C, four separate categories of compounds could be identified based on the two-dimensional combination of their transactivation and transrepression abilities. Muraglitazar, troglitazone, and SP2 were both strong transactivators and transrepressors (TA/TR) whereas tesaglitazar, rosiglitazone, and pioglitazone were strong transactivators but weak transrepressors (TA/tr). Interestingly, although both SP1 and MBX-102 were equally weak
transactivators, they fell into two distinct categories because only MBX-102 displayed a strong ability to transrepress MCP-1 secretion (ta/TR), whereas SP1 was the weakest transrepressor (ta/tr).

**Discussion**

MBX-102 is the (−) enantiomer of halofenate and is currently in clinical development as a next generation insulin sensitizer. It displays glucose-lowering activity in diabetic patients (35), yet its mechanism of action is unclear. In this report, we provide evidence that MBX-102 is a selective partial agonist of PPAR-γ that differs from TZDs structurally, mechanistically, and preclinically. MBX-102 has a unique PPAR-γ activation profile and, in preclinical models, has potent insulin sensitization and antidiabetic activities without the side effects observed with PPAR-γ full agonists.

Our *in vitro* studies demonstrate that MBX-102 is a selective ligand and activator of PPAR-γ having no ability to activate either PPAR-α or PPAR-δ. MBX-102 is a partial PPAR-γ agonist that has weak transactivation potential and antagonizes rosiglitazone in cell-based settings. Binding to tyrosine 473 in helix 12 of the PPAR-γ LBD is required for the activity of full agonists, and absence of this interaction is a hallmark feature of partial PPAR-γ agonists (24, 32). Similar functional potency of MBX-102 acid with wild-type and mutated Y473A PPAR-γ in our reporter gene assay further supports its partial agonist status. These studies suggest that the binding site for MBX-102 acid in the PPAR-γ LBD overlaps with, but differs from, that of rosiglitazone, and we have confirmed this by solving the x-ray crystal structure of MBX-102 acid-bound PPAR-γ LBD (data not shown).

*In vitro* and *in vivo* studies demonstrate that MBX-102 is a potent insulin sensitizer and that short-term treatment with MBX-102 efficiently lowers glucose in several diabetic rodent models. Although commonly used for the assessment of partial PPAR-γ agonists (23, 36–40), short-term studies are not adequate for observing adverse events that require chronic drug exposure. Thus, long-term comparative studies are necessary to demonstrate an improved safety profile over the currently marketed drugs. In long-term studies in the insulin-resistant obese ZF rat model, MBX-102 treatment enhanced insulin sensitivity to a similar degree as rosiglitazone. Unlike rosiglitazone, MBX-102 did not affect body or heart weight and caused much smaller increases in adipose tissue masses, suggesting that MBX-102 possesses minimal adipogenic capacity *in vivo.*
This is in agreement with our in vitro data, which demonstrate that MBX-102 has reduced ability to drive murine and human adipogenesis and reduced ability to up-regulate key lipogenic genes in adipose cells. Because these effects on adipose tissue are characteristic of the full PPAR-γ agonists and are believed to be important for their insulin sensitization actions (41), our results raise an interesting question. In clinical and/or preclinical studies, treatment with TZDs have been shown to cause an adipose tissue remodeling, consisting of a decrease in intraabdominal fat and an increase in sc fat (42–47) that contains small, newly differentiated, insulin-responsive adipocytes (48, 49). Therefore, it would be of great interest to evaluate whether a partial PPAR-γ agonist such as MBX-102, which appears unable to change the overall adipose mass, also causes adipose tissue remodeling. Further studies are necessary to answer this question, including careful analysis of body composition, individual adipose depot mass, and adipocyte cell size distribution.

Exposure of rodent or human white adipocytes either in culture or in vivo to full PPAR-γ agonists leads to increased mitochondrial mass, induction of mitochondrial genes such as UCP-1, Cox8b, Cox7a1, and genes in the fatty acid oxidation pathway, resulting in enhanced oxygen consumption and lipid oxidation (50–52). Because both our in vitro and in vivo gene expression studies were rather limited, additional studies are needed to determine whether a partial PPAR-γ such as MBX-102 also has the ability to modulate the expression levels of these mitochondrial genes in adipose cells. Assessing whether MBX-102 treatment can positively modulate energy expenditure in future experiments would also be important.

Ligand-dependent nuclear hormone receptor activation turns on gene expression through the dissociation of corepressors and sequential recruitment of coactivators (5). PPAR-γ partial agonists have weaker coactivator recruitment activity compared with full agonists (23, 27). We have shown this to be true for MBX-102 using FRET and ChIP analyses. Weak recruitment of coactivators by MBX-102 acid is not due to weak interaction of MBX-102 acid with PPAR-γ because MBX-102 acid induced efficient dissociation of the corepressors NCoR and SMRT. As proposed for other partial PPAR-γ agonists (8, 23), the markedly decreased adipogenic capacity of MBX-102 could be explained in part by its limited ability to recruit pro-adipogenic coactivators such as TIF2, CBP, P300, and TRAP220 (53). Differential coactivator recruitment is a likely mechanistic explanation for the differential gene expression profiles of MBX-102 and rosiglitazone.

The adipogenic activity of TZDs is associated with a reciprocal decrease in osteoblast differentiation and subsequent bone loss in rodents (8, 23, 54–58). Decreased in vitro adipogenic capacity is highlighted for most partial PPAR-γ agonists (4, 16, 23–27), but little information is available on their bone-related effects. Currently marketed TZDs have adverse skeletal actions that include decreased bone formation, accelerated bone loss, and increased risk of fracture (13–15, 59–61). Unlike rosiglitazone, MBX-102 has weak adipogenic activity in multipotent stem cells and, in these same cells, does not inhibit osteoblast differentiation. These data predict that the increase in fractures observed in women treated with TZDs will not be observed with MBX-102. Because PPAR-γ has recently been shown to regulate osteoclastogenesis in mice (62), preclinical investigations on the effects of MBX-102 on osteoclast differentiation and bone resorption are needed to better predict how MBX-102 will affect bone safety in a clinical setting.

Inflammation is a critical component in the pathogenesis of insulin resistance and the metabolic syndrome (63). Macrophages are a primary source of proinflammatory factors such as TNFα, IL-6, and MCP-1. Recent studies have shown that PPAR-γ plays an important role in the regulation of macrophage infiltration and activation in adipose tissue and atherosclerotic plaques (20, 21, 64), and TZDs have been shown to repress the expression of a number of nuclear factor-kB-dependent inflammatory genes (2, 22). MBX-102 is a potent transrepressor, and our data suggest this effect is partially PPAR-γ dependent and partially PPAR-γ independent, based on the cytokine assessed. Although activation of PPAR-δ by high concentrations of PPAR-γ agonists has been cited as a possible explanation for the PPAR-γ-independent repression of inflammatory genes in PPAR-γ-deficient macrophages (65), it can be ruled out here because MBX-102 has no ability to transactivate PPAR-δ in cell-based reporter assays. Surprisingly, rosiglitazone was able to partially transrepress the cytokines tested in our PPAR-γ-deficient macrophages. Although PPAR-γ protein was dramatically down-regulated in our knockout cells, we cannot claim it was fully abolished. Therefore, we cannot exclude the possibility that rosiglitazone was transrepressing inflammatory
genes through very low levels of remaining PPAR-γ protein in the knockout cells.

The molecular mechanisms by which MBX-102 transrepresses inflammatory mediators have yet to be established. PPAR-γ transrepresses inflammatory activation of macrophages by binding to nuclear receptor/corepressor complexes on the promoters of nuclear factor-κB target genes (22). Association of PPAR-γ at these sites requires ligand-dependent sumoylation and inhibits the exchange of corepressors for coactivators upon inflammatory stimulation. Thus, ligand-bound, sumoylated PPAR-γ transrepresses activation of inflammatory genes. For example, rosiglitazone and the partial agonist GW0072 both inhibit inflammation-induced inducible nitric oxide synthase expression and corepressor removal at the inducible nitric oxide synthase promoter (22). Although only rosiglitazone has been reported to induce PPAR-γ sumoylation, other transrepression-capable PPAR-γ ligands, such as MBX-102 acid, presumably induce PPAR-γ sumoylation as well.

One interesting aspect of this study relates to the transrepression ability of the various compounds tested. Our data demonstrate that strong transrepression activity is not necessarily a characteristic of partial selective PPAR-γ agonists. For example, SP1 is a weak transactivator and also a weak transrepressor. The unique profile of MBX-102 (i.e. weak transactivator and strong transrepressor) demonstrates, for the first time, that it is possible to separate transactivation and transrepression activities of PPAR-γ ligands and may explain why such a weak transactivator can retain full antidiabetic activity with reduced side effects.

Taken together, the evidence presented here strongly supports the claim that MBX-102 is an optimized, selective partial PPAR-γ agonist exhibiting full insulin sensitization activity with minimal adipogenic capacity both in vitro and in vivo. MBX-102 does not inhibit osteoblastogenesis or cause heart weight gain, highlighting its potential for lacking the negative skeletal and cardiovascular side effects seen with the currently marketed drugs. In agreement with these preclinical results, phase 2a clinical trial data indicate that MBX-102 significantly lowers plasma glucose levels in the absence of side effects such as weight gain and edema that are observed with the currently used pharmacological agents (35). This clinical trial was conducted using insulin-treated diabetic patients who are particularly sensitive to the TZD-induced side effects. Thus, these reported results obtained with MBX-102 are in stark contrast to those observed with the currently marketed TZDs. MBX-102 may therefore represents the next generation of insulin sensitizer and holds promising therapeutic potential in the treatment of type 2 diabetes.

**Materials and Methods**

**Chemicals**

MBX-102, SP1, SP2 (selective partial PPAR-γ agonist 2), rosiglitazone maleate, tesaglitazar, muraglitazar, and GW501516 were synthesized at Metabolex (Hayward, CA). Troglitazone and GW7647 were obtained from Sigma-Aldrich (St. Louis, MO).

**Cell-based reporter assay**

The LBD for human PPAR-γ (amino acids 172-476) was obtained by PCR and cloned into pFA-CMV plasmid (Stratagene, La Jolla, CA) to generate a Gal 4-human PPAR-γ LBD chimera. Gal4-human PPAR-γ Y473A LBD was generated by site-directed mutagenesis (QuikChange Site-Directed Mutagenesis Kit; Stratagene) as per manufacturer’s instructions using the following oligonucleotides: forward: CGCTCCTG-CAGGAGATCGCAAGGACTTGTACTAG and reverse: CTAGTACAGGCTTGGCAGATCTCCTGCAGGAGCG. HEK-293T cells were transfected with either Gal 4-human PPAR-γ LBD or Gal4-human PPAR-γ Y473A LBD, pFR-Luciferase, and Lac-z plasmids using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) and incubated for 4 h before treatment with compound for 20–24 h. Expression was assayed using the Steady-Glo assay system (Promega Corp., Madison, WI) according to manufacturer’s instructions. Fluorescence emission (excitation, 485 nm; emission, 535 nm) was measured after addition of 100 μL of 10 μM Fluorescein di-bromo-galactopyranoside (Invitrogen) in assay buffer (2.1 mM KH₂PO₄, 310.3 mM NaCl, 5.9 mM Na₂HPO₄·7H₂O, 20 mM KCl, 2 mM MgSO₄, 0.2% Triton X-100). Each experimental condition was performed in triplicate. The data were normalized for each well by dividing the luminescence measurement by the fluorescence measurement. Dose-response curves were generated and EC₅₀ values were calculated using Prism version 5.01 (GraphPad Software, Inc., San Diego, CA).

**Binding assay**

The binding between test compounds and the human PPAR-γ LBD was measured using the PolarScreen PPAR-γ Competitor Assay (Invitrogen) using the manufacturer’s recommended protocol.

**FRET PPAR-γ coregulator peptide interaction assay**

FRET PPAR-γ coregulator peptide interaction assays were performed using the LanthaScreen TR-FRET PPAR-γ Coactivator Assay Kit (catalog no. PV4548, Invitrogen, Carlsbad, CA) as per manufacturer’s instructions. Human coregulator peptides were synthesized and conjugated to fluorescein by Invitrogen: TRAP220/DRIP (thyroid hormone receptor-associated protein 220, included in the PV4548 kit), CBP (catalog no. PV4596), PGC1α (catalog no. PV4421), SRC1 (nuclear receptor coactivator 1, catalog no. PV4582), SRC2 (nuclear receptor coactivator 2, catalog no. PV4586), NCoR (CoR2, catalog no. PV4624), SMRT (silencing mediator for retinoid and thyroid-hormone receptors, catalog no. PV4423). MBX-102 acid or rosiglitazone was diluted to 2× in assay buffer and 10 μM was added to 384-well black plates. Glutathione-S-transferase-PPAR-γ LBD was diluted to 20 nM in assay buffer and 5 μM was added to the wells. A mixture of Tb anti-glutathione-S-transferase antibody (20 nM) and fluorescein-peptide (0.5 μM) was prepared in assay buffer and 5 μM was added to the wells. The plates were covered, shaken briefly, and incubated at room temperature for 4 h. TR-FRET signal was measured using a PerkinElmer fluorescence counter (BMG Labtech, Durham, NC). The data were calculated as the ratio of the emission intensity of the acceptor (fluorescine: λ = 520 nm) divided by the emission intensity of the donor (Tb: λ = 490 nm). Dose-response curves for each coregulator were done in quadruplicate and the EC₅₀/IC₅₀ values were generated using Prism version 5.01 (GraphPad). Coactivator data were analyzed as a percentage of the maximum rosiglitazone response. Corepressor data were analyzed as a percentage of the maximum recruitment in the absence of ligand. Experiments for CBP, PGC1α, SRC1, and NCoR were repeated twice (n = 2). Experiments for TRAP220 and TIF2 were repeated four times (n = 4). Experiments for SMRT were repeated five times (n = 5).

**Glucose uptake and gene silencing in 3T3-L1 adipocytes**

**Cell culture and electroporation**

3T3-L1 fibroblasts were plated into growth medium [DMEM supplemented with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin] and grown to confluence for 7 d, with media changes every 2–3
d. Differentiation into adipocytes was induced by incubating the cells in DMEM supplemented with 10% FBS, 1% penicillin-streptomycin, 698 nM bovine insulin, 518 nM IBMX, and 248 nM dexamethasone. 3T3-L1 adipocytes (4 d of age) were transferred with siRNA duplexes by electroporation. PPAR-γ siRNA sequence was derived from Katayama et al. (66), and siRNA duplexes, including a scramble control siRNA, were synthesized by Dharmacon (Lafayette, CO). Briefly, 4-d-old differentiated adipocytes were electroporated with 10 nmol of PPAR-γ siRNA duplexes or 10 nmol of scramble control siRNA duplexes using a BTX generator 830 electroporator (settings: 170 V, 7 msec, two pulses). After electroporation, cells were resuspended in 4 ml of DMEM/10% medium, counted and plated into 96-well plates (for glucose uptake assay) or 24-well plates (for cell viability and protein and RT-PCR analysis). Electroporated cells were incubated for 8 h at 37 °C, 5% CO2. The cells were then treated for 16 h with DMSO, MBX-102 acid (100 μM), rosiglitazone (1 μM), or Endothelin-1 (ET-1, 1 nM, obtained from Sigma-Aldrich) prepared in DMEM/10% FBS media. The final concentration of DMSO in all treatments was 0.05%.

2-Deoxy-D-[3H] glucose uptake assay

Glucose uptake activity was determined by measuring the uptake of 2-deoxy-D-[3H] glucose (Amersham/GE, Piscataway, NJ). Briefly, 3T3-L1 adipocytes (electroporated and compound treated as above) were washed once with PBS, two times with fat cell buffer (FCB: 125 mM NaCl, 5 mM KCl, 1.8 mM CaCl2, 2.6 mM MgSO4, 25 mM HEPES, 2 mM pyruvate and 2% BSA, 0.2 μM sterile filtered) and were serum starved by incubation with FCB at 37 °C for 30 min. Insulin was prepared at the indicated concentrations in FCB, added to the cells, and incubated for 20 min at 37 °C. Glucose uptake was initiated by the addition of 2-deoxy-D-[3H] glucose (0.083 μCi/ml) and 1 mM 2-deoxy-D-glucose (Sigma-Aldrich) in FCB and incubated for 10 min at 37 °C. Glucose uptake was terminated by removing the contents of the wells and washing the cells three times with cold PBS. The cells were disrupted with scintillation solution, and 2-deoxy-D-[3H] glucose retained by the cells was counted (MicroBeta Trilux 1450; PerkinElmer, Boston, MA). Cell viability was assessed independently with the CellTitre-Glo Luminous Cell Viability Assay Kit (Promega, Madison, WI) as per manufacturer’s instructions. Glucose uptake was quantified by normalizing the glucose uptake measurement for each compound treatment to the corresponding cell viability value. The fold induction of glucose uptake was calculated by normalizing all values against the average value of the scramble control basal value (taken as 1-fold).

PPAR-γ protein levels

After experimental treatments, PPAR-γ and actin levels were assessed by Western blot using anti-PPAR-γ antibodies and anti-actin, horseradish peroxidase (HRP) antibodies (1:250 dilution, goat polyclonal anti-Actin, HRP conjugated (sc-1616), Santa Cruz Biotechnology) followed by antirabbit, HRP antibodies. Mouse adipocyte cultures and treatment for gene expression analysis on a custom quantitative nuclease protection multiplex array. Two independent experiments were performed using two different cell lots (lots L042503ab and L060403T). Final results were pooled, and the fold change vs. vehicle was calculated for each compound treatment.

Human adipocyte cultures and treatment for gene expression analysis

Differentiated human adipocytes were obtained from Zen-Bio and were treated for 48 h with DMSO (0.1%), MBX-102 acid (150 μM), or rosiglitazone (1 μM). After treatment, the cells were harvested for gene expression analysis using a quantitative nuclease protection technology (HTG, Inc., Tucson, AZ). Briefly, the cells were harvested with HTG proprietary lysis buffer, and cell lysates were shipped to HTG for mRNA measurement on a custom quantitative nuclease protection multiplex array. Resulting DNA fragments were visualized by ethidium bromide gel electrophoresis.
lipogenesis (adipogenic marker) and alkaline phosphatase activity (early osteoblast marker), cells were seeded in 96-well plates at a density of 10,000 cells per well. Two days after reaching confluence the cells were treated with 200 nM insulin, 500 nM ATRA (Sigma-Aldrich) and varying concentrations of compound. Fresh media and compounds were added at d 4, and lipogenesis and alkaline phosphatase activity was assessed at d 8. At d 8 cells were washed with PBS followed by hypotonic lysis in the presence of 1% Triton X-100 at 4°C for determining alkaline phosphatase (ALP) activity. For triglyceride accumulation, cells were lysed in 1% digitonin (Sigma-Aldrich) after being washed in PBS. Total triglyceride accumulation was determined using Trigent reagent (Sigma-Aldrich) by measuring absorbance at 540 nm. Alkaline phosphatase activity was measured using p-nitrophenol as a substrate (phosphatase substrate, Sigma-Aldrich) by measuring absorbance at 405 nm. Cell density and viability were measured with a nonradioactive cell proliferation assay [CellTitre 96 Non-Radioactive Cell Proliferation Assay (Promega Corp., Madison, WI)].

In vivo studies

The Metabolic Institutional Animal Care and Use Committee approved all animal care and experimental procedures described below. All animals were housed in temperature (22 ± 3°C) and humidity (55 ± 4%) controlled rooms, with 12-h light (0600–1800 h) dark cycle. Unless specified otherwise, mice were housed four to five mice per cage, and rats were housed two rats per cage and were allowed ad libitum access to tap water and Purina Rodent Chow (stock no. 5001 4.5% fat; Nestle). Rats were housed two rats per cage and were allowed to recover at least for 2 d. All animals were housed in temperature (22°C) and humidity (55%) controlled rooms. In vivo chronic efficacy study

In vivo chronic efficacy study

Male 9-wk-old Zucker Lean (ZL) and Zucker Fatty (ZF) rats were obtained from Charles River Laboratories. Vehicle and drug suspensions were administered to the rats daily by oral gavage for 50 d. Eight rats were assigned to each of the following groups: ZL Vehicle (5 ml/kg), ZF Vehicle (5 ml/kg), ZF + rosiglitazone maleate (0.5, 1, 3, 10, and 30 mg/kg) and ZF + MBX-102 (3, 10, 30, 60, and 100 mg/kg). Body weight and food intake were recorded every 2–4 d. An oral glucose tolerance test was performed at d 43 of dosing (supplemental Fig. 2 and supplemental methods). Blood samples (~250 µl) were collected to measure plasma glucose and insulin levels. The insulin resistance index was calculated by multiplying glucose by insulin values (glucose × insulin) at each time point. The glucose and insulin area under the curves were calculated according to the trapezoidal rule. The insulin resistance area under the curve was calculated by applying the trapezoidal rule to the insulin resistance index values obtained for each time point, as described above. Blood and tissue samples were collected at study end (d 51) by cardiac puncture after a 6-h fast to measure insulin, triglycerides, and adiponectin.

Reagents and assays

Plasma glucose levels were measured using the method of Trinder (Glucose Oxidase G7016; Peroxidase P8125; Sigma Chemical Co.). Plasma triglycerides were measured using a triglyceride diagnostic kit (kit 344, Sigma Chemical Co.), plasma insulin and adiponectin levels were determined using a rat Insulin EIA kit (catalog no. 80-INSRTU-E10; ALPCO Diagnostics, Salem, NH) and rat adiponectin EIA kit (catalog no. 44-ADPRT-E01; ALPCO Diagnostics) respectively, according to the instructions provided by the manufacturer.

Short-term in vivo efficacy studies

For the ob/ob study, 8-wk-old male ob/ob mice (The Jackson Laboratory, Bar Harbor, ME) were used. Vehicle (5 ml/kg, 1% carboxymethyl cellulose), rosiglitazone maleate (10 mg/kg), or MBX-102 (125 mg/kg) was administered by oral gavage daily for 12 d. For the db/db study, 11-wk-old male db/db mice were used. Vehicle (5 ml/kg), rosiglitazone maleate (10 mg/kg), or MBX-102 (250 mg/kg) was administered by oral gavage for 9 d. For the ZDF rat study, 9-wk-old male ZDF rats (Charles River Laboratories, Inc., Wilmington, MA) were used. Vehicle (5 ml/kg), rosiglitazone maleate (4 mg/kg), or MBX-102 (100 mg/kg) was administered by oral gavage for 11 d. At the end of treatment, blood samples were collected after a 6 h fast by tail nip.

Clamp studies

Male ZDF rats were obtained from Harlan Laboratories (San Diego, CA) at 8 wk of age. ZDF rats were single housed and allowed access ad libitum to tap water and chow (Purina 5008 diet; Ralston Purina Co., St. Louis, MO). ZDF rats were screened into three groups with similar mean plasma glucose levels. ZDF rats were cannulated in the jugular vein and the carotid artery and were allowed to recover at least for 2 d. The following day, test compounds were prepared at 2 mg/ml in DMEM/10% FBS. Medium was aspirated from the cells, and 250 µl/well fresh DMEM/10% FBS followed by 250 µl/well 2× compound was added to the wells. The final concentration of DMSO was 0.1% in all conditions tested. The cells were incubated with compound for 72 h and then harvested in Qiagen Lysis Reagent (Qiagen, Chatsworth, CA). Total RNA was isolated and RT-PCR (Taqman) was performed as described above using the following ABI probe
gene expression assay mixes for FABP4 (catalog no. Mm00445880_m1) and Arbp (catalog no. Mm00725448_s1).

Statistical analysis

Data are expressed as mean ± SEM. Prism software (GraphPad version 5.01) was used for all statistical analysis. Unless specified otherwise in the figure legends, one-way ANOVA followed by Tukey’s or Dunnett’s multiple comparison tests, or two-way ANOVA followed by Bonferroni post tests was used to assess statistical differences between groups.

Acknowledgments

We thank Carl Mondon, Vanina Barreiro, Bindu Pandey, James Tang, Apurva Chandalia, and Judy Udove (Metabolex, Inc., Hayward, CA) for excellent technical assistance with the in vitro studies. We thank Andrea Bell, Zhonghao Liu, and Paul Lee (Metabolex, Inc.) for their help with the in vitro studies. We thank Jerry Olefsky (University of California San Diego) and Morris Birnbaum (University of Pennsylvania, Philadelphia, PA) for their insightful review of the manuscript.

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Disclosure Summary: F.M.G. is employed by Metabolex, Inc. and has an equity interest in Metabolex, Inc. F.Z. was previously employed by Metabolex, Inc. and has an equity interest in Metabolex, Inc. Y.M. has an equity interest in Metabolex, Inc. F.Z. was previously employed by Metabolex, Inc., 1 DNA Way, South San Francisco, California 94080.

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