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PPARα agonist fenofibrate improves diabetic nephropathy in *db/db* mice

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Peroxisome proliferator-activated receptor α (PPARα) is a member of the ligand-activated nuclear receptor superfamily, and plays an important role in lipid metabolism and glucose homeostasis. The purpose of this study is to determine whether the activation of PPARα by fenofbrate would improve diabetes and its renal complications in type II diabetes mellitus. Male C57 BLKS *db/db* mice and *db/m* controls at 8 weeks of age were divided to receive either a regular diet chow (*db/db*, $n=8$; *db/m*, $n=6$) or a diet containing fenofibrate (*db/db*, $n=8$; *db/m*, $n=7$). Mice were followed for 8 weeks. Fenofibrate treatment dramatically reduced fasting blood glucose ($P<0.001$) and HbA1c levels ($P<0.001$), and was associated with decreased food intake ($P<0.01$) and slightly reduced body weight. Fenofibrate also ameliorated insulin resistance ($P<0.001$) and reduced plasma insulin levels ($P<0.05$) in *db/db* mice. Hypertrophy of pancreatic islets was decreased and insulin content markedly increased ($P<0.05$) in fenofibrate-treated diabetic animals. In addition, fenofibrate treatment significantly reduced urinary albumin excretion ($P<0.001$). This was accompanied by dramatically reduced glomerular hypertrophy and mesangial matrix expansion. Furthermore, the addition of fenofibrate to cultured mesangial cells, which possess functional active PPARα, decreased type I collagen production. Taken together, the PPARα agonist fenofibrate dramatically improves hyperglycemia, insulin resistance, albuminuria, and glomerular lesions in *db/db* mice. The activation of PPARα by fenofibrate in mesangial cells may partially contribute to its renal protection. Thus, fenofibrate may serve as a therapeutic agent for type II diabetes and diabetic nephropathy.

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The worldwide prevalence of type II diabetes is rapidly increasing, and diabetic nephropathy is projected to become most common cause of end-stage renal disease and cardiovascular events in the industrialized world. Insulin resistance is a central and pathogenic feature of type II diabetes[1–3] contributing to the development of obesity, dyslipidemia, hypertension, and cardiovascular disease.[4] Hyperglycemia has also recently been demonstrated to be a principal causative factor in the development of micro- and macrovascular complications in diabetic patients.[5,6] Furthermore, dyslipidemia associated with increased plasma triglycerides and decreased plasma high-density lipoprotein cholesterol together with hypertension represent two additional important risk factors associated with cardiorenal complications.

Peroxisome proliferator-activated receptor α (PPARα) is a member of the nuclear hormone receptor superfamily of fatty acid activated transcription factors.[7,8] PPARα binds to a specific peroxisome proliferators response element (PPRE) in the promoter element of target genes, forming a heterodimer with the 9-*cis*-retinoic acid receptor RXRα. PPARα target genes include several key enzymes actively involved in lipid metabolism. PPARα is particularly abundant in tissues exhibiting high levels of energy metabolism, including the brown fat tissue, liver, kidney and heart, and to a lesser extent in skeletal muscle.[9,10] Although its role in lipid metabolism is most firmly established, it has recently been found that PPARα may also play an important role in enhancing insulin action.[11,12] These findings suggest that PPARα ligands might provide novel therapeutic agents for the treatment of type II diabetes.

Here we report the effect of fenofibrate, a specific PPARα ligand, on hyperglycemia, insulin resistance, and diabetic nephropathy in type II diabetic *db/db* mice. We report that PPARα activation by fenofibrate improves insulin sensitivity, glucose control, and diabetic nephropathy associated with decreased urine albumin excretion and attenuated glomerular mesangial matrix accumulation.

RESULTS

Food intake and body weight

Compared to untreated *db/db* mice, daily food consumption in fenofibrate-treated *db/db* mice was initially not different,

but subsequently significantly decreased after 4 weeks of treatment ($P<0.05$, Table 1). After 2 months of treatment with fenofibrate, the body weight of *db/db* mice was slightly reduced compared with that of control *db/db* mice ($P<0.05$) (Figure 1a).

Glucose, HbA1c, insulin, and Homeostasis model assessment ($HOMA_{IR}$) index

Fenofibrate treatment dramatically improved glycemic control in *db/db* mice so that both blood sugar and HbA1c levels were reduced to levels similar to that seen in *db/m* mice (Figure 1b and c). Compared to untreated *db/db* mice, serum insulin levels in *db/db* mice receiving fenofibrate treatment were significantly reduced after 8 weeks' fenofibrate treatment (2.6 ± 1.2 vs 4.5 ± 0.9 ng/ml, $P<0.05$) (Figure 1d). Surprisingly, we noticed a late increase in serum insulin levels in untreated *db/m* mice at 16 weeks of age that was also blocked by fenofibrate treatment (Figure 1d). Similarly, *db/db* mice receiving fenofibrate treatment exhibited improved insulin sensitivity reflected by significantly lower $HOMA_{IR}$ indexes compared to that in untreated *db/db* animals (2.2 ± 1.3 vs 16.6 ± 3.9, $P<0.001$). Decreased $HOMA_{IR}$ indexes were also observed at the end of study in *db/m* mice treated with fenofibrate (data not shown).

Pancreatic histology

Untreated *db/db* mice exhibited marked hyperplasia and hypertrophy (Figure 2a). In contrast, fenofibrate-treated *db/db* mice showed dramatically reduced islet size to values within the range of that in *db/m* mice ($P<0.01$) (Figure 2b). Immunostaining study further demonstrated that insulin content as reflected by insulin-positive area per islet was significantly increased in pancreatic islets in *db/db* mice treated with fenofibrate treatment compared to untreated mice ($P<0.05$) (Figure 2c). No change in islet size and insulin immunoreactivity was found in fenofibrate treated *db/m* mice.

Table 1 | Effects of fenofibrate on body weight, food intake, and lipid profiles in nondiabetic *db/m* and diabetic *db/db* mice

	db/db	*db/db*-Feno	*db/m*	*db/m*-Feno
Body weight (g)				
Baseline	42.5 ± 1.4	41.9 ± 1.7	24.5 ± 1.8	25.7 ± 2.4
Final	53.4 ± 3.7	47.0 ± 4.0*	26.8 ± 1.4	24.5 ± 3.9
Food intake (g)				
Baseline	4.5 ± 0.7	5.2 ± 0.8	0.8 ± 0.2	1.0 ± 0.3
Final	4.1 ± 0.8	2.2 ± 0.6**	1.1 ± 0.3	0.7 ± 0.3
Lipid profiles TC (mg/dl)				
Baseline	177.1 ± 19.5	173.3 ± 13.8	111.9 ± 32.9	120.2 ± 32.0
Final	159.0 ± 15.9	273.2 ± 35.6*	101.9 ± 9.4	$209.5\pm21.9^{\#}$
VLDL	12.5 ± 2.7	13.3 ± 3.6	ND	ND
LDL	13.1 ± 3.0	10.3 ± 3.0	ND	ND
HDL	140.4 ± 12.4	189.6 ± 10.1*	ND	ND
TG (mg/dl)				
Baseline	154.3 ± 16.3	165.7 ± 16.7	94.3 ± 24.8	109.4 ± 21.4
Final	153.2 ± 26.1	156.1 ± 46.7	99.4 ± 7.9	$117.9\pm25.4^{\#}$

HDL, high-density lipoprotein; LDL, low-density lipoprotein; TC, total cholesterol; TG, triglyceride; VLDL, very low-density lipoprotein; ND, not done. *$P<0.05$, **$P<0.01$ vs *db/db* mice and $^{\#}P<0.05$ vs *db/m* mice.

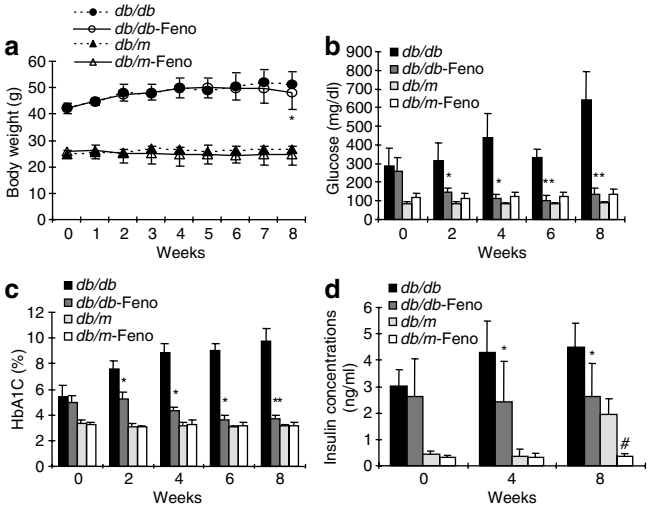

Figure 1 | Changes in (a) body weight, (b) fasting blood glucose, (c) HbA1c, and (d) plasma insulin concentrations in nondiabetic *db/m* and diabetic *db/db* mice treated without or with fenofibrate for 2 months starting at age of 8 weeks. Overnight fasting blood glucose, HbA1c, plasma insulin levels, and body weight were determined as described in 'Materials and Methods'. *$P<0.05$ and **$P<0.001$ vs *db/db* mice, $^{\#}P<0.05$ vs *db/m* mice.

Serum lipid levels

Serum triglyceride levels appeared to be lower in *db/db* mice, but slightly increased in *db/m* mice following 8 weeks' treatment (Table 1). In contrast, serum total cholesterol levels increased in both *db/db* and *db/m* mice after 2 months treatment with fenofibrate ($P<0.05$). The lipoprotein profile showed that the major cholesterol subfraction affected by fenofibrate was comprised of increased high-density lipoprotein-cholesterol with little change in very low-density lipoprotein- or low-density lipoprotein-cholesterol levels in *db/db* mice (Table 1).

Blood chemistry

Blood $Na+$, $K+$, $Cl-$, hematocrit, and creatinine levels showed no significant difference among the groups. Compared to untreated *db/db* mice, fenofibrate-treated *db/db* mice exhibited a slight increased in blood urea nitrogen (BUN) ($P<0.05$) (Table 2). The anion gap in treated *db/db* mice was significantly higher than that in untreated *db/db* mice (13.4 ± 1.8 vs 10.6 ± 2.1, $P<0.02$), suggesting ketosis could contribute to unmeasured anions in treated *db/db* mice.

Organ weight

Fenofibrate treatment of *db/db* or *db/m* mice did not affect kidney weight (Table 3). In contrast, fenofibrate treatment induced dramatic hepatomegaly and reduced epididymal adipose tissue mass in both *db/db* and *db/m* mice. These

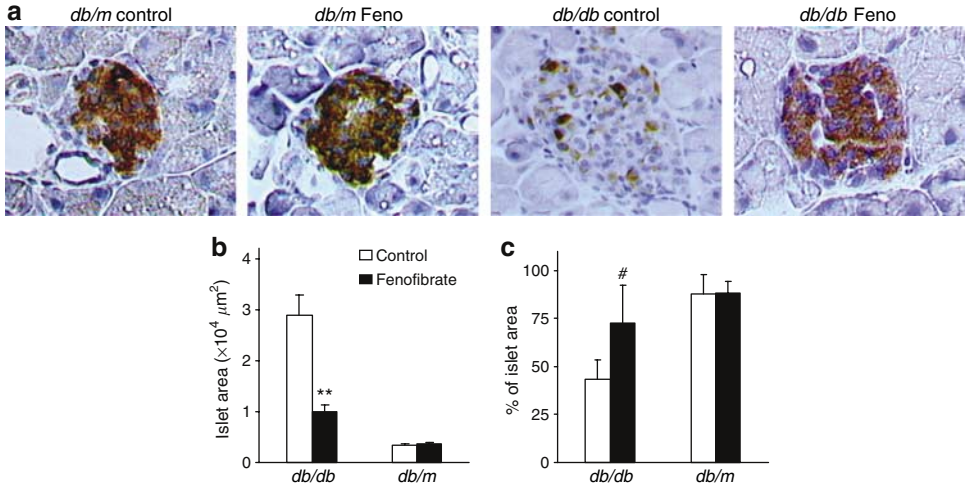

Figure 2 | Pancreatic islets in diabetic *db/db* mice treated with fenofibrate. (**a**) Insulin immunoreactivity in pancreatic islets in *db/db* and *db/m* mice treated with or without fenofibrate (Original magnification: × 200). More insulin positive cells were found in fenofibrate treated *db/db* mice. (**b**) Quantitative analysis showing that marked improvement of pancreatic islet hypertrophy in *db/db* mice receiving fenofibrate treatment compared to untreated *db/db* animals. (**c**) Quantitative comparison of insulin immunoreactivity (% insulin staining-positive area per islet) in pancreatic islets between control *db/db* mice and fenofibrate-treated *db/db* mice. $^{**}P<0.001$, $^{\#}P<0.05$, vs control *db/db* mice; $n=5$ (fenofibrate-treated *db/db* mice) and $n=6$ (untreated control *db/db* mice).

Table 2 | Influences of fenofibrate on blood chemistry in nondiabetic *db/m* and diabetic *db/db* mice

	db/db	*db/db*-feno	*db/m*	*db/m*-feno
BUN (mg/dl)	21.6±4.2	27.3±4.3*	25.6±4.6	30.0±5.0
Creatinine (μg/ml)	1.08±0.21	0.86±0.13	1.08±0.11	1.30±0.10
Anion gap	10.6±2.1	13.4±1.8*	ND	ND
Hematocrit (%)	49.0±2.3	48.1±2.2	45.2±2.7	47.0±3.2

*$P<0.05$ vs *db/db* group.

Table 3 | Kidney, liver, heart, and epididymal fat tissue weight (per 100 g body weight) in nondiabetic *db/m* and diabetic *db/db* mice treated without or with fenofibrate

	db/db	*db/db*-feno	*db/m*	*db/m*-feno
Kidney	0.48±0.04	0.49±0.10	0.54±0.09	0.53±0.08
Liver	5.19±0.16	10.69±0.81**	4.57±0.21	12.9±2.34##
Heart	0.28±0.05	0.35±0.08*	0.50±0.04**	0.54±0.09#
Epididymal	5.48±0.40	4.83±0.40*	0.69±0.16**	0.56±0.28#

*$P<0.05$ and **$P<0.001$ vs *db/db* mice; #$P<0.05$ and ##$P<0.001$ vs *db/m* mice.

changes were more prominent in *db/db* mice. Fenofibrate also increased cardiac weight in both *db/db* and *db/m* mice ($P<0.05$).

Urine volume and albuminuria

Untreated *db/db* mice consumed more water (data not shown) and exhibited greater urine volume than control *db/m* mice (Figure 3a). Following fenofibrate treatment for 2 weeks, water intake (data not shown) and urine output rapidly decreased to levels seen in *db/m* mice ($P<0.001$, Figure 3a). Untreated *db/db* mice exhibited a persistent increase in urine albumin excretion. However, after treatment with fenofibrate for 2 weeks, urine albumin excretion decreased to levels comparable to that in *db/m* mice. Albuminuria in *db/db* mice was persistently reduced throughout the treatment period ($P<0.01$, Figure 3b). Fenofibrate treatment in *db/m* mice did not exhibit any change in water intake (data not shown), urine output (Figure 3a), or albuminuria (Figure 3b).

Renal histological examination

Marked glomerular mesangial expansion in *db/db* mice was improved by fenofibrate treatment (Figure 4a and b). Glomerulometric determinations further showed significantly decreased glomerular surface area in fenofibrate-treated *db/db* mice ($P=0.03$) as well as a tendency for reduced mesangial area ($P=0.07$, Figure 4b).

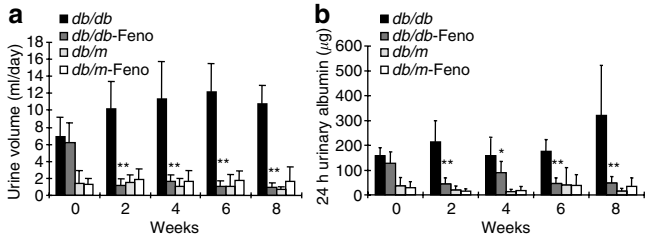

Figure 3 | Fenofibrate decreases (a) urine volume and (b) urine albumin excretion in diabetic *db/db* mice. *db/db* and *db/m* mice were treated with or without fenofibrate (Feno) for 2 months. Urine volume was collected and measured using the metabolic cages every 2 weeks. Urinary albumin excretion was analyzed using the method described in 'Materials and Methods'. *$P<0.01$ and **$P<0.001$ vs. *db/db* mice.

Expression of PPARα in freshly isolated glomeruli and cultured mesangial cells

PPARα mRNA was detected in freshly isolated glomeruli, cultured MCT (a murine renal proximal tubule cell line) cells,

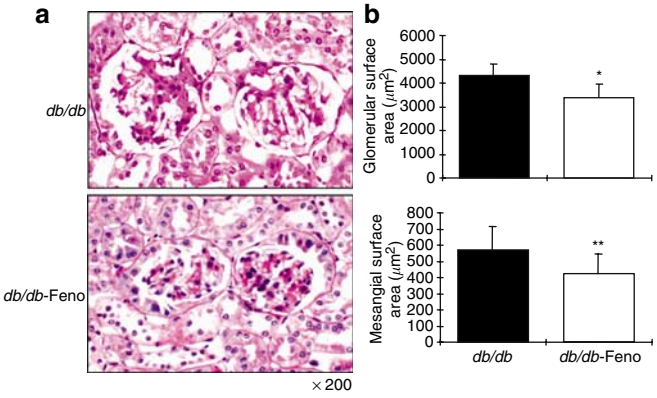

Figure 4 | (a) Renal morphology in *db/db* mice treated with (lower panel) or without (upper panel) fenofibrate. Kidney sections (5 μm) were stained with PAS (Original magnification: × 200). *Note*: reduced glomerular matrix accumulation in fenofibrate-treated *db/db* mouse glomeruli compared to untreated *db/db* mice; (**b**) glomerular surface area and mesangial surface area assessed by glomerulometry. *Note*: decreased glomerular surface area (upper) and a trend towards decreased mesangial surface area (lower) in *db/db* mice receiving 2-month fenofibrate treatment. *$P = 0.03$ and **$P = 0.07$ vs *db/db* group.

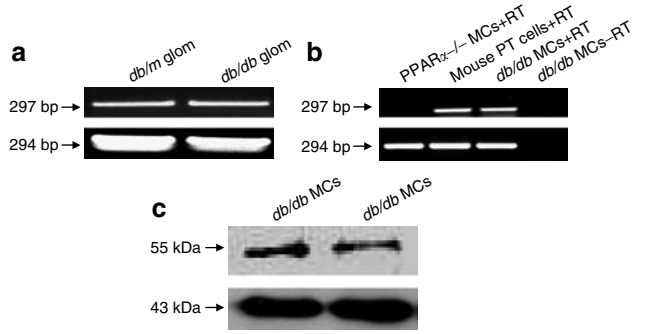

Figure 5 | Analysis of PPARα expression and activity in freshly isolated glomeruli and mesangial cells cultured from *db/db* mice. (**a**) Reverse transcriptase-polymerase chain reaction analysis of PPARα mRNA in 15 freshly isolated glomeruli dissected from a *db/m* mouse and a *db/db* mouse. A single band of 297 bp was observed in both genotypes. β-Actin was utilized as an internal control. (**b**) Total RNA from primarily cultured *db/db* mouse mesangial cells, MCT cells (a murine proximal tubule cell line), and PPARα gene-deficient mesangial cells was extracted using Tri Reagent and used for polymerase chain reaction analysis of PPARα mRNA with or without reverse-transcription. The 297 bp of polymerase chain reaction fragments shown were sequenced and confirmed to be mouse PPARα. (**c**) Western blot analysis was performed using 100 μg of whole-cell lysate from primarily cultured *db/db* mouse mesangial cells. The arrow indicated ~55 kDa bands recognized by a specific PPARα antibody in two distinct mesangial cell lines derived from two individual *db/db* mice. β-Actin was utilized as an internal control.

as well as mesangial cells from *db/db* mice, as assessed by reverse transcriptase-polymerase chain reaction (Figure 5a and b). PPARα mRNA levels were increased by 1.4-fold in the kidneys of *db/db* mice compared to *db/m* mice ($P < 0.05$, $n = 3$), as assessed by real-time polymerase chain reaction analysis. PPARα protein expression was also evident by Western blot in two *db/db* mice mesangial cell lines (Figure 5c).

Functional PPARα activity and inhibitory effect of fenofibrate on high glucose-induced type I collagen production in cultured mesangial cells

PPRE3X luciferase reporter analysis demonstrated that the fenofibrate significantly increased luciferase activity in *db/db* mouse mesangial cells (Figure 6a). Cells cultured under high glucose exhibited more type I collagen production than in cells cultured with normal glucose. Treatment with fenofibrate (10 μM) significantly suppressed collagen I production stimulated by high glucose (Figure 6b).

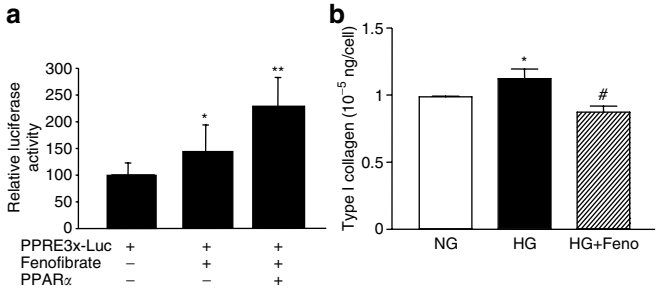

Figure 6 | Functional PPARα activity in cultured mesangial cells and inhibitory effect of PPARα activation on high glucose-induced type I collagen production. (**a**) PPRE3X luciferase reporter assay showing fenofibrate treatment significantly increased luciferase activity in cultured *db/db* mouse mesangial cells with or without PPARα overexpression. Data were presented as mean ± s.d., *$P < 0.05$ vs control; **$P < 0.001$ vs control, $n = 8$; (**b**) activation of PPARα suppresses high-glucose-mediated type I collagen expression. Treatment of primarily cultured *db/db* mouse mesangial cells for 3 days with high-glucose (30 mM) significantly increased type I collagen production as assessed by enzyme linked immunosorbent assay. Fenofibrate, a PPARα agonist, suppressed the high-glucose-mediated increase in type I collagen protein production. *$P < 0.05$ vs cells cultured with normal glucose (5 mM); #$P < 0.01$ vs cells treated with high-glucose. Values are means ± s.d. ($n = 6$) in a single experiment representative of two independent experiments.

DISCUSSION

The present studies demonstrate that the PPARα agonist fenofibrate improves insulin resistance, glucose control, and adiposity in a mouse model of type II diabetic *db/db* mice. Fenofibrate treatment also reduces 24-h urinary albumin excretion and improves renal histopathologic changes, including reduced glomerular hypertrophy and mesangial matrix expansion in *db/db* mice. These beneficial renal effects of fenofibrate appear to be associated both with its insulin-sensitizing effect as well as a direct action on cultured glomerular mesangial cells. These findings suggest that PPARα may represent a potential therapeutic target in treating type II diabetes and its renal complications.

Accumulating evidence suggests that PPARα activators may improve insulin resistance in type 2 diabetic animals[12] and patients with the insulin resistance syndrome.[13] Multiple mechanisms have been postulated regarding the

hypoglycemic and insulin-sensitizing effect of PPARα agonists. PPARα activators have been found to increase hepatic fatty acid catabolism, resulting in decreased systemic and tissue free fatty acid content.[14] Fibrates have also been reported to reduce the triglyceride content in skeletal muscle, which has been correlated with improved insulin sensitivity.[12,15] Finally, PPARα activation suppresses monocyte production of inflammatory cytokines including interleukin-6 and TNF-α, thereby improving insulin resistance.[16–18] A recent report by Koh *et al.*[12] showed that fenofibrate treatment prevents the development of diabetes in OLETF rats by reducing adiposity, improving peripheral insulin action, and exerting beneficial effects on pancreatic β-cells. Here we report that activation of PPARα by fenofibrate also improved glycemic control in *db/db* mice by attenuating insulin resistance, reducing pancreatic islet hypertrophy, enhancing islet insulin expression, and increasing high-density lipoprotein-cholesterol. Taken together, these studies are consistent with the possibility that PPARα activators may provide a novel therapeutic approach for treating insulin resistance and type II diabetes.

The *db/db* mouse is characterized by a G-to-T point mutation of the leptin receptor gene, leading to abnormal receptor splicing and defective signaling of leptin.[19,20] The present studies provide confirmation that the PPARα agonist fenofibrate decreases food intake by a mechanism independent of leptin action. This finding is in sharp contrast to thiazolidinedione PPARγ activators, which increase body weight and adipose tissue mass.[21–23] The mechanisms by which fenofibrate decreases body weight in *db/db* mice remain unclear. In addition to reduced food intake, hypercatabolism induced by fenofibrate may also play a role.[12,24] This is supported by the observation that the BUN levels, BUN/creatinine ratio, and the anion gap were greater in *db/db* mice receiving fenofibrate than in control *db/db* mice. In present studies, it is also unexpected to notice that there was only slight improvement in plasma triglyceride levels in *db/db* mice receiving 2-month fenofibrate treatment. A previous study reports that fenofibrate slightly but significantly lowers serum triglyceride in *db/db* mice treated for 14 days.[25] The difference in findings might be a function of different durations, dosages, and genetic backgrounds studied.

Treatment of rodents with peroxisome proliferators including fibrate can cause liver enlargement via PPARα activation. In the present study, we also observed the hepatomegly as well as cardiomegaly in *db/m* and *db/db* mice receiving fenofibrate treatment. However, histological examination excluded the contribution of steatosis and fibrosis. In fact, fibrate treatment has been reported to be effective in preventing myocardial fibrosis, steatosis, and hepatic fibrosis in several rodent models.[26–28] Most importantly, the hepato-proliferative effect of fibrate does not appear to occur in humans, possibly due to species difference and low PPARα activity.[29]

In present studies, within 2 weeks of fenofibrate treatment, urinary albumin excretion was significantly reduced in diabetic mice and remained low throughout the 2-month period of fenofibrate treatment. The early beneficial effect may also reflect renal hemodynamic changes rather than being directly attributed to renal histological improvement, since fenofibrate has been shown to be able to modulate nitric oxide and eicosanoid production.[30–32] In contrast, the long-term improvement may be associated with improved renal structural features, as supported by the findings that fenofibrate-treated *db/db* mice exhibited decreased glomerular volume and attenuated matrix deposition. Although a profound reduction in albuminuria and a modest improvement in renal histology were evident after 2 months of treatment, studies of longer duration may be able to demonstrate more profound renal protection by fenofibrate.

At present the mechanisms by which PPARα agonists improve diabetic nephropathy remain unclear. Both indirect metabolic effects and direct renal effect seem likely. Improved glucose control and reduced hyperinsulinemia associated with fenofibrate treatment may contribute to reduced albuminuria and improved renal glomerular lesions in *db/db* mice.[33,34] In addition, direct renal actions appear to be involved in beneficial renal effect of fenofibrate in diabetic nephropathy. This possibility is supported by the fact that PPARα activator fenofibrate suppressed exaggerated type I collagen production in high-glucose treated mesangial cells. Therefore, direct renal action may also play an important role in mediating renoprotective effect of fenofibrate in diabetic nephropathy.

In summary, the present studies show the PPARα agonist fenofibrate markedly improves hyperglycemia and insulin resistance in *db/db* mice without inducing weight gain or adiposity. Treatment with fenofibrate also results in marked renoprotective effect in these animals. Our studies suggest that PPARα could serve as an important therapeutic target for treating type II diabetes and diabetic nephropathy as well.

MATERIALS AND METHODS

Animals

Six-week-old male C57BLKS/J *db/db* and *db/m* mice were purchased from Jackson Labs and housed under a standard condition. Fenofibrate (0.2%, w/w, Sigma, St Louis, MO, USA) was mixed into the standard chow diet, and provided to *db/db* mice ($n = 8$) and age- and gender-matched *db/m* mice ($n = 7$) for 2 months starting at age of 8 weeks. Control *db/db* mice ($n = 8$) and control *db/m* mice ($n = 6$) received normal mouse chow for 8 weeks. In total, 250–300 mg/kg/day of fenofibrate were administered in treated *db/db* and *db/m* group.

Measurement of serum parameters

Blood was collected following an overnight fast. Blood glucose and HbA1c levels were measured using HemoCue B-Glucose kit (HemoCue AB, Angelholm, Sweden) and DCA 2000+ HbA1c kit (Bayer, Elkhart, IN, USA), respectively. Plasma insulin levels were measured using radioimmunoassay kit (Linco Reasearch, St Charles, MO, USA). Blood BUN and hematocrit and serum creatinine were

measured using iStat-Kit (HESKA, Fort Collins, MO, USA) and HPLC, respectively.[35] Serum lipid profile was measured using GPO-Trinder kit (Sigma, St Louis, MO, USA) and FPLC. $HOMA_{IR}$ index was calculated as follows: Fasting glucose (mmol/l) × fasting insulin (mU/l)/22.5.

Measurements of urinary parameters

A 24-h urine collection was obtained using metabolic cages. Urine albumin and creatinine concentrations were measured by an immunoassay and the Jaffe alkaline picrate reaction (DCA 2000 + Analyzer, Bayer, Elkhart, IN, USA).

Light microscopy and immunostaining

Kidney and pancreas samples were fixed in 4% formaldehyde. Histology was assessed following HE or PAS staining. Pancreatic samples were also stained with insulin antibody (1:100, Zymed). The surface area of staining of islet was quantified using morphometric software.[36] To examine the effect of fenofibrate on glomerular volume and matrix area, glomerulometry analysis was utilized using PAS-stained kidney sections as previously reported.[37]

Cell culture

Murine mesangial cells from a *db/db* mouse were cultured with some modifications as previously reported[38] and characterized by positive staining for α-smooth muscle actin and negative staining for vWF and cytokeratin.

Reverse transcriptase-polymerase chain reaction

Total RNA was extracted from glomeruli of male *db/db* and *db/m* mice, mesangial cells isolated from wild-type and PPARα null mice and MCT cells, a mouse proximal tubule cell line[39] using Tri Reagent (MRC, Cincinnati, OH, USA). Expression of PPARα was determined by reverse transcriptase-polymerase chain reaction using a specific set of primers: 5′-CGT TCC AGC CCT TCC TCA GTC AGC-3′ (sense) and 5′-GAC ATC CCG ACA GAC AGG CAC TTG-3′ (antisense). In addition, real-time polymerase chain reaction was utilized to assess the mRNA levels of PPARα mRNA in male *db/m* and *db/db* mice (8-week-old, $n = 3$).

Western blots

Samples containing equal amounts of protein (100 μg) were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred onto nitrocellulose membranes, and incubated with a rabbit anti-PPARα antibody (Santa Cruz). The specificity of the antibody used was further confirmed by an immunoprecipitation study using an additional polyclonal antibody from Sigma (Cat# P0869).

Transient transfections and peroxisome proliferators response element-luciferase reporter assays

Mesangial cells were transfected with PPREx3 TK-Luc[38,40] (Qiagen Inc., Valencia, CA, USA). After incubation for 24 h, the transfection mixture was replaced with complete media containing either vehicle or fenofibrate (10 μM). After 24 h, cells were harvested in 1 × luciferase lysis buffer (Dual Luciferase Kit, Promega) and relative light units were determined using a luminometer (Mono light 2010, Analytical Luminescence Laboratory, San Diego, CA, USA).

Type I collagen enzyme linked immunosorbent assay

Mesangial cells (2×10^4) were seeded in each well of a 24-well plate. After washing cells three times with 1 × phosphate-buffered saline, normal (5 mM) or high glucose (30 mM) medium containing 0.1% fetal bovine serum was added to the cells in the presence or absence of 10 μm fenofibrate for 72 h. Media and cell lysate were collected for determination of type I collagen production by enzyme linked immunosorbent assay as previously described.[38] Final values were normalized for cell numbers.

Statistical analysis

The data expressed as means ± s.d. Significance of difference between two groups was evaluated using Student's *t*-test. For multiple comparisons, one-way analysis of variance was used to evaluate differences among groups. A *P*-value of < 0.05 was considered statistically significant.

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