PPARδ Activation Protects Endothelial Function in Diabetic Mice

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Recent evidence highlights the therapeutic potential of peroxisome proliferator–activated receptor-δ (PPARδ), a member of the PPAR family, in regulating vascular function, insulin sensitivity, and lipid metabolism. PPARδ activation improves endothelial function in diabetic and obese mice. PPARδ agonists (e.g., GW1516) were used to investigate whether PPARδ activation improves endothelial function in diabetic and obese mice. PPARδ knockout (KO) and wild-type (WT) mice were used as diabetic mouse models, compared with PPARδ KO and WT mice on normal diet and db/db mice. Endothelium-dependent relaxation (EDR) was measured by wire myograph. Flow-mediated vasodilatation (FMD) was measured by pressure myograph. Nitric oxide (NO) production was examined in primary endothelial cells from mouse aortae. PPARδ agonist GW1516 restored EDRs in mouse aortae under high-glucose conditions or in db/db mouse aortae ex vivo. After oral treatment with GW1516, EDRs in aortae and FMDs in mesenteric resistance arteries were improved in obese mice in a PPARδ-specific manner. The effects of GW1516 on endothelial function were mediated through phosphatidylinositol 3-kinase (PI3K) and Akt with a subsequent increase of endothelial nitric oxide synthase (eNOS) activity and NO production. The current study demonstrates an endothelial-protective effect of PPARδ agonists in diabetic mice through PI3K/Akt/eNOS signaling, suggesting the therapeutic potential of PPARδ agonists for diabetic vasculopathy. Diabetes 61:3285–3293, 2012

Peroxisome proliferator–activated receptor-δ (PPARδ) is the least studied isoform of PPARs, and it is ubiquitously expressed in tissues such as liver, brain, skin, and adipose (1). Recently, the role of PPARδ in obesity and diabetes has been examined by using the loss-of-function approach or synthetic PPARδ ligands. Although it was reported that PPARδ deficiency may lead to reduced adipogenesis (2), the PPARδ knockout (KO) mouse is more prone to weight gain on a high-fat diet, whereas the PPARδ transgenic mouse is protected against obesity and lipid accumulation (3,4). PPARδ agonists GW501516/GW1516, GW0742, and L-165041 can improve the lipid profile in obese animal models through increasing levels of HDL and decreasing LDL cholesterol and triglycerides (5,6).

PPARδ also regulates glucose homeostasis and insulin signaling in various tissues (7–9). PPARδ activation in db/db mice improves hepatic and peripheral insulin sensitivity by increasing glucose consumption in the liver (10). GW0742 treatment or hepatic overexpression of PPARδ attenuates fatty liver and nephropathy in diabetic mice (11,12). In human subjects, GW1516 enhances the HDL level and facilitates triglyceride clearance in healthy individuals by upregulation of fatty acid oxidation in skeletal muscle (13). GW1516 can also lower plasma levels of triglyceride, LDL cholesterol, and insulin in obese men (14). In general, PPARδ is beneficial against obesity, insulin resistance, and metabolic syndrome.

The metabolic functions of PPARδ are likely to be associated with cardiovascular benefits in diabetes. PPARδ is an important transcriptional factor in myocardial metabolism (15,16). PPARδ activation inhibits oxidative stress and inflammation and prevents myocardial hypertrophy in diabetic mice (17). However, the direct effects of PPARδ activation on vascular processes such as angiogenesis and endothelial function are less studied. PPARδ is expressed in endothelial cells (18). Importantly, prostacyclin, which can be released by the endothelium, promotes proangiogenic function in a PPARδ-dependent manner (19). PPARδ agonists enhance the regenerative capacity of endothelial progenitor cells (20,21) and promote endothelial cells from apoptosis (22). PPARδ agonists also inhibit vascular inflammation and reduces atherosclerotic lesions in mouse models (23–26). These experimental observations suggest that PPARδ may play a positive role in vascular activities such as angiogenesis, apoptosis, vascular inflammation, and endothelial vasodilatory function.

Notably, the effect of the PPARδ activator GW1516 to enhance vasoculogenesis is reported to be mediated by the phosphatidylinositol 3-kinase/Akt (PI3K/Akt) signaling pathway (20,21). GW0742 can induce vasodilatation through PI3K/Akt and reduce blood pressure in hypertensive rats (27,28). Up to date, no study has examined the possible role of PPARδ in endothelial dysfunction related to diabetes and obesity. Therefore, the current study investigated the effect of PPARδ activation on endothelial dysfunction in diabetic mice and determined whether or not PPARδ could contribute to the vascular benefit of PPARδ activation.

RESEARCH DESIGN AND METHODS

Animal protocols. Male C57BL6 mice, leptin receptor KO (db/db), with their lean db/+ littermates (both at the age of 12–14 weeks) (PPARδ KO mice and PPARδ WT [wild type]) generated from C57BL6N × Sv129 background were used for this study. PPARδ KO mice were generated as described previously (1). This mouse line has been verified by several studies.
Glucose, whereas 25 mmol/L of mannitol was used as the normal osmotic control. After the incubation period, the rings were transferred to a chamber filled with fresh Krebs solution and mounted in a myograph for measurement of changes in isometric force. Acetylcholine, phenylephrine, and SNP were dissolved in water and others in dimethyl sulfoxide (DMSO).

**Functional assay by wire myograph.** After mice were killed, thoracic aortae were removed and placed in oxygenated ice-cold Krebs solution that contained (mmol/L): 119 NaCl, 4.7 KCl, 2.5 CaCl₂, 1 MgCl₂, 25 NaHCO₃, 1.2 KH₂PO₄, and 11 D-glucose. Changes in isometric tone of the aortic rings were recorded in myograph (Danish Myo Technology, Aarhus, Denmark). The rings were stretched to an optimal baseline tension of 3 mN and then allowed to equilibrate for 60 min before the experiment commenced. Rings were first contracted with 60 mmol/L KCl and rinsed in Krebs solution. After several washouts, phenylephrine (1 μmol/L) was used to produce a steady contraction, and acetylcholine (Ach) (10 μmol/L to 1 μmol/L) was added cumulatively to induce endothelium-dependent relaxation (EDR). Endothelium-independent relaxation to sodium nitroprusside (SNP) was performed in aortic rings, with endothelium removed by gently rubbing with fine forceps.

**Ex vivo culture of mouse aortic rings.** Mouse thoracic aortic rings (2 mm in length) were dissected in sterile PBS and incubated in Dulbecco’s modified Eagle’s medium (DMEM; Gibco, Gaithersburg, MD) supplemented with 10% FBS (Gibco), plus 100 U/mL penicillin and 100 μg/mL streptomycin. Drugs, including GW1516 (PPARγ agonist, 0.1 μmol/L; Alexis Biochemicals, Lausen, Switzerland), GW0742 (PPARδ agonist, 0.1 μmol/L; Tocris Bioscience, Bristol, U.K.), GSK0660 (PPARγ antagonist, 1 μmol/L; Sigma-Aldrich, St. Louis, MO), LY294002 (PI3K inhibitor, 5 μmol/L; Tocris), wortmannin (PI3K inhibitor, 0.1 μmol/L; Sigma-Aldrich), and Akt inhibitor V (API-2/triciribine, 5 μmol/L; Sigma-Aldrich) were individually added into the culture medium that bathed the aortic rings. High-glucose (HG) conditions were achieved by the addition of 25 mmol/L glucose, whereas 25 mmol/L of mannitol was used as the normal control.

**Primary culture of mouse aortic endothelial cells.** The method was modified based on the early reported procedures (35). In brief, mice were anesthetized with an intraperitoneal injection of pentobarbital sodium (40 mg/kg). Heparin (100 units/mL in PBS) was infused into the circulation from the left ventricle. The aortae were dissected in DMEM and incubated with collagenase. After mice were killed, thoracic aortae were dissected and cannulated between two glass cannulas as previously described (30). The vessel diameter was monitored by a Zeiss Axiovert 40 microscope, model 110P, with video camera monitored with the MyoView software (Danish Myo Technology, Aarhus, Denmark). Contraction was induced by 10 μmol/L phenylephrine after the vessel stabilized at 100 mmHg intraluminal pressure, and flow-mediated vasodilatation (FMD) was induced by pressure change, which equals −15 dynes/cm² shear stress. After washout, insulin-induced vasodilatation was examined in phenylephrine-contracted arteries. Passive dilation was tested at the end of experiment in the Ca²⁺-free Krebs solution with 2 mmol/L EGTA. FMD data were expressed as percent of diameter changes = (flow-induced dilation − Phe tone)/(passive dilation − Phe tone).

**Ex vivo adenoviral gene transfer in mouse aorta.** The infection protocol in mouse aortic rings ex vivo was described previously (31). Adenovirus encoding constitutively activated PI3K (Ad-CA-PI3K) and constitutively activated Akt (Ad-CA-Akt) were described previously (32). Adenovirus encoding dominant-negative Akt (Ad-DN-Akt) was described previously (33). An adenovirus carrying green fluorescent protein (Ad-GFP) was used as the internal control (34). Aortic segments were infected with adenovirus (5 × 10⁹ pfu) for 4 h in FBS-free DMEM and then transferred to DMEM with 10% FBS with the addition of 25 mmol/L mannitol (NG), or 25 mmol/L glucose (HG) and/or GW1516 (0.1 μmol/L), for 6 h. Transfection efficiency was verified by monitoring the green fluorescence of Ad-GFP on the endothelial side while also preparing mouse aorta under confocal microscope.

**Western blot showing the effect of GW1516 and the antagonists of its action on eNOS and Akt phosphorylations in primary MAECs treated with HG (30 mmol/L; 36 h).** eNOS, 140 kDa; Akt, 60 kDa. Results are means ± SEM of six mice. *P < 0.05 vs. NG; #P < 0.05 vs. HG; †P < 0.05 vs. HG+GW1516.
for 36 h before the measurement of nitric oxide (NO) using laser confocal fluorescence microscopy.

**Transient transfection of mouse aortic endothelial cells.** Mouse aortic endothelial cells (MAECs) were transfected with either a constitutively active Akt plasmid (CA-Akt), a dominant-negative Akt construct (DN-Akt), or control plasmid by electroporation using the Nucleofector II machine (Amaxa/Lonza, Walkersville, MD) according to the procedure provided by the manufacturer. DNA plasmids were provided by Dr. Wu Zhengu (Hong Kong University of Science and Technology, Hong Kong, China) (36). About 70% of endothelial cells were successfully transfected using these protocols, as indicated by control transfection using a GFP-expressing pCAGGS vector.

**Measurement of NO production.** Fluorimetric measurements were performed on primary MAECs using the Olympus Fluoview FV1000 laser scanning confocal system. 4-Amino-5-methylamino-2',7'-difluorofluorescein diacetate (DAF-FM DA; Molecular Probes, Eugene, OR) was used as the NO indicator. The amount of NO in response to 1 μmol/L A23187 was evaluated by measuring the fluorescence intensity excited at 495 nm and emitted at 515 nm. The cells were stimulated with the calcium ionophore A23187 because there was no calcium or NO signal in response to ACh in the cultured MAECs. Changes in intracellular NO production were displayed as relative fluorescence intensity (F1/F0, where F0 = control and F1 = after A23187).

**Western blotting.** Protein samples prepared from mouse aortae or MAEC homogenates were electrophoresed through a 7.5 or 10% SDS-PAGE gel and transferred onto an immobilon-P polyvinylidene difluoride membrane (Millipore Corp., Bedford, MA). Non-specific binding sites were blocked with 1% BSA in 0.05% Tween-20 TBS. The blots were incubated overnight at 4°C with the primary antibodies anti-phospho-endothelial nitric oxide synthase (eNOS) at Ser1177 (1:1,000; Upstate Biotechnology, Lake Placid, NY); anti-phospho-Akt at Ser473 and Thr308, anti-Akt1 (1:1,000; Cell Signaling Technology, Danvers, MA), and anti-eNOS (1:1,000; BD Transduction Laboratory, San Diego, CA), followed by horseradish peroxidase–conjugated secondary antibody (DakoCytomation, Carpinteria, CA). Anti-GAPDH (1:5,000; Ambion, Cambridge, U.K.) was used to normalize the protein loading.

**Statistics.** Results represent means ± SEM from different groups. The protein expression was quantified by densitometer (FluorChem; Alpha Innotech, San Leandro, CA), normalized to GAPDH, and then compared with control. Comparisons among groups were made using ANOVA followed by an unpaired Student t test. The P values <0.05 were accepted to indicate statistically significant differences.

**RESULTS**

PI3K/Akt contributes to the beneficial effect of PPARδ agonists on endothelium-dependent vasodilatation impaired by HG. Both PPARδ agonist GW0742 (0.1 μmol/L)
and GW1516 (0.1 μmol/L) augmented EDRs that were impaired by exposure to HG in aortae from C57BL/6J mice (Fig. 1A). The effect of GW1516 was abolished by coinoculation with PPARδ antagonist GSK0660 (1 μmol/L) (Fig. 1B). Coinoculation with LY294002 (PI3K inhibitor, 5 μmol/L), wortmannin (PI3K inhibitor, 0.1 μmol/L), or Akt inhibitor V (Akt inhibitor, 5 μmol/L) also diminished the effect of GW1516 to restore EDRs in aortic rings exposed to HG (Fig. 1B and C). GW1516 or GW0742 did not alter EDR in aortic rings in NG (Supplementary Fig. 1). In HG-treated (30 mmol/L, 36 h) primary MAECs, eNOS phosphorylation at Ser1177 and Akt phosphorylation at Ser473 and Thr308 were decreased. These effects were reversed by GW1516 (0.1 μmol/L). Coinoculation with GSK0660, LY294002, or wortmannin inhibited the effect of GW1516 (Fig. 1D–G). Specificity of GW1516 was further confirmed in PPARδ WT and KO mice (Supplementary Fig. 2).

PPARδ agonist enhances the NO production in MAECs. In MAECs, the addition of the Ca²⁺ ionophore A23187 (0.1 μmol/L) induced a rise of the DAF-FM DA fluorescence, which reflects the level of NO production in

![Graphs and images](https://example.com/graphs)

**FIG. 3.** The effect of Akt activity inhibition on the EDRs in mouse aortae and NO production in MAECs. Effects of Ad-GFP (A), Ad-DN-Akt (B), Ad-CA-PI3K (C), and Ad-CA-Akt (D) on EDRs in mouse aortae treated with GW1516 (0.1 μmol/L) and/or HG (30 mmol/L, 36 h). Mouse aortae were exposed to adenovirus for 4 h in serum-free DMEM, and then changed to DMEM with 10% FBS, before vasoreactivity was measured in the myograph. Effects of CA-Akt (E) or DN-Akt (F) transfection on NO production in MAECs coincubated with GW1516 (0.1 μmol/L) and/or HG (30 mmol/L, 36 h). MAECs were transiently transfected with CA-Akt or DN-Akt plasmid by electroporation. G: Summarized data using the area under the curve (AUC) starting from the addition of A23187 for 120 s of E and F. H: Representative Western blot to show the effect of CA-Akt and DN-Akt on Akt and eNOS phosphorylation in MAECs. p-eNOS (Ser1177) and eNOS, 140 kDa; p-Akt (Thr308 and Ser473) and Akt, 60 kDa. Experiments were repeated five times using MAECs from different mice. Results are means ± SEM of five to six experiments. *P < 0.05 vs. NG from each group; #P < 0.05 vs. HG from each group.
the NG group, which was similar in cells treated with GW1516 in the NG group (Fig. 2A, B, and E). HG (30 mmol/L, 36 h) reduced NO production, which was restored by cotreatment with 0.1 μmol/L GW1516 (Fig. 2A, B, and E). GW0742 at 0.1 μmol/L produced a similar effect as GW1516 in HG-treated MAECs, whereas GSK0660 (1 μmol/L) antagonized the effect of GW1516 (0.1 μmol/L) (Fig. 2A, C, and E). Coincubation with LY294002 (5 μmol/L), wortmannin (0.1 μmol/L), or Akt inhibitor V (5 μmol/L) also inhibited the effect of GW1516 (Fig. 2A, D, and E).

**Inhibition of Akt activity diminishes the effect of GW1516 on mouse aortae and MAECs.** Ad-GFP served as control to verify that adenoviral infection did not affect the EDRs of mouse aortae (Fig. 3A). Inhibition of Akt activity by Ad-DN-Akt, the adenovirus expressing dominant-negative Akt, abolished the effect of GW1516 on EDRs in HG-treated aortae from C57BL/6 mice (Fig. 3B). Conversely, increasing PI3K or Akt activity by Ad-CA-PI3K or Ad-CA-Akt, the adenoviruses expressing constitutively active PI3K or Akt, reversed HG-impaired EDRs in mouse aortae (Fig. 3C and D). To further confirm the role of Akt in the effect of GW1516 on NO production in MAECs, we found that CA-Akt slightly increased NO production in HG-treated MAECs (Fig. 3E and G) without affecting other groups, as compared with data presented in Fig. 2B. Suppression of Akt activity by DN-Akt inhibited the restoration of NO production by GW1516 in HG-treated MAECs (Fig. 3F and G). DN-Akt suppressed, whereas CA-Akt increased, Akt phosphorylation (Fig. 3H) at basal level. CA-Akt also increased eNOS phosphorylation stimulated by GW1516 (Fig. 3H).

**PPARδ agonists improve endothelial function in aortae from db/db mice.** Treatment with PPARδ agonists GW1516 (0.1 μmol/L, 24 h) or GW0742 (0.1 μmol/L, 24 h) markedly improved EDRs, which were impaired in aortae from db/db mice (Fig. 4A). Coincubation with GSK0660 (1 μmol/L) antagonized the beneficial effect of GW1516 (0.1 μmol/L) (Fig. 4A). Coincubation with LY294002 (5 μmol/L), wortmannin (0.1 μmol/L), or Akt inhibitor V (5 μmol/L) also attenuated the beneficial effect of GW1516 on db/db mouse aortae (Fig. 4B). To examine the effect of PPARδ activation in vivo, GW1516 was given by oral gavage (5 mg/kg/day, 7 days) to db/db mice. EDRs in aortae from db/db mice were significantly reduced as compared with those from db/m+ mice. The impaired EDRs were improved after GW1516 treatment (Fig. 4C). By contrast, endothelium-independent relaxations to SNP were similar in db/m+, db/db, and db/db treated with GW1516 (Fig. 4D).

**FIG. 4.** The effect of GW1516 treatment on endothelial function in db/db mice. A: PPARδ agonist GW0742 (0.1 μmol/L, 24 h) or GW1516 (0.1 μmol/L, 24 h) improved EDRs in aortae from db/db mice. The beneficial effect of GW1516 was abrogated by coincubation with GSK0660 (PPARδ antagonist, 1 μmol/L). B: The beneficial effect of GW1516 in aortae from db/db mice is abrogated by LY294002 (5 μmol/L), wortmannin (0.1 μmol/L), or Akt inhibitor V (5 μmol/L). C: GW1516 treatment (5 mg/kg/day, 7–10 days) improved EDRs in aortae from db/db mice, without affecting endothelium-independent relaxations to SNP (D). E and F: GW1516 treatment increased phosphorylations of eNOS and Akt in db/db mouse aortae. Results are means ± SEM of n mice (n specified in Supplementary Table 1). *P < 0.05 vs. db/m+; #P < 0.05 vs. db/db.
Phosphorylations of eNOS at Ser1177 and of Akt at Thr308 were restored in aortae from GW1516-treated db/db mice (Fig. 4E and F).

**GW1516 treatment improves FMD and insulin-induced vasodilatation in arteries from DIO mice.** FMD was measured in mesenteric resistance arteries of normal or DIO C57BL/6 mice and of db/db mice. FMD was induced by a pressure change of 20 mmHg, which equals an initial shear stress of ~15 dynes/cm² in pressurized arteries. FMD was improved in mesenteric resistance arteries from DIO or db/db mice treated with GW1516 (Fig. 5A–C). GW1516 treatment also enhanced insulin-induced vasodilatation in arteries from DIO mice (Fig. 5B). ACh-induced relaxation in aortae or FMD in resistance arteries was unaffected in control mice treated with GW1516 (Fig. 5C and Supplementary Fig. 1).

GW1516 treatment in vivo improves endothelial function in DIO mice in a PPARδ-specific manner. PPARδ KO and PPARδ WT mice were fed with a high-fat diet for 12 weeks. GW1516 was administered by oral gavage (5 mg/kg/day, 7 days). EDRs were impaired in aortae from DIO PPARδ KO or DIO PPARδ WT mice compared with control mice of the same genotype on a normal diet (Fig. 6A and B). GW1516 treatment in vivo restored EDRs in aortae from DIO PPARδ WT mice (Fig. 6A), but not in those from DIO PPARδ KO mice (Fig. 6B). Again, endothelium-independent relaxations to SNP were similar in all groups (Fig. 6C and D). Reduced eNOS and Akt phosphorylation upon high-fat feeding in DIO mice was restored after GW1516 treatment only in aortae from PPARδ WT mice, not in those from PPARδ KO mice (Fig. 6E and F).

**FIG. 5.** GW1516 treatment improves FMD and insulin-induced vasodilatation in pressurized mesenteric arteries of diet-induced obese mice. GW1516 was administered by oral gavage (5 mg/kg/day, 7–10 days) to DIO C57BL/6 and db/db mice. A: Representative image of mesenteric resistance arteries from control C57BL/6 mice pressurized at 100 mmHg. B: Representative trace of FMD from each group. Pressure rise was induced in stepwise order (20–100 mmHg). Phe (10 μmol/L) was added after arteries stabilized at 100 mmHg to induce contraction before FMD was examined. C: Summarized FMD response in arteries from all the groups. D: Summarized data of insulin-induced vasodilatation in mesenteric resistance arteries from C57BL/6 control and DIO mice. Results are means ± SEM of four to five mice. *P < 0.05 vs. control or db/m+; #P < 0.05 vs. vehicle.
We demonstrated that PPARδ activation improved endothelial function in diabetic mice through the activation of PI3K/Akt. PPARδ agonists GW1516 and GW0742 improve EDRs in aortae that were impaired by a 36-h exposure to HG.

First, the effects of PPARδ ligands were PPARδ specific, which was verified by using three approaches: 1) two selective PPARδ agonists, GW1516 and GW0742, exhibited similar effects in improving EDRs in arteries and in augmenting the NO production in MAECs; 2) the selective PPARδ antagonist GSK0660 blocked the beneficial effect of GW1516; and 3) the beneficial effect of GW1516 was absent in arteries from PPARδ KO mice. Second, the effect of PPARδ activation in mouse aortae and in MAECs is mediated through PI3K/Akt signaling based on the following observations: 1) the effect of PPARδ agonists to improve NO production in arteries was reversed by pharmacological inhibitors of both PI3K and Akt; 2) the PPARδ agonist–stimulated phosphorylation of Akt and eNOS and the PPARδ-mediated NO production in MAECs were also sensitive to PI3K/Akt inhibition; 3) the suppression of Akt activity by DN-Akt diminished the effect of GW1516 to restore impaired EDRs in mouse aorta and attenuated the effect of GW1516 to enhance NO production as well as phosphorylation of eNOS and Akt in MAECs exposed to HG; and 4) adenoviral constructs encoding the constitutively active Akt or PI3K restored the impaired EDRs in mouse aortae and augmented NO production in MAECs exposed to HG. Finally, we documented the beneficial effects of PPARδ activation on endothelial function by showing that oral GW1516 treatment improves EDRs and FMDs in arteries from db/db and DIO PPARδ WT but not KO mice.

Although PPARδ is expressed in vascular cells (3,18), the role of PPARδ in the regulation of cardiovascular function has not been fully characterized. It is known that PPARδ activation induces angiogenesis and vasculogenesis (19,21). In mouse models of atherosclerosis, PPARδ activation decreases the size of atherosclerotic lesions and suppresses the expression of endothelial adhesion molecules (24,26,37). However, there is no study examining the impact of PPARδ agonists on diabetic vasomotor dysfunction. The current study provides novel evidence demonstrating that PPARδ agonists can restore endothelium-dependent dilatory function in isolated aortae from diabetic mice after high-fat diet (DIO). GW1516 was administered by oral gavage (5 mg/kg/day, 7–10 days) to DIO PPARδ WT and age-matched PPARδ KO fed on high-fat diet for 3 months. Effects of GW1516 treatment on EDRs in aortae from PPARδ WT (A) and PPARδ KO (B) after high-fat diet–induced obesity compared with control mice on normal diet. Endothelium-independent relaxations to SNP in aortae from PPARδ WT (C) and PPARδ KO (D). E and F: Phosphorylation of eNOS and Akt in aortae from DIO PPARδ WT mice increased after GW1516 treatment, an effect that was not observed in those from DIO PPARδ KO mice. Results are means ± SEM of n mice (n specified in Supplementary Table 1). *P < 0.05 vs. control (normal diet) from each group; #P < 0.05 vs. DIO from each genotype.
the current study, glucose metabolism was not significantly affected by in vivo GW1516 treatment in db/db mice and in DIO PPARδ WT and KO mice, although the triglyceride level decreased after GW1516 treatment, which might also contribute to the beneficial effect on vascular function (Supplementary Table 1). Furthermore, a beneficial effect of PPARδ agonists was observed using ex vivo organ culture of isolated mouse aortic segment, as well as cultured endothelial cells, where ambient lipid and glucose levels were constant. In addition, unlike the previous study, which showed that GW1516 decreased GLUT1 activation and expression (38), in the current study, GLUT1 activation and expression were not altered in the endothelial cells by GW1516 (Supplementary Fig. 4). The results from these experiments strongly suggest that PPARδ agonists have a direct effect on endothelium-dependent vasodilatation independent of any effects on lipid or glucose metabolism.

Existing evidence indicates that PPARs benefit endothelial function. Although the function of PPARα is less studied, PPARγ agonists pioglitazone and rosiglitazone improve EDR in resistance arteries and reduce the angiotensin II-induced hypertension in rats (39). PPARα and PPARγ agonists inhibit the thrombin-activated endothelin-1 synthesis in vitro (40). PPARγ also elevates NO bioavailability by increasing its synthesis (41), through activation of p38 MAPK (42), and/or by decreasing its degradation by superoxide anions (43). PPAR agonists also improve vascular function in diabetic patients (44,45). Both animal and clinical studies suggest that PPARs can be potential targets for pharmaceutical intervention in the protection of endothelial function in diabetes. Given the recently reported adverse effect of rosiglitazone on cardiovascular outcomes in diabetic patients (46,47), PPARδ could be an alternative target for the treatment of atherosclerosis, hypertension, and other cardiovascular events in diabetic patients. The current study shows that the activation of the P3K/Akt pathway is essential for the beneficial effect of PPARδ agonists on endothelial function. The PPARδ agonist promotes cell survival through P3K/Akt-dependent mechanisms (48). The interaction between PPARδ and P3K/Akt was also found in other cell type (49). A recent study described that GW1516 promotes vasculogenesis through genomic transcription and nongenomic activation of P3K/Akt via interaction with p85α, a regulatory subunit of P3K (21). In addition, GW0742 and L-165041 at higher concentrations (>1 μmol/L) can directly induce vasodilatation, NO generation, and eNOS phosphorylation, which are partially related to activation of the P3K/Akt pathway in the rat aorta (27).

The present data supported the aforementioned observations that P3K/Akt is one of the most likely downstream mechanisms for the vascular-protective effects of PPARδ activation. Furthermore, we have provided evidence that the benefit of PPARδ on vasodilatation requires the activation of P3K/Akt. In addition, GW1516 did not alter p38, ERK, endothelin-1 activity, or caspase activity in aortae or endothelial cells (Supplementary Figs. 5–8).

Recently, a beneficial effect of GW1516 on dyslipidemia in humans has been observed (13,14). However, the cardiovascular safety and outcome of PPARδ ligands is still under investigation. The current study indicates that PPARδ activation is likely to reduce the risk of adverse cardiovascular events, as it improves the vasodilator function of the endothelium exposed to HG conditions in vitro or ex vivo. These novel findings may help to enhance the prospective of the use of safe PPARδ ligands in combating vascular dysfunction in diabetes and obesity.

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X.Y.T. and W.T.W. designed and conducted the experiments, analyzed the data, and prepared the manuscript. N.W. and Y.H. designed the experiments and prepared the manuscript. Y. Lu, W.S.C., J.L., L.L., and Y.Li. conducted the experiments. S.S.-T.L. provided the transgenic mice. Z.Y.C., J.P.C., and X.Y. assisted with discussion and reviewed the manuscript. Y.H. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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