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European Journal of Pharmacology 572 (2007) 40–48
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Comparison of vascular relaxation, lipolysis and glucose uptake by peroxisome proliferator-activated receptor-γ activation in *+db/+m* and *+db/+db* mice

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Received 16 February 2007; received in revised form 22 May 2007; accepted 23 May 2007
Available online 14 June 2007

Abstract

In this study, we determined the *in vitro* effect of peroxisome proliferator-activated receptor-γ (PPAR-γ) activation on the aortic relaxation, lipolysis and insulin-induced [^{3}H]-glucose uptake of the abdominal (omental) adipocytes of the non-diabetic (*+db/+m*) and obese/diabetic (*+db/+db*) mice. The expression of PPAR-γ (mRNA and protein) in aorta and adipose tissues was evaluated and compared. Cumulative application of ciglitazone, pioglitazone and troglitazone (PPAR-γ agonists) caused a concentration-dependent aortic relaxation (sensitive to 2-chloro-5-nitro-*N*-phenylbenzamide (GW9662) (1 μM, a selective PPAR-γ antagonist) and N^{ω}-nitro-L-arginine methyl ester (L-NAME) (20 μM, a nitric oxide synthase inhibitor)) with a maximum relaxation of ~30% (3 μM) in *+db/+m* mice, whereas no relaxation was observed in *+db/+db* mice. All PPAR-γ agonists examined did not alter the basal lipolysis of both species, but forskolin caused a concentration-dependent lipolysis, with a greater magnitude observed in *+db/+m* mice. Insulin (0.1 and 1 μM) caused an enhancement of [^{3}H]-glucose uptake into adipocytes with a greater magnitude in *+db/+m* mice. In contrast, none of the PPAR-γ agonists tested (0.1, 1 and 10 μM) altered the basal and the insulin (0.1 μM)-induced [^{3}H]-glucose uptake into adipocytes of both species. In addition, there was no difference in PPAR-γ expression (mRNA and protein) in the aorta and adipose tissues between the species. In conclusion, our results demonstrate that PPAR-γ is present in the abdominal (omental) adipose tissue and thoracic aorta. An acute activation of PPAR-γ produced a small (~30%) aortic relaxation (nitric oxide/endothelium-dependent) of *+db/+m* mice. However, all PPAR-γ agonists examined have no acute effect on lipolysis and the insulin-induced glucose uptake into adipocytes of both *+db/+m* and *+db/+db* mice.

Keywords: Peroxisome proliferator-activated receptor-γ; Aortic relaxation; Lipolysis; Glucose uptake; *+db/+db* mice

1. Introduction

The increasing prevalence of diabetes and cardiovascular diseases has become a serious health-service burden to our societies. Hypertension is a common observation of non-insulin-dependent diabetes mellitus and it has been suggested due to the abnormal reactivity of vascular tissues under diseased conditions e.g. an increased contractility and/or impaired dilatation (Chau et al., 2003; Okon et al., 2003; Katakam et al., 2005). Free fatty

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doi:10.1016/j.ejphar.2007.05.070

acids released from the adipose tissues during lipolysis caused aberrant responses of the vascular systems (Sainsbury et al., 2004) that may account for the progression/development of different cardiovascular diseases observed in diabetic patients.

Recent evidence suggested that the peroxisome proliferator-activated receptor-gamma (PPAR-γ) activation has an important link between obesity and atherosclerosis (Kersten, 2002; Marx et al., 1999). PPAR-γ is abundantly expressed in adipose tissues and is important in fat cells differentiation (Freake, 1999) and lipid storage (Tontonoz et al., 1994). More importantly, a gene mutation encoding for PPAR-γ has been suggested associated with extreme obesity (Freake, 1999). In over-weight patients, the basal expression of PPAR-γ is not altered, and a defect in PPAR-γ functions has been suggested for the development of body fatness (Rieusset et al., 1999). Paradoxically, *ob/ob* mice fed with rosiglitazone (a PPAR-γ agonist) resulted in an increase in body weight (Wilson-Fritch et al., 2004), and it is probably related to PPAR-γ activation-mediated adipogenesis (Tontonoz et al., 1994). However, it is not known whether PPAR-γ are involved in adipose tissue lipolysis and the modulation of diabetic conditions on PPAR-γ-mediated lipolysis remains to be determined.

In addition, PPAR-γ receptors are found in different tissues/organs including blood vessels (Buchanan et al., 1995; Ryan et al., 2004). A decade ago, thiazolidinediones, a new class of anti-diabetic agents, have been shown binding to and activating PPAR-γ (Lehmann et al., 1995). Activation of PPAR-γ resulted in an in vitro vascular relaxation (Ryan et al., 2004) and a decrease in blood pressure of rats (Buchanan et al., 1995). The vascular relaxation effect of PPAR-γ agonists observed in different tissues of normal animals has been suggested involving various ion channels gatings modulation (Knock et al., 1999). In addition, it is unknown whether the vascular effects, if any, of the PPAR-γ agonists are altered under the diabetic conditions despite the fact that these agents are currently used in treating Type II diabetes mellitus (Saltiel and Olefsky, 1996). Given the important characteristic of thiazolidinediones on insulin's action (i.e. insulin sensitization), it is important to elucidate the effect, if any, of PPAR-γ agonists on insulin-induced response (e.g. glucose uptake) in adipocytes.

In the present study, the diabetic (+*db*/+*db*) mice were used as these animals are commonly used models for obesity and non-insulin-dependent diabetes mellitus research (Mukherjee et al., 1997; Leckstrom et al., 1999; Winters et al., 2000; Lam et al., 2006). It is important to indicate that the therapeutic/beneficial effects of PPAR-γ agonists on most diabetic patients and animal models are obvious only after a chronic (long-term) use. However, it is well-documented that there are common metabolic and physiological changes (e.g. oedema, increase in body weight) observed after a long-term treatment with thiazolidinediones (Sotiropoulos et al., 2006). In this study, we therefore evaluated the acute effects of PPAR-γ agonists so as to avoid physiological changes occurred (after a long-term administration) that can complicate an accurate interpretation of our results. We examined and compared the acute *in vitro* vasodilatation response, lipolytic effect and glucose uptake caused by the PPAR-γ activation, as well as the underlying mechanism(s) involved in both lean (+*db*/+*m*) and obese/diabetic (+*db*/+*db*) mice.

2. Methods and materials

2.1. Animals

C57BL/KsJ mice (female; 6 months old) (non-diabetic (+*db*/+*m*) mice: 24.5±2.3 g; diabetic (+*db*/+*db*) mice: 58.2±3.4 g were housed under a 12:12-h light–dark cycle (7:00 am, ON; 7:00 pm, OFF) (relative humidity: 55–60%; temperature: 22±1 °C) and were given standard chow (Leiter et al., 1981) and water ad libitum before they were sacrificed by cervical dislocation. The +*db*/+*db* mice displayed typical phenotypes of obesity, hyperinsulinemia and hyperglycemia, as reported by our group previously (Lam et al., 2006). The Animal Experimentation Ethics Committee of The Chinese University of Hong Kong (HKSAR, China) approved all experiments performed in this study (approval no: 04/054/MIS). The recommendations from the Declaration of Helsinki and the internationally accepted principles for the use of experimental animals were adhered to. Every effort was made to limit animal suffering and to limit the number of animals used in these experiments.

2.2. Thoracic aorta and abdominal (omental) fat tissues isolation

Thoracic aorta (length: ~12 mm; O.D. ~0.8 mm) were dissected from animals immediately after sacrificed. Fat and connective tissues around the thoracic aorta were carefully removed under the dissecting stereo-microscope (Leica, Germany). Care was taken not to touch the lumen of the thoracic aorta during dissection to ensure the endothelium intact, unless otherwise stated. Four aortic rings (1 mm in length) were obtained from each aortic preparation and only one ring was used for each drug treatment.

The abdomen of each mouse was opened, and the abdominal (omental) adipose tissue (a metabolically active fat depot) was harvested. In general, the amount of adipose tissue collected from each +*db*/+*m* mouse was ~0.4–0.7 g (n=50), whereas ~4.2–6.0 g (n=50) was collected from each +*db*/+*db* mouse.

2.3. Isometric tension measurement

The thoracic aortic ring was mounted in a 5-ml small-vessel wire-myograph (DMT, Denmark) containing physiological salt solution (gassed with 16% O_2/6% CO_2 balanced with N_2, pO_2 ~ 100 mmHg) of the following composition (mM): NaCl 118, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 11 and $CaCl_2$ 1.8 (pH 7.4, 37 °C). To exclude the involvement of cyclo-oxygenase cascade, indomethacin (1 μM, a non-selective cyclo-oxygenase inhibitor) was included in physiological salt solution throughout the experiments. In some preparations, the role of nitric oxide synthase (NOS) and endothelium was evaluated by N^{ω}-nitro-L-arginine methyl ester (L-NAME) (20 μM, a NOS-inhibitor) and mechanical rubbing of the lumen of the aorta with a stainless-steel wire.

The preparations were equilibrated under the resting tension of 6.0±0.3 mN, as described previously (Okon et al., 2003; Lam et al., 2006) in the bath solution for 90 min. Resting tension was re-adjusted, if necessary, before commencing the experiments. All blockers used in this study were allowed to incubate with the preparation for 30 min before the construction of the concentration–response curve. Increasing concentrations of individual relaxant were administered at half-log increments at the plateau (i.e. steady-state) of the previous response. The response at each concentration of drug added (expressed as final bath concentration) was measured using the MacLab Chart v 3.6 program (AD Instruments, Australia). Relaxation in response to individual agent was expressed as % of the phenylephrine (1 μM) (~90% of maximum contractile response elicited by 1 μM phenylephrine observed in individual specie; $n=5$ for each specie)-induced tone (Okon et al., 2003; Lam et al., 2006), and 100% relaxation was considered when the active tone returned to the baseline level.

2.4. Adipocytes preparation and lipolysis measurement

After harvesting, the abdominal (omental) adipose tissue from each mouse was incubated immediately in Krebs'-Ringer buffer with collagenase (0.5 mg/ml) (Sigma-Aldrich, USA) at 37 °C in a shaker bath for 90 min. After enzymatic treatment, adipocytes were collected and allowed to incubate in Krebs'-Ringer buffer (with the supplement of 3% bovine serum albumin) with different concentrations of drugs at 37 °C for 150 min. The number of adipocytes harvested after enzymatic dissociation was counted under the phase-contrast microscope after trypan blue staining using a hematocytometer. The amount of glycerol released was measured after drug treatment using the commercial free glycerol determination kit (Sigma-Aldrich, USA) according to the manufacturer's protocols. The amount of glycerol released (expressed as μg glycerol/1×10^6 adipocytes) was used as an index of lipolysis for comparison.

2.5. Measurement of 2-deoxy-[^{3}H]-glucose uptake

All experiments were performed in HEPES-buffered Ringer's solution containing (mM): 135 NaCl; 5 KCl; 3.33 NaH_2PO_4; 0.83 Na_2HPO_4; 1.0 $CaCl_2$; 1.0 $MgCl_2$ and 5 HEPES (pH 7.4). Aliquots of adipocytes suspension (100 μl) were exposed to different concentrations of thiazolidinediones (ciglitazone, pioglitazone and troglitazone) for 150 min followed by 100 nM insulin for 30 min at 37 °C. Uptake measurements were initiated by the addition of 2-deoxy-[^{3}H]-glucose (10 μM, 1 μCi/ml). After 10 min incubation, the uptake process was terminated by the

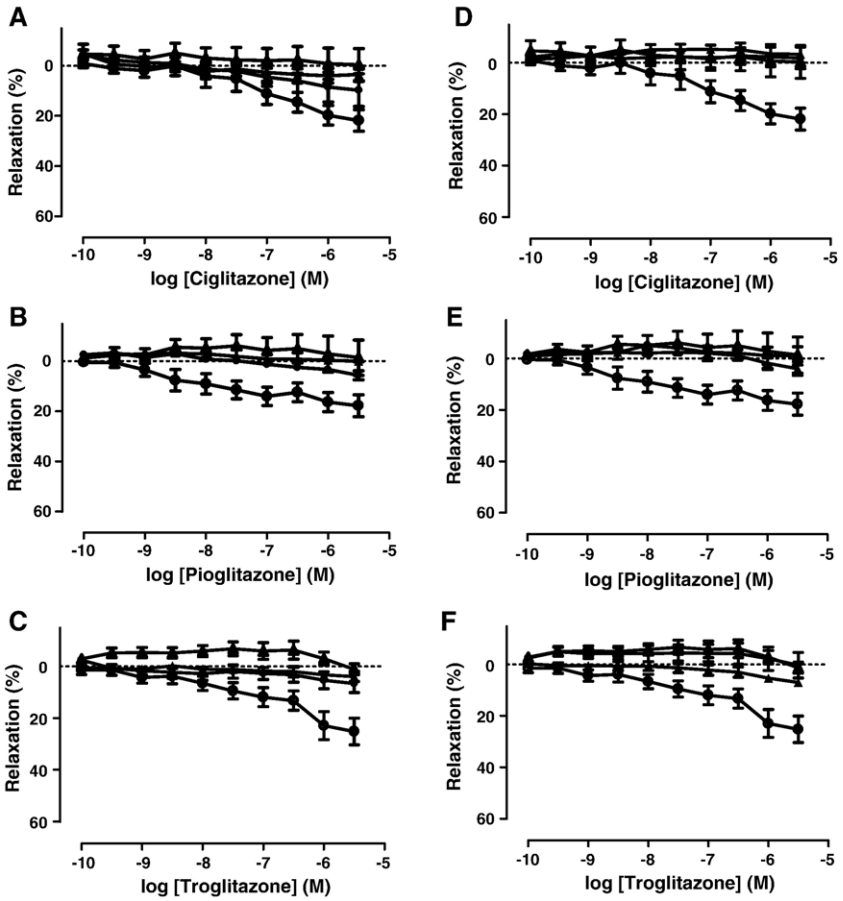

Fig. 1. Cumulative concentration–response curves of ciglitazone, pioglitazone and troglitazone in phenylephrine pre-contracted isolated thoracic aorta, in the absence (open symbols: ○, △) and the presence (closed symbols: ●, ▲) of GW9662 (1 μM, left panels) and L-NAME (20 μM, right panels), of +*db*/+*m* mice (○, ●) and +*db*/+*db* mice (△,▲)). Results are expressed as means±S.E.M.

addition of cold HEPES-buffered Ringer solution (400 μl) containing 50 μM cytochalasin B and 100 μM phloretin. Cells were separated from the uptake buffer by centrifugation (15,000 ×*g*, 1 min) through silicone oil. The radioactivity associated with adipocytes was measured using a β-scintillation counter. Non-specific uptake of 2-deoxy-[^{3}H]-glucose was determined by the addition of 50 μM cytochalasin B and 100 μM phloretin 30 min prior to 2-deoxy-[^{3}H]-glucose administration. Protein content of adipocytes was determined spectrophotometrically using the bicinchoninic acid assay (Pierce Biochemicals, USA).

2.6. Determination of mRNA expression of PPAR-γ

Thoracic aorta (endothelium removed) and the omental adipocytes were isolated from *+db/+m* and *+db/+db* mice as described in previous sections. Total RNA was prepared using the RNeasy minikit (Qiagen, USA) according to the manufacturer's protocols. The integrity of RNA was checked by electrophoresis in a 1% agarose gel containing ethidium bromide. The RNA concentration was measured spectrophotometrically. cDNA was synthesized from isolated total RNA using an oligo (dT) primer and ThermoScript™ RT-PCR system (Invitrogen, USA). Briefly, 1 μg of total RNA was transcribed into cDNA in a final vol. of 20 μl reaction mixture containing 1 μg total RNA, 10 μl H_2O and 1 μl thermoscript reverse transcriptase. After 60 min incubation at 55 °C, the reaction was terminated by incubation at 85 °C for 10 min. Finally, 1 μl Rnase H was added and the reaction was incubated at 37 °C for another 20 min. The reverse transcription products were used immediately in the polymerase chain reaction (PCR) experiments. Primers used for PCR were designed based on the sequence available in the GenBank database (Mural et al., 2002). The following primers were used: PPAR-γ receptor: 5′-GGCGAGGGCGATCTTGACAG-3′ (forward); 5′-AGCAGGTTGTCTTGGATGT-3′ (reverse). The PCR products were examined on 1% agarose gel containing ethidium bromide.

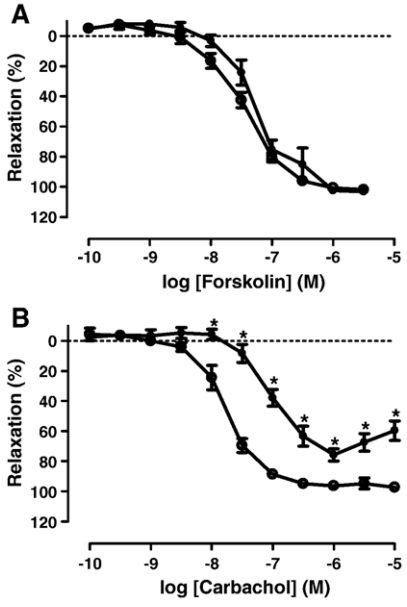

Fig. 2. Cumulative concentration–response curves of forskolin and carbachol in phenylephrine pre-contracted isolated thoracic aorta (endothelium intact) of *+db/+m* mice (○) and *+db/+db* mice (●). Results are expressed as means± S.E.M., and * $P<0.05$ compared to *+db/+m* mice (○).

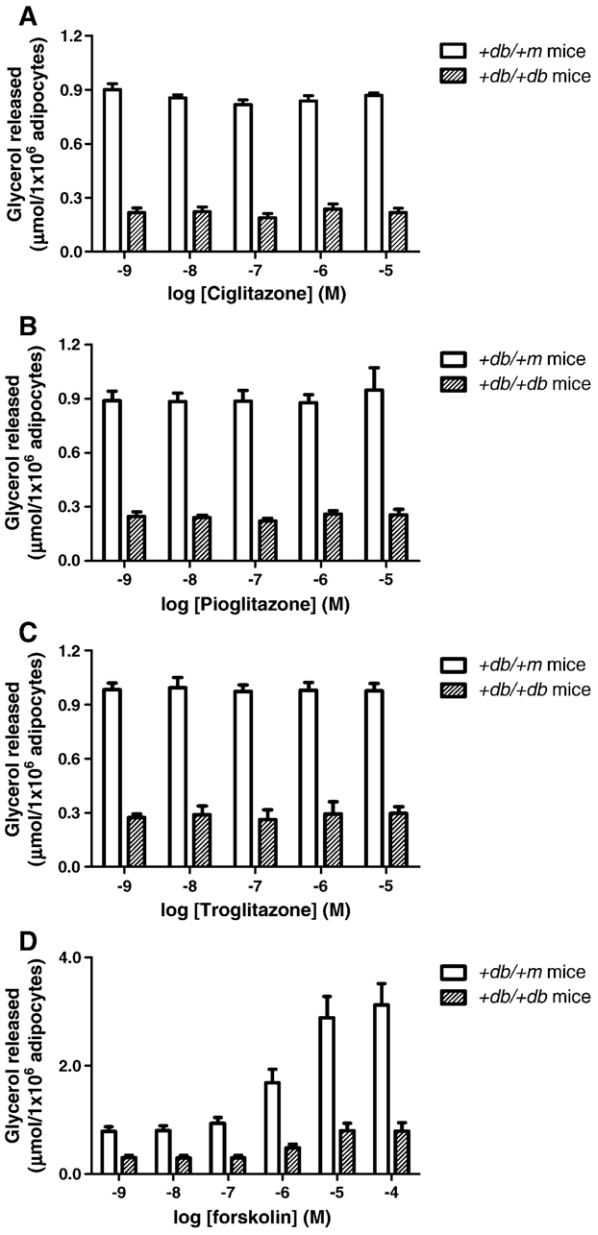

Fig. 3. Concentration–response curves of lipolysis of the abdominal (omental) adipocytes (measured as glycerol released: μg/1×10^6 adipocytes) elicited by (A) ciglitazone, (B) pioglitazone, (C) troglitazone and (D) forskolin of *+db/+m* mice (open bar) and *+db/+db* mice (hatched bar). Results are expressed as means±S.E.M.

2.7. Western immunoblot analysis

Thoracic aorta (endothelium removed) and abdominal (omental) fat tissues were homogenized in the presence of

protease inhibitors to obtain extracts of proteins. Protein concentrations were determined using BCA™ protein assay kit (Pierce, USA). Samples (25 μg of protein per lane) were loaded onto a 10% SDS-polyacrylamide gel electrophoresis gel. After electrophoresis (180 V, 60 min), the separated proteins were transferred (12 mA, 60 min) to polyvinylidene difluoride membrane (PerkinElmer™ Life Sciences, USA). Non-specific sites were blocked with 5% non-fat dry milk for 120 min, and the blots were then incubated with anti-PPAR-γ antibody, 1:1000 (Santa Cruz Biotechnology, USA) overnight at 4 °C. Anti-mouse HRP conjugated IgG, 1:1000 (DakoCytomation, Denmark) was used to detect the binding of its correspondent antibody. Membranes were stripped and re-blotted with anti-β actin antibody, 1:6000 (Sigma-Aldrich, USA) to verify an equal loading of protein in each lane. The protein expression was detected with Western Lightning Chemiluminescence Reagent Plus (PerkinElmer Life Sciences, USA) and quantified using NIH Image analysis programme.

2.8. Chemicals

Physiological salts (GR grade) for preparing Krebs' solution for isometric tension measurement were obtained from Merck (Darmstadt, Germany). L-phenylephrine hydrochloride, carbamylcholine chloride, forskolin, ciglitazone, pioglitazone, troglitazone, indomethacin and N^{ω}-nitro-L-arginine methyl ester (L-NAME) were obtained from Sigma-Aldrich Co. (USA). 2-Chloro-5-nitro-*N*-phenylbenzamide (GW 9662) was obtained from Tocris Cookson (UK) and 2-deoxy-[^{3}H]-glucose was purchased from Amersham (USA). Insulin was purchased from Affinity BioReagents, Inc. (USA).

2.9. Statistical analysis

Data are expressed as means ± S.E.M.; *n* refers to number of mice from which thoracic aorta or adipose tissue was taken for experiments. Statistical comparisons were performed using

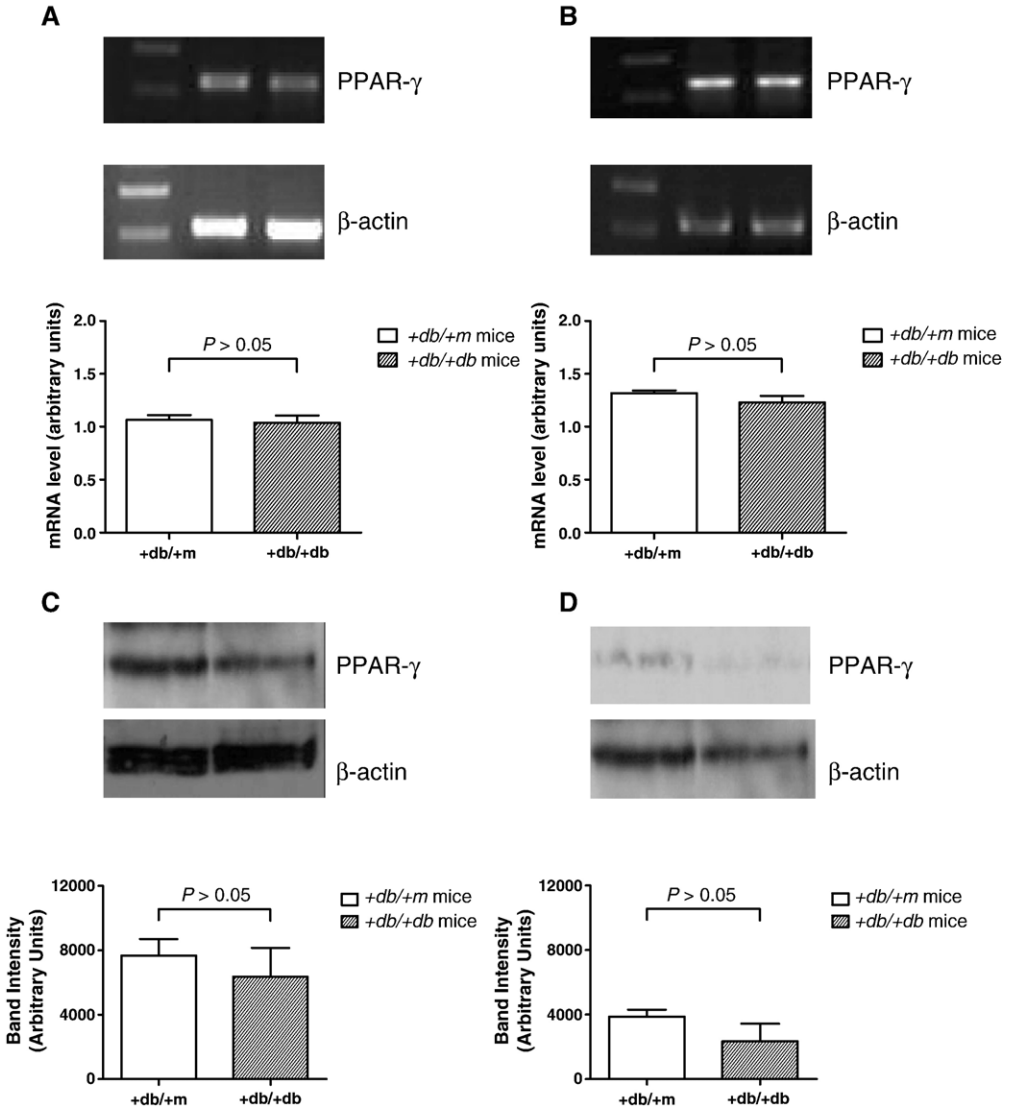

Fig. 4. Summary of PPAR-γ expression in the thoracic aorta (A: mRNA, C: protein) and the abdominal (omental) adipocytes (B: mRNA, D: protein) of +*db*/+*m* mice (open bar) and +*db*/+*db* mice (hatched bar). Quantification of mRNA (RT-PCR) and protein (Western blotting) of PPAR-γ was normalized to β-actin content for comparison. Results are expressed as mean (arbitrary units) ± S.E.M. of three independent experiments.

analysis of variance (ANOVA) or Student's *t*-test, where appropriate. Difference was considered to be statistically significant at $P<0.05$.

3. Results

3.1. Comparison of the aortic relaxation of +db/+m and +db/+db mice

After the phenylephrine (1 μM)-induced contraction reached a steady-state condition, individual PPAR-γ agonists (ciglitazone, pioglitazone and troglitazone) (0.1 nM–3 μM) was added cumulatively to the aortic preparation. All PPAR-γ agonists elicited a concentration (0.1 nM–3 μM)-dependent aortic relaxation of *+db/+m* mice with ~25–30% maximum relaxation at 3 μM ($n=7$–8). However, there was no apparent relaxation response elicited by all PPAR-γ agonists over the same concentration range in *+db/+db* mice ($n=7$–8) (Fig. 1). The PPAR-γ agonist-induced relaxation of aorta was inhibited by 2-chloro-5-nitro-*N*-phenylbenzamide (GW 9662) (1 μM, a selective PPAR-γ receptor blocker) ($n=5$) (Fig. 1A, B and C) and N^{ω}-nitro-L-arginine methyl ester (L-NAME, 20 μM) ($n=6$) (Fig. 1D, E and F), applied alone. In endothelium-denuded preparations, PPAR-γ agonists caused no apparent relaxation in both *+db/+m* and *+db/+db* mice ($n=4$–5) (data not shown).

Cumulative application of carbachol (an acetylcholine analogue) (0.1 nM–10 μM) and forskolin (an adenylyl cyclase activator) (0.1 nM–3 μM) caused a concentration-dependent relaxation with ~100% relaxation at 3 μM ($n=5$–6) (Fig. 2). However, a smaller magnitude of relaxation caused by carbachol was observed in *+db/+db* mice compared to that was observed in *+db/+m* mice (Fig. 2B). In contrast, forskolin (0.1 nM–3μM) caused a similar magnitude of relaxation irrespective of the type of mice used (Fig. 2A).

3.2. Comparison of the lipolytic effect of ciglitazone, pioglitazone, troglitazone and forskolin in +db/+m and +db/+db mice

Three PPAR-γ agonists (ciglitazone, pioglitazone and troglitazone) (1 nM–10 μM) were evaluated on the lipolysis response of the abdominal (omental) adipocytes. The basal (i.e. without drug challenge) glycerol release in *+db/+m* mice was ~3 times higher than that in *+db/+db* mice (~0.8 μmol/1 × 10^6 adipocytes versus ~0.3 μmol/1 × 10^6 adipocytes). However, all three PPAR-γ agonists tested failed to alter the basal lipolysis in both *+db/+m* and *+db/+db* mice ($n=5$ for each PPAR-γ agonist) (Fig. 3A, B and C). In contrast, forskolin (1 nM–100 μM) elicited a concentration-dependent lipolysis and a ~4-fold increase in glycerol release occurred at 100 μM (compared

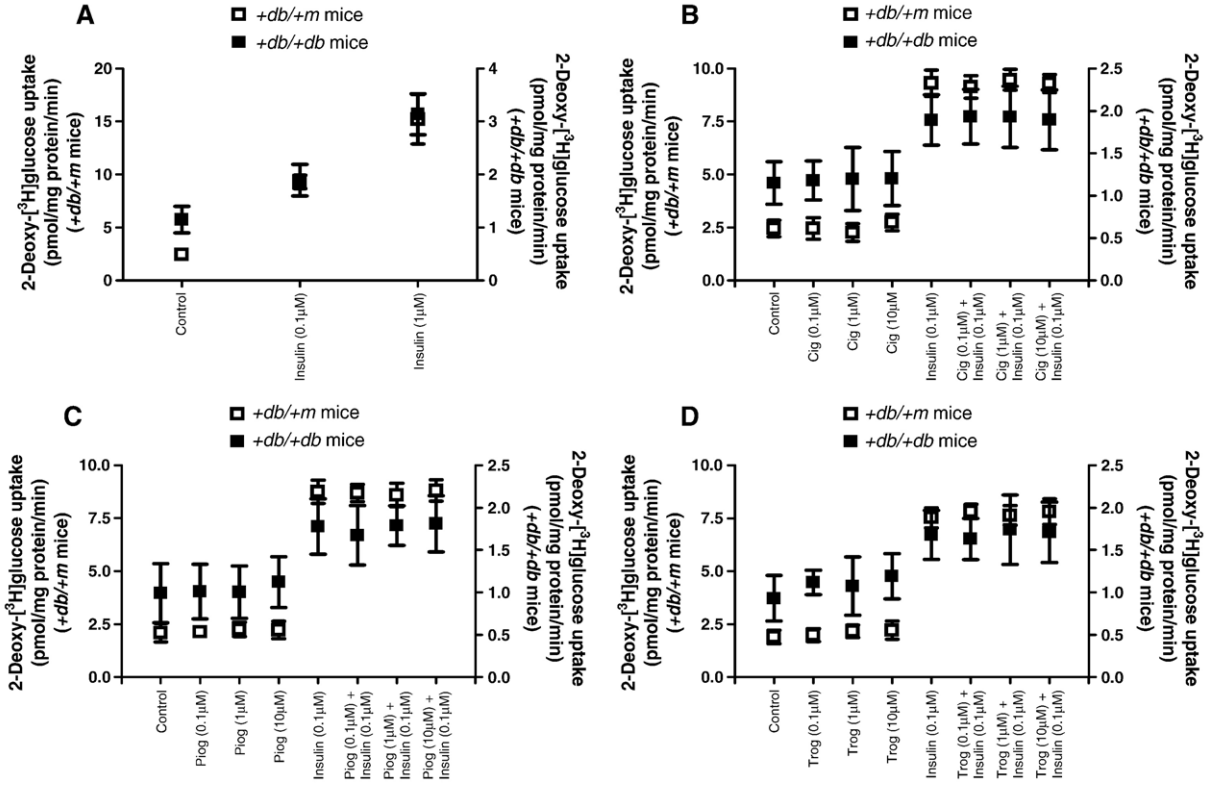

Fig. 5. (A) Stimulation of 2-deoxy-[^{3}H]-glucose uptake by insulin (0.1 μM and 1 μM) into adipocytes of *+db/+m* mice (□), $n=4$) and *+db/+db* mice (■, $n=4$). (B). Effects of ciglitazone (0.1 μM–10 μM), (C) pioglitazone (0.1 μM–10 μM) and (D) troglitazone (0.1 μM–10 μM) on 2-deoxy-[^{3}H]-glucose uptake into adipocytes, with or without stimulation by 0.1 μM insulin. (*+db/+m* mice (□), $n=4$; *+db/+db* mice (■), $n=4$). Results are expressed as means ± S.E.M. of three independent experiments.

to the respective drug-free controls) in both strains of mice ($n=5$), with a greater magnitude of lipolysis observed in $+db/+m$ mice, compared to $+db/+db$ mice (Fig. 3D).

3.3. Determination of PPAR-γ (mRNA and protein) expression

There was no difference in PPAR-γ mRNA expression in both the thoracic aorta (arbitrary units: 1.066±0.063 ($+db/+m$ mice) versus 1.037±0.957 ($+db/+db$ mice)) ($n=3$) (Fig. 4A) and the abdominal adipose tissues (arbitrary units: 1.318±0.043 ($+db/+m$ mice) versus 1.232±0.104 ($+db/+m$ mice)) ($n=3$) (Fig. 4B) ($P>0.05$). In addition, there was no apparent difference in PPAR-γ protein expression in the thoracic aorta ($+db/+m$ mice versus $+db/+db$ mice) (Fig. 4C) and the abdominal adipocytes ($+db/+m$ mice versus $+db/+db$ mice) (Fig. 4D).

3.4. Glucose uptake in adipocytes

Under basal (i.e. drug-free) conditions, the rate of 2-deoxy-[^{3}H]-glucose uptake into the omental adipocytes of $+db/+m$ mice was higher than that in $+db/+db$ mice (2.450±0.396 versus 1.151±0.252 pmol/mg protein/min) (Fig. 5A). In both species, insulin (0.1 and 1 μM) caused a concentration-dependent enhancement of 2-deoxy-[^{3}H]-glucose uptake into the adipocytes (insulin 0.1 μM: $+db/+m$ mice, 9.312±0.621 pmol/mg protein/min; $+db/+db$ mice, 1.893±0.297 pmol/mg protein/min) (insulin 1 μM: $+db/+m$ mice, 15.230±2.347 pmol/mg protein/min; $+db/+db$ mice, 3.140±0.387 pmol/mg protein/min) (Fig. 5A). However, ciglitazone (0.1, 1 and 10 μM) (Fig. 5B), pioglitazone (0.1, 1 and 10 μM) (Fig. 5C) and troglitazone (0.1, 1 and 10 μM) (Fig. 5D), applied alone, did not alter the basal 2-deoxy-[^{3}H]-glucose uptake, and all PPAR-γ agonists tested failed to modify insulin (0.1 μM)-induced 2-deoxy-[^{3}H]-glucose uptake into the adipocytes of both species (Fig. 5B, C and D).

4. Discussion

In our study, we have demonstrated that all three peroxisome proliferator-activated receptor-γ (PPAR-γ) agonists (ciglitazone, pioglitazone and troglitazone), applied acutely, elicited a cumulative concentration-dependent relaxation of isolated thoracic aorta of $+db/+m$ mice, but not $+db/+db$ mice. It is consistent with a pervious study in which activation of PPAR-γ by rosiglitazone caused a vasodilatation of the carotid artery of the hypertensive transgenic mice (Ryan et al., 2004). In addition, pioglitazone reduced the blood pressure in fructose-fed and chow-fed rats (Buchanan et al., 1995). In our study, inhibition of PPAR-γ agonist-induced relaxation by 2-chloro-5-nitro-*N*-phenylbenzamide (GW9662, a selective PPAR-γ blocker) (Seargent et al., 2004) suggesting that PPAR-γ activation plays an essential role in the relaxation response.

Reduction of reactive oxygen species production is one of the possible mechanisms that has been suggested involved in PPAR-γ agonist-mediated vasodilatation (Majithiya et al., 2005). A reduction of reactive oxygen species generation results in an enhancement of vasodilatation by increasing the bioavailability of nitric oxide. It has been demonstrated that troglitazone improved flow-mediated vasodilatation of the brachial artery in obese human subjects by reducing reactive oxygen species generated from lipid peroxidation (Garg et al., 2000). In our study, our data suggest that the PPAR-γ-induced relaxation was endothelium-dependent where the relaxation was abolished by the administration of N^{ω}-nitro-L-arginine methyl ester (L-NAME) (20 μM, a nitric oxide synthase inhibitor). Although the relaxation elicited by PPAR-γ was rather modest with ~30% at 3 μM, it is important to point out that instead of acting indirectly via a reduction of reactive oxygen species generation (at least not after an acute administration of PPAR-γ agonists as demonstrated in the present study), activation of the PPAR-γ receptor was directly involved in the aortic vasodilatation.

In addition, our results demonstrate that the carbachol-induced endothelium-dependent relaxation was impaired in $+db/+db$ mice. This finding is consistent with previous reports in which nitric oxide/endothelium-dependent relaxation of aorta was smaller (over the same concentration range) in $+db/+db$ mice than that was observed in the age-matched non-diabetic ($+db/+m$) mice (Oyama et al., 1986; Matsumoto et al., 2004; Lam et al., 2006). Perhaps, it may explain the absence of the PPAR-γ agonists-induced, endothelium-dependent relaxation observed in $+db/+db$ mice as the function of nitric oxide/endothelium is curtailed which is associated with an increased expression of caveolin-1 protein in $+db/+db$ mice, as reported by our group previously (Lam et al., 2006).

Various studies have demonstrated that PPAR-γ is expressed in different tissues including vascular smooth muscle cells (Ricote et al., 1998; Marx et al., 2004). Therefore, it is possible that a different expression of PPAR-γ between normal and diseased subjects is responsible for a different magnitude of the vascular responses observed. In fact, it has been demonstrated that the mRNA expression of PPAR-γ is higher in the aorta and mesenteric artery of the Spontaneously Hypertensive rats (SHR) compared to the normotensive Wistar-Kyoto rats (WKY) (Xiong et al., 2005). Our data, in contrast, demonstrated that there is no significant difference in the PPAR-γ expression (mRNA and protein) of the thoracic aorta of both strains of mice. Perhaps, it is possible that a malfunction of the downstream signalling cascade could contribute to the absence of the vascular relaxation elicited by PPAR-γ agonists in $+db/+db$ mice. However, we have reported (Lam et al., 2006) that only acetylcholine- but not sodium nitroprusside-mediated relaxation was blunted in $+db/+db$ mice. We therefore considered whether other intracellular signalling cascade (e.g. adenylyl cyclase)-mediated relaxation was altered or not under the diabetic conditions. Interestingly, forskolin (an adenylyl cyclase activator) elicited a similar magnitude of relaxation in both $+db/+m$ and $+db/+db$ mice. Our results therefore further strengthened the conclusion that the endothelium/nitric oxide cascade of the vascular tissues was attenuated in $+db/+db$ mice.

The functions and mechanisms of the PPAR-γ cascade of the adipose tissue are controversial. Previous reports have suggested

that PPAR-γ plays an essential role in lipogenesis (Kubota et al., 1999; Camp et al., 2001), and the regulatory role of PPAR-γ on lipogenesis has been demonstrated in heterozygous PPAR-γ mutant mice (Kubota et al., 1999). On a high-fat diet, the PPAR-γ heterozygous mice gained a lesser weight with a significantly smaller fat store than the wild-type mice.

Although the contribution of PPAR-γ on lipogenesis has been extensively studied, surprisingly, the direct effect of PPAR-γ agonist on lipolysis has not received much attention. So far, most effort has been focused on how PPAR-γ agonist altered the lipolysis phenomenon elicited by different lipolytic agents. It has been shown that insulin (100 nmol/l, 2 day-incubation)-stimulated human adipose tissue lipolysis. However, a combination of insulin (1–1000 nmol/l) with rosiglitazone (10 nmol/l) did not alter this lipolysis response (McTernan et al., 2002). In our study, there was a ~3-fold higher basal lipolysis in *+db/+m* mice compared to that in *+db/+db* mice. It is tempting to speculate that an attenuation of the basal as well as the agonist-induced lipolysis plays an important role in the development of obesity. Unfortunately, all PPAR-γ agonists tested failed to alter the basal lipolysis in both *+db/+m* and *+db/+db* mice suggesting that an acute activation by PPAR-γ agonists produced no apparent lipolysis of the omental adipocytes.

In obese Zucker rats (Gorla-Bajszczak et al., 2000), the PPAR-γ expression in white adipose tissue was 2-fold higher compared to the lean controls. Consistent with previous studies (Vidal-Puig et al., 1996; Shimoike et al., 1998), PPAR-γ (mRNA and protein) is expressed in the adipose tissue of both *+db/+m* and *+db/+db* mice and there was no significant difference in the expression of PPAR-γ between these species.

In view of the well-documented insulin-sensitizing properties of PPAR-γ agonists after a long-term treatment (Bhatia and Viswanathan, 2006; Wang and Tafuri, 2003), we have therefore evaluated the modulation by PPAR-γ agonists of the basal and insulin-induced glucose uptake into adipocytes. Similar to all results obtained in this study, insulin-elicited a greater magnitude of glucose uptake into the adipocytes of *+db/+m* mice, compared to *+db/+db* mice. However, none of the PPAR-γ agonists examined modified the basal and insulin-induced glucose uptake into the adipocytes of both species. The lack of an acute effect of PPAR-γ agonists on glucose uptake into adipocytes is consistent to previous studies showing that at least 24 h of treatment is required for PPAR-γ agonists to up-regulate glucose transporters (Shintani et al., 2001). However, it is important to point out that the lack of acute effects of PPAR-γ agonists on insulin sensitivity (i.e. glucose uptake) observed in our study cannot rule out the chronic effects of PPAR-γ agonists on insulin's actions which are well-documented.

In conclusion, our study for the first time compares the in vitro acute effect of PPAR-γ agonists on blood vessel (aorta) and adipocytes activities of the non-diabetic (*+db/+m*) and diabetic (*+db/+db*) mice. Interestingly, there is a generalized depression of the biological responses (relaxation, lipolysis and glucose uptake) evaluated in tissues of *+db/+db* mice. Despite the fact that PPAR-γ is expressed in both the aorta and the omental adipose tissue of both species, it only contributes minimally to the acute responses of both blood vessel (relaxation) and omental adipocytes (lipolysis and glucose uptake) in both *+db/+m* and +db/+db mice.

Acknowledgements

We are grateful to Li Ka Shing Institute of Health Sciences (Faculty of Medicine, The Chinese University of Hong Kong) for financial supports (to YW Kwan). This project is financially supported by the RGC Earmarked Grants of Hong Kong SAR (Ref.: 4107/01M and Ref.: 4166/02M), and Direct Grants for Research (The Chinese University of Hong Kong) (Reference no.: 2401149; Project code: 2041231). SW Seto and TY Lam are recipients of Postgraduate Studentships of The Chinese University of Hong Kong. The provision of the Student Campus Work Scheme by the Chou's Foundation Fund and the Student Campus Work Scheme (Shaw College, The Chinese University of Hong Kong) is appreciated.

References

Bhatia, V., Viswanathan, P., 2006. Insulin resistance and PPAR insulin sensitizers. Curr. Opin. Investig. Drugs 7, 891–897.

Buchanan, T.A., Meehan, W.P., Jeng, Y.Y., Yang, D., Chan, T.M., Nadler, J.L., Scott, S., Rude, R.K., Hsueh, W.A., 1995. Blood pressure lowering by pioglitazone. Evidence for a direct vascular effect. J. Clin. Invest. 96, 354–360.

Camp, H.S., Chaudhry, A., Leff, T., 2001. A novel potent antagonist of peroxisome proliferator-activated receptor gamma blocks adipocyte differentiation but does not revert the phenotype of terminally differentiated adipocytes. Endocrinology 142, 3207–3213.

Chau, W.H., Lee, W.H., Lau, W.H., Kwan, Y.W., Au, A.L., Raymond, K., 2003. Role of Na^+/H^+ exchanger in acetylcholine-mediated pulmonary artery contraction of spontaneously hypertensive rats. Eur. J. Pharmacol. 464, 177–187.

Freake, H.C., 1999. A genetic mutation in PPAR gamma is associated with enhanced fat cell differentiation: implications for human obesity. Nutr. Rev. 57, 154.

Garg, R., Kumbkarni, Y., Aljada, A., Mohanty, P., Ghanim, H., Hamouda, W., Dandona, P., 2000. Troglitazone reduces reactive oxygen species generation by leukocytes and lipid peroxidation and improves flow-mediated vasodilatation in obese subjects. Hypertension 36, 430–453.

Gorla-Bajszczak, A., Siegrist-Kaiser, C., Boss, O., Burger, A.G., Meier, C.A., 2000. Expression of peroxisome proliferator-activated receptors in lean and obese Zucker rats. Eur. J. Endocrinol. 142, 71–78.

Katakam, P.V.G., Tulbert, C.D., Snipes, J.A., Erdos, B., Miller, A.W., Busija, D.W., 2005. Impaired insulin-induced vasodilation in small coronary arteries of Zucker obese rats is mediated by reactive oxygen species. Am. J. Physiol. 288, H854–H860.

Kersten, S., 2002. Peroxisome proliferator activated receptors and obesity. Eur. J. Pharmacol. 12, 223–234.

Knock, G.A., Mishra, S.K., Aaronson, P.I., 1999. Differential effects of insulin-sensitizers troglitazone and rosiglitazone on ion channels in rat vascular myocytes. Eur. J. Pharmacol. 368, 103–109.

Kubota, N., Terauchi, Y., Miki, H., Tamemoto, H., Yamauchi, T., Komeda, K., Satoh, S., Nakano, R., Ishii, C., Sugiyama, T., Eto, K., Tsubamoto, Y., Okuno, A., Murakami, K., Sekihara, H., Hasegawa, G., Naito, M., Toyoshima, Y., Tanaka, S., Shiota, K., Kitamura, T., Fujita, T., Ezaki, O., Aizawa, S., Kadowaki, T., 1999. PPAR gamma mediates high-fat diet-induced adipocyte hypertrophy and insulin resistance. Mol. Cell 4, 597–609.

Lam, T.Y., Seto, S.W., Lau, Y.M., Au, L.S., Kwan, Y.W., Ngai, S.M., Tsui, K.W., 2006. Impairment of the vascular relaxation and differential expression of caveolin-1 of the aorta of diabetic +db/+db mice. Eur. J. Pharmacol. 546, 134–141.

Leckstrom, A., Lundquist, I., Ma, Z., Westermark, P., 1999. Islet amyloid polypeptide and insulin relationship in a longitudinal study of the genetically obese (*ob*/*ob*) mouse. Pancreas 18, 266–273.

Lehmann, J.M., Moore, L.B., Smith-Oliver, T.A., Wilkison, W.O., Wilson, T.M., Kliewer, S.A., 1995. An antidiabetic thiazolidinedione is a highly affinity ligand for peroxisome proliferator-activated receptor γ. J. Biol. Chem. 270, 12953–12956.

Leiter, E.H., Coleman, D.L., Eisenstein, A.B., Strack, I., 1981. Dietary control of pathogenesis in C57BL/KsJ *db/db* diabetes mice. Metabolism 30, 554–562.

Majithiya, J.B., Paramar, A.N., Balaraman, R., 2005. Pioglitazone, a PPARgamma agonist, restores endothelial function in aorta of streptozotocin-induced diabetic rats. Cardiovasc. Res. 66, 150–161.

Marx, N., Bourcier, T., Sukhova, G.K., Libby, P., Plutzky, J., 1999. PPARγ activation in human endothelial cells increases plasminogen activator inhibitor type-1 expression. PPAR-γ as a potential mediator in vascular disease. Arterioscler. Thromb. Vasc. Biol. 19, 546–551.

Marx, N., Duez, H., Fruchart, J.C., Staels, B., 2004. Peroxisome proliferator-activated receptors and atherogenesis: regulators of gene expression in vascular cells. Circ. Res. 94, 1168–1178.

Matsumoto, T., Wakabayashi, K., Kobayashi, T., Kamata, K., 2004. Alterations in vascular endothelial function in the aorta and mesenteric artery in type II diabetic rats. Can. J. Physiol. Pharm. 82, 175–182.

McTernan, P.G., Harte, A.L., Anderson, L.A., Green, A., Smith, S.A., Holder, J.C., Barnett, A.H., Eggo, M.C., Kumar, S., 2002. Insulin and rosiglitazone regulation of lipolysis and lipogenesis in human adipose tissue in vitro. Diabetes 51, 1493–1498.

Mukherjee, R., Davies, P.J.A., Crombie, D.L., Bischoff, E.D., Cesario, R.M., Jow, L., Hamann, L.G., Boehm, M.F., Mondon, C.E., Nadzan, A.M., Paterniti Jr., J.R., Heyman, R.A., 1997. Sensitization of diabetic and obese mice to insulin by retinoid X receptor agonists. Nature 386, 407–410.

Mural, R.J., Adams, M.D., Myers, E.W., Smith, H.O., Miklos, G.L., Wides, R., Halpern, A., Li, P.W., Sutton, G.G., Nadeau, J., Salzberg, S.L., Holt, R.A., Kodira, C.D., Lu, F., Chen, L., Deng, Z., Evangelista, C.C., Gan, W., Heiman, T.J., Li, J., Li, Z., Merkulov, G.V., Milshina, N.V., Naik, A.K., Qi, R., Shue, B.C., Wang, A., Wang, J., Wang, X., Yan, X., Ye, J., Yooseph, S., Zhao, Q., Zheng, L., Zhu, S.C., Biddick, K., Bolanos, R., Delcher, A.L., Dew, I.M., Fasulo, D., Flanigan, M.J., Huson, D.H., Kravitz, S.A., Miller, J.R., Mobarry, C.M., Reinert, K., Remington, K.A., Zhang, Q., Zheng, X.H., Nusskern, D.R., Lai, Z., Lei, Y., Zhong, W., Yao, A., Guan, P., Ji, R.R., Gu, Z., Wang, Z.Y., Zhong, F., Xiao, C., Chiang, C.C., Yandell, M., Wortman, J.R., Amanatides, P.G., Hladun, S.L., Pratts, E.C., Johnson, J.E., Dodson, K.L., Woodford, K.J., Evans, C.A., Gropman, B., Rusch, D.B., Venter, E., Wang, M., Smith, T.J., Houck, J.T., Tompkins, D.E., Haynes, C., Jacob, D., Chin, S.H., Allen, D.R., Dahlke, C.E., Sanders, R., Li, K., Liu, X., Levitsky, A.A., Majoros, W.H., Chen, Q., Xia, A.C., Lopez, J.R., Donnelly, M.T., Newman, M.H., Glodek, A., Kraft, C.L., Nodell, M., Ali, F., An, H.J., Baldwin-Pitts, D., Beeson, K.Y., Cai, S., Carnes, M., Carver, A., Caulk, P.M., Center, A., Chen, Y.H., Cheng, M.L., Coyne, M.D., Crowder, M., Danaher, S., Davenport, L.B., Desilets, R., Dietz, S.M., Doup, L., Dullaghan, P., Ferriera, S., Fosler, C.R., Gire, H.C., Gluecksmann, A., Gocayne, J.D., Gray, J., Hart, B., Haynes, J., Hoover, J., Howland, T., Ibegwam, C., Jalali, M., Johns, D., Kline, L., Ma, D.S., MacCawley, S., Magoon, A., Mann, F., May, D., McIntosh, T.C., Mehta, S., Moy, L., Moy, M.C., Murphy, B.J., Murphy, S.D., Nelson, K.A., Nuri, Z., Parker, K.A., Prudhomme, A.C., Puri, V.N., Qureshi, H., Raley, J.C., Reardon, M.S., Regier, M.A., Rogers, Y.H., Romblad, D.L., Schutz, J., Scott, J.L., Scott, R., Sitter, C.D., Smallwood, M., Sprague, A.C., Stewart, E., Strong, R.V., Suh, E., Sylvester, K., Thomas, R., Tint, N.N., Tsonis, C., Wang, G., Wang, G., Williams, M.S., Williams, S.M., Windsor, S.M., Wolfe, K., Wu, M.M., Zaveri, J., Chaturvedi, K., Gabrielian, A.E., Ke, Z., Sun, J., Subramanian, G., Venter, J.C., Pfannkoch, C.M., Barnstead, M., Stephenson, L.D., 2002. A comparison of whole-genome shotgun-derived mouse chromosome 16 and the human genome. Science 296, 1661–1671.

Okon, E.B., Szado, T., Laher, I., McManus, B., van Breemen, C., 2003. Augmented contractile response of vascular smooth muscle in a diabetic mouse model. J. Vasc. Res. 40, 520–530.

Oyama, Y., Kawasaki, H., Hattori, Y., Kanno, M., 1986. Attenuation of endothelium-dependent relaxation in aorta from diabetic rats. Eur. J. Pharmacol. 132, 75–78.

Ricote, M., Huang, J., Fajas, L., Li, A., Welch, J., Najib, J., Witztum, J.L., Auwerx, J., Palinski, W., Glass, C.K., 1998. Expression of the peroxisome proliferator-activated receptor gamma (PPARgamma) in human atherosclerosis and regulation in macrophages by colony stimulating factors and oxidized low density lipoprotein. Proc. Natl. Acad. Sci. 23, 7614–7619.

Rieusset, J., Andreelli, F., Auboeuf, D., Roques, M., Vallier, P., Riou, J.P., Auwerx, J., Laville, M., Vidal, H., 1999. Insulin acutely regulates the expression of the peroxisome proliferator-activated receptor-γ in human adipocytes. Diabetes 48, 699–705.

Ryan, M.J., Didion, S.P., Mathur, S., Faraci, F.M., Sigmund, C.D., 2004. PPAR-γ agonist rosiglitazone improves vascular function and lowers blood pressure in hypertensive transgenic mice. Hypertension 443, 661–666.

Sainsbury, C.A.R., Sattar, N., Connell, J.M.C., Hillier, C., Petries, J.R., 2004. Non-esterified fatty acids impair endothelium-dependent vasodilation in rat mesenteric resistance vessels. Clin. Sci. 107, 625–629.

Saltiel, A.R., Olefsky, J.M., 1996. Thiazolidinediones in the treatment of insulin resistance and type II diabetes. Diabetes 45, 1661–1669.

Seargent, J.M., Yates, E.A., Gill, J.H., 2004. GW9662, a potent antagonist of PPARgamma, inhibits growth of breast tumour cells and promotes the anticancer effects of the PPARgamma agonist rosiglitazone, independently of PPARgamma activation. Br. J. Pharmacol. 143, 933–937.

Shimoike, T., Yanase, T., Umeda, F., Ichino, I., Takayanagi, R., Nawata, H., 1998. Subcutaneous or visceral adipose tissue expression of the PPAR-gamma gene is not altered in the fatty (fa/fa) Zucker rat. Metabolism 47, 1494–1498.

Shintani, M., Nishimura, H., Yonemitsu, S., Ogawa, Y., Hayashi, T., Hosoda, K., Inoue, G., Nakao, K., 2001. Troglitazone not only increases GLUT4 but also induces its translocation in rat adipocytes. Diabetes 50, 2296–2300.

Sotiropoulos, K.B., Clermont, A., Yasuda, Y., Rask-Madsen, C., Mastumoto, M., Takahashi, J., Vecchia, K.D., Kondo, T., Aiello, L.P., King, G.L., 2006. Adipose-specific effect of rosiglitazone on vascular permeability and protein kinase C activation: novel mechanism for PPARγ agonist's effects on edema and weight gain. FASEB J. 20, E367–E380.

Tontonoz, P., Hu, E., Graves, R.A., Budavari, A.I., Spiegelman, B.M., 1994. mPPAR gamma 2: tissue-specific regulator of an adipocyte enhancer. Genes Dev. 8, 1224–1234.

Vidal-Puig, A., Jimenez-Linan, M., Lowell, B.B., Hamann, A., Hu, E., Spiegelman, B., Flier, J.S., Moller, D.E., 1996. Regulation of PPAR gamma gene expression by nutrition and obesity in rodents. J. Clin. Invest. 97, 2553–2561.

Wang, M., Tafuri, S., 2003. Modulation of PPARgamma activity with pharmaceutical agents: treatment of insulin resistance and atherosclerosis. J. Cell. Biochem. 1, 38–47.

Wilson-Fritch, L., Nicoloro, S., Chouinard, M., Lazar, M.A., Chui, P.C., Leszyk, J., Straubhaar, J., Czech, M.P., Corvera, S., 2004. Mitochondrial remodelling in adipose tissue associated with obesity and treatment with rosiglitazone. J. Clin. Invest. 114, 1281–1289.

Winters, B., Mo, Z., Brooks-Asplund, E., Kim, S., Shoukas, A., Li, D., Nyhan, D., Berkowitz, D.E., 2000. Reduction of obesity, as induced by leptin, reverses endothelial dysfunction in obese (Lep^{ob}) mice. J. Appl. Physiol. 89, 2382–2390.

Xiong, C., Mou, Y., Zhang, J., Fu, M., Chen, Y.E., Akinbami, M.A., Cui, T., 2005. Impaired expression of PPAR gamma protein contributes to the exaggerated growth of vascular smooth muscle cells in spontaneously hypertensive rats. Life Sci. 77, 3037–3048.