# Rosiglitazone (BRL49653), a PPARγ-selective agonist, causes peroxisome proliferator-like liver effects in obese mice

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**Abstract** **The PPAR (peroxisome proliferator activated receptor) transcription factors are ligand-activated nuclear receptors that regulate genes involved in lipid metabolism and homeostasis. PPARα is preferentially expressed in liver and PPARγ preferentially in adipose tissue. Activation of PPARα leads to peroxisome proliferation and increased β-oxidation of fatty acids in rodents. PPARγ-activation leads to adipocyte differentiation and improved insulin signaling of mature adipocytes. Both PPAR receptors are believed to be functional targets for treatment of hyperlipidemia in man. We have treated obese diabetic mice (*ob*/*ob*), which have highly elevated levels of plasma triglycerides, glucose and insulin, for 1 week with WY14,643 (180 μmol/kg/day), a selective PPARα agonist, or rosiglitazone (BRL49653; 2.5 μmol/kg/day), a selective PPARγ agonist. The doses used produce a similar therapeutic effect in both treatment groups (lowering of triglycerides and glucose). High resolution two-dimensional gel electrophoresis of livers showed that WY14,643 and rosiglitazone both produced changes in expression pattern of many proteins involved in peroxisomal fatty acid β-oxidation. However, similar experiments performed in lean mice showed significant up-regulation of these proteins only with WY14,643 treatment. Furthermore, the proteins up-regulated by the drugs in obese mice had a higher basal expression in obese controls compared to the lean littermates. Liver PPARγ mRNA levels were determined and we observed that PPARγ2 mRNA levels were elevated in obese mice compared to lean littermates. As PPARα and PPARγ recognize similar DNA response elements, it is likely that the effects of rosiglitazone on PPARα responsive genes in livers of the *ob*/*ob* mice are mediated by PPARγ2.**—Edvardsson, U., M. Bergström, M. Alexandersson, K. Bamberg, B. Ljung, and B. Dahllöf. **Rosiglitazone (BRL49653), a PPARγ-selective agonist, causes peroxisome proliferator-like liver effects in obese mice.** ***J. Lipid Res.*** **1999.** 40: **1177–1184.**

**Supplementary key words** BRL49653 • WY14,643 • peroxisome proliferation • PPAR • proteomics • *ob*/*ob* • obese mice • insulin resistance • two-dimensional gel electrophoresis

Peroxisome proliferator activated receptors (PPAR) are nuclear transcription factors that heterodimerize with RXRα and activate a multitude of genes involved in lipid metabolism (1–3). There are three PPAR subtypes known to date with different tissue distribution; PPARα is highly expressed in liver and kidney and PPARδ is ubiquitously expressed. The PPARγ protein exists in two isoforms, γ1 and γ2. Profiling their relative abundance revealed that PPARγ1 is expressed in several tissues, whereas PPARγ2 is preferentially expressed in adipose tissue (1, 3–6). The γ1 and γ2 isoforms arise from differential promoter usage, which results in 30 additional N-terminal amino acids in PPARγ2. Also, a third PPARγ mRNA transcript has recently been reported (7). This transcript gives rise to a protein identical to PPARγ1 and is preferentially expressed in adipose tissue and large intestine. It has been shown that fatty acids and/or fatty acid metabolites are activators of PPARs (8–12) and it is possible to view PPARs as physiological sensors of intracellular lipid/fatty acid concentrations (1, 2, 10).

In rodent livers, activation of PPARα promotes increased fatty acid oxidation in peroxisomes, mitochondria and microsomes, and induces proliferation of peroxisomes (4, 13–15). Several proteins involved in lipid metabolism, such as lipoprotein lipase, apolipoproteins A-I, A-II and C-III, and cytochrome P450 4A, are regulated by PPARα (1, 15, 16). Ligand activation of PPARγ leads to adipocyte differentiation in cell culture as well as in whole animals (3, 17–22). In white adipose tissue, expression of adipocyte fatty acid binding protein (aFABP) (18) and expression of insulin receptors increase upon PPARγ activation (21, 23). Other proteins, such as TNFα, TNFα receptor (24), and leptin (25–27), are down-regulated in

Abbreviations: 3KCT, 3-ketoacyl CoA thiolase; ACO, acyl CoA oxidase; CYP4A, cytochrome P450-4A; aFABP, adipocyte fatty acid binding protein; PBE, peroxisomal bifunctional enzyme; PPAR, peroxisome proliferator activated receptor; PPRE, peroxisome proliferator response element; TZD, thiazolidinedione.

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adipocytes by PPARγ-activation. Taken together, activation of either PPARα or PPARγ may act to withdraw lipids and fatty acids from the circulation through increased oxidation or storage, respectively (1, 2).

It is therefore adequate that both PPARα and PPARγ may represent functional targets for hypolipidemic agents. Thus, fibrates, used for clinical treatment of severe hypertriglyceridemia, have been found to activate PPARα (4, 13) and in humans, a major effect is thought to be down-regulation of apolipoprotein C-III (2, 28, 29), an inhibitor of peripheral lipolysis. Peroxisome proliferation, as a result of PPARα activation, is considered a rodent-specific phenomenon and has not been convincingly detected in humans (30). Thiazolidinediones (TZD) (23, 31, 32) are a new class of insulin sensitizers and antidiabetic agents, with substantial effects on circulating lipids in diabetic rodents, and PPARγ has been shown to be a molecular target of TZD action (9, 33). The first TZD in clinical use is troglitazone, which recently was registered in the US for treatment of non-insulin-dependent diabetes mellitus (34). Rosiglitazone (BRL49653), a more potent PPARγ-activator, is currently near registration (35).

Reporter gene assays in transfected cell lines have shown that TZD are highly selective PPARγ-agonists (3, 12, 33). Thus, primary effects of TZD are expected in tissues expressing PPARγ. Previously, we have used proteomics (high-resolution two-dimensional electrophoresis and mass spectrometry) to characterize the effect of the peroxisome proliferator and PPARα agonist WY14,643 on the livers of obese mice (36). These experiments showed that WY14,643 induced expression of most enzymes involved in the peroxisomal fatty acid β-oxidation. Now, we have characterized the effects of both rosiglitazone and WY14,643 on the same set of proteins in the livers of both obese and lean mice. We found that whereas WY14,643 produced a similar response in both strains, rosiglitazone showed peroxisome proliferator-like effects only in obese mice. When compared to lean littermates, expression analysis of mRNA showed that obese mice have elevated liver PPARγ2 levels. We conclude that obesity in this model leads to an up-regulation in the liver of the PPARγ2 isoform and suggest that rosiglitazone causes peroxisome proliferator-like effects by activating liver PPARγ2 in obese mice.

## MATERIALS AND METHODS

### Materials

The IsoDalt system was from Hoefer (San Francisco, CA). Equipment for isoelectric focusing (IEF), IPGphor, Immobiline DryStrips (18 cm, pH 3–10 NL (non-linear)), IPG-buffer 3–10 NL, and Drystrip cover fluid were purchased from Amersham Pharmacia Biotech (Uppsala, Sweden). Iodoacetamide and sodium thiosulfate were from Sigma (St. Louis, MO). Silver nitrate, formaldehyde, and sodium carbonate were from Merck (Darmstadt, Germany). Nonidet P-40 (NP-40) was from United States Biochemical Corp. (Cleveland, OH). Duracryl (30%, 0.65% Bis) and all other chemicals were electrophoresis grade, obtained from ESA Inc. (Chelmsford, MA). WY14,643 was purchased from Sigma (St. Louis, MO) and rosiglitazone was obtained from Medicinal Chemistry, Astra Hässle AB.

### Animals and drug treatment

Obese mice (*ob/ob*; Umeå) and their lean littermates (+/?) were from Bomholtgård Breeding and Research Centre, Denmark (genotyping of lean mice was not considered critical for this study and for practical reasons we therefore used +/? lean control mice). The 7-week-old animals were treated orally once daily for 1 week with WY14,643 (180 μmol/kg/day) or rosiglitazone (2.5 μmol/kg/day). On the last day of the experiment the mice were anesthetized in $CO_2$ and exsanguinated via a carotid artery. The blood was collected in vials containing EDTA.

### Metabolic effects

In plasma, insulin was analyzed using a rat insulin RIA kit (RI-13K; Linco, St. Louis, MO) and triglycerides (TG) and glucose were analyzed spectrophotometrically on the Cobas Mira plus (Hoffman la Roche, Basel, CH) using Calibrator Human (07 3718 6; Roche, Basel, CH) as calibrant. For TG the enzymatic kit "Triglycerides/Glycerol Blanking" (450032; Boehringer Mannheim, Indianapolis, IN) was used, and for glucose "Glucose HK" (07 3672 4; Roche, Basel, CH).

### Sample preparation for two-dimensional electrophoresis

The apical ends of the left liver lobes were rapidly removed, frozen in liquid nitrogen, and stored at −150°C until subsequent electrophoretic analysis. Liver samples were weighed and homogenized with a glass/Teflon homogenizer (5 strokes at 400 rpm) in 8 volumes of solubilizing solution (8 M Urea, 0.3% (w/v) dithiothreitol (DTT), 2% (v/v) NP-40, and 2% (v/v) IPG-buffer 3–10 NL). To remove solid tissue, the homogenate was centrifuged at 100 000 *g* for 30 min at 15°C. The supernatant was carefully removed and immediately frozen at −70°C.

### First dimension (isoelectric focusing)

Electrophoresis of mouse liver proteins was performed on individual samples, four from each treatment group and from lean controls, and six from obese controls. Immobiline DryStrips (18 cm, pH 3–10 NL (non-linear)) were used for isoelectric focusing. Each strip was rehydrated for 12 h in 350 μl of rehydration solution containing 4 μl (~100 μg) of solubilized liver protein. The rehydration solution consisted of 8 M urea, 2% (v/v) NP-40, 0.3% (w/v) DTT, and 0.5% (v/v) IPG-buffer 3–10 NL. The strips were run under a layer of Drystrip cover fluid, at 20°C, in a IPGphor unit according to the manufacturer's instructions. The focusing was carried out at 500 V; 1 h, 1000 V; 1 h and 8000 V; 9 h to reach a total of 74 kVh.

### Equilibration of IPG gel strips

After IEF the strips were equilibrated 2 × 15 min with gentle shaking (37). The first equilibration solution contained 30% glycerol (w/v), 6 M urea, 2% SDS, 50 mM Tris/HCl, pH 8.8, 65 mM DTT, and a trace of bromophenol blue as tracking dye. The second equilibration was carried out in same solution, except that DTT was replaced by 260 mM iodoacetamide.

### Second dimension (SDS-PAGE)

The gels used in this study were continuous 14% T, 0.3% C gels in the format of 23 × 20 × 0.1 cm. After the equilibration, each IEF strip was drained on a filter paper and immersed in SDS running buffer (24 mM Tris base, 0.2 M glycine, and 0.1% SDS) before it was sealed at the top of the second dimension gel with 1% agarose in SDS running buffer. Electrophoresis was performed in the IsoDalt tank (Hoefer) at 100 V for ~19 h, until the tracking dye reached the anodic end of the gels.



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### Silver staining

Analytical gels were silver stained according to Shevchenko et al. (38) with some modifications. The gels were fixed in 50% ethanol, 5% acetic acid for 1 h, washed in 50% ethanol for 30 min and additionally 60 min with water to remove the remaining acid. Thereafter, the gels were sensitized by a 1-min incubation in 0.02% sodium thiosulfate and rinsed with two changes of distilled water for 1 min each. After rinsing, the gels were incubated in 0.1% silver nitrate for 30 min. After incubation, the gels were rinsed twice with distilled water for 1 min and then developed in 0.04% formaldehyde, 2% sodium carbonate. When the developer turned yellow (~30 sec) it was discarded and replaced with fresh solution. When desired intensity of staining was achieved (~3.5 min), the development was terminated by discarding the reagent, followed by washing with 5% acetic acid for 5–10 min. Finally, the gels were washed in water and stored in sealed plastic bags at 4°C.

### Image analysis

Silver-stained gels were imaged using a cooled CCD camera-based instrument, Fluor-S™ MultiImager (Bio-Rad). Raw scans were processed by the 2D software PDQuest (PC version 5.1) according to the following procedure: make gel image, ball background subtraction (radius 80), two times averaging smooth, spots detected and fitted to Gaussian volumes (39). After manual landmarking of approximately 140 spots in each gel image, the spot patterns of the different gels were automatically matched to each other and each spot was given a unique identification number (SSP). Individual quantifications of resolved proteins were normalized to the total intensity of detected spots in each gel. To find spots that differed quantitatively between the control and treated groups, the average intensities (n = 4–6) of resolved spots were compared using the statistical and quantitative functions within the PDQuest software.

### RNA isolation

Fresh liver samples were rinsed in PBS and frozen immediately in liquid $N_2$. Total RNA was prepared using RNA-Stat 60 (Tel-Test, Inc., Friendswood, TX) according to the manufacturer's suggestions. Concentration and yield were determined by optical density measurement at 260 nm and the integrity of the RNA was verified by agarose gel electrophoresis and quantification of the 28S and 18S ribosomal bands.

### Ribonuclease protection assay (RPA)

Nucleotides 1–323 (position 1 is the A in the initial ATG) from mouse PPARγ2 in a pBluescript vector (Stratagene, La Jolla, CA) served as template for PPARγ probe synthesis. $^{32}$P-labeled antisense probe was generated using Promega in vitro transcription kit and the probe was purified by PAGE.

The RPAII kit (Ambion Inc., Austin, TX) was used for mRNA quantification. Briefly, 20 μg total liver RNA or 5 μg total RNA from white adipose tissue was hybridized at 45°C overnight to 80,000 cpm probe. The samples were subsequently treated with an RNaseT1/RNaseA mix at 37°C for 30 min to digest single-stranded RNA prior to separation on a 6% denaturing polyacrylamide gel. Radioactivity on the dried gel was visualized using a STORM™ phosphorimager (Molecular Dynamics, Sunnyvale, CA).

## RESULTS

### Drug treatment and therapeutic effects

Male obese and lean mice were treated for 1 week with WY14,643 (180 μmol/kg/day) or rosiglitazone (2.5 μmol/kg/day), doses that were previously determined by dose–response experiments to be equally effective in male obese mice regarding lowering of plasma triglycerides and glucose (data not shown). In **Fig. 1** we show that both substances again caused similar reductions in plasma triglycerides and glucose in obese insulin-resistant mice, to levels of the lean control mice. In lean insulin-sensitive mice, drug treatment did not significantly affect triglyceride or glucose levels.

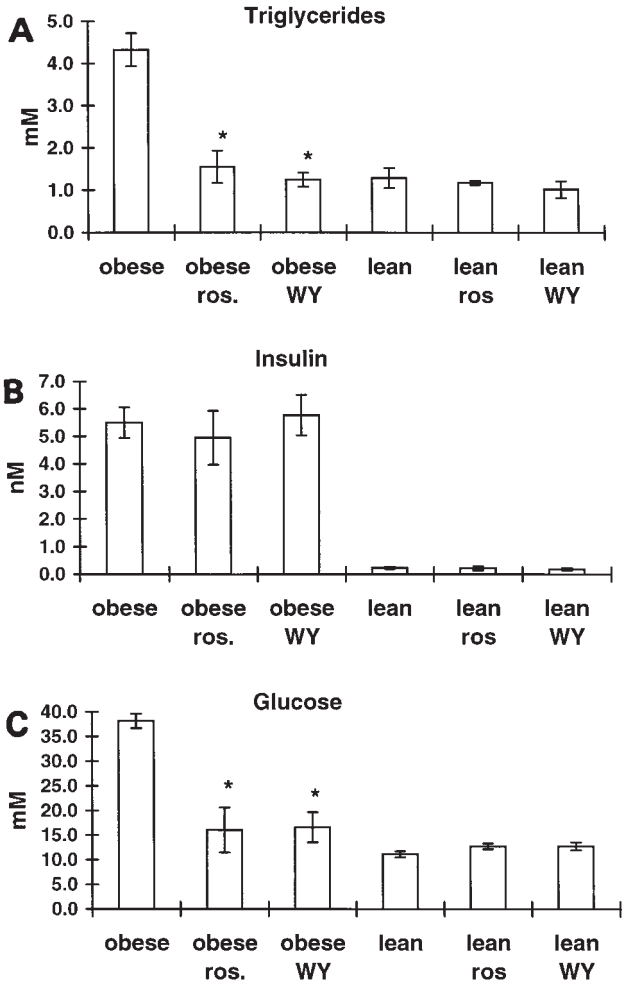


**Fig. 1.** Therapeutic effects of rosiglitazone and WY14,643. Plasma levels of triglycerides (A), insulin (B), and glucose (C) in obese and lean mice after 1 week treatment with rosiglitazone (2.5 μmol/kg/day) or WY14,643 (180 μmol/kg/day) in comparison to untreated obese and lean animals. Values are mean ± SE, n = 4 (obese controls, n = 6). * $P < 0.05$ using Student's *t*-test for each treated group versus obese or lean controls.

### Two-dimensional gel electrophoresis

Liver samples, 4 from each treatment group and 6 from obese controls, were homogenized in rehydration buffer and analyzed by two-dimensional electrophoresis as described in Materials and Methods. The images of the 26 gels were captured and analyzed by using the PDQuest software as described in Materials and Methods. Previously, we have characterized the dominant response to WY14,643 in obese mice and found that 16



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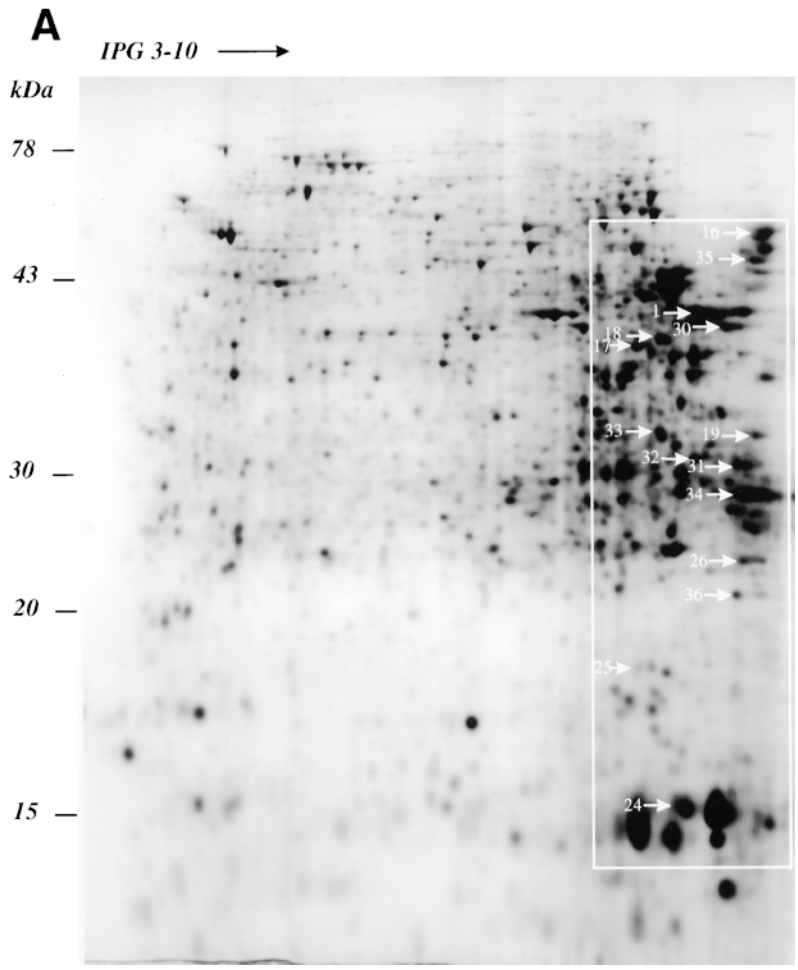


**Fig. 2**

spots were clearly up-regulated by the treatment. These spots were identified by in-gel tryptic digestion and mass spectrometry (MALDI-TOF and MS/MS) as described (36). Of these spots, 14 were identified as polypeptides from acyl CoA oxidase (ACO), peroxisomal bifunctional enzyme (PBE), and 3-ketoacyl thiolase (3KCT), enzymes which are central components of the peroxisomal fatty acid β-oxidation. Furthermore, one spot was identified as adipocyte fatty acid binding protein, aFABP, which may be viewed as a substrate carrier for this metabolic pathway, and one spot was identified as HMG-CoA synthase which is not directly involved in peroxisomal fatty acid β-oxidation. In this study, we focused on the regulation of the 15 spots that are components of, or are related to (aFABP), peroxisomal fatty acid β-oxidation. These spots are located in the basic region of the gels covering a pI range from 3 to 10 (see the master gel in **Fig. 2A**). Representative gels of liver samples from untreated, rosiglitazone- and WY14,643-treated obese mice are shown as cut-outs from 2D-gels in Fig. 2B. By visual inspection of the gels, we found that both rosiglitazone and WY14,643 apparently up-regulated most of these 15 spots.

Thereafter, semiquantitative data obtained from computer-aided image analysis were analyzed and we found that in obese animals, out of these 15 spots, WY14,643 and rosiglitazone induced significant up-regulation of 14 and 9 spots, respectively (**Fig. 3A**). As each enzyme is represented by several spots, a common finding in two-dimensional electrophoresis most likely representing cleavage and degradation occurring in vivo, it is important to note that rosiglitazone did affect at least one spot from all of the three identified peroxisomal enzymes. To explain that several spots, originating from one protein, are not regulated in a coordinated manner by pharmacological treatments would require complete knowledge of the primary sequence of each spot, as well as an understanding of each activation/degradation pathway. Such an analysis has not been possible to perform in this study. In lean mice, rosiglitazone did not cause any significant changes whereas WY14,643 caused significant up-regulation of 15 spots (Fig. 3B). It is interesting to note that all spots representing ACO, PBE, and 3KCT had a higher basal expression in obese compared to lean mice (Fig. 3C), but this was not the case for aFABP. Thus, rosiglitazone caused a similar, but somewhat less pronounced, induction of peroxisomal enzymes in obese mice as compared to WY14,643. In



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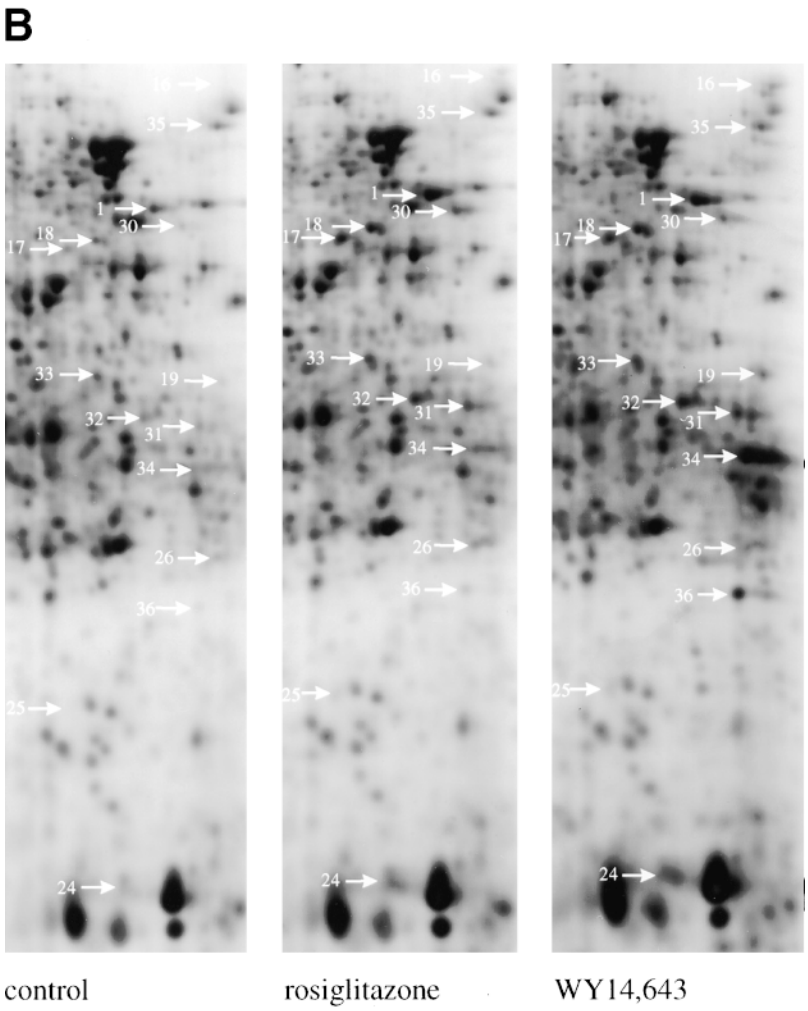


**Fig. 2.** (A) Two-dimensional master gel with the 15 spots characterizing the major peroxisome proliferator-like response in obese mice: (1) 3-ketoacyl-CoA thiolase, (16) peroxisomal bifunctional enzyme, (17) 3-ketoacyl-CoA thiolase, (18) 3-ketoacyl-CoA thiolase, (19) peroxisomal bifunctional enzyme, (24) aFABP, (25) acyl CoA oxidase, (26) acyl CoA oxidase, (30) 3-ketoacyl-CoA thiolase, (31) peroxisomal bifunctional enzyme, (32) 3-ketoacyl-CoA thiolase, (33) acyl CoA oxidase, (34) peroxisomal bifunctional enzyme, (35) peroxisomal bifunctional enzyme, (36) peroxisomal bifunctional enzyme. (B) Enlarged sections, cut-out as indicated by the box in (A), from representative gels of liver samples from obese control, rosiglitazone- and WY14,643-treated mice. Arrows indicate spots up-regulated by the rosiglitazone and/or the WY14,643 treatment.

contrast, only WY14,643 induced this metabolic pathway in lean mice.

### Liver expression of PPARγ mRNA

PPARα and PPARγ show similar binding to the peroxisomal proliferator response element (PPRE) of ACO (20). It is therefore conceivable that PPARγ, if expressed in liver, will bind to the same PPREs as PPARα and hence mimic PPARα-mediated gene regulation. Therefore, we decided to compare mRNA expression levels of PPARγ1 and PPARγ2 in livers from the lean and obese mice. As can be seen in **Fig. 4**, lean mice expressed only low levels of PPARγ1 and barely detectable amounts of PPARγ2. However, obese animals showed markedly elevated levels of the PPARγ2 isoform, whereas the PPARγ1 levels remained low. Thus, the peroxisome proliferator-like liver effects of rosiglitazone in obese mice may be due to activation of PPARγ2.

## DISCUSSION

In our efforts to map the mechanisms of action of antihyperlipidemic drugs, we first characterized the response of the selective PPARα activator WY14,643 in livers from obese mice (36). We found the expected induction of central components of the peroxisomal fatty acid β-oxidation pathway, a response typical for peroxisome proliferators. Now, we asked whether the TZD rosiglitazone, reported to be a selective PPARγ-agonist (9, 33), may cause similar effects as WY14,643 in lean and obese mice.

The results are summarized as follows. *i*) At equally



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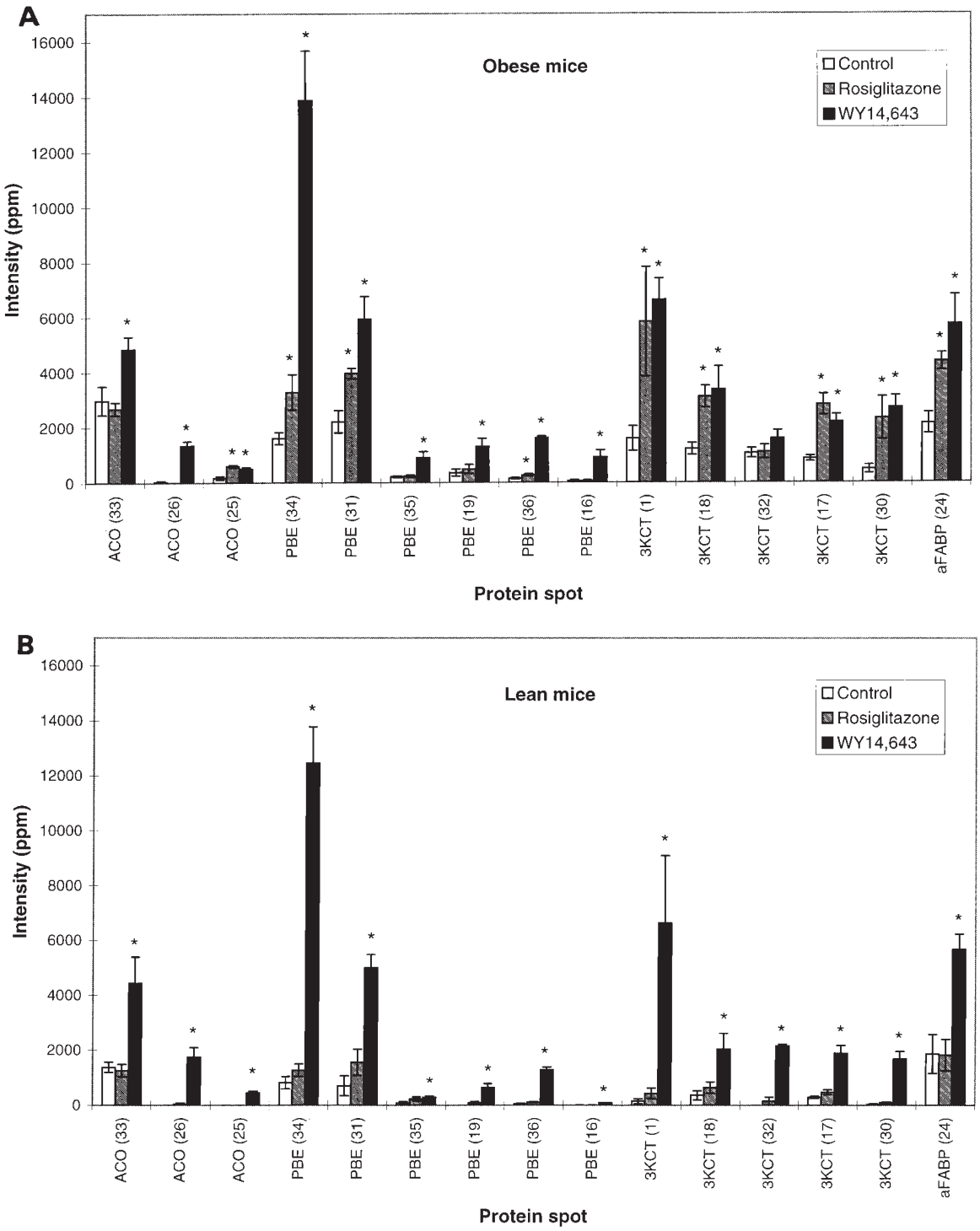


**Fig. 3**

effective doses in obese mice (with regard to correction of hypertriglyceridemia and hyperglycemia), rosiglitazone and WY14,643 induce similar responses identified as up-regulation of peroxisomal fatty acid β-oxidation. *ii*) In lean mice, WY14,643 exerted effects similar to those in obese mice whereas rosiglitazone had no effect on the studied proteins. *iii*) Livers from obese mice contain more PPARγ2 mRNA than livers from lean mice. To conclude, our findings suggest that the elevated levels of liver PPARγ2 in obese mice may render these animals susceptible to a peroxisome proliferator-like activation by rosiglitazone. In relation to this, it is interesting to note that others have found elevated PPARγ expression in livers from obese mice fed a high fat-containing diet (40). Also, a recent report on PPARα knockout mice, which become obese, showed an increased expression of liver PPARγ in fat-filled liver cells from males, whereas the expression of aFABP, adipoQ, and hormone-sensitive



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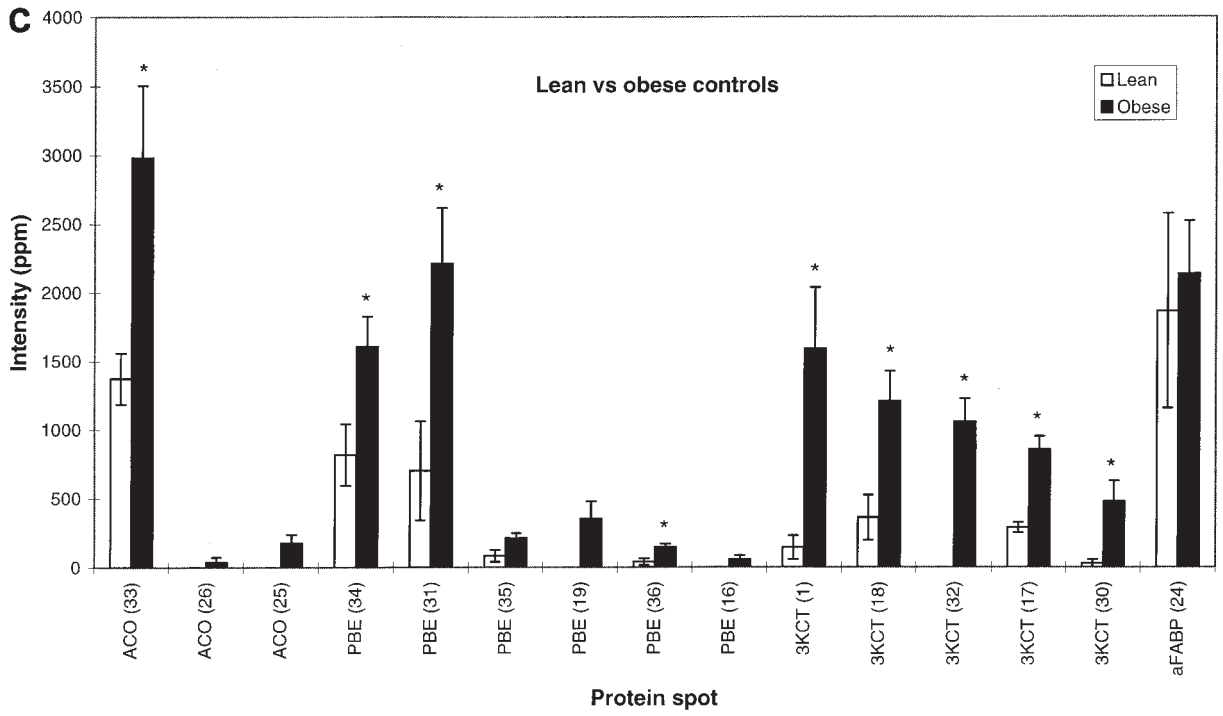


**Fig. 3.** A, B: Semi-quantitative data of 15 spots involved in peroxisomal fatty acid β-oxidation up-regulated by rosiglitazone (2.5 μmol/kg/day, hatched bars) or WY14,643 (180 μmol/kg/day; black bars) compared to control (white bars), (A: obese mice; B: lean mice). C: Comparison of the expression levels of the same proteins between untreated lean (white bars) and obese mice (black bars). The ordinate represents numbers given by the PDQuest software (ppm; normalized to the total intensity of all detected spots on each gel). ACO, acyl CoA oxidase; PBE, peroxisomal bifunctional enzyme; 3KCT, 3-ketoacyl thiolase; aFABP, adipocyte fatty acid binding protein. Numbers in brackets refer to corresponding numbers on the master gel in Fig. 2A. Values are mean ± SE, n = 4 (obese controls, n = 6). *$P < 0.05$ using Student's *t*-test for each treated group versus obese (A) or lean controls (B), and obese versus lean (C).

lipase remained unchanged (41). The results are in line with our data concerning the hepatic expression of PPARγ and aFABP in obesity. Thus, obesity and high-fat feeding regulate PPARγ levels in mice, and this may determine the response to PPAR activators in vivo. In particular, the PPARγ2 isoform may be the one that is regulated in obesity. To understand the pharmacodynamic effects of PPAR activators in humans, it will be important to investigate possible regulatory effects of obesity on hepatic PPARγ expression in human subjects.

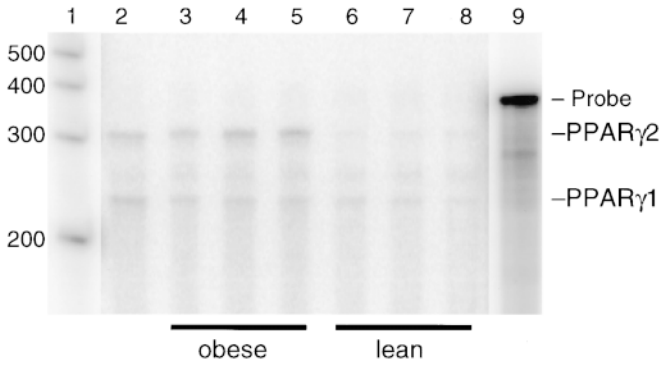


**Fig. 4.** PPARγ1 and PPARγ2 expression in lean and obese mice quantified by RPA. Five μg total white adipose tissue RNA from obese mice (lane 2) or 20 μg total liver RNA from 3 obese (lanes 3–5) or 3 lean (lanes 6–8) animals was hybridized to a $^{32}$P-labeled antisense probe, digested with a RNaseT1/RNaseA mix, and separated on a 6% denaturing polyacrylamide gel. The probe contained the nucleotides 1–323, counting from the initiating ATG in PPARγ2, plus vector sequence to account for a total of 379 nt (lane 9, undigested probe). PPARγ1 mRNA protects a 233 nt fragment, PPARγ2 protects a 323 nt fragment. Lane 1 contains a molecular size marker.

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## REFERENCES

1. Wahli, W., O. Braissant, and B. Desvergne. 1995. Peroxisome proliferator activated receptors–transcriptional regulators of adipogenesis, lipid metabolism and more. *Chem Biol.* **2:** 261–266.
2. Schoonjans, K., B. Staels, and J. Auwerx. 1996. Role of the peroxisome proliferator-activated receptor (PPAR) in mediating the effects of fibrates and fatty acids on gene expression. *J. Lipid Res.* **37:** 907–925.
3. Spiegelman, B. M. 1998. PPAR-gamma—adipogenic regulator and thiazolidinedione receptor. *Diabetes.* **47:** 507–514.
4. Issemann, I., and S. Green. 1990. Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. *Nature.* **347:** 645–650.
5. Tontonoz, P., E. Hu, R. A. Graves, A. I. Budavari, and B. M. Spiegel-



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man. 1994. mPPAR gamma 2: tissue-specific regulator of an adipocyte enhancer. *Genes & Dev.* **8:** 1224–1234.
6. Braissant, O., F. Foufelle, C. Scotto, M. Dauca, and W. Wahli. 1996. Differential expression of peroxisome proliferator-activated receptors (PPARs)—tissue distribution of PPAR-α, -β, and -γ in the adult rat. *Endocrinology.* **137:** 354–366.
7. Fajas, L., J. C. Fruchart, and J. Auwerx. 1998. PPAR-gamma-3 mRNA—a distinct PPAR-gamma mRNA subtype transcribed from an independent promoter. *FEBS Lett.* **438:** 55–60.
8. Yu, K., W. Bayona, C. B. Kallen, H. P. Harding, C. P. Ravera, G. McMahon, M. Brown, and M. A. Lazar. 1995. Differential activation of peroxisome proliferator-activated receptors by eicosanoids. *J. Biol. Chem.* **270:** 23975–23983.
9. Forman, B. M., P. Tontonoz, J. Chen, R. P. Brun, B. M. Spiegelman, and R. M. Evans. 1995. 15-Deoxy-delta(12,14)-prostaglandin J(2) is a ligand for the adipocyte determination factor PPAR-gamma. *Cell.* **83:** 803–812.
10. Forman, B. M., J. Chen, and R. M. Evans. 1997. Hypolipidemic drugs, polyunsaturated fatty acids, and eicosanoids are ligands for peroxisome proliferator-activated receptors alpha and delta. *Proc. Natl. Acad. Sci. USA.* **94:** 4312–4317.
11. Devchand, P. R., H. Keller, J. M. Peters, M. Vazquez, F. J. Gonzalez, and W. Wahli. 1996. The PPAR-alpha-leukotriene B-4 pathway to inflammation control. *Nature.* **384:** 39–43.
12. Kliewer, S. A., J. M. Lenhard, T. M. Willson, I. Patel, D. C. Morris, and J. M. Lehmann. 1995. A prostaglandin J(2) metabolite binds peroxisome proliferator-activated receptor gamma and promotes adipocyte differentiation. *Cell.* **83:** 813–819.
13. Issemann, I., R. A. Prince, J. D. Tugwood, and S. Green. 1993. The peroxisome proliferator-activated receptor:retinoid X receptor heterodimer is activated by fatty acids and fibrate hypolipidaemic drugs. *J. Mol. Endocrinol.* **11:** 37–47.
14. Frøyland, L., L. Madsen, H. Vaagenes, G. K. Totland, J. Auwerx, H. Kryvi, B. Staels, and R. K. Berge. 1997. Mitochondrion is the principal target for nutritional and pharmacological control of triglyceride metabolism. *J. Lipid Res.* **38:** 1851–1858.
15. Simpson, A. 1997. The cytochrome P450 4 (CYP4) family. *Gen. Pharmacol.* **28:** 351–359.
16. Schoonjans, K., J. Peinadoonsurbe, A. M. Lefebvre, R. A. Heyman, M. Briggs, S. Deeb, B. Staels, and J. Auwerx. 1996. PPAR-alpha and PPAR-gamma activators direct a distinct tissue-specific transcriptional response via a PPRE in the lipoprotein lipase gene. *EMBO J.* **15:** 5336–5348.
17. Hiragun, A., M. Sato, and H. Mitsui. 1988. Preadipocyte differentiation in vitro: identification of a highly active adipogenic agent. *J. Cell. Physiol.* **134:** 124–130.
18. Kletzien, R. F., S. D. Clarke, and R. G. Ulrich. 1992. Enhancement of adipocyte differentiation by an insulin-sensitizing agent. *Mol. Pharmacol.* **41:** 393–398.
19. Tontonoz, P., E. D. Hu, and B. M. Spiegelman. 1994. Stimulation of adipogenesis in fibroblasts by PPAR gamma 2, a lipid-activated transcription factor. *Cell.* **79:** 1147–1156.
20. Brun, R. P., P. Tontonoz, B. M. Forman, R. Ellis, J. Chen, R. M. Evans, and B. M. Spiegelman. 1996. Differential activation of adipogenesis by multiple PPAR isoforms. *Genes & Dev.* **10:** 974–984.
21. Young, P. W., M. A. Cawthorne, P. J. Coyle, J. C. Holder, G. D. Holman, I. J. Kozka, D. M. Kirkham, C. A. Lister, and S. A. Smith. 1995. Repeat treatment of obese mice with BRL 49653, a new potent insulin sensitizer, enhances insulin action in white adipocytes. Association with increased insulin binding and cell-surface GLUT4 as measured by photoaffinity labeling. *Diabetes.* **44:** 1087–1092.
22. Hallakou, S., L. Doare, F. Foufelle, M. Kergoat, M. Guerre-Millo, M. F. Berthault, I. Dugail, J. Morin, J. Auwerx, and P. Ferre. 1997. Pioglitazone induces in vivo adipocyte differentiation in the obese Zucker Fa/Fa rat. *Diabetes.* **46:** 1393–1399.
23. Chang, A. Y., B. M. Wyse, and B. J. Gilchrist. 1983. Ciglitazone, a new hypoglycemic agent. II. Effect on glucose and lipid metabolisms and insulin binding in the adipose tissue of C57BL/6J-ob/ob and -+/? mice. *Diabetes.* **32:** 839–845.
24. Hofmann, C., K. Lorenz, S. S. Braithwaite, J. R. Colca, B. J. Palazuk, G. S. Hotamisligil, and B. M. Spiegelman. 1994. Altered gene expression for tumor necrosis factor-α and its receptor during drug and dietary modulation of insulin resistance. *Endocrinology.* **134:** 264–270.
25. Macdougald, O. A., C. S. Hwang, H. Y. Fan, and M. D. Lane. 1995. Regulated expression of the obese gene product (Leptin) in white adipose tissue and 3T3-L1 adipocytes. *Proc. Natl. Acad. Sci. USA.* **92:** 9034–9037.
26. Zhang, B., M. P. Graziano, T. W. Doebber, M. D. Leibowitz, S. White-Carrington, D. M. Szalkowski, P. J. Hey, M. Wu, C. A. Cullinan, P. Bailey, B. Lollmann, R. Frederich, J. S. Flier, C. D. Strader, and R. G. Smith. 1996. Down-regulation of the expression of the obese gene by an antidiabetic thiazolidinedione in Zucker diabetic fatty rats and db/db mice. *J. Biol. Chem.* **271:** 9455–9459.
27. Rentsch, J., and M. Chiesi. 1996. Regulation of ob gene mRNA levels in cultured adipocytes. *FEBS Lett.* **379:** 55–59.
28. Staels, B., N. Vudac, V. A. Kosykh, R. Saladin, J. C. Fruchart, J. Dallongeville, and J. Auwerx. 1995. Fibrates downregulate apolipoprotein C-III expression independent of induction of peroxisomal acyl coenzyme a oxidase—a potential mechanism for the hypolipidemic action of fibrates. *J. Clin. Invest.* **95:** 705–712.
29. Hertz, R., J. Bisharashieban, and J. Bartana. 1995. Mode of action of peroxisome proliferators as hypolipidemic drugs—suppression of apolipoprotein C-III. *J. Biol. Chem.* **270:** 13470–13475.
30. Ashby, J., A. Brady, C. R. Elcombe, B. M. Elliott, J. Ishmael, J. Odum, J. D. Tugwood, S. Kettle, and I. F. Purchase. 1994. Mechanistically based human hazard assessment of peroxisome proliferator-induced hepatocarcinogenesis. *Hum. Exp. Toxicol.* **13:** S1–117.
31. Colca, J. R., and D. R. Morton. 1990. Antihyperglycaemic thiazolidinediones: ciglitazone and its analogues. *In* New Antidiabetic Drugs. C. J. Bailey and P. R. Flatt, editors. Eldered Smith-Gordon, London. 255–261.
32. Cantello, B. C. C., M. A. Cawthorne, G. P. Cottam, P. T. Duff, D. Haigh, R. M. Kindley, C. A. Lister, S. A. Smith, and P. L. Thurlby. 1994. [[Omega-(heterocyclylamino)alkoxy]benzyl]-2, 4-thiazolidinediones as potent antihyperglycemic agents. *J. Med. Chem.* **37:** 3977–3985.
33. Lehmann, J. M., L. B. Moore, T. A. Smith-Oliver, W. O. Wilkison, T. M. Willson, and S. A. Kliewer. 1995. An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor gamma (PPAR-gamma). *J. Biol. Chem.* **270:** 12953–12956.
34. Johnson, M. D., L. K. Campbell, and R. K. Campbell. 1998. Troglitazone—review and assessment of its role in the treatment of patients with impaired glucose tolerance and diabetes mellitus. *Ann. Pharmacother.* **32:** 337–348.
35. Patel, J., E. Miller, and R. Patwardhan. 1998. Rosiglitazone (BRL49653) monotherapy has significant glucose lowering effect in type 2 diabetic patients. *Diabetes.* **47:** A17.
36. Edvardsson, U., M. Alexandersson, F. Nilsson, H. Brockenhuus von Löwenhielm, A-C. Nyström, B. Ljung, and B. Dahllöf. 1999. A proteome analysis of livers from obese (*ob/ob*) mice treated with the peroxisome proliferator WY14,643. *Electrophoresis.* **20:** 935–942.
37. Görg, A., G. Boguth, C. Obermaier, A. Posch, and W. Weiss. 1995. Two-dimensional polyacrylamide gel electrophoresis with immobilized pH gradients in the first dimension (IPG-Dalt): the state of the art and the controversy of vertical versus horizontal systems. *Electrophoresis.* **16:** 1079–1086.
38. Shevchenko, A., M. Wilm, O. Vorm, and M. Mann. 1996. Mass spectrometric sequencing of proteins silver-stained polyacrylamide gels. *Anal. Chem.* **68:** 850–858.
39. Garrels, J. I. 1989. The QUEST system for quantitative analysis of two-dimensional gels. *J. Biol. Chem.* **264:** 5269–5282.
40. Vidalpuig, A., M. Jimenezlinan, B. B. Lowell, A. Hamann, E. Hu, B. Spiegelman, J. S. Flier, and D. E. Moller. 1996. Regulation of PPAR gamma gene expression by nutrition and obesity in rodents. *J. Clin. Invest.* **97:** 2553–2561.
41. Costet, P., C. Legendre, J. More, A. Edgar, P. Galtier, and T. Pineau. 1998. Peroxisome proliferator-activated receptor α-isoform deficiency leads to progressive dyslipidemia with sexually dimorphic obesity and steatosis. *J. Biol. Chem.* **273:** 29577–29585.



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