Peroxisome Proliferator Activated Receptor-Mediated Pathways
Are Altered in Hepatocyte-Specific Retinoid X Receptor α Deficient Mice

Yu-Jui Yvonne Wan‡, Yan Cai, William Lungo, Paul Fu, Joseph Locker§,
Samuel French, Henry M. Sucov¶

‡Department of Pathology, Harbor-UCLA Medical Center, Torrance, CA
§Department of Pathology, Albert Einstein College of Medicine, Bronx, NY
¶Department of Biochemistry & Molecular Biology and Cell & Neurobiology, Institute for Genetic Medicine, Keck School of Medicine, University Southern California, Los Angeles, CA

Running title: The functional role of hepatocyte RXRα in PPARα –mediated pathways

Correspondence:
Yu-Jui Yvonne Wan, Ph.D.
Department of Pathology
Harbor-UCLA Medical Center
1000 West Carson Street
Torrance CA, 90509
Tel: 310-222-3876
Fax: 310-782-6649
E-mail: agarose@ucla.edu
SUMMARY

Retinoid x receptor α (RXRα) serves as an active partner of peroxisome proliferator activated receptor (PPARα). In order to dissect the functional role of RXRα and PPARα in PPARα-mediated pathways, the hepatocyte RXRα deficient mice have been challenged with physiological and pharmacological stresses—fasting and Wy14,643, respectively. The data demonstrate that RXRα and PPARα deficiency are different in several aspects. At the basal untreated level, RXRα deficiency resulted in marked induction of apolipoprotein AI and CIII (apoAI and apoCIII) mRNA levels and serum cholesterol and triglyceride levels, which was not found in PPARα–null mice. Fasting induced PPARα activation was drastically prevented in the absence of hepatocyte RXRα. Wy14,643-mediated pleiotropic effects were also altered due to the absence of hepatocyte RXRα. Hepatocyte RXRα deficiency did not change the basal acyl-CoA oxidase, medium chain acyl-CoA dehydrogenase, and malic enzyme mRNA levels. However, the inducibility of those gene by Wy14,643 was markedly reduced in the mutant mouse livers. In contrast, the basal cytochrome P450 4A1, liver fatty acid-binding protein, and apoAI and CIII mRNA levels were significantly altered in the mutant mouse livers, but the regulatory effect of Wy14,643 on expression of those genes remained the same. Wy14,643 induced hepatomegaly was partially inhibited in hepatocyte RXRα deficient mce. Wy14,643 induced hepatocyte peroxisome proliferation was preserved in the absence of hepatocyte RXRα. These data suggested that in comparison to PPARα, hepatocyte RXRα has its unique role in lipid homeostasis and that the effect of RXRα, β, and γ is redundant in certain aspects.
INTRODUCTION

Peroxisome proliferators including herbicides, plasticizers, hypolipidemic drugs (fibrates), and leukotriene D4 inhibitors play a crucial role in hepatocyte proliferation. The most potent peroxisome proliferator is Wy14,643. These agents cause profound peroxisome proliferation in hepatocytes resulting in hepatomegaly and hepatoma and a rapid transcription of genes encoding the enzymes involved in fatty acid metabolism (for reviews see 1-4). Peroxisome proliferators exert their pleitropic responses via PPAR\(\alpha\), a member of the nuclear hormone receptor superfamily (5-10). Besides peroxisome proliferator, PPAR\(\alpha\) can also be activated by certain conditions such as starvation, high fat diet, diabetes mellitus under which increased fatty acids are delivered to the liver (11-13).

RXRs are the required active heterodimeric partners of PPARs (14). Thus, RXR, PPAR, and their ligands are all actively involved in regulating liver gene expression, fatty acid metabolism, lipid transport, and hepatocyte proliferation. Among the three types of RXR, RXR\(\alpha\) is the predominant one expressed in the liver.

Absence of PPAR\(\alpha\) expression in knockout mice prevents the induction of hepatocyte peroxisome proliferation and of fatty acid synthesizing enzymes and \(\beta\) oxidizing enzymes by Wy14,643 (15-17). In addition, PPAR\(\alpha\) deficiency leads to elevated serum cholesterol levels in young adult mice and increased serum triglyceride levels and steatosis in aging mice (18). There is no in vivo model available with which to compare the role of RXR\(\alpha\) with PPAR\(\alpha\), because of embryonic lethality.
caused by a fetal cardiac phenotype in RXRα-null mice (19-21). RXRβ and RXRγ-null mice have no apparent consequence on the liver (21). Furthermore, RXRα is strongly implicated in postnatal liver physiology and regulation of liver gene (22-28). To understand the biological role of RXRα in the liver, we have generated hepatocyte-specific RXRα knockout mice using a cre/loxP recombination system (29). In this study, we further characterized the impact of RXRα in PPARα mediated pathways.

EXPERIMENTAL PROCEDURES

Mouse- A line of mice in which the RXRα gene is conditionally mutated by introduction of loxP sites into introns flanking exon 4 of the RXRα gene was provided by Dr. K. Chien (UCSD) (30). This modified allele is fully functional, in those animals which are homozygous for this allele are normal and viable. Moreover, this mutated allele was used to specifically ablate RXRα function in the cardiomyocyte lineage (30). To abolish RXRα function in the hepatocytes, the albumin promoter/enhancer was employed to express cre recombinase. Dr. M. A. Magnuson (Vanderbilt Medical Center) provided this albumin-cre transgenic line, which provides liver specific expression. Hepatocyte-specific RXRα knockout was established by crossing albumin-cre transgene with the RXRα flox/flox background (29). PPARα-null mice were generously provided by Dr. Frank Gonzalez (15).

4-chloro-6-(2,3-sylidine)-pyrimidinylthio)acetic acid (Wy14,643) was purchased commercially
Pelleted mouse chow, which composed of 21.4% protein, 55% carbohydrates, 4% fat, 6.7% ash, 4% fiber, and less than 10% moisture, was commercially prepared containing either 0.0% (control) or 0.1% (wt/wt) Wy14,643 (Bioserv, Frenchtown, NJ). For all the experiments 10-16 weeks old male mice were used. Mice were fed either control or Wy14,643 diet ad libitum for 10 days. For starvation experiment, mouse chow was removed from mice for 48 hours. Animals were housed in groups of two or three in plastic microisolator cages at 25°C with a 12-h light/12-h dark cycle.

At the end of the treatment, animals were weighed and anaesthetized with pentobarbital (60 mg/kg, ip). Blood samples were obtained by intracardiac puncture. Blood triglycerides and cholesterol levels were determined by automated analysis. The liver was removed immediately, weighed, frozen in liquid nitrogen, and processed for RNA extraction. Part of liver was fixed by formalin and 1.5% glutaraldehyde for light and electron microscopy analysis, respectively.

**Northern Blot Hybridization**- Molecular aspects of hepatocyte-specific RXRα mutation were evaluated by northern blotting analysis of RNA levels in the liver for the expression of PPARα target genes. The gene probes used were apoAI and CIII (provided by Dr. J. Auwerx), liver fatty acid-binding protein (provided by Dr. J. Gordon), malic enzyme (provided by Dr. G. Brent), acyl-CoA oxidase (31, provided by Dr. T. Osumi), medium chain acyl-CoA dehydrogenase (12, provided by Dr. D. Kelly), CYP4A1 (32, provided by Dr. F. Gonzalez), and catalase (33, purchased from American Type Culture Collection).
For northern analysis, hepatocyte and liver total RNA was extracted by the guanidinium isothiocyanate method (34). Twenty μg of total RNA per lane was resolved by electrophoresis on a 1.2 % agarose gels containing 2.2 M formaldehyde and then transferred to nylon membranes by capillary blotting. cDNA fragments were labeled by random priming and hybridized to membranes in 7% (w/v) SDS, 0.5 M sodium phosphate, pH 6.5, 1 mM EDTA, and 1 mg/ml bovine serum albumin at 68°C overnight. The membranes were washed twice in 1% SDS, 50 mM NaCl, and 1 mM EDTA at 68°C for 15 minutes each and autoradiographed using intensifying screens. Four animals from each group were studied for each gene. The amount of mRNA expressed was quantitated by densitometry and then normalized with the level of 18S rRNA to obtain mean and standard deviation. Statistical relevance of discrepancies between groups was evaluated by Student's t-test.

RESULTS

Inhibition of fasting induced PPARα activation in hepatocyte RXRα deficient mouse- Prolonged starvation induces dramatic changes in metabolism, including the release of large amounts of fatty acids from the adipose tissue, followed by fatty acid oxidation in the liver. It has been demonstrated that PPARα mediates the adaptive response to fasting (11-13). To analyze the role of RXRα in fasting activated PPARα pathways, PPARα-null mic, hepatocyte RXRα deficient mice, and wild type controls were deprived from food for 48 hours and then the expression of PPARα target genes in the livers was examined by northern hybridization.

In mice fed the control diet, PPARα deficiency caused a reduction in the level of acyl-CoA oxidase
(AOX) and cytochrome P450 4A1 (CYP4A1) mRNA encoding the key enzymes involved in fatty acid β- and ω-oxidation pathways (Fig. 1). PPARα deficiency also resulted in a decreased expression of liver fatty acid binding protein (LFABP) mRNA and a weak induction of apoA1 mRNA. In comparison, RXRα deficiency resulted in inhibition of expression of CYP4A1 and LFABP mRNA. The level of AOX and medium chain acyl-CoA dehydrogenase (MCAD) mRNA remained unchanged. The most striking difference between the PPARα and RXRα deficient mice was that the expression of apoAI and apoCIII mRNA was markedly increased in the absence of RXRα, whereas the induction was very weak, if there was any, in the PPARα-null mice (Fig. 1).

In wild type mice, starvation caused significant induction of PPARα target gene except for the apoCIII gene (Fig.1). PPARα deficiency completely abolished PPARα target gene activation induced by starvation. The reduced expression of AOX, MCAD, CYP4A1, and LFABP genes in fasted PPARα-null mice suggested that the transcription of these genes was dependent on PPARα in the fasting state. RXRα deficiency had a similar effect; starvation induced PPARα activation was prevented in the absence of RXRα. Starvation only caused a weak induction of MCAD and CYP4A1 mRNA in RXRα deficient mice (1.8- and 4-fold induction, respectively) compared with wild-type mice (10- and 20-fold induction, respectively); this weak effect caused by starvation probably was due to the presence of RXRβ and γ. These data unambiguously proved that in vivo in the hepatocyte, the effect of PPARα and RXRα is coupled. In addition, hepatocyte RXRα has a unique effect in regulating the expression of apolipoprotein genes in vivo.
Alteration of peroxisome proliferator induced PPARα activation in hepatocyte RXRα deficient mouse- To further analyze the role of RXRα in PPARα/RXRα-mediated pathways; the expression of the PPARα/RXRα target genes was examined in Wy14,643 treated mice. Wild-type and RXRα deficient mice were treated with Wy14,643 (0.1%, wt/wt) for 10 days. Total liver RNA was extracted for analyzing the expression of PPARα target genes. The results of two representative mouse liver samples from two mice are shown in Figure 2. Consistent with the data demonstrated in Figure 1, the basal AOX, MCAD, and malic enzyme mRNA level remained unchanged in mutant mouse livers. After Wy14,643 treatment, the expression of AOX, MCAD, and malic enzyme mRNA (50X, 20X, 50X, respectively) in the wild type mouse livers was significantly induced (Fig. 2). In contrast, the inductions were markedly reduced due to hepatocyte RXRα deficiency (only 2- to-5-fold induction). The expression of the catalase gene was not affected by Wy14,643 in wild-type and mutant mouse livers (Fig. 2). These data indicate that at the physiological level, PPARα/RXRα or RXRα/RXRα do not regulate the basal transcription of the AOX, MCAD, and malic enzyme gene in vivo and that only exogenous ligand (Wy14,643)-activated PPARα/RXRα can regulate the expression of these genes. The weak residual inducibility of these genes by Wy14,643 in the mutant mouse livers may be due to the presence of RXRβ and γ.

In contrast to the AOX, MCAD, and malic enzyme genes, the basal transcription of the CYP4A1 and LFABP genes can be controlled by PPARα/RXRα at the physiological level. CYP4A1 and LFABP mRNA level was reduced about 3-fold in RXRα deficient mouse livers compared with the wild-type livers (Fig. 3). However, the inducibility of these two genes by Wy14,643 remained the same in
mutant mouse livers (Fig. 3). After Wy14,643 administration, there was a 50- and 10-fold induction of CYP4A1 and LFABP mRNA level, respectively, in both wild-type and mutant mouse livers. These data suggest that the basal transcription of the CYP4A1 and LFABP genes is constitutively regulated by PPARα/RXRα or RXRα/RXRα through endogenous ligands such as polyunsaturated fatty acids or 9-cis-retinoic acid *in vivo*. Therefore, in the absence of RXRα, these genes are expressed at a reduced level. However, when pharmacological levels of exogenous ligands are present, the availability of RXRβ and γ is sufficient to mediate the inductive effect of Wy14,643.

To further understand the role of RXRα in regulating cholesterol and lipid homeostasis, the expression of apoA1 and apoCIII mRNA was examined in Wy14,643 treated mice. In normal cells, PPARα agonists suppress the expression of these genes. RXRα is involved in the basal transcription of the apolipoprotein genes because the basal mRNA levels in normally fed mice were increased in the absence of RXRα (Fig. 4). However, the inhibitory effect of Wy14,643 on apolipoprotein gene expression remained in the absence of hepatocyte RXRα.

Taken together, the expression pattern of these PPARα target genes can be divided into two groups. In the first group exemplified by the AOX, MCAD, and malic enzyme genes, these genes’ basal mRNA level remains unchanged in mutant mouse liver, but the inducibility of the gene by Wy14,643 is decreased remarkably. In the second group, which includes the CYP4A1, LFABP, apoAI and apoCIII genes, the basal mRNA level is altered in the absence of RXRα, but the regulatory effect of Wy14,643 on gene expression remains unchanged in mutant mouse liver.
Reduction of serum cholesterol and triglyceride level by Wy14,643 in hepatocyte-specific RXRα deficient mouse- As a hypolipidemic drug (1-4), Wy14,643 reduces serum cholesterol and triglyceride level. These effects were tested in the hepatocyte RXRα deficient mice. As shown in figure 5, basal serum triglyceride and cholesterol levels were elevated in the RXRα deficient mice, which is consistent with the northern data (Fig. 1 and 4) demonstrating the induction of apoAI and CIII mRNA in the mutant mouse livers. Administration of Wy14,643 reduced serum triglyceride and cholesterol level not only in wild-type but also in mutant mice. Therefore, Wy14,643 still can exert its hypolipidemic effect even when RXRα is not expressed in the hepatocyte.

Reduced hepatomegaly in the hepatocyte RXRα deficient mice fed Wy14,643 diet- It is well characterized that Wy14,643 causes liver enlargement due to hypertrophy and hyperplasia (hepatomegaly) of hepatocytes (1-4). Furthermore, clofibrate and Wy14,643-induced hepatomegaly is not found in PPARα-null mice (15). In our system, the data was reproducible where Wy14,643 also produced a marked increase in liver weight in the wild-type mouse. The liver/body weight ratio of the wild-type mice increased 2.4-fold after 10 days of Wy14,643 feeding compared with mice fed a standard control diet (Table 1). In contrast, the liver/body ratio of hepatocyte RXRα deficient mouse only increased by 1.6-fold after Wy14,643 treatment. Therefore, the hepatomegaly caused by treatment with the peroxisome proliferator was partially prevented when RXRα was absent.

Morphological analysis of hepatocyte-specific RXRα deficient mice fed control and Wy14,643
Using light and electron microscopy, the liver morphology of the wild type and RXRα-deficient mice was evaluated (Fig. 6). Compared with wild-type mouse livers, RXRα-deficient mouse livers had normal morphology under light and electron microscope (Fig. 6a-d). Treatment of wild-type mice with Wy14,643 resulted in pale pink staining enlarged cells which had increased homogeneous cytoplasm. The cytoplasmic rough endoplasmic reticulum was strikingly reduced (Fig. 6e). Further, the number and the size of peroxisome were significantly increased after the administration of Wy14,643 as demonstrated by electron microscopy (Fig. 6f). In contrast, under light microscopy, the mutant mouse liver contain both normal and enlarged cells after administration of Wy14,643 (Fig. 6g). Electron microscopy revealed that Wy14,643 still induced hepatocyte peroxisome proliferation in RXRα-deficient mice (Fig. 6h).

**DISCUSSION**

Using biochemical and morphological analyses, we have analyzed the hepatic role of RXRα and demonstrated both essential and redundant effects of RXRα in RXRα/PPARα-mediated pathways. Hepatocyte RXRα is crucial for basal lipid and cholesterol homeostasis since serum cholesterol and triglyceride levels are elevated in normally fed mice lacking RXRα. RXRα deficiency can partially prevent the hepatomegaly effect of peroxisome proliferator. Hepatocyte RXRα is essential for maintaining the physiological level of CYP4A1, LFABP, apoAI, and apoCIII. Hepatocyte RXRα deficiency also significantly prevents starvation and Wy14,643-induced PPARα activation. Mice with hepatocyte RXRα deficiency are unable to increase the capacity for cellular fatty acid utilization
in the context of short-term starvation. However, hepatocyte RXRα deficiency neither prevents hepatocyte peroxisome proliferation nor the hypolipidemic effect of the peroxisome proliferators. Since the RXRα, β, and γ genes are expressed in different types of liver cells including parenchyma, endothelial, Kupffer, and stellate cells (35, 36), the presence of RXRα in the liver cells other than hepatocytes as well as the redundant role of RXRs could explain why hepatocyte RXRα deficient mice are still responsive to Wy14,643.

The hepatocyte-specific RXRα deficient mice allow us to compare the functional roles of RXRα with PPARα. Phenotype comparison between the hepatocyte RXRα deficient and PPARα-null mice (15-18) is summarized in Table 2. In PPARα knockout mice, basal serum cholesterol level is elevated to the same extent (1.6-fold induction) as in the hepatocyte specific RXRα knockout mice. However, young adult male PPARα-null mice have normal serum triglyceride and apoCIII level (16, 18). Serum triglyceride level only elevates in aged animals (6-12-month-old), and the level is higher in females (2-fold induction) than males (1.5-fold induction) (18). In contrast, in hepatocyte RXRα deficient mice, a 1.7-fold induction of serum triglyceride level and a remarkable induction of apoCIII gene expression were observed in 2-month-old male mice. The early induction in serum triglyceride level defines the unique and important role of hepatocyte RXRα in controlling lipid homeostasis. It is possible that the effect of RXRα in regulating apoCIII gene expression and serum triglyceride level is mediated through dimerization with PPARγ rather than PPARα.

In PPARα-null mice, peroxisome proliferators such as clofibrate and Wy14,643 are completely
unable to induce hepatomegaly and hepatocyte peroxisome proliferation, and have no effect in regulating the expression of PPAR\(\alpha\) target genes including AOX, bifunctional enzymes, CYP4A1, CYP4A3, LFABP, apoAI, and apoCIII (15-17). These data suggest that the effect of PPAR\(\alpha\) is unique in peroxisome proliferator-mediated pathways, and that PPAR\(\beta\) and \(\gamma\) cannot replace PPAR\(\alpha\). In contrast, \textit{in vivo}, the roles of RXR\(\alpha\), \(\beta\), and \(\gamma\) appear to be at least partially redundant.

Based on our results, the PPAR\(\alpha\)/RXR\(\alpha\) target genes can be categorized into several groups. The first group of genes includes AOX and malic enzyme. The basal transcriptional rate of these genes is controlled by PPAR\(\alpha\), but not by RXR\(\alpha\). The second group of genes is CYP4A1, LFABP, and apoAI. Within this group, the basal transcriptional rate of the genes is constitutively maintained by PPAR\(\alpha\) as well as by RXR\(\alpha\) through endogenous ligands. The third group of gene includes apoCIII. The basal transcriptional rate of the apoCIII gene is controlled by RXR\(\alpha\), but not by PPAR\(\alpha\). Since RXR\(\alpha\) controls the basal transcription of the CYP4A1, LFABP, and apoAI genes, but has no effect on the AOX, MCAD, and malic enzyme genes, these data suggest that \textit{in vivo} at the physiological level RXR\(\alpha\) is crucial for microsomal \(\omega\)-hydroxylation of fatty acids, fatty acid transport, and cholesterol and fatty acid homeostasis, whereas RXR\(\alpha\) may only become important for AOX and MCAD-mediated fatty acid \(\beta\)-oxidation and malic enzyme-mediated lipogenesis when pharmacological dose of PPAR\(\alpha\) ligand is employed.

Even though RXR\(\beta\) and \(\gamma\) are able to substitute RXR\(\alpha\), the total amount of RXRs is critical in mediating the action of RXRs because in the absence of RXR\(\alpha\), fatty acid is not utilized efficiently in
response to starvation and Wy14,643 can not fully exert its effects. RXR dimerizes with more than ten different kinds of receptor. Activation one of these RXR-mediated pathways might alter other pathways in opposite directions. When the pool of RXRs is decreased, many RXR-mediated regulatory pathways may be impaired. Based on our data, it seems that the level of RXR, rather than the type of RXR, has a major impact in mediating the effect of peroxisome proliferator. It is crucial to understand the regulation of the RXR genes.

RXR can be freely activated in permissive heterodimers with PPAR (37) although it also can be silent in non-permissive heterodimers with the thyroid hormone receptor or the vitamin D receptor (38). It would be interesting to test if 9-cis-retinoic acid has the same effect as Wy14,643 on RXRα deficient mice. 9-Cis retinoic acid can activate RXR/RAR and RXR/RXR, and that would further deprive the availability of RXR to PPARα. Therefore, challenge the mutant mice with 9-cis retinoic acid may produce more phenotypes.

Taken together, nuclear factors might have unique, redundant, synergistic, or antagonistic effects. These effects depend on the relative level of the receptors, presence of hormones, or the pathological condition. Comprehension of the regulation of liver gene transcription provides insight into the understanding of the molecular mechanisms leading to liver physiology, function, development, differentiation as well as proliferation.

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FIGURE LEGENDS

Fig. 1  Inhibition of starvation induced PPARα target gene expression in PPARα-null mice and hepatocyte RXRα deficient mice.

Representative northern blots demonstrate the expression of AOX, MCAD, CYP4A1, LFABP, apoAI and apoCIII mRNA in the livers of wild-type, PPARα deficient, and hepatocyte RXRα deficient mice fed regular diet (control) or starved for 48 hours (fasting). Total RNA (20 µg) from mouse livers were electrophoresed and hybridized with the indicated cDNA probes. The relative fold changes of the message levels after normalization to 18S rRNA level are indicated below each panel.
Fig. 2  Inhibition expression of AOX, MCAD, and malic enzyme mRNA induced by Wy14,643 in hepatocyte RXRα deficient mouse livers.

Representative northern blots demonstrate the expression of AOX, MCAD, malic enzyme, and catalase mRNA in the livers of wild-type and hepatocyte RXRα deficient mice fed control or Wy14,643 (0.1%) rodent diet for 10 days. Total RNA (20 µg) from mouse livers were electrophoresed and hybridized with the indicated cDNA probes. The relative fold changes of the message levels after normalization to 18S rRNA level are indicated below each panel.

Fig. 3  Reduction of basal CYP4A1 and LFABP mRNA level in hepatocyte RXRα deficient mouse livers.

Total RNA (20 µg) was extracted from representative livers of wild-type and hepatocyte RXRα deficient mice fed control or Wy14,643 (0.1%) rodent diet for 10 days. Northern blot hybridization was performed using the indicated cDNA probes. The relative fold changes of the message levels after normalization to 18S rRNA level are indicated below each panel.

Fig. 4  The expression of the apoAI and CIII genes in wild type and hepatocyte RXRα deficient mouse livers.

Representative northern blots demonstrate the expression of apoAI and CIII mRNA in the livers of wild-type and hepatocyte RXRα deficient mice fed control or Wy14,643 (0.1%) rodent diet for 10 days. Total RNA (20 µg) was electrophoresed and hybridized with the
indicated cDNA probes. The relative fold changes of the message levels after normalization to 18S rRNA level are indicated below each panel.

Fig. 5  Triglycerides and cholesterol levels after Wy14,643 treatment in RXRα (+/+) and (-/-) mice. Each value represents the mean ± S.D. of 4 mice. When existence, statistically significant differences between treated and untreated animals of the same genotype (*), as well as between wild type and deficient mice (**) are indicated by asterisks (P < 0.05).

Fig. 6.  Light (364X) and electron micrographs (2,652X) of livers from wild-type (a, b, e, f) and hepatocyte RXRα deficient (c, d, g, h) mice fed control (a, b, c, d) and Wy14,643 (0.1%, wt/wt, e, f, g, h) rodent diet for 10 days.
# TABLES

Table 1. Liver/body weight ratio of wild-type (RXRα +/+ ) and hepatocyte
RXRα deficient (RXRα -/- ) mice.

Mice were fed either control diet or 0.1% Wy14,643 for 10 days.
Results are the means ± S.D. of four determinations.

|                 | RXRα (+/+ ) | RXRα (-/- ) |
|-----------------|-------------|-------------|
| Control         |             |             |
| Wy14,643        |             |             |
| Liver/body weight | 0.048 ± 0.005 | 0.118 ± 0.010^ |
|                 | 0.046 ± 0.005 | 0.080 ± 0.008^ |

*P<0.05
Table 2. Phenotype comparison between hepatocyte-specific RXRα deficient and PPARα-null mice.

| Phenotypes                                      | Hepatocyte RXRα Deficient Mouse | PPARα-null Mouse |
|-------------------------------------------------|---------------------------------|------------------|
| Hepatomegaly                                    | Yes                             | No               |
| Peroxisome Proliferation Induced by PP          | Yes                             | No               |
| Serum Cholesterol Level                         | Induced (1.6X)                  | Induced (1.6X)   |
| Serum Triglyceride Level in Young Adult Male Mice | Induced (1.7X)                  | No change        |
| Serum Triglyceride Level in Aged Mice (6-12-month-old) | Not done                       | Induced (higher in females (2X) than males (1.5X)) |

| Gene Expression                  | Hepatocyte RXRα Deficient Mouse | PPARα-null Mouse |
|----------------------------------|---------------------------------|------------------|
| Acyl-CoA Oxidase                 | No change                       | Decrease         |
| Medium chain acyl-CoA dehydrogenase | No change                       | No change         |
| Malic Enzyme                     | No change                       | Decrease         |
| CYP4A1                           | Decrease                        | Decrease         |
| Liver Fatty Acid-binding Protein | Decrease                        | Decrease         |
| ApoAI                            | Increase                        | Increase         |
| Apo CIII                         | Increase                        | No change        |

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Figure 1
Figure 2

| RXRα (+/+) | RXRα (-/-) |
|------------|------------|
| Control    | Control    |
| Wy14,643   | Wy14,643   |
| 1          | 1          |
| 50         | 1          |
| 1          | 2          |
| 20         | 2          |
| 1          | 5          |
| 50         | 1          |
| 1          | 1          |
| 1          | 1          |

- AOX
- MICAD
- Malic Enzyme
- Catalase
- 18S rRNA
Figure 3

| RXRα (+/+) | RXRα (-/-) |
|------------|------------|
| Control    | Wyl14,643  | Control    | Wyl14,643  |
| [Image]    | [Image]    | [Image]    | [Image]    |

- CYP 4A1
- LFABP
- 18S rRNA
Figure 4

[Diagram showing Western blots for RXRα (+/+) and RXRα (-/-) mice with control and Wy14,643 conditions. The blots are labeled with proteins Apo AI, Apo CIII, and 18S rRNA.]
Figure 5

The bar graph shows the effect of RXRα and Wy14,643 on triglycerides and cholesterol levels. The graph illustrates a significant increase in triglyceride levels and a decrease in cholesterol levels with the addition of RXRα and Wy14,643.
Peroxisome proliferator activated receptor alpha-mediated pathways are altered in hepatocyte-specific retinoid X receptor alpha deficient mice
Yu-Jui Yvonne Wan, Yan Cai, William Lungo, Paul Fu, Joseph Locker, Samuel French and Henry M. Sucov

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