

# PPAR $\alpha$ and PPAR $\gamma$ activators direct a distinct tissue-specific transcriptional response via a PPRE in the lipoprotein lipase gene

Kristina Schoonjans,  
Julia Peinado-Onsurbe<sup>1</sup>,  
Anne-Marie Lefebvre, Richard A. Heyman<sup>2</sup>,  
Michael Briggs<sup>2</sup>, Samir Deeb<sup>3</sup>, Bart Staels  
and Johan Auwerx<sup>4</sup>

U325 INSERM, Département d'Athérosclérose, Institut Pasteur, 59019 Lille, France, <sup>1</sup>Department of Biochemistry and

Molecular Biology, University of Barcelona, Barcelona, Spain,

<sup>2</sup>Ligand Pharmaceuticals, San Diego, CA 92121, USA and

<sup>3</sup>Departments of Medicine and Genetics, University of Washington, Seattle, WA 98195, USA

<sup>4</sup>Corresponding author

**Increased activity of lipoprotein lipase (LPL) may explain the hypotriglyceridemic effects of fibrates, thiazolidinediones and fatty acids, which are known activators (and/or ligands) of the various peroxisome proliferator-activated receptors (PPARs). Treatment with compounds which activate preferentially PPAR $\alpha$ , such as fenofibrate, induced LPL expression exclusively in rat liver. In contrast, the antidiabetic thiazolidinedione BRL 49653, a high affinity ligand for PPAR $\gamma$ , had no effect on liver, but induced LPL expression in rat adipose tissue. In the hepatocyte cell line AML-12, fenofibric acid, but not BRL 49653, induced LPL mRNA, whereas in 3T3-L1 preadipocytes, the PPAR $\gamma$  ligand induced LPL mRNA levels much quicker and to a higher extent than fenofibric acid. In both the *in vivo* and *in vitro* studies, inducibility by either PPAR $\alpha$  or  $\gamma$  activators, correlated with the tissue distribution of the respective PPARs: an adipocyte-restricted expression of PPAR $\gamma$ , whereas PPAR $\alpha$  was expressed predominately in liver. A sequence element was identified in the human LPL promoter that mediates the functional responsiveness to fibrates and thiazolidinediones. Methylation interference and gel retardation assays demonstrated that a PPAR $\alpha$  or  $\gamma$  and the 9-*cis* retinoic acid receptor (RXR) heterodimers bind to this sequence **-169 TGCCCTTCCCCC -157**. These data provide evidence that transcriptional activation of the LPL gene by fibrates and thiazolidinediones is mediated by PPAR-RXR heterodimers and contributes significantly to their hypotriglyceridemic effects *in vivo*. Whereas thiazolidinediones predominantly affect adipocyte LPL production through activation of PPAR $\gamma$ , fibrates exert their effects mainly in the liver via activation of PPAR $\alpha$ .**

**Keywords:** atherosclerosis/gene expression/lipoproteins/nuclear receptors/peroxisomes

## Introduction

Peroxisome proliferator-activated receptors (PPARs) are members of the superfamily of nuclear hormone receptors

that function as ligand-dependent transcription factors. Upon ligand activation they regulate the expression of genes containing specific response elements, called PPRES (Osumi *et al.*, 1991; Tugwood *et al.*, 1992). Three receptor subtypes of PPARs, termed  $\alpha$ ,  $\delta$  (or  $\beta$ ) and  $\gamma$ , have been identified (Isseman and Green, 1990; Dreyer *et al.*, 1992; Gottlicher *et al.*, 1992; Schmidt *et al.*, 1992; Sher *et al.*, 1993; Zhu *et al.*, 1993; Kliewer *et al.*, 1994; Tontonoz *et al.*, 1994a; Amri *et al.*, 1995; Aperlo *et al.*, 1995). These receptors heterodimerize with the retinoid X receptor (RXR) and alter the transcription of target genes after binding to PPRES, which consist of a hexameric nucleotide direct repeat of the recognition motif (TGACCT) spaced by one nucleotide (DR-1). After activation, PPARs control the expression of genes implicated in intra- and extracellular lipid metabolism, such as the genes encoding enzymes involved in the peroxisomal  $\beta$ -oxidation pathway (Osumi *et al.*, 1991; Tugwood *et al.*, 1992; Zhang *et al.*, 1992; Bardot *et al.*, 1993; Marcus *et al.*, 1993; Alvarez *et al.*, 1994), cytochrome P450 4A6 (Muerhoff *et al.*, 1992; Palmer *et al.*, 1994), 3-hydroxy-3-methylglutaryl-CoA synthase (Rodriguez *et al.*, 1994), medium chain acyl-CoA dehydrogenase (Gulick *et al.*, 1994), adipocyte fatty acid binding protein aP2 (Tontonoz *et al.*, 1994a), apolipoproteins A-I and A-II (Vu-Dac *et al.*, 1994, 1995) and the acyl-CoA synthetase gene (Schoonjans *et al.*, 1995).

The transcriptional activity of the PPAR subtypes is enhanced by a multitude of chemical compounds, including fatty acids, thiazolidinedione antidiabetic agents, prostaglandins, peroxisome proliferators and fibrate hypolipidemic drugs. Whereas all these compounds are known to activate PPARs, only for PPAR $\gamma$  has a ligand been identified. PPAR $\gamma$  directly binds antidiabetic thiazolidinediones (Forman *et al.*, 1995; Lehmann *et al.*, 1995) and prostaglandin derivatives (Forman *et al.*, 1995; Kliewer *et al.*, 1995), but not the other activators. Fatty acids more selectively activate the PPAR $\delta$  type, whereas fibrates are more selective as PPAR $\alpha$  activators (Kliewer *et al.*, 1994). Furthermore, the fibrate Wy-14,643 was shown to have almost no activity on PPAR $\gamma$  (Yu *et al.*, 1995). In addition to ligand selectivity for the PPAR subtypes, the expression patterns of the various PPAR subtypes are also distinct. PPAR $\alpha$  is predominantly expressed in liver, heart, kidney, intestinal mucosa and brown adipose tissue, all tissues with high catabolic rates of fatty acids and peroxisomal metabolism (Isseman and Green, 1990; Beck *et al.*, 1992; Braissant *et al.*, 1995). PPAR $\delta$  is abundantly and ubiquitously expressed, whereas PPAR $\gamma$  presents a much more restricted expression (Braissant *et al.*, 1995). In fact, the mPPAR $\gamma$ 2 isoform is predominantly expressed in adipose tissue (Zhu *et al.*, 1993; Tontonoz *et al.*, 1994a,b). The pivotal role of mPPAR $\gamma$ 2 in adipocyte differentiation was elegantly demonstrated by the induction of an adipocyte phenotype in fibroblast and muscle cells overexpressing

mPPAR $\gamma$ 2 (Tontonoz *et al.*, 1994a). Furthermore, several key adipocyte-specific genes are induced by PPAR activators and contain functional PPREs in their regulatory sequences (Schoonjans *et al.*, 1993b, 1995; Tontonoz *et al.*, 1994b, 1995). Finally, arachidonic acid-derived PPAR $\gamma$  ligands are potent inducers of adipocyte differentiation (Gaillard *et al.*, 1989; Forman *et al.*, 1995; Kliewer *et al.*, 1995).

Fibrates, fish oils and thiazolidinediones are known to lower serum triglyceride levels (Grundy and Denke, 1990; Ikeda *et al.*, 1990; Schonfeld, 1994), but the exact mechanism by which they affect triglyceride levels is currently unknown. Whereas clinical studies suggested that part of the beneficial effects of fibrates and fish oils on lipoprotein metabolism is due to the induction of lipoprotein lipase (LPL; EC 3.1.1.34) activity (Heller and Harvengt, 1983; Sommariva *et al.*, 1983; Gnasso *et al.*, 1986; Weisweiler, 1989; Levy *et al.*, 1993; Schonfeld, 1994; reviewed in Grundy and Denke, 1990; Schonfeld, 1994), it is not known whether thiazolidinediones have a similar effect. LPL catalyzes the hydrolysis of triglycerides in lipoprotein particles into fatty acids and monoacylglycerol (reviewed in Auwerx *et al.*, 1992). Hence, the action of LPL is crucial both in energy metabolism (supply of free fatty acids) and in lipoprotein metabolism (conversion of triglyceride-rich lipoproteins). Tissue-specific expression of the LPL gene is complex and not fully understood at present. Typically, human LPL is absent in undifferentiated tissues and cells and its expression is induced upon differentiation in adipose tissue, muscle, macrophages and the mammary gland. In liver, LPL is only expressed before weaning and extinguished during liver differentiation and development (Staels and Auwerx, 1992).

Given this pivotal role of LPL in lipid and energy metabolism, we investigated the effects of two distinct chemical classes of PPAR activators, i.e. fibrates (PPAR $\alpha$ -specific) and the antidiabetic thiazolidinediones (PPAR $\gamma$ -specific), on tissue-specific LPL gene expression in the rat. Fibrate treatment induced LPL expression exclusively in liver, whereas BRL 49653, the high affinity ligand for PPAR $\gamma$ , had no effect on liver, but induced adipocyte LPL expression. In both the *in vivo* and *in vitro* studies, inducibility by either PPAR $\alpha$  or PPAR $\gamma$  activators correlated with the tissue-specific distribution of the respective PPARs. This transcriptional induction of the LPL gene by fibrates and thiazolidinediones is mediated through binding of PPAR-RXR heterodimers to a functional PPRE sequence in the human LPL gene promoter. The regulation of LPL by fibrates and thiazolidinediones may contribute to the beneficial triglyceride lowering effect of these substances.

## Results

### **PPAR $\alpha$ and PPAR $\gamma$ activators induce LPL mRNA and activity selectively in liver and adipose tissue *in vivo***

Since the hypotriglyceridemic activity of fibrates and thiazolidinedione antidiabetic agents may be mediated by inducing LPL activity, we measured LPL mRNA and activity levels in various rat tissues after administration of these compounds. Organ and body weights and lipid values of rats are presented in Table I. Fenofibrate (0.5%

w/w ~0.5 g/kg/day), a selective PPAR $\alpha$  activator, lowered both serum triglyceride and cholesterol levels (Table I). In rats treated with fenofibrate, liver weight increased considerably after a 14 day treatment interval, whereas total body weight and weights of the other organs did not change significantly. LPL mRNA, which is normally undetectable in adult rat liver, was strongly induced in rat liver (Figure 1A and B), confirming our previous observations (Staels and Auwerx, 1992). In contrast, treatment with fenofibrate did not change LPL mRNA levels in adipose tissue or heart, two major sites of LPL production (Figure 1A and B). In heart and adipose tissue, no change in LPL activity was detected, whereas a strong induction of LPL activity was detected in liver (Figure 1C;  $P < 0.01$ ). A similar tissue-selective induction of liver LPL mRNA was observed after treatment of rats with other fibrates, such as clofibrate and gemfibrozil (data not shown).

Administration of compound BRL 49653 (5 mg/kg/day for 7 days) also reduced serum triglyceride levels, but did not affect serum cholesterol concentration (Table I). Treatment with BRL 49653 resulted in a significant increase in the weight of epididymal adipose tissue from  $3.5 \pm 0.5$  g to  $5.8 \pm 0.9$  g (adipose tissue/body weight increased from  $0.99 \pm 0.13\%$  to  $1.51 \pm 0.23\%$ ). Total body weight had a tendency to increase, whereas the weight of the other organs remained stable. BRL 49653 treatment resulted in a significant induction of adipose tissue LPL mRNA and activity levels (Figure 1;  $P < 0.05$ ). In contrast, liver LPL mRNA and activity levels remained unchanged. Although heart LPL mRNA did not change, heart LPL activity levels decreased significantly (Figure 1;  $P < 0.05$ ). This decrease in heart LPL activity was consistently observed, since in an independent experiment LPL activity decreased upon treatment with BRL 49653 (10 mg/kg/day for 7 days) from  $531 \pm 47$  to  $279 \pm 39$  mU/g ( $n = 4$ ,  $P < 0.05$ ), despite the absence of changing LPL mRNA levels (data not shown).

### **BRL 49653 induces LPL mRNA specifically in pre-adipocyte cells, whereas fibrates induce LPL mRNA in cells of hepatic origin**

To study the cellular and molecular mechanism of this induction, we investigated the regulation of LPL gene expression by fibrates and BRL 49653 in hepatocyte and adipocyte cell lines. LPL mRNA was measured in AML-12 cells, a mouse hepatocyte cell line with a differentiated phenotype, after stimulation with fenofibric acid or BRL 49653. Fenofibric acid induced LPL mRNA at a minimal dose of 100  $\mu$ M (data not shown), whereas a maximal response was seen at 500  $\mu$ M (Figure 2A). Time course experiments demonstrated a maximal effect within 24 h (data not shown). In contrast, the PPAR $\gamma$  ligand BRL 49653 had no effect on LPL mRNA levels in these cells (Figure 2A).

To examine LPL regulation in adipocyte-like cell lines, 3T3-L1 pre-adipocyte cells were grown until confluency in medium containing fetal calf serum. Confluent cells were transferred to medium containing delipidated serum in order to maintain a more fibroblast-like phenotype, thus avoiding excessive spontaneous differentiation (Chawla and Lazar, 1994). Under these conditions, 3T3-L1 cells continue to proliferate and maintain *c-myc* gene expression,

**Table 1.** Body weight, organ weights and plasma lipid values in rats treated with BRL 49563 or fenofibrate

	Body weight (g)	Adipose tissue weight (g)	Liver weight (g)	Triglycerides (mg/dl)	Cholesterol (mg/dl)
Control	351 ± 19	3.5 ± 0.5	14.4 ± 1.2	167 ± 19	73 ± 2
Fenofibrate (~0.5 g/kg/day)	361 ± 12	3.3 ± 0.5	19.8 ± 1.7 <sup>a</sup>	114 ± 17 <sup>a</sup>	47 ± 9 <sup>a</sup>
BRL 49563 (5 mg/kg/day)	383 ± 15	5.8 ± 0.9 <sup>a</sup>	15.5 ± 1.4	88 ± 30 <sup>a</sup>	77 ± 14

<sup>a</sup>Values significantly different from control rats by Student's *t*-test ( $P < 0.05$ ).

a marker for their proliferative state (Chawla and Lazar, 1994). Next, these cells were challenged with either fenofibric acid (500  $\mu$ M) or BRL 49563 (10  $\mu$ M) (Figure 2B and 2C). LPL mRNA was induced 5-fold after a 1 day treatment with BRL 49563, attaining a maximal 9-fold induction after 3 days. Fenofibric acid induced LPL mRNA levels only 3-fold after a 4 day lag period. aP2 gene expression in 3T3-L1 cells showed a parallel pattern to LPL expression after BRL 49563 or fenofibric acid treatment in 3T3-L1 cells (data not shown). The ob 1771 pre-adipocyte cell line (Negrel *et al.*, 1978) was also analyzed. Addition of BRL 49563 induced LPL mRNA levels in this cell line, whereas fenofibric acid had only a weak effect (Figure 2C).

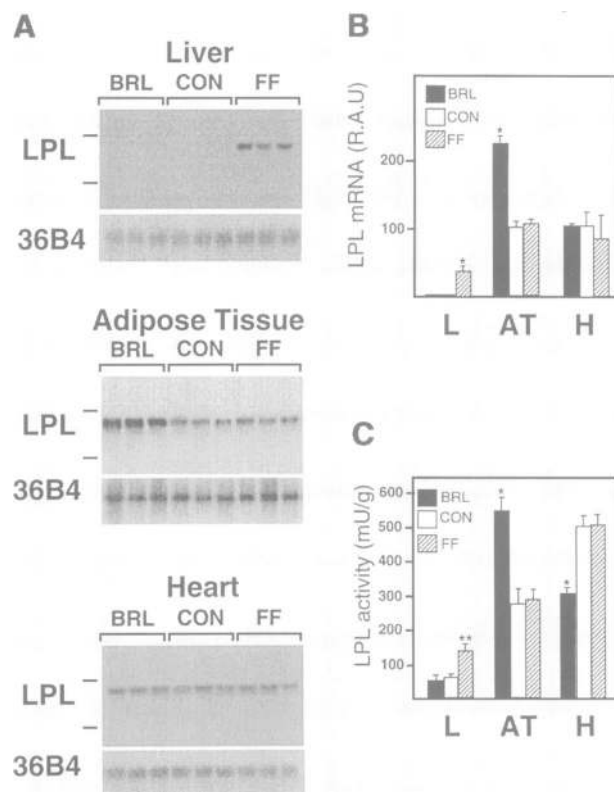
#### **PPAR $\alpha$ is expressed in liver and heart, whereas PPAR $\gamma$ expression is restricted to adipose tissue**

To further characterize the tissue-specific regulatory effects of BRL 49563 and fenofibric acid on adipose tissue and liver respectively, the expression of PPAR $\alpha$  and PPAR $\gamma$  in human and rat tissues was analyzed (Figure 3A). Rat adipose tissue expressed the highest level of PPAR $\gamma$ . PPAR $\gamma$  was also expressed in human adipose tissue and in the pre-adipocyte 3T3-L1 cells, although at lower abundance. PPAR $\alpha$  showed a reciprocal pattern of expression, and was not detected in adipose tissue. PPAR $\alpha$  mRNA levels were highest in rat liver, with lower levels being present in human liver as well as in the differentiated AML-12 hepatocyte cell line. Interestingly, the size of the mRNA for PPAR $\alpha$  was substantially larger in human liver relative to its size in rat liver or the murine AML-12 cells.

We further analyzed whether PPAR $\alpha$  and PPAR $\gamma$  mRNA expression was regulated by their activators and/or ligands (Figure 3B). PPAR $\alpha$  mRNA levels did not change significantly after treatment of animals with fenofibric acid or BRL 49563 in the tissues analyzed. In contrast, adipose tissue PPAR $\gamma$  mRNA levels showed a tendency to increase after the addition of BRL 49563.

#### **LPL cis-regulatory sequences mediate the induction of LPL gene expression by fibrates and thiazolidinediones**

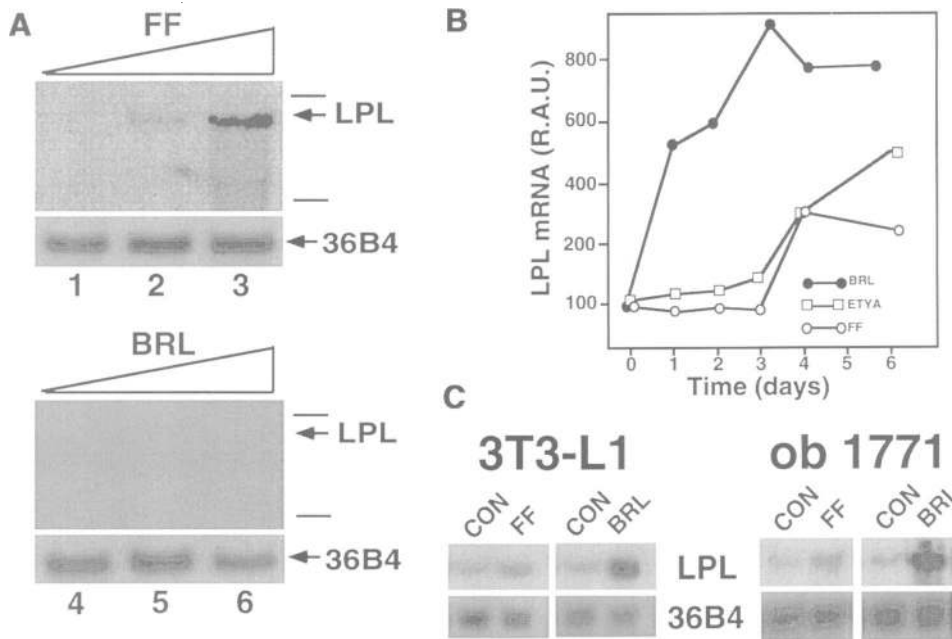
We next investigated whether LPL mRNA induction, observed *in vivo* and in the cell culture experiments, occurred at the transcriptional level. Therefore, LPL promoter constructs hooked to the chloramphenicol acetyltransferase (CAT) reporter were transiently transfected into murine 3T3-L1 cells and into the mouse hepatocyte-derived cell lines BWTG3 and AML-12. The LPL promoter deletion constructs PN-CAT, XN-CAT and AN-CAT contain the 5' upstream regulatory sequence of the human LPL promoter from -1719 to +136, -852 to +136 and -230 to +136 bp respectively (Figure 4A). In BWTG3



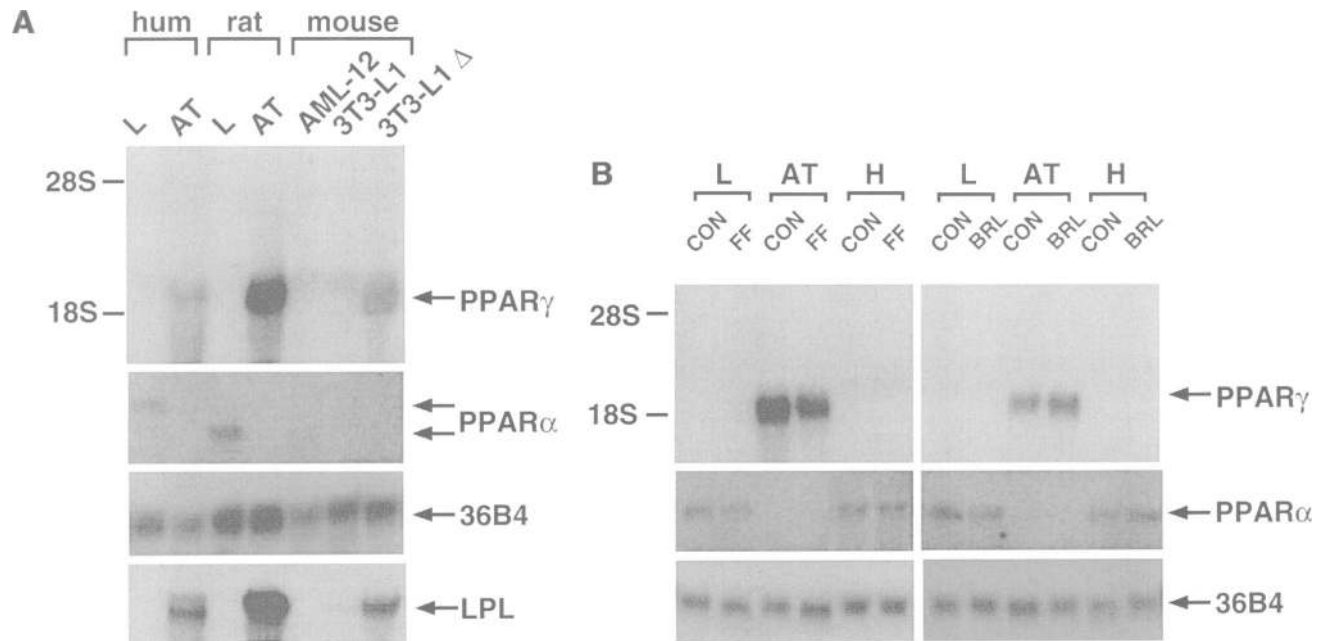
**Fig. 1.** Tissue-selective induction of LPL mRNA (A and B) and activity (C) in rat liver, adipose tissue and heart by fenofibrate and BRL 49563 respectively. (A) Expression of LPL mRNA in liver, epididymal adipose tissue and heart of animals treated with fenofibrate (FF; 0.5% w/w for 7 days, ~0.5 g/kg/day) or BRL 49563 (BRL; 5 mg/kg/day for 7 days). The blots were stripped and re-hybridized with human acidic ribosomal phosphoprotein 36B4 control cDNA. Animal treatment and preparation and analysis of RNA are described in Materials and methods. (B) Comparison of LPL mRNA levels in liver (L), epididymal adipose tissue (AT) and heart (H) of control rats and rats treated with fenofibrate (FF) or BRL 49563 (BRL). LPL mRNA is quantified as described in Materials and methods. Asterisks indicate values statistically significant from control by Student's *t*-test ( $P < 0.05$ ). (C) LPL activity values in liver (L), epididymal adipose tissue (AT) and heart (H) of control rats and rats treated with fenofibrate (FF) or BRL 49563 (BRL). LPL activity assay is described in Materials and methods. Asterisks indicate values statistically significant from control by Student's *t*-test (\* $P < 0.05$ , \*\* $P < 0.01$ ).

cells, only the AN-CAT construct was activated following the addition of fenofibric acid. However, upon co-transfection with a vector that expresses mouse PPAR $\alpha$  (pSG5-mPPAR $\alpha$ ), induction of all three reporter constructs, PN-CAT, XN-CAT and AN-CAT, independent of the presence of fenofibric acid was observed (Figure 4B). In another hepatocyte cell line, AML-12, data similar to the transfection data in BWTG3 were obtained (data not shown).

Upon co-transfection of 3T3-L1 cells with the LPL



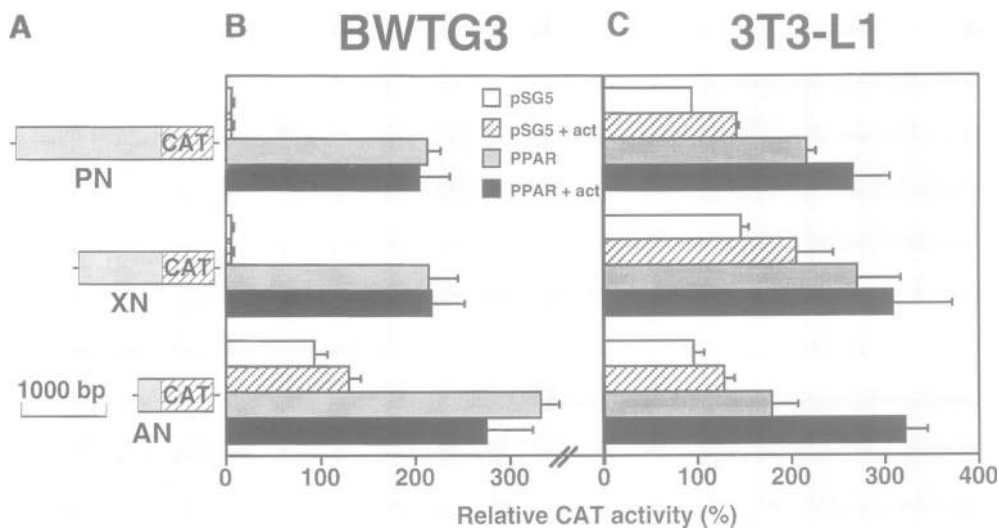
**Fig. 2.** Regulation of LPL mRNA expression in AML-12 hepatocytes (A), in 3T3-L1 preadipocytes (B and C) and in ob 1771 pre-adipocytes (C) by fibrates, thiazolidinediones and fatty acids. (A) AML-12 hepatocytes. Dose-response of LPL mRNA induction in AML-12 cells treated for 24 h with the indicated concentrations of fenofibric acid (FF; lanes 1-3, 0, 250 and 500  $\mu$ M) or BRL 49653 (BRL; lanes 4-6, 0, 1 and 10  $\mu$ M). A probe for 36B4 was used as a control. Cells were grown and mRNA analysis performed as described in Materials and methods. (B) 3T3-L1 cells. Pre-adipocytes were grown until confluency in regular medium containing fetal calf serum. Upon confluency they were changed to medium containing delipidated serum according to the protocol of Chawla *et al.* (1994). Cells were then challenged for different times with either fenofibric acid (FF; 500  $\mu$ M), 5,8,11,14-eicosatetraynoic acid (ETYA; 100  $\mu$ M) or BRL 49653 (BRL; 10  $\mu$ M). mRNA was isolated and analyzed by dot blot hybridization. (C) 3T3-L1 and ob 1771 cells. 3T3-L1 cells were treated exactly as mentioned in (B). RNA isolated 3 days after treatment with either fenofibric acid or BRL 49653 was analyzed by Northern blot analysis. Undifferentiated ob 1771 cells were challenged for 3 days with either fenofibric acid (FF; 500  $\mu$ M) or BRL 49653 (BRL; 10  $\mu$ M).



**Fig. 3.** Expression of the different PPAR forms in adipose tissue and liver. (A) Comparison of PPAR $\alpha$  and PPAR $\gamma$  expression in liver and adipose tissue from rat and human and in mouse AML-12 and 3T3-L1 cell lines. The blots were re-hybridized with 36B4 and LPL cDNAs. Preparation and analysis of RNA is described in Materials and methods. The arrows indicate the various mRNA species. Note the difference in size between human and rodent PPAR $\alpha$  mRNA. (B) Expression of PPAR $\gamma$ , PPAR $\alpha$  and 36B4 mRNA in liver (L), epididymal adipose tissue (AT) and heart (H) of animals treated with fenofibrate (FF; 0.5% w/w for 14 days) or BRL 49653 (BRL; 5 mg/kg/day for 7 days). Animal treatment and preparation and analysis of RNA is described in Materials and methods. The arrows indicate the various mRNA species.

promoter constructs in the presence of pSG5-haPPAR $\gamma$ . LPL promoter activity was also induced (Figure 4C). In this cell line, BRL 49653 alone also induced expression of

the LPL promoter, indicating the presence of endogenous PPAR. These results suggest a functional role for the various PPARs in activation of the LPL gene by BRL



**Fig. 4.** Induction of the LPL gene by peroxisome proliferators is mediated by the LPL 5'-URS. **(A)** Schematic structure of the different human LPL promoter deletion constructs. **(B)** Regulation of deletions in 5' flanking sequences of the LPL gene by fenofibric acid and/or mPPAR $\alpha$  (PPAR) in BWTG3 mouse hepatoma cells. Cells were transfected with the LPL promoter deletion constructs, indicated in (A), either in the presence or absence of co-transfected pSG5 vector plasmid (pSG5), pSG5-mPPAR $\alpha$  expression vector (PPAR) and/or fenofibric acid (500  $\mu$ M). The results represent the mean  $\pm$  SD of a triplicate experiment. The value of CAT activity observed in 3T3-L1 cells transfected with the PN-CAT construct and the pSG5 empty expression vector was arbitrarily set as 100% (see C). CAT activity was measured and expressed relative to this value. **(C)** Regulation of deletions in 5' flanking sequences of the LPL gene by BRL 49653 (10  $\mu$ M) and haPPAR $\gamma$  in 3T3-L1 cells. Legends are identical to Figure 2B. The results represent the mean  $\pm$  SD of three individual experiments. The activity of PN-CAT in absence of any stimulus was taken as 100%.

49653 and fibrates in BWTG3, AML-12 and 3T3-L1 cells. Furthermore, they demonstrate that a *cis*-acting regulatory sequence is located within the first 230 bp upstream from the transcription initiation site which mediates this effect.

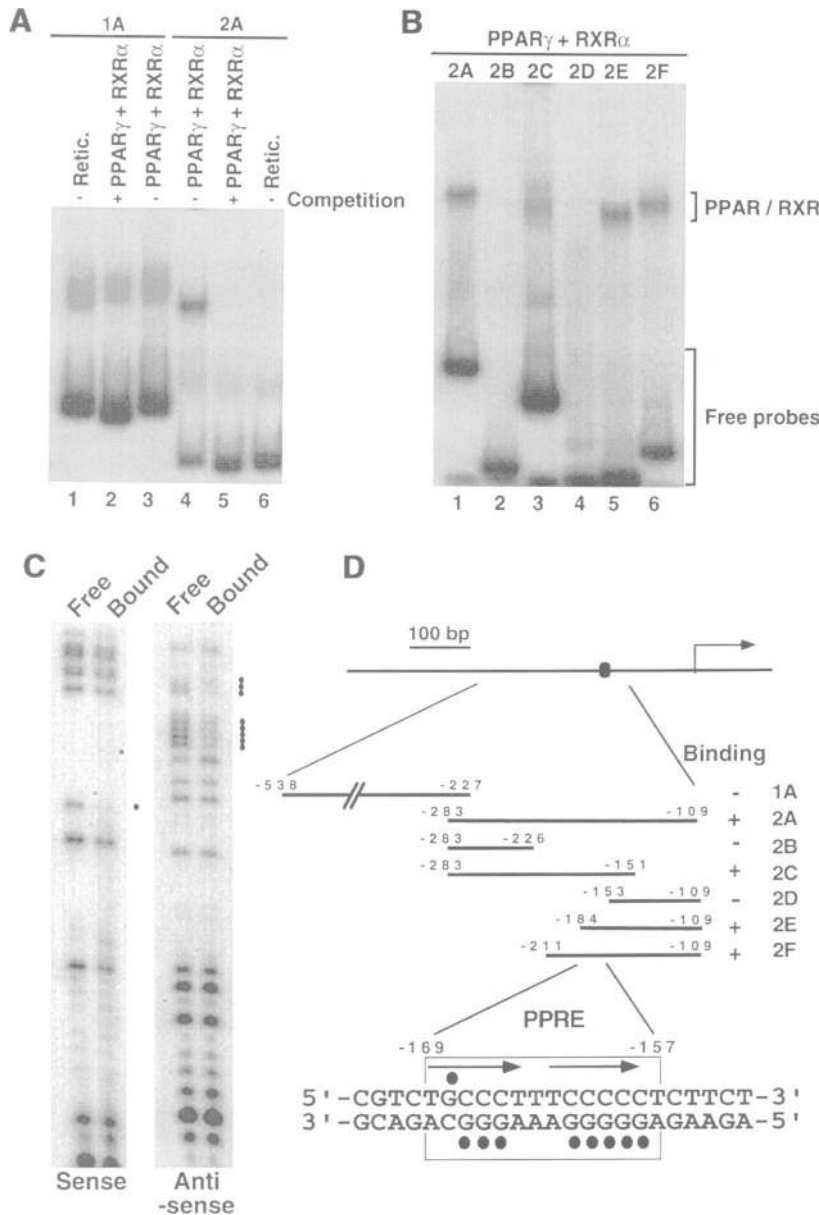
#### Localization of a PPRE in the LPL promoter

Results of transfections with nested deletions of the LPL promoter localized the PPRE within 230 bp upstream from the transcription initiation site of the LPL gene (AN region). In a first attempt to map the potential PPRE in the AN region, two overlapping DNA fragments, 1A and 2A (Figure 5D), each containing a different segment of the AN fragment, were PCR amplified. These fragments were labeled and tested for binding to *in vitro* translated haPPAR $\gamma$  and mRXR $\alpha$  by electrophoretic mobility shift assays (EMSA) (Figure 5A). Fragment 2A, which spans nucleotides -283 to -109, proved to be the only fragment which could specifically bind to haPPAR $\gamma$  and mRXR $\alpha$  heterodimers. Therefore, the regulatory region containing the potential PPRE is restricted to 174 bp. Subsequently, five smaller DNA fragments generated by PCR amplification encompassing different parts of the 2A fragment were synthesized and analyzed for their capacity to bind haPPAR $\gamma$  and mRXR $\alpha$  heterodimers (Figure 5B). The results narrowed the potential binding site of PPAR-RXR heterodimers to a small region located between -184 and -154 (Figure 5B and D). When haPPAR $\gamma$  was replaced by *Xenopus* PPAR $\alpha$ , a similar, albeit slightly less intense, binding was observed in EMSA (data not shown). Methylation interference analysis of fragment 2F confirmed that haPPAR $\gamma$ -mRXR $\alpha$  was able to bind to this region (Figure 5C) and revealed that one methylated guanidine on the sense strand (at -168) and eight methylated guanidines on the antisense strand (from bases -167 to -157) interfered with binding of the haPPAR $\gamma$ -mRXR $\alpha$  protein complex.

An oligonucleotide containing the LPL<sub>wt</sub> PPRE, as defined by physical mapping, was synthesized and used

in EMSA. This oligonucleotide was capable of binding haPPAR $\gamma$ -mRXR $\alpha$  heterodimers in EMSA (Figure 6, lane 1). Similar binding data were obtained when xPPAR $\alpha$  was used instead of haPPAR $\gamma$  and mRXR $\alpha$  was replaced by mRXR $\beta$  (data not shown), demonstrating that the LPL<sub>wt</sub> PPRE element was capable of binding different types of PPAR-RXR heterodimers. Homodimers of haPPAR $\gamma$  or mRXR $\alpha$ , however, were incapable of binding to this oligonucleotide (data not shown). When increasing concentrations of unlabeled LPL<sub>wt</sub> PPRE were added as competitor, binding of the haPPAR $\gamma$ -mRXR $\alpha$  heterodimer to labeled LPL<sub>wt</sub> PPRE was almost completely inhibited (Figure 6A, lanes 2-5). In comparison, addition of similar concentrations of the LPL<sub>mut</sub> PPRE (Figure 6A, lanes 6-9) or an unrelated unlabeled oligonucleotide (data not shown) did not result in competition. An unlabeled oligonucleotide comprising the PPRE of acyl-CoA oxidase (ACO) competed more efficiently with binding of haPPAR $\gamma$ -mRXR $\alpha$  heterodimers when compared with the LPL<sub>wt</sub> PPRE oligonucleotide (Figure 6A, compare lanes 3 and 11). A second cross-competition experiment was performed, using the ACO PPRE as probe (Figure 6B). Whereas the addition of unlabeled ACO oligonucleotide (100-fold molar excess) almost completely inhibited the ability of haPPAR $\gamma$ -mRXR $\alpha$  heterodimers to bind to the ACO PPRE, addition of unlabeled LPL<sub>wt</sub> PPRE induced a somewhat lower level of competition. Hence, the results of both the methylation interference assay and the EMSA experiments unequivocally demonstrate the capacity of PPAR-RXR to physically bind to the LPL PPRE localized between -169 and -157 in the human LPL promoter. This region contains two motifs related to the consensus intracellular receptor binding half-site that are arranged as direct repeats with a spacing of one nucleotide (Osumi *et al.*, 1991; Tugwood *et al.*, 1992).

The mouse LPL gene also contains a sequence TGCCCTTTCCCT at -169 to -157 which is similar to the



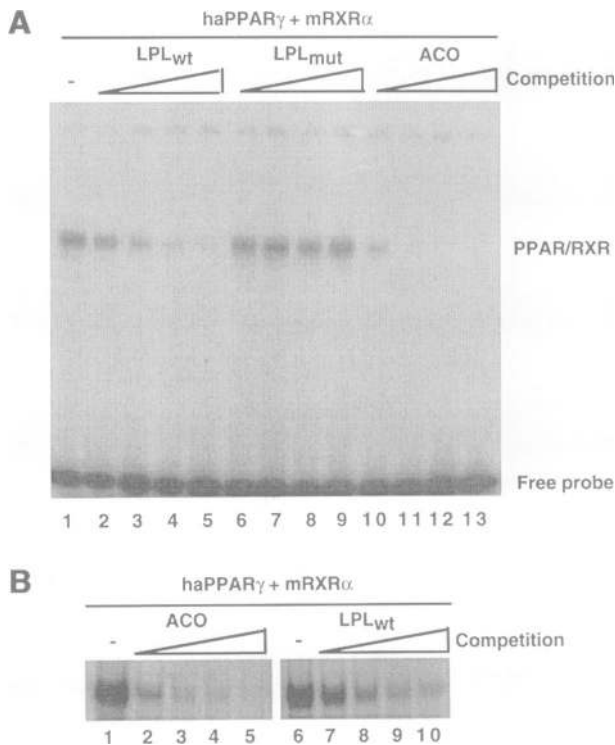
**Fig. 5.** Definition and mapping of a PPAR binding site in the LPL promoter. (A) EMSA assay was performed on PCR fragments 1A (-538 to -227) and 2A (-283 to -109) in the presence of *in vitro* translated mRXR $\alpha$  and haPPAR $\gamma$  exactly as described in Materials and methods. Competition was performed with cold 1A and 2A PCR fragments. (B) EMSA assay was performed on smaller PCR fragments, depicted schematically in (D), in the presence of *in vitro* translated mRXR $\alpha$  and haPPAR $\gamma$  exactly as described in Materials and methods. (C) Methylation interference assays were performed exactly as described in Materials and methods. The reactions using labeled sense and antisense strands are indicated. Contact interference sites of guanidine residues with the protein complex are indicated by a dot. (D) A scheme which depicts the LPL 5' regulatory region and the different PCR fragments used in the EMSA in (A) and (B). In addition, a graphic depiction of the LPL PPRE and the methylated guanidines interfering with binding of the protein complex are shown in (C).

identified PPRE in the human LPL gene. In order to demonstrate that this mouse sequence could also bind haPPAR $\gamma$ -mRXR $\alpha$  heterodimers, we compared binding of these heterodimers with the mouse and human LPL<sub>wt</sub> PPRES in EMSA (Figure 7). Interestingly, the mouse LPL<sub>wt</sub> PPRES bound haPPAR $\gamma$ -mRXR $\alpha$  heterodimers with higher affinity (Figure 7A). The somewhat higher affinity of haPPAR $\gamma$ -mRXR $\alpha$  heterodimers for the mouse LPL PPRES was confirmed in a cross-competition experiment, where an unlabeled oligonucleotide comprising the mouse LPL<sub>wt</sub> PPRES (Figure 7B, lanes 2-4) competed more efficiently with binding of haPPAR $\gamma$ -mRXR $\beta$  heterodimers relative to the human LPL<sub>wt</sub> PPRES oligonucleotide

(Figure 7B, lanes 8-10). The presence of a PPRES in the mouse LPL gene translates into a strong inducibility of the mouse LPL gene by various PPAR activators either *in vitro* in cultured mouse cell lines, such as 3T3-L1, ob 1771 and AML-12 cells (Figure 2), or *in vivo*, as demonstrated by the induction of liver LPL mRNA after treatment of mice with fenofibrate (Figure 7C; 0.5% w/w for 14 days).

#### **The sequence identified in the LPL promoter is a functional PPRES**

We next investigated whether the element identified could mediate the effects of BRL 49653, fibrates and PPAR on



**Fig. 6.** PPAR-RXR heterodimers bind to the LPL PPRE. (A) PPAR-RXR heterodimers bind to the LPL<sub>wt</sub> PPRE. EMSA was performed on end-labeled LPL<sub>wt</sub> PPRE oligonucleotide in the presence of *in vitro* translated mRXR $\alpha$  and haPPAR $\gamma$  (lane 1). Competition experiments were performed in the presence of 10-, 50-, 100- or 200-fold molar excess of either unlabeled LPL<sub>wt</sub> PPRE (lanes 2-5), LPL<sub>mut</sub> PPRE (lanes 6-9) or ACO PPRE (lanes 10-13) oligonucleotides. (B) EMSA assays were performed using end-labeled ACO PPRE oligonucleotide in the presence of *in vitro* translated mRXR $\alpha$  and haPPAR $\gamma$  (lanes 1 and 6). Competition experiments were performed in the presence of 10-, 50-, 100- and 200-fold molar excess of either cold ACO PPRE (lanes 2-5) or LPL<sub>wt</sub> PPRE (lanes 7-10) oligonucleotides.

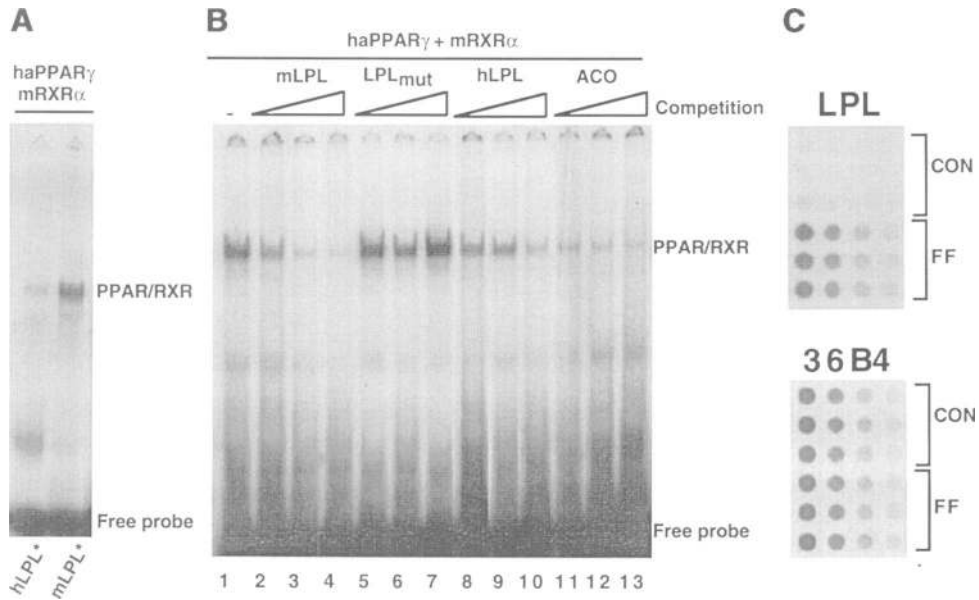
transcription of the LPL gene. To this end, the wild-type PPRE in the AN-CAT construct was mutated (AN-PPRE<sub>mut</sub>-CAT; Figure 8A). The activities of the wild-type and mutated AN-PPRE-CAT constructs were compared after transfection into 3T3-L1 cells (Figure 8B). Treatment of 3T3-L1 cells with either BRL 49653 (10  $\mu$ M) or fenofibric acid (250  $\mu$ M) was unable to induce expression of AN-PPRE<sub>mut</sub>-CAT in either the presence or absence of co-transfected pSG-haPPAR $\gamma$ . These results are in contrast to those observed with the AN-PPRE<sub>wt</sub>-CAT reporter, whose expression was induced by the above-mentioned activators (Figures 4 and 8). Fenofibric acid was a considerably less potent inducer of AN-CAT activity in 3T3-L1 cells than the PPAR $\gamma$  ligand BRL 49653. In AML-12 hepatocyte cells no induction of AN-PPRE<sub>mut</sub>-CAT was also obtained after fenofibric acid (250  $\mu$ M) and/or mPPAR $\alpha$  co-transfection (data not shown). This was in contrast to the results obtained with the AN-PPRE<sub>wt</sub>-CAT reporter with the same inducers.

3T3-L1 pre-adipocyte cells most likely contain low levels of PPARs (below the detection limit of Northern blot hybridization) which can mask the effects of co-transfected PPARs. Therefore, we transfected the wild-type and mutated AN-PPRE-CAT constructs into HeLa cells, which contain very low amount of the different PPARs (Figure 8C). In contrast to 3T3-L1 cells, treatment

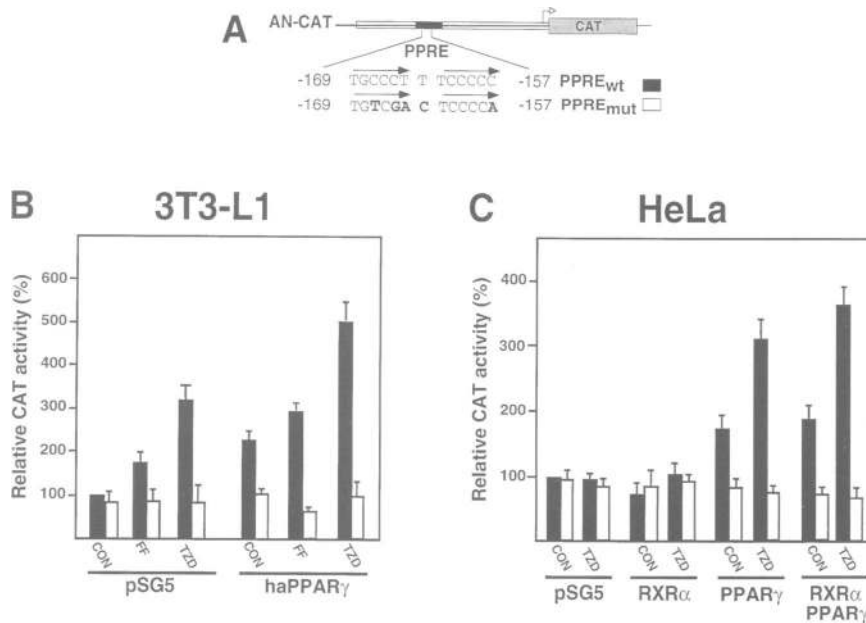
of HeLa cells with BRL 49653 (10  $\mu$ M; Figure 8C) was unable to induce expression of AN-PPRE<sub>wt</sub>-CAT. The AN-PPRE<sub>wt</sub>-CAT reporter was, however, induced upon co-transfection of either haPPAR $\gamma$  by itself or the combination of haPPAR $\gamma$  and mRXR $\alpha$ . This effect was further enhanced by addition of the PPAR $\gamma$  ligand BRL 49653. Treatment of HeLa cells with BRL 49653 was unable to induce expression of AN-PPRE<sub>mut</sub>-CAT in either the presence or absence of co-transfected haPPAR $\gamma$ .

## Discussion

The distinct tissue-specific expression pattern of the different PPARs as well as their distinct activation by various activators and/or ligands suggests a highly specific function for each PPAR type. This investigation aimed to define the molecular mechanisms by which two well-established groups of synthetic PPAR activators, i.e. fibrates (considered to preferentially activate PPAR $\alpha$ ) and the anti-diabetic thiazolidinediones (PPAR $\gamma$  ligands), affect LPL expression in a tissue-specific manner and to investigate the relative contribution of the various PPAR subtypes to these regulatory events. We have chosen to use LPL as a target gene in these studies because a number of clinical studies suggested that the triglyceride lowering action of fibrates and polyunsaturated fatty acids is associated with an increase in LPL activity (Schonfeld *et al.*, 1982; Heller and Harvengt, 1983; Sommariva *et al.*, 1983; Gnasso *et al.*, 1986; Weisweiler, 1989; Ikeda *et al.*, 1990; Levy *et al.*, 1993). LPL is a key enzyme in the metabolism of triglyceride-rich lipoproteins and plays the role of a gatekeeper in energy metabolism by controlling the generation of fatty acids. In contrast to other genes involved in the determination of serum triglyceride levels, such as apolipoprotein C-III, which is only expressed in the liver and intestine, LPL is expressed in multiple tissues, including adipose tissue, liver, heart, mammary gland, macrophages and brain. Results obtained in this study using cultured hepatocyte and pre-adipocyte cell lines, as well as *in vivo* data obtained in rat adipose tissue or rat and mouse liver, establish that LPL mRNA and activity levels can be regulated in a tissue-specific fashion by PPAR $\alpha$  activators and PPAR $\gamma$  ligands. Transient transfection assays of the LPL 5' regulatory sequences indicated that LPL induction was mediated by a response element, located between -157 and -169 in the human LPL gene. This region contains two motifs which are somewhat related to the consensus steroid/thyroid hormone receptor half-site TGACCT, arranged as direct repeats separated by one nucleotide (DR-1), previously identified as mediating responsiveness to the PPARs in several genes involved in lipid metabolism (Osumi *et al.*, 1991; Muerhoff *et al.*, 1992; Tugwood *et al.*, 1992; Zhang *et al.*, 1992; Bardot *et al.*, 1993; Marcus *et al.*, 1993; Alvarez *et al.*, 1994; Gulick *et al.*, 1994; Palmer *et al.*, 1994; Rodriguez *et al.*, 1994; Vu-Dac *et al.*, 1994, 1995; Schoonjans *et al.*, 1995). A sequence highly comparable with the PPRES of the human LPL gene (TGCCCTTTCCCC) (Deeb and Peng, 1989) is found in the mouse LPL gene (TGCCCTTTCCCCT) (Hua *et al.*, 1991), indicating that these rather atypical PPRES-like sequences might have an important regulatory role. We showed that PPAR-RXR heterodimers bind to the response element in the human and mouse



**Fig. 7.** PPAR-RXR heterodimers bind to the mouse LPL PPRE. (A) PPAR-RXR heterodimers bind with a higher affinity to the mouse LPL PPRE when compared with the human LPL PPRE. EMSA was performed on end-labeled oligonucleotides representing either the mouse or human LPL<sub>wt</sub> PPREs in the presence of an identical amount of *in vitro* translated mRXR $\alpha$  and haPPAR $\gamma$ . (B) Competition experiments were performed in the presence of 10-, 50- and 100-fold molar excess of either unlabeled mLPL<sub>wt</sub> PPRE (lanes 2-4), hLPL<sub>mut</sub> PPRE (lanes 5-7), hLPL<sub>wt</sub> PPRE (lanes 8-10) or ACO PPRE (lanes 11-13) oligonucleotides and using an end-labeled mLPL<sub>wt</sub> PPRE oligonucleotide in the presence of *in vitro* translated mRXR $\alpha$  and haPPAR $\gamma$  (lane 1). (C) Expression of LPL mRNA in mouse liver of animals treated with fenofibrate (FF; 0.5% w/w for 14 days). The amount of RNA spotted was equivalent, as demonstrated by re-hybridization of the dot blot with the 36B4 control cDNA. Animal treatment and preparation and analysis of RNA are described in Materials and methods.



**Fig. 8.** Localization of a functional PPRE in the human LPL promoter. (A) Scheme depicting the AN-PPRE<sub>wt</sub>-CAT and AN-PPRE<sub>mut</sub>-CAT vectors. The bases mutated in AN-PPRE<sub>mut</sub>-CAT are indicated in bold. (B) The AN-PPRE<sub>wt</sub>-CAT (indicated in black) and AN-PPRE<sub>mut</sub>-CAT (indicated in white) vectors were co-transfected into 3T3-L1 cells with either the pSG5 plasmid or pSG5-haPPAR $\gamma$  and cells were treated with 10  $\mu$ M BRL 49653 (TZD), 250  $\mu$ M fenofibrate (FF) or vehicle (CON). Experiments were performed as described in Materials and methods. The results represent the mean  $\pm$  SD of three independent experiments. The CAT activity of AN-PPRE<sub>wt</sub>-CAT in the presence of co-transfected pSG5 was arbitrarily set to 100%. (C) The AN-PPRE<sub>wt</sub>-CAT (indicated in black) and AN-PPRE<sub>mut</sub>-CAT (indicated in white) vectors were co-transfected into HeLa cells with either the pSG5, pSG5-mRXR $\alpha$ , pSG5-haPPAR $\gamma$  or pSG5-mRXR $\alpha$  and pSG5-haPPAR $\gamma$  plasmids and cells were treated with 10  $\mu$ M BRL 49653 (TZD) or vehicle (CON). Experiments were performed as described in Materials and methods. The results represent the mean  $\pm$  SD of three independent experiments. The CAT activity of AN-PPRE<sub>wt</sub>-CAT in the presence of co-transfected pSG5 was arbitrarily set to 100%.

LPL promoter, albeit with a weaker affinity than to the classical ACO PPRE. The LPL<sub>wt</sub> PPRE is furthermore activated by PPARs, thiazolidinediones and fibric acids.

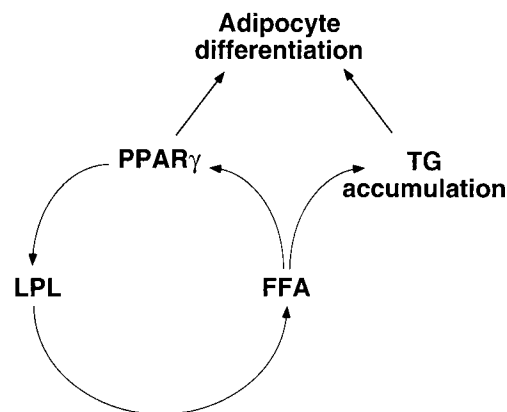
The transmission of PPAR regulatory signals through such 'non-consensus' PPREs, as present in the LPL gene and as recently identified by Brun *et al.* (1996) in the aP2



gene, indicates that careful analysis of candidate PPRE-like sequences is necessary before conclusions can be drawn about their functionality.

The tissue-selective effects of the PPAR activators/ligands are highly intriguing and provide insight into their effects on triglyceride metabolism. Fibrate treatment induced LPL expression strongest in liver, whereas BRL 49653 had no effect on liver, but induced adipocyte LPL expression. These compounds had no effect on heart LPL mRNA levels. In both the *in vivo* and *in vitro* studies, inducibility of LPL mRNA expression by either PPAR $\alpha$  or PPAR $\gamma$  activators correlated with the tissue-specific distribution of the respective PPARs. This selectivity of PPAR $\alpha$  and PPAR $\gamma$  activators, together with the tissue-specific expression of the respective receptors, allows tissue-selective activation of the LPL gene. In this context, we need to address the minimal effects which fenofibrate exerts on both LPL mRNA levels as well as LPL promoter activity in 3T3-L1 cells. Several mechanisms, which are not mutually exclusive, can explain this observation. It is possible that the addition of fibrates to pre-adipocytes in culture results in the production of endogenous PPAR $\gamma$  ligands, such as prostaglandins (Forman *et al.*, 1995; Kliewer *et al.*, 1995), which then could activate PPAR $\gamma$  and induce LPL gene transcription. The slower kinetics of LPL mRNA induction after fenofibrate treatment are consistent with this hypothesis. Alternatively, fibrates, such as fenofibrate (Aperlo *et al.*, 1995) or Wy 14643 (Dreyer *et al.*, 1993; Marcus *et al.*, 1993; Tontonoz *et al.*, 1994a,b; Kliewer *et al.*, 1995; Lehmann *et al.*, 1995; Yu *et al.*, 1995), are reported to activate PPAR $\gamma$ , although to a lower extent than PPAR $\gamma$  ligands. Identification of the natural ligands for all PPAR types, as well as the identification of high affinity synthetic agonists and antagonists, will most likely result in chemicals which will be more selective than the currently available compounds, allowing target gene activation in a specific tissue of interest while limiting undesired side-effects in other tissues. These compounds might have interesting therapeutic features, especially if one considers the context of the widespread use of fibrates in the treatment of diet-resistant hyperlipidemia and the potential usefulness of thiazolidinediones in treatment of non-insulin-dependent diabetes. In addition to studies aimed at defining new agonists and antagonists, it is of equal importance to understand the distinct structural features of the ligand binding domain of the PPAR subtypes, which allow similar and related receptors to be activated with a high degree of specificity by certain ligands or activators.

The paradoxical relationship existing between LPL mRNA (Figure 1A and B) and activity (Figure 1C) in the heart after treatment with BRL is very puzzling. In view of the constant levels of LPL mRNA, it is relatively unlikely that this reduction in LPL activity is mediated by a change in LPL gene transcription induced by PPAR $\gamma$ . Furthermore, with Northern blot analysis no PPAR $\gamma$  mRNA was detected in rat heart tissue, making it unlikely that PPAR $\gamma$  is a major player, although the presence of minute quantities of PPAR $\gamma$  mRNA, which might be sufficient to mediate this effect, might be overlooked by this technique. We believe, however, that it is more likely that BRL 49653 has other effects beyond the well-established activation of PPAR $\gamma$ . Recent studies suggest



**Fig. 9.** Schematic representation of the interdependent role of LPL and PPAR $\gamma$  in adipocyte differentiation.

that several signal transduction pathways are affected by thiazolidinediones, most notably protein tyrosine phosphatase activity (Maegawa *et al.*, 1995) and calcium fluxes (Buchanan *et al.*, 1995). Further studies are, however, necessary to delineate which signaling pathway is responsible for this observed discrepancy.

The demonstration of a PPRE in the LPL gene has important implications for adipocyte physiology. The adipose-restricted PPAR $\gamma$ 2 type can promote pre-adipocyte determination as well as terminal differentiation (Tontonoz *et al.*, 1994a,b). In fact, PPAR $\gamma$ 2 mRNA is itself induced in the earliest steps of adipocyte differentiation before the induction of early marker genes such as LPL. Many of these genes induced during adipocyte differentiation encode proteins involved in lipid storage and metabolism. The PPAR-mediated induction of LPL expression hence explains the mechanism by which the differentiation-linked induction of LPL occurs. The observation that the LPL gene appears to be slightly more efficiently activated by PPAR $\gamma$  than by PPAR $\alpha$  (data not shown) is consistent with the specific role of PPAR $\gamma$  in adipose differentiation (Tontonoz *et al.*, 1994b; Brun *et al.*, 1996). The increase in lipolytic capacity as a result of induction of LPL by PPAR $\gamma$  will result in increased delivery of fatty acids to the adipocytes. This points to the possible existence of a positive regulatory feedback loop involving PPAR $\gamma$  and LPL and aim at promoting and maintaining the mature adipocyte phenotype (Figure 9). In fact, it was recently reported that in addition to the thiazolidinediones, certain fatty acid-derived prostaglandin derivatives, whose delivery to the cell is increased by LPL, bind to and/or activate PPAR $\gamma$  (Forman *et al.*, 1995; Kliewer *et al.*, 1995; Lehmann *et al.*, 1995; Yu *et al.*, 1995). This hypothesis is supported by the observation that fatty acids (including arachidonic acid-derived prostaglandins) and fatty acid analogs induce expression of adipocyte-specific genes and enhance adipocyte conversion (Gaillard *et al.*, 1989; Amri *et al.*, 1991; Distel *et al.*, 1992; Chawla and Lazar, 1994; Tontonoz *et al.*, 1994a,b). In addition to being potent PPAR activators (Gottlicher *et al.*, 1992; Keller *et al.*, 1993; Kliewer *et al.*, 1994; Forman *et al.*, 1995; Yu *et al.*, 1995), LPL-derived fatty acids will provide the necessary building blocks for triglyceride accumulation, ultimately enhancing adipocyte differentiation. The PPAR-mediated activation of LPL expression in cells of the adipogenic

lineage might furthermore be responsible for the previously reported capacities of thiazolidinediones to induce adipocyte differentiation and induce the development of obesity (Hiragun *et al.*, 1988; Ikeda *et al.*, 1990; Sparks *et al.*, 1991; Kletzien *et al.*, 1992; Sandouk *et al.*, 1993; Batchvarova *et al.*, 1995; Hu *et al.*, 1995; Martin *et al.*, 1995; Teboul *et al.*, 1995; Brun *et al.*, 1996). In this context, it is interesting to note that the PPAR $\gamma$ -mediated effects of BRL 49653 on LPL expression are acting in concert with reduced *ob* mRNA and leptin levels and the associated increase in caloric uptake enhancing energy storage in the adipocytes observed with this compound (De Vos *et al.*, 1996; Zhang *et al.*, 1996).

One remaining question is the relationship between PPAR $\gamma$ , thiazolidinediones and insulin resistance. It is tempting to speculate that the increase in LPL activity in adipose tissue is related to the antidiabetic effects of the thiazolidinediones. Due to the enhanced triglyceride clearance in adipose tissue, less triglycerides will become available to be hydrolyzed to fatty acids in the vascular bed of the muscle. In view of the inhibitory effects of fatty acids on insulin-mediated glucose metabolism (Randle *et al.*, 1961), the decrease in fatty acids delivered to the muscle cells might be responsible for the improvement in insulin sensitivity of this tissue. The apparent inverse relationship between certain adipose tissue depots and insulin sensitivity is another argument supporting the relation between adipose tissue, fatty acids and insulin resistance. In fact, in contrast to the improvement in insulin resistance seen with thiazolidinediones, which have a tendency to increase the adipose tissue mass, lipodystrophic syndromes, characterized by the absence or a decrease in lipid storage in adipocytes, are characterized by extreme insulin resistance (Moller and Flier, 1991; Flier, 1995).

The physiological importance of the induction of LPL mRNA and activity in adult liver and in hepatic cells is unclear at present. LPL activity in the liver can be involved in the clearance of triglyceride-rich lipoprotein particles, a phenomenon stimulated by fibrates (reviewed in Auwerx *et al.*, 1992). Furthermore, the induction of LPL in the liver may facilitate peroxisome proliferation, a well-known phenomenon in rodents (Lock *et al.*, 1989; Osmundsen *et al.*, 1991). In fact, LPL will generate fatty acids which are taken up by the hepatocyte and might overwhelm its capacity for  $\beta$ -oxidation, ultimately enhancing fibrate-induced peroxisome proliferation. However, it is becoming increasingly evident that PPARs play an important role not only in the control of intracellular lipid metabolism and  $\beta$ -oxidation, but also in a wide range of activities, such as lipid transport and lipoprotein physiology. We have previously shown that PPARs control the expression of both human apoA-I and apoA-II, two major protein components of HDL (Vu-Dac *et al.*, 1994, 1995). Fibrates, thiazolidinediones, fatty acids and PPARs may also influence the metabolic fate of triglyceride-rich apoB-containing particles by stimulating LPL expression. This stimulation of LPL production in liver by fibrates occurs in addition to the decrease in hepatic production of apoC-III, which by itself has a beneficial effect on plasma triglyceride levels (Hertz *et al.*, 1995; Staels *et al.*, 1995). Indeed, due to the reduced apoC-III concentration, both LPL-mediated lipolysis (Wang *et al.*, 1985) and receptor-

mediated clearance of triglyceride-rich particles (Aalto-Setälä *et al.*, 1992; de Silva *et al.*, 1994) will be improved, resulting in lowering of plasma triglyceride levels.

In conclusion, LPL mRNA and activity levels can be regulated in a tissue-specific fashion by PPAR $\alpha$  activators and PPAR $\gamma$  ligands. The induction of LPL mRNA by these compounds is mediated by the transcription factor PPAR, which interacts with a PPRE in the LPL promoter. In adipose tissue, the increase in LPL production after treatment with thiazolidinediones will enhance the clearance of plasma triglycerides and provide the (pre-)adipocytes with additional fatty acids, which can further stimulate the transactivation capacity of PPAR or which can be stored in the form of triglycerides. In the liver, enhanced production of LPL, together with reduced production of apoCIII (Staels *et al.*, 1995), may contribute to the hypolipidemic action of these compounds. This tissue-selective induction of LPL gene transcription by activators of different PPARs demonstrates the feasibility of the development of highly specific PPAR subtype-specific agonists and antagonists, which can be used as drugs.

## Materials and methods

### Materials

BRL 49653 and fenofibric acid were kind gifts of Dr Larry Hamann (Ligand Pharmaceuticals, San Diego, CA) and Dr Alan Edgar (Laboratoires Fournier, Daix, France).

### Recombinant plasmids

The construction of the LPL promoter-containing CAT vectors PN (-1719 to +136), XN (-852 to +136) and AN (-230 to +136) was described elsewhere (Schoonjans *et al.*, 1993a). Site-directed mutagenesis of the LPL PPRE was accomplished by introduction of a *SalI* restriction site into AN-CAT. Briefly, two restriction site-containing PCR fragments, *HindIII*-5'AN PPRE<sub>1/2</sub>-*SalI* (PCR 1) and *SalI*-PPRE<sub>1/2</sub> AN3'-*BamHI* (PCR 2), were synthesized using the primers 5'-AATAAGCTTCCGGGT-AGAGTGAACCCCTT-3' and 5'-GGAGTCGACAGACGGAAAAA-TTTGCTTTGTT-3' (PCR 1) and primers 5'-TCTGTGCACTCCAC-TCTTCTCGTTGGCAG-3' and 5'-AATGGATCCGGCTGAGCCGG-AGGCTGGAGA-3' (PCR 2) and subcloned into pBLCAT5 (Luckow and Schütz, 1987). The expression vectors pSG5-haPPAR $\gamma$  (Aperlo *et al.*, 1995), pSG5-mPPAR $\alpha$  (Isseman and Green, 1990), pSG5-mRXR $\alpha$  (Leid *et al.*, 1992) and pSG5-mRXR $\beta$  (Leid *et al.*, 1992) were described previously.

### Animal studies

Adult (95-days old) Sprague-Dawley rats were housed in groups and acclimated to a 12:12 h day/night illumination cycle (light from 8 a.m. to 8 p.m.). Rats were divided into groups of a minimum of three animals each and were treated for either 7 or 14 days. The first group received BRL 49653 (5 mg/kg/day) by gavage. The second group of animals received 0.5% w/w fenofibrate (-0.5 g/kg/day) mixed with their food, whereas the third group of animals served as controls and received 10% carboxymethylcellulose (vehicle for gavage) by gavage. In a separate experiment, adult C57B16 male mice were fed for 14 days with either a control chow ( $n = 3$ ) or the same chow containing 0.5% w/w fenofibrate. At the end of the treatment period all animals were weighed and sacrificed by exsanguination under ether anesthesia between 8.00 and 10.00 a.m. Epididymal adipose tissue (in rats) and liver (in rats and mice) were removed, weighed, rinsed with 0.9% NaCl and frozen in liquid nitrogen until RNA preparation.

### Tissue biopsies

Omental adipose tissue was obtained from non-obese adult subjects undergoing elective surgery. All subjects had fasted overnight before surgery (between 8.00 and 10 a.m.) and received i.v. saline infusion. They had given informed consent and the project was approved by the Ethics Committee of the Institut Pasteur of Lille. Adipose tissue was immediately frozen in liquid nitrogen until RNA preparation. Human

liver specimens were collected from physically healthy multiorgan donors for transplantation who died after severe traumatic brain injury. Permission to use the remaining, non-transplanted part of donor liver for scientific research was obtained from the Ethics Committee.

#### Cell culture and transient transfection assays

The mouse hepatoma and pre-adipocyte cell lines, BWTG3 (Schoonjans *et al.*, 1993a), ob 1771 (Negrel *et al.*, 1978) and 3T3-L1 (ATCC), were maintained in Dulbecco's modified Eagle's minimal essential medium (DMEM) supplemented with 10% delipidated and charcoal-treated fetal calf serum (FCS), L-glutamine and antibiotics, unless stated otherwise. AML-12 mouse hepatocytes (Wu *et al.*, 1994) were maintained in DMEM/Ham's F-12 supplemented with 10% delipidated and charcoal-treated FCS, insulin, transferrin and selenium (ITS; Collaborative Research), dexamethasone (0.1  $\mu$ M) and gentamycin (50  $\mu$ g/ml). Fenofibric acid, BRL 49563 (both in DMSO) and fatty acids (in ethanol) were added to the medium at the appropriate concentrations and times indicated. Control cells received vehicle only. Fatty acids were complexed to serum albumin contained in delipidated and charcoal-treated FCS by preincubation for 45 min at 37°C. Transfections and CAT assays were carried out exactly as described previously (Vu-Dac *et al.*, 1994).

#### RNA analysis

RNA preparation, Northern blot hybridizations and quantification of total cellular RNA were performed as described previously (Staels and Auwerx, 1992). A human LPL cDNA (Auwerx *et al.*, 1989), mPPAR $\alpha$  (Isseman and Green, 1990), haPPAR $\gamma$  (Aperlo *et al.*, 1995) and human acidic ribosomal phosphoprotein 36B4 (Masiakowski *et al.*, 1982) were used as probes.

#### Measurements of plasma lipids and LPL activity

Plasma lipids (cholesterol and triglycerides) were measured colorimetrically (490 nm) using a microtiter plate reader and reagents from Bio-Merieux (Marcy-l'Étoile, France). LPL activity was measured in tissue extracts according to the procedure of Ramirez (Ramirez *et al.*, 1985). One unit of enzyme activity was defined as the amount of enzyme that releases 1  $\mu$ mol oleate/min at 25°C.

#### Electrophoretic mobility shift assays (EMSA) and oligonucleotide sequences

haPPAR $\gamma$  (Aperlo *et al.*, 1995), xPPAR $\alpha$  (Dreyer *et al.*, 1992), mRXR $\alpha$  (Leid *et al.*, 1992) and mRXR $\beta$  (Leid *et al.*, 1992) proteins were synthesized *in vitro* in rabbit reticulocyte lysate (Promega). Molecular weights and quality of the *in vitro* translated proteins were verified by SDS-PAGE. PPAR (2  $\mu$ l) and/or RXR (2  $\mu$ l) were incubated for 15 min on ice in a total volume of 20  $\mu$ l with 1 ng probe, 2.5  $\mu$ g poly(dI-dC) and 1  $\mu$ g herring sperm DNA in binding buffer (10 mM Tris-HCl, pH 7.9, 40 mM KCl, 10% glycerol, 0.05% Nonidet P-40 and 1 mM DTT). For competition experiments, increasing amounts (from 10- to 200-fold molar excess) of cold oligonucleotide ACO PPRE (Tugwood *et al.*, 1992) (5'-GATCCCGAACGTGACCTTTGCTCCTGGTCCC-3'), hLPL-PPRE (5'-GATCCGTCTGCCCTTCCCTCTTCA-3'), mLPL-PPRE (5'-GATCCGTCTGCCCTTCCCTCTTCA-3') and/or hLPL<sub>mut</sub>-LPL (5'-GATCCGTCTGCCCTTCCCTCTTCA-3') were included just before adding T4 PNK end-labeled oligonucleotide or PCR fragment. DNA-protein complexes were separated by electrophoresis on a 4% polyacrylamide gel in 0.25 $\times$  TBE buffer at 4°C (Fried and Crothers, 1983). PCR fragments 1A (-538 to -227) and 2A (-304 to -109) were synthesized using sense and antisense primers 5'-TCG-AGCTTTGTCCATCACAATAAGC-3', 5'-CGCGGATCGATACCGGGGGCCCCCTGTCTAAG-3' and 5'-TCGAGAAAACAGGTGATTGTGAGT-3', 5'-TCGAGAAGCTTTGAGCAAACA-3' respectively. PCR fragments 2B and 2C were synthesized using the 2A sense primer and antisense primers 1A and 5'-GAAGAGGGGAAAGGGCAGAC-3' respectively. PCR fragments 2D, 2E and 2F were synthesized using the sense primers 5'-GATCCTCTCGTTGGCAGGGTTGATCCT-3', 5'-GCAAATTTTCTCTAGAGTCTGCCCTTTC-3' and 5'-TAAGCTA-AGCTCTAGAACAGGAGCCT-3' respectively and the 2C antisense primer.

#### Methylation interference assay

PCR fragments overlapping the -211/-109 LPL promoter region were [ $\gamma$ -<sup>32</sup>P]ATP end-labeled either on the sense or the antisense strand. After partial methylation of DNA probes, a 5-fold up-scaled preparative EMSA assay was performed as described above. The protein-bound and protein-free bands were cut from the gel and probes were electrophoresed on a DEAE membrane, eluted and precipitated. Purified probes were treated

with 1 M piperidine at 95°C for 30 min. Piperidine was removed by evaporation and DNA samples were dissolved in formamide and electrophoresed on a 8% acrylamide-7 M urea sequencing gel in 1 $\times$  TBE.

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