

Novel Roles of Liver X Receptors Exposed by Gene Expression Profiling in Liver and Adipose Tissue

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ABSTRACT

Liver X receptor (LXR) α and LXR β are nuclear oxysterol receptors whose biological function has so far been elucidated only with respect to cholesterol and lipid metabolism. To expose novel biological roles for LXRs, we performed genome-wide gene expression profiling studies in liver and white and brown adipose tissue from wild-type (LXR $\alpha^{+/+}\beta^{+/+}$) and knockout mice (LXR $\alpha^{-/-}\beta^{-/-}$) treated with a synthetic LXR agonist. By an adapted statistical analysis, we detected 319 genes significantly regulated by LXR agonist treatment in wild-type but not in knockout mice, fulfilling most stringent criteria with an overall confidence of 94%. Down-regulation of essential enzymes of gluconeogenesis in liver could point to possible beneficial ef-

fects of LXR agonists in diabetes mellitus. LXR agonist treatment also altered expression of genes involved in steroid hormone synthesis and growth hormone receptor signaling, emphasizing a potential impact on endocrine function. Notably, LXR agonist treatment up-regulated CYP4A10 and CYP4A14 together with cytochrome P450 reductase, indicating a possible enhancement of microsomal lipid peroxidation. In conclusion, these gene expression profiling data identify novel areas of regulation by LXRs and provide a highly valuable basis for further research on the biological functions of these nuclear receptors and the pharmacological characteristics of their ligands.

Liver X receptors (LXRs) are nuclear receptors whose endogenous ligands are distinct oxysterols (Janowski et al., 1996; Lehmann et al., 1997). In liver and adipose tissue, LXR α (NR1H3) is highly expressed and predominant over its more ubiquitously expressed paralog LXR β (NR1H2) (Repa and Mangelsdorf, 1999). LXRs have primarily been shown to be directly implicated in regulation of lipid metabolic pathways. LXRs are key regulators of certain aspects of cholesterol metabolism; they regulate bile acid synthesis in mice by stimulating expression of its rate-limiting enzyme CYP7A1 (Peet et al., 1998; Repa et al., 2000b). In addition, ATP-binding cassette transporters ABCG5 and ABCG8, which limit intestinal absorption and promote biliary excretion of sterols (Berge et al., 2000), are directly up-regulated by LXRs (Repa et al., 2002). By inducing expression of ABCA1 and ABCG1, LXRs enhance cholesterol efflux from macrophages

(Costet et al., 2000; Schwartz et al., 2000; Venkateswaran et al., 2000a,b; Chawla et al., 2001). Because reverse cholesterol efflux is an essential mechanism for macrophages to prevent formation of foam cells, a characteristic of atherosclerotic lesions, LXR $\alpha^{-/-}\beta^{-/-}$ mice accumulate foam cells in the aorta (Schuster et al., 2002). In addition, lipoprotein metabolism is influenced by LXRs by regulating the expression of apolipoprotein E (Laffitte et al., 2001b), lipoprotein lipase (Zhang et al., 2001), as well as human cholesterol ester transfer protein (Luo and Tall, 2000). Notably, LXRs control the expression of sterol regulatory element-binding protein (SREBP)-1c, a key transcription factor for several lipogenic enzymes (Repa et al., 2000a; Schultz et al., 2000; DeBose-Boyd et al., 2001).

Although LXR function has been elucidated in detail with respect to cholesterol and lipid metabolism, other biological roles of LXRs remain to be discovered. Because LXRs mediate certain actions of insulin on gene expression (Tobin et al., 2002), gene regulation by LXRs is particularly interesting in primary insulin target tissues such as liver and adipose tissue. Moreover, because LXR agonists are currently developed (e.g., to interfere with the development of atherosclerosis), LXR effects on gene expression are highly valuable for

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ABBREVIATIONS: LXR, liver X receptor; ABC, ATP-binding cassette; SREBP, sterol regulatory element-binding protein; RT-PCR, reverse transcriptase polymerase chain reaction; PPAR, peroxisome proliferator-activated receptor; PEPCK, phosphoenolpyruvate carboxykinase; WAT, white adipose tissue; BAT, brown adipose tissue; CAR, constitutive active receptor.

prediction of potential side effects and to identify other applications of these drugs.

However, a genome-wide picture of how LXRs regulate gene expression in metabolically important tissues is still lacking. We therefore analyzed genome-wide expression profiles in liver and white and brown adipose tissue from mice treated with a synthetic LXR agonist using high-density oligonucleotide microarrays representing $\approx 12,000$ genes. Parallel treatment of wild-type and $\text{LXR}\alpha^{-/-}\beta^{-/-}$ knockout mice enabled us to exclude genes also regulated in LXR-deficient animals suggesting activation of other receptors by the drug. Moreover, by comparison with data from knockout mice, we could directly estimate the high confidence of the obtained expression profiling data.

Materials and Methods

Animal Treatment and RNA Preparation. Wild-type ($\text{LXR}\alpha^{+/+}\beta^{+/+}$) and $\text{LXR}\alpha^{-/-}\beta^{-/-}$ mice were generated by gene targeting in our laboratory as previously described in detail (Alberti et al., 2001; Schuster et al., 2002). The mice used in this study were Sv129/C57BL/6 hybrids finally backcrossed in C57BL/6 mice for three generations. Mice were housed on a 12-h/12-h light/dark cycle in the specific pathogen-free animal unit at the University Hospital at Huddinge. For the experiment, male mice, 10 to 12 months of age, had free access to water and an experimental diet based on a low-fat standard rodent diet (R36; Lactamin AB, Vadstena, Sweden). The diet was either mixed with vehicle alone (ethanol; “control-treated”) or supplemented with 0.025% (w/w) of the synthetic LXR agonist T0901317 (“agonist treated”; Repa et al., 2000a) and was extensively dried to evaporate any traces of ethanol. Three wild-type and three knockout mice were assigned to each experimental group and treated for 7 days. Mice were sacrificed and liver, epididymal white adipose tissue, and interscapular brown fat pads were snap frozen in liquid nitrogen and kept at -80°C until isolation of RNA. The experiment was approved by the local ethics committee for animal experiments and the Guidelines for the Care and Use of Laboratory Animals were followed.

Total RNA was prepared by disrupting tissues in TRIzol reagent (Invitrogen, Carlsbad, CA) with a tissue homogenizer followed by RNA isolation according to the manufacturer's instructions. Total RNA samples were repurified (RNeasy mini kit; QIAGEN, Valencia, CA) and checked for integrity by agarose gel electrophoresis.

Microarray Experiment. Probe synthesis from total RNA samples from individual mice, hybridization, detection and scanning were performed according to standard protocols from Affymetrix Inc. (Santa Clara, CA). Fifteen micrograms of labeled cRNA were hybridized to each mouse U74Av2 genechip (Affymetrix), and scanning was performed after biotin/avidin/phycoerythrin amplification according to Affymetrix standard protocols.

Data Analysis. Scanned data files were analyzed using Microarray Suite v5.0 software (Affymetrix). All chips were normalized to target intensity 100 to make them comparable with each other. Genes were selected for analysis if they achieved a significant “present” call by the default parameters of the Affymetrix software, and the geometric mean of expression levels was ≥ 40 signal intensity units in all three animals of agonist-treated or control-treated mice of a given genotype. Genes with mean signal intensities ≥ 40 and < 100 units were called “low expressed” as opposed to “highly expressed” genes.

To assess the change in one gene between agonist and control treatment, the Microarray Suite v5.0 software performs a Wilcoxon signed-rank test using information from all probe pairs (15–20 per gene) in the two samples giving a “change call” when detecting significant differences (increase, decrease). Although the Affymetrix estimate of the overall expression level of a particular gene is also based on multiple probe pairs, the pair-wise comparison uses much

more information than is available in the estimated expression levels. Hence, we modeled a “coincidence call” based on the number of significant pair-wise comparisons between all three agonist and control-treated samples (nine comparisons in total) by simulation of a null distribution. Because the nine comparisons were statistically not completely independent of each other, the p values were calculated based on a Cauchy (t_1) distribution applied to one million sextuplets of random samples. According to this coincidence analysis, genes regulated by agonist treatment in the same direction (either increase or decrease) in at least eight of the nine comparisons (allowing one “no-change” call) were assigned genes with high probability of regulation ($p < 0.00001$). Genes with regulation in the same direction in five to seven comparisons (allowing two to four no-change calls, or one opposite change call \pm one no-change call) were assigned those with lower probability of regulation ($p < 0.01$). The ratio of the geometric mean expression levels between agonist- and control-treated animals (“fold change”) was used to estimate the extent of its regulation with down-regulation expressed as negative numbers. Because all known LXR target genes were up-regulated at least 1.6-fold in our study, genes with regulation of less than ± 1.5 -fold were excluded.

According to their probability of regulation by LXR and their expression levels, genes were assigned to two groups. “Index genes” were identified in individual tissues by high expression and highest probability of regulation in coincidence analysis. Other index genes were assigned if genes with lower probability of regulation in coincidence analysis and/or low expression level were regulated in the same direction in at least two tissues. To increase sensitivity to detect LXR-mediated regulation in functional groups of genes, genes regulated in single tissues with lower probability of regulation in coincidence analysis and/or low expression levels were included as “additional genes”. Genes that fulfilled these criteria for index or additional genes, respectively, but were regulated in knockout mice ($\text{LXR}\alpha^{-/-}\beta^{-/-}$) with at least low probability ($p < 0.01$) in the same direction as in wild-type mice ($\text{LXR}\alpha^{+/+}\beta^{+/+}$) were excluded because of suspected regulation by receptors other than LXRs.

Quantitative Real-Time RT-PCR. One microgram of RNA was treated with DNase (amplification grade; Invitrogen) before reverse transcription into cDNA by Superscript II (Invitrogen) using random hexamer priming. Expression of specific mRNAs was quantitated by Taqman real-time reverse transcriptase-polymerase chain reaction (RT-PCR) normalized to 18S (Applied Biosystems, Foster City, CA). Sequences for primers and probes are shown in Table 1.

Results and Discussion

Gene Expression Profiling Overview. To elucidate regulation of gene expression by LXRs, we analyzed gene expression profiles from liver as well as white and brown adipose tissue from a total of 12 individual mice treated p.o. with a synthetic LXR agonist or a control diet for 7 days. Because even subtle metabolic alterations may be physiologically relevant, conventional procedures to identify regulated genes by relatively high threshold levels for the magnitude of change were not considered appropriate to detect metabolically significant regulation. Therefore, we adapted a statistical analysis to evaluate coincident changes in the expression of individual genes using pair-wise comparisons on the probe level between agonist and control-treated animals to yield highest confidence of the data. According to their probability of regulation by LXRs and their expression levels, we assigned index genes with high probability of regulation and high expression, and additional genes with less stringent criteria as described in detail under *Materials and Methods*. After exclusion of genes regulated in knockout mice in the same direction (increase or decrease) as in wild-type mice, 319

index genes (2.6% of investigated genes) were assigned in wild-type mice (Table 2). Most of them (303) fulfilled the criteria for index genes in individual tissues; the others were regulated with lower probability but in the same direction in at least two different tissues (not shown). Most index genes were found in liver (167) followed by brown adipose tissue (117), whereas a markedly lower number of index genes was detected in white adipose tissue (54). Index genes were categorized in functional groups and subgroups; 308 additional genes (2.5% of investigated genes) were also assigned to these groups. Figure 1 gives an incomplete list of most interesting index genes categorized according to their proposed function. The complete list of regulated index and additional genes is electronically available as Supplemental Fig. 3 (<http://molpharm.aspetjournals.org/cgi/content/full/62/6/1299/DC1>). Many functional groups revealed rather uniform up- or down-regulation of most included genes. Notably, all LXR target genes known for the analyzed tissues or cell types [CYP7A1 (Peet et al., 1998; Repa et al., 2000b), ABCA1 (Costet et al., 2000; Schwartz et al., 2000; Venkateswaran et al., 2000a; Chawla et al., 2001), ABCG1 (Venkateswaran et al., 2000a,b), apolipoprotein E (Laffitte et al., 2001b), lipoprotein lipase (Zhang et al., 2001), fatty acid synthase (Joseph et al., 2002), and SREBP-1c (Repa et al., 2000a; Schultz et al., 2000; DeBose-Boyd et al., 2001)] appeared as index genes in our analyses indicating also a substantial sensitivity of the analytical approach. Quantitative real-time RT-PCR of several index and additional genes revealed a high reliability of the data concerning direction and extent of regulation (Fig. 2).

Data Quality Assessment. Genome-wide expression profiling data are often taken with caution because of uncertainties concerning data quality. Inclusion of agonist and control-treated knockout mice lacking both LXRs ($LXR\alpha^{-/-}\beta^{-/-}$) enabled us to assess the confidence of our data (i.e., an estimate of the number of genes truly regulated in vivo) by applying the same selection criteria separately on data from knockout and wild-type animals (Table 3). For index genes, confidence was found to be 87 to 98%, depending on the tissue. When subtract-

ing individual genes regulated in both wild-type and control mice in the same direction, indicating a true regulation in vivo but by receptors other than LXRs, overall confidence of the data was 94%. This means that only 1 of 16 index genes is wrongly assigned to be regulated by LXRs. This extraordinarily high data confidence confirms the reliability of the analytical approach. To detect regulation of whole groups of functionally related genes, we also assigned so-called additional genes applying less stringent criteria. As expected, confidence was lower for additional genes (overall 76%). Thus, index genes can be regarded as individual genes truly regulated by LXR agonist treatment, whereas additional genes are assigned to indicate regulation of functional groups of genes.

Expression Profiling Data Indicate Novel Biological Roles of LXRs. LXRs have been shown to exert a role in fatty acid synthesis and metabolism, particularly by up-regulating the central transcription factor SREBP-1c in all three tissues. Although the probe for SREBP-1 included both variants 1a and 1c, real-time quantitative RT-PCR with variant specific probes revealed predominant regulation of SREBP-1c (Fig. 2A). Expression of adipose-tissue specific fatty acid binding protein (aFABP), an important adipocyte marker gene, was not changed in adipose tissues as confirmed by real-time RT-PCR (Fig. 2B). Besides up-regulation of the acidic pathway of bile acid synthesis by sterol-7-hydroxylase (CYP7A1; 11-fold), a known LXR target gene in mice (Lehmann et al., 1997; Peet et al., 1998; Chiang et al., 2001), there was a concomitant down-regulation of the alternative pathway (CYP7B1) and sterol-12-hydroxylase (CYP8B1; 2-fold), the latter indicating altered balance between cholic and chenodeoxycholic acid.

Genes involved in fatty acid synthesis (fatty acid synthase, malic enzyme, acyl-CoA thioesterase) were 4- to 6-fold up-regulated in liver of LXR agonist-treated mice together with some genes involved in lipid synthesis, particularly glycerol-3-phosphate acyltransferase. This result is reflected by a considerable increase in hepatic triglyceride content in LXR agonist-treated wild-type mice as reported previously (Schultz et al., 2000). Increased mRNA expression of several peroxisomal proteins, particularly a multifunctional β -oxidation protein that was up-regulated up to >8-fold, indicates a shift from mitochondrial to peroxisomal lipid metabolism in liver and adipose tissues. Moreover, up-regulation of peroxisomal proteins emphasizes that certain effects of peroxisome proliferator-activated receptor (PPAR) α and PPAR γ agonists occur via up-regulation of LXR α (Tobin et al., 2000; Chawla et al., 2001; Chinetti et al., 2001). Increased gene expression of apolipoproteins, lipoprotein lipase, and phospholipid transfer protein suggests enhanced serum lipoprotein metabolism. Several genes involved in mitochondrial energy me-

TABLE 1

Primers and probes

All probes were 5'-labeled with carboxyfluorescein and 3'-labeled with carboxytetramethylrhodamine (5'-3').

SREBP-1a	
Primers	GGCCGAGATGTGCGAACT AAGTCACTGTCTTGGTTGTTGATGA
Probe	TTTGAACGACATCGAAGACATGCTCCA
SREBP-1c	
Primers	GGACCACGGAGCCATGG GGAAGTCACTGTCTTGGTTGTTGA
Probe	TTGCACATTGTAAGACATGCTCCAGCTC
PEPCK	
Primers	AAATCCGGCAAGGCGC TCTGGTGCCACCTTCTCTCC
Probe	CAGCGATCTCTGATCCAGACCTTCCAA
11 β -HSD-1	
Primers	AAGCAGAGCAATGCGAGCAT GAGCAATCATAGGCTGGGTCTAT
Probe	CGTCATCTCTCTCTGGCTGGGAA
Dexras1	
Primers	GATCCGCGGCGAAGTCT GGCGGGAACGGATGATT
Probe	CCAGTTGGACATCTGGACACATCCGG
Adipocyte-specific fatty acid binding protein	
Primers	GAATTGATGAAATCACCGCA CTCTTTATTGTGGTTCGACTTTCC
Probe	ACGACAGGAAGGTGAAGAGCATCATAAC

TABLE 2

Selection of genes regulated by LXR agonist treatment in wild-type mice

Shown are the number of genes regulated in wild-type mice excluding those regulated in the same direction in $LXR\alpha^{-/-}\beta^{-/-}$ mice; overall numbers exclude genes regulated in the same direction in $LXR\alpha^{-/-}\beta^{-/-}$ mice as in wild-type mice in at least one tissue. Numbers in parentheses represent the number of genes that fulfill all other criteria for selection but are regulated in parallel in wild-type and knockout mice; overall numbers include genes regulated in parallel in $LXR\alpha^{-/-}\beta^{-/-}$ mice as in wild-type mice in at least one tissue.

	Liver	WAT	BAT	Overall
Index genes	167 (12)	54 (0)	117 (0)	319 (12)
Add. Genes	176 (11)	114 (1)	97 (1)	308 (13)

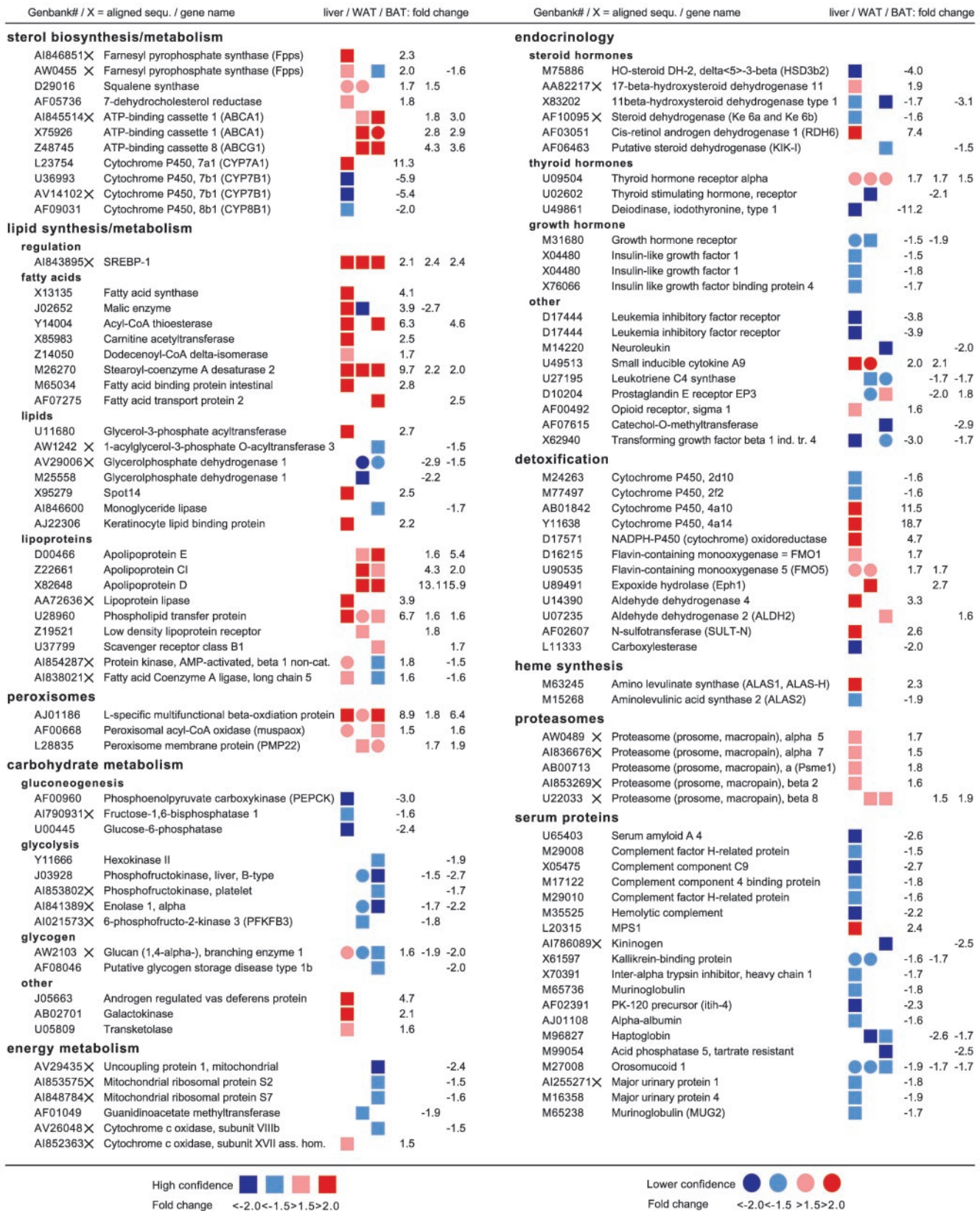


Fig. 1. Gene regulation by LXR agonist treatment in liver and white and brown adipose tissue: incomplete list of index genes. Wild-type ($LXR\alpha^{+/+}\beta^{+/+}$) mice were treated p.o. with an LXR agonist or a control diet for 7 days followed by gene expression profiling analysis of liver, white and brown adipose tissue. Index genes were selected as detailed under *Materials and Methods*. The GenBank accession number refers to the origin of the tested sequence that was aligned to later identified genes as indicated by a cross. Colored squares and circles represent genes fulfilling criteria for high and lower confidence in individual tissues, respectively, with colors referring to the direction and the extent of change by the LXR agonist treatment. The geometric mean of the fold change in gene expression by LXR agonist treatment is given as color-coded symbols and numbers in the order liver, WAT, BAT.

tabolism were down-regulated in brown adipose tissue. Notably, uncoupling protein 1 was down-regulated by approximately 2-fold, pointing to improved efficiency of energy metabolism by LXR agonist treatment.

A novel role of LXRs in regulation of carbohydrate metabolism was indicated by striking down-regulation of the key enzymes of gluconeogenesis in liver, namely phosphoenolpyruvate carboxykinase (PEPCK), fructose-1,6-bisphosphatase and glucose-6-phosphatase. Down-regulation of

PEPCK in liver of wild-type mice (−3-fold) was confirmed by quantitative real-time RT-PCR (Fig. 2C). Down-regulation of hepatic gluconeogenesis is one of the most important roles of insulin and its failure is a major problem in diabetic patients. Thus, the data could indicate probable beneficial effects of LXR agonists in diabetes mellitus. On the other hand, expression of essentially all regulated glycolytic enzymes was decreased in adipose tissues except the negative regulator pyruvate-dehydrogenase kinase 4. Isoform 3 of 6-phospho-

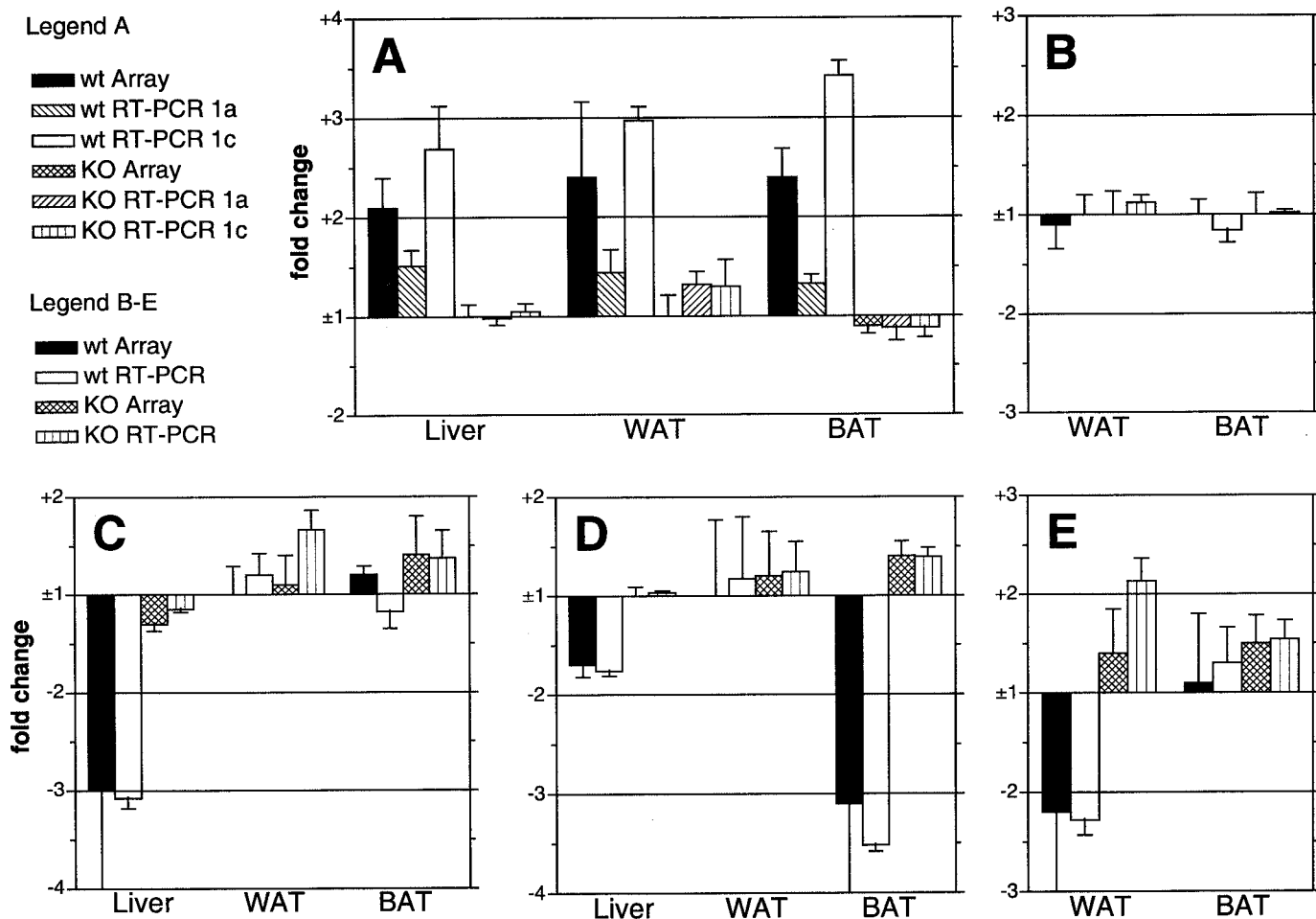


Fig. 2. Confirmation of gene expression profiling data by quantitative real-time RT-PCR. RNA samples from individual mice prepared for gene profiling experiments were analyzed by quantitative real-time RT-PCR for expression of SREBP-1 (A; RT-PCR specific for variants SREBP-1a and -1c as indicated in legend A), adipocyte-specific fatty acid binding protein (aFABP; B), PEPCK (C), 11 β -HSD-1 (D), and Dexras1 (E). "Fold change" denotes the ratio of gene expression between LXR agonist and control-treated wild-type (wt) or LXR $\alpha^{-/-}\beta^{-/-}$ knockout mice (KO) as evaluated by microarray analysis (Array) and real-time RT-PCR (means \pm S.E.M.).

TABLE 3

Confidence of gene expression profiling data: gene regulation by LXR agonist treatment in wild-type and LXR $\alpha^{-/-}\beta^{-/-}$ mice

Genes were selected by the same criteria within wild type (wt) and LXR $\alpha^{-/-}\beta^{-/-}$ knockout mice (KO) to compare the number of regulated genes in either genotype. Genes with regulation in the same direction in LXR $\alpha^{-/-}\beta^{-/-}$ mice are included unless stated otherwise. The level of confidence (i.e. an estimate of the number of genes truly regulated *in vivo*) is calculated as 100% minus the number of genes regulated in KO mice divided by the number of genes regulated in wt mice. Because regulation in the same direction in KO and wild-type mice suggests true regulation *in vivo* but by receptors other than LXRs, these genes were subtracted from the number of genes regulated in the same direction in KO mice before calculation of the overall confidence level.

	Liver		WAT		BAT		Overall		
	wt	KO	wt	KO	wt	KO	wt	KO	KO w/o wt
Index genes	179	23	54	4	117	2	331	32	20 ^a
Confidence		87%		93%		98%		90%	94%
Add. genes	187	44	114	46	98	11	321	95	77 ^b
Confidence		76%		60%		89%		70%	76%

^a Twelve genes (all in liver) were excluded because of parallel changes in wild-type mice in the same tissue.

^b Eighteen genes were excluded because of parallel changes in wild-type mice in the same tissue (13 in liver, 4 in WAT, 1 in BAT).

fructo-2-kinase/fructose-2,6-bisphosphatases, enzymes that control glycolysis by generating the most potent activator of 6-phosphofructo-1-kinase, was down-regulated in WAT as well, but the direction of its activity is mainly regulated by phosphorylation (Pilkis et al., 1995). Overall, LXR agonist treatment seems to down-regulate gluconeogenesis in liver as well as glycolysis in peripheral tissues, such as adipose tissues, indicating reduced carbohydrate metabolism.

Novel roles of LXRs in endocrine regulation were indicated by regulation of several enzymes involved in steroid hormone synthesis, eicosanoid metabolism, and thyroid hormone function. Two abundant 3β -hydroxysteroid dehydrogenase- $\Delta 5/\Delta 4$ -isomerases, which catalyze a crucial step in the synthesis of steroid hormones, were strongly down-regulated selectively in livers of wild-type mice (one as index gene). One additional 3β -hydroxysteroid dehydrogenase- $\Delta 5/\Delta 4$ -isomerase (HSD3B5) was the most strongly down-regulated mRNA (-36 -fold; not shown) but had to be excluded because of a relatively small but significant decrease in $LXR\alpha^{-/-}\beta^{-/-}$ mice (-2.9 -fold) suggesting minor regulation also by other pathways. These data strongly suggest alterations in steroid hormone homeostasis after LXR agonist treatment. The LXR-mediated down-regulation of 11β -hydroxysteroid dehydrogenase type 1, the key enzyme for local production of glucocorticoids (Sandeep and Walker, 2001), was confirmed by quantitative RT-PCR (Fig. 2D) and is further characterized elsewhere (Stulnig et al., 2002). In contrast, *cis*-retinol androgen dehydrogenase 1, which regenerates dihydrotestosterone from its catabolite 5-androstan- $3\alpha,17\beta$ -diol but may also be implicated in generation of 9-*cis*-retinoic acid (Chai et al., 1997), was 7-fold up-regulated in LXR agonist-treated wild-type mice. Because 9-*cis*-retinoic acid is an agonist of the general nuclear receptor heterodimer partner RXR (Rastinejad, 2001), LXR activation could synergize with numerous other RXR heterodimer partners by inducing production of its ligand. In addition, LXR agonist treatment induced its own up-regulation (suppl. Fig. 3, cat. transcription) as part of an autoregulatory loop at the transcriptional level which was reported selectively for the human gene (Laffitte et al., 2001a; Li et al., 2002).

LXR agonist treatment also interfered with peptide hormones in adipose tissue and liver. Expression of the adipocyte hormone leptin was down-regulated 2-fold in white adipose tissue of agonist-treated mice (suppl. Fig. 3, cat. endocrinology-cytokines), suggesting a possible impact of LXRs on body weight control (Friedman and Halaas, 1998). Expression of growth hormone receptor and its major effector protein insulin-like growth factor 1 was decreased by at least 1.5-fold (Fig. 1 and suppl. Fig. 3, cat. endocrinology). Because growth hormone is a major opponent of insulin action in liver, down-regulation of the growth hormone pathway may add to the role of LXRs in glucose homeostasis described above. Notably, not only growth hormone receptor but also epidermal growth factor receptor expression was strongly down-regulated (approximately -7 -fold) by LXR agonist treatment in liver (suppl. Fig. 3, cat. proliferation).

LXR agonist treatment markedly altered expression of genes involved in various aspects of signal transduction and regulation of transcription in both directions. Proteins involved in signaling via small as well as heterotrimeric G-proteins were altered in liver as well as adipose tissues. Dexras1, the murine homolog of human activator of G-protein signaling, was strikingly down-regulated in WAT of

wild-type mice as confirmed by quantitative real-time RT-PCR (Fig. 2E), indicating interaction with heterotrimeric G-protein signaling at different levels in this tissue. Moreover, Dexras1 is involved in nitric oxide signaling, at least in brain (Fang et al., 2000), and could hence contribute to the pathogenesis of insulin resistance (Pilon et al., 2000). Notably, LXRs influence expression of many transcription factors, including $LXR\alpha$, as mentioned above, but also $PPAR\alpha$ and retinoid X receptor γ , although selectively in BAT. In conclusion, LXR agonist treatment leads to complex changes in several pathways of cell signaling whose net outcomes can be defined only by functional evaluation based on these results.

Besides several cytochrome P450 enzymes that were also induced in knockout mice (not shown), indicating effects of receptors other than LXRs, two cytochrome P450 genes (*CYP4A10*, *CYP4A14*) were up-regulated by LXR agonist treatment by more than 10-fold selectively in wild-type mice (most of them listed under cat. detoxification) together with increased expression of cytochrome-P450 reductase. Because $LXR\alpha$ is a direct target gene of $PPAR\alpha$ and $PPAR\gamma$ (Tobin et al., 2000; Chawla et al., 2001; Chinetti et al., 2001), the known increased expression of *CYP4A10* and *CYP4A14* after peroxisome proliferator treatment (Heng et al., 1997) could involve induction of $LXR\alpha$. Deficiency in *CYP4A14* results in elevated blood pressure because of increased levels of androgens in male mice (Holla et al., 2001), emphasizing a potential interference of LXRs with androgen metabolism and blood pressure regulation particularly in male subjects. *CYP4A10* and *CYP4A14* are not only up-regulated in models for diabetes and obesity (Enriquez et al., 1999; Sakuma et al., 2001) but also are major microsomal lipid peroxidases that probably exert a pathophysiologic role in nonalcoholic steatohepatitis (Leclercq et al., 2000). The coordinate up-regulation of cytochrome P450 reductase selectively in wild-type mice emphasizes the functional significance of LXR-mediated up-regulation of *CYP4A10* and *CYP4A14* and underlines the conclusion that LXRs and their agonists could be involved in the development of common liver disorders.

Amino levulinate synthase 1 (*ALAS1*) was up-regulated more than 2-fold, indicating that LXR agonists could trigger exacerbations of hepatic porphyrias by stimulating heme synthesis for new cytochrome P450 proteins. Interestingly, phenobarbital, an agonist of the constitutive active receptor (CAR), up-regulates *ALAS1* and two enzymes involved in sterol biosynthesis (7-dehydrocholesterol reductase, squalene oxidase) in wild-type as well as $CAR^{-/-}$ mice, but increases expression of *CYP4A10* and *CYP4A14* selectively in $CAR^{-/-}$ mice (Ueda et al., 2002). These and several other genes were regulated in parallel by LXR agonist treatment in wild-type but not $LXR\alpha^{-/-}\beta^{-/-}$ mice (Fig. 1 and suppl. Fig. 3) suggesting a possible cross talk between phenobarbital action and LXRs.

Beyond alterations in the expression of particular genes, LXR agonist treatment had an impact on overall protein metabolism. Expression of proteasome components was increased, particularly in liver, indicating that LXRs stimulate protein turnover. In contrast, expression of several chaperones was down-regulated in liver and brown adipose tissue. Several serum proteins were down-regulated in liver by LXR agonist treatment, including complement components (Fig. 1 and suppl. Fig. 3). Also, a number of matrix genes, including many procollagens, were uniformly down-regulated by LXR agonist treatment, particularly in white adipose tissue

(suppl. Fig. 3), suggesting an impact of LXR agonists on connective tissue architecture.

Microarray data are not intended to prove regulation of a particular gene but to give a genome-wide overview of specific pathways that can then be studied in detail. Because of the high quality of this knockout-controlled data, as revealed by a confidence of 94% for index genes, this compilation of regulated genes represents a highly valuable basis for further research. Because sustained regulation of genes is important for pathogenesis and treatment of metabolic and endocrine disorders, gene expression profiles studying long-term treatment (7 days) as performed here provide most relevant information.

In conclusion, the genome-wide expression profiling data presented here elucidated novel roles of LXRs, particularly in regulating carbohydrate metabolism, several aspects of endocrine homeostasis, particularly steroid hormone synthesis, cytochrome P450 gene expression, including lipid peroxidation processes, and protein turnover. Thus, these data on gene regulation by LXRs considerably enhance our understanding of the biological roles of LXRs in important insulin target organs such as liver and adipose tissues. Although more detailed analyses including functional data have to be awaited, our data could indicate a possible use of LXR agonists in diabetes mellitus and endocrine disorders.

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