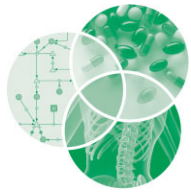
Advanced Review



Lipid-sensing nuclear receptors in the pathophysiology and treatment of the metabolic syndrome

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Metabolic syndrome (MS) is a cluster of different diseases, namely central obesity, hypertension, hyperglycemia, and dyslipidemia, together with a pro-thrombotic and pro-inflammatory state. These metabolic abnormalities are often associated with an increased risk for cardiovascular disease (CVD) and cancer. Dietary and lifestyle modifications are currently believed more effective than pharmacological therapies in the management of MS patients. Nevertheless, the relatively low grade of compliance of patients to these recommendations, as well as the failure of current therapies, highlights the need for the discovery of new pharmacological and nutraceutic approaches. A deeper knowledge of the patho-physiological events that initiate and support the MS is mandatory. Lipid-sensing nuclear receptors (NRs) are the master transcriptional regulators of lipid and carbohydrate metabolism and inflammatory responses, thus standing as suitable targets. This review focuses on the physiological relevance of the NRs (peroxisome proliferator-activated receptors, liver X receptors, and farnesoid X receptor) in the control of whole-body homeostasis, with a special emphasis on lipid and glucose metabolism, and on the relationships between metabolic unbalances, systemic inflammation, and the onset of CVD. Future perspectives and possible clinical applications are also presented. *WIREs Syst Biol Med* 2011 DOI: 10.1002/wsbm.137

INTRODUCTION

Metabolic Syndrome

Metabolic Syndrome (MS) is a cluster of clinical disorders including central obesity, hypertension, hyperinsulinemia and insulin resistance (leading to impaired fasting glycemia and glucose tolerance and to type 2 diabetes), atherogenic dyslipidemia [low levels of high-density lipoprotein cholesterol (HDL-c), high levels of triglycerides (TG), and low-density lipoprotein (LDL) particles], inflammation, and a pro-thrombotic state.[1,2] When clustered together, these clinical conditions may amplify or confer additive risk for the development of cardiovascular disease (CVD). Over the past decade, several classification systems have been proposed to describe the clinical features of MS.[1,3–6] The National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATPIII)[1] is currently accepted as the most accurate definition in terms of sensitivity and specificity. Although the etiology of MS is still unknown, there is consensus that both genetic background and unhealthy lifestyle habits (insufficient physical exercise and calorie overload) contribute coordinately to the onset of MS, resulting in an increased risk for CVD, cancer, and thrombosis.[2] There is a general agreement that lifestyle modifications may have the potential to more effectively ameliorate most of the clinical features of MS. Nevertheless, several pharmacological approaches, including thiazolidinediones (TDZ),

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cannabinoid CB1 receptor antagonists, ezetimibe, and fibrates, have been extensively used. There is an increasing interest in elucidating the metabolic pathways underlying the maintenance of energy homeostasis and, in this regard, nuclear receptors (NRs) and their role in the metabolic homeostasis have received great attention over the past decade. The purpose of this review is to provide insights into the metabolic pathways governed by lipid-sensing NRs, and endorse them as feasible and promising therapeutic targets in the management of MS.

Nuclear Receptors

NRs are key players in the coordination of the development, metabolism, circadian rhythms, cell growth, and differentiation. NRs are transcription factors transducing different signals into modulation of gene transcription,[7] thus participating in the control of all complex processes in living organisms. NRs show considerable specificity in their activation and tissue-specific expression,[8] and they can work as monomers, homodimers, and heterodimers.[7] In the human genome 48 NRs have been identified, whereas in rodents there are 49.[7] NRs are called 'orphans' when the endogenous ligands are unknown,[9,10] and 'true orphans' when NRs regulate the transcription independent of binding to specific ligands.[11,12] Some NRs are regulated by small lipophilic ligands [i.e., hormones, vitamins, dietary lipids, bile acids (BAs), xenobiotics, etc.].[9,12,13] In this review, we focus on the 'adopted orphan' lipid sensors, namely the peroxisome proliferator-activated receptors (PPARs), the liver X receptors (LXRs), and the farnesoid X receptor (FXR), which all form heterodimers with the retinoid X receptors (RXR). NRs are characterized by a conserved modular structure including an amino-terminal ligand-independent activation function domain (AF-1), a DNA-binding domain (DBD), and a region involved in protein–protein interaction and transcriptional activation of target-gene expression. The DBD is composed of two zinc-finger motifs, which bind a specific hormone response element (HRE).[14,15] HREs contain the canonical sequence AGGTCA that can be present alone or repeated. Thus, HREs can differ in terms of extension, duplications, and orientation of the repeats (direct, inverted, or everted)[7]; as a consequence, HREs can be selective for a given NR or for a class of receptors. The ligand-binding domain (LBD) contains a ligand-dependent activation function-2 (AF-2) motif that mediates coactivator recruitment.[13] In the absence of ligand, the LBD of many NRs is bound to transcriptional corepressor complexes that cause chromatin condensation and gene silencing. Ligand binding to NRs induces a change in NR three-dimensional conformation, which results in the dissociation of corepressors and in a subsequent recruitment of tissue-specific coregulators (allowing a fine-tuning of the physiologic response to ligand binding) and transcription machineries.[7,11,12]

PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS

Peroxisome proliferator-activated receptors are ligand-inducible transcription factors acting as 'fatty acid sensors' to control metabolic programs and to regulate energy homeostasis.[16] PPARs form obligate heterodimers with RXRs and bind to specific consensus DNA sites termed PPAR response elements (PPREs). PPREs are composed of direct repeats (DRs) of hexameric sequences AGGTCA, interspaced only by a single nucleotide spacer (DR-1 motif), located in the promoter/enhancer region of target genes. X-ray crystal analyses revealed that, compared with other NRs, the PPAR ligand-binding pocket is unusually large (about 1300 Å) and can accommodate a wide diversity of natural and synthetic compounds, including native and modified (oxidized and nitrated) fatty acids (FA), eicosanoids, derivatives of polyunsaturated FA, fibrates, and TDZ.[17–23] Three PPAR isotypes exist in mammals: PPARα (NR1C1), PPARβ/δ (NR1C2), and PPARγ (NR1C3). Although the three PPAR subtypes share a high degree of sequence and structure homology, they are characterized by a very specific tissue distribution and unique physiological functions.[24] In the following paragraphs, we focus on the physiological functions of PPAR subtypes with a special emphasis on those related to the key features of MS (i.e., elevated TG, low HDL-c, and glucose imbalance).

PPARα

In the late 1960s peroxisome proliferation and hepatomegaly were first reported occurring in rat livers when treated with clofibrate or related compounds (termed peroxisome proliferators) and, several years later (1990), the first receptor for these molecules was cloned and named PPARα.[25] PPARα acts as master transcriptional regulator of FA utilization and is prominently expressed in the liver and, to a lesser extent, in kidney, heart, skeletal muscle, small intestine, brown adipose tissue (BAT), immune cells, and endothelium.[26,27] Fibrates bind to PPARα in the high micromolar range (that may explain why large doses are required for clinical use) and to a lesser extent to PPARγ and PPARβ/δ contributing to some of the metabolic effects. Given the ability of PPARα agonists

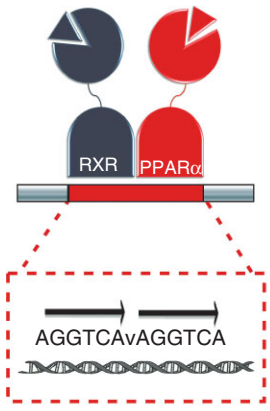
to modulate both TG and HDL metabolism,[28,29] fibrates are currently used for the management of primary hypertriglyceridemia, combined hyperlipidemia, type 3 dyslipoproteinemia, and lipid abnormalities associated with type 2 diabetes and MS.[30]

PPARα and Triglyceride Metabolism

The major physiological function of PPARα is to promote FA utilization, and the first PPRE has been identified in the 5′-flanking sequence of the rat acyl-CoA oxidase (ACOX) gene.[31] PPARα target genes are involved in FA transport and uptake [FA transport protein (FATP), CD36, and carnitine-palmitoyltransferase 1 (CPT-1)] and β-oxidation (ACOX, thiolase, acyl-CoA-dehydrogenase, cytochrome P450-ω-hydroxylase), and their transcriptional activation contributes to FA homeostasis in lipid-metabolizing tissues such as liver, heart, and muscle (Figure 1).[32–36] Accordingly, PPARα knockout animals exhibited fatty liver phenotype (steatosis), peripheral tissue lipid accumulation, adipocytes hypertrophy, and late onset obesity (despite a stable caloric intake).[37–39] Several proteins involved in the apolipoprotein (apo) B-containing lipoprotein metabolism, such as lipoprotein lipase (LPL), apoCIII, apoAV, and proprotein convertase subtilisin/type 9 (PCSK9) are PPARα target genes.[40–45] The well-documented TG-lowering properties of PPARα agonists result from both an increased lipolysis and clearance of TG-rich lipoproteins (via activation of LPL and inhibition of apoCIII) and a reduced availability of free FA (FFAs) for TG synthesis (via enhanced β-oxidation). PPARα activation influences the circulating levels of apoB100-containing lipoproteins (via increased VLDL-apoB100 catabolism), as well as their distribution profile (via lower levels of small dense LDL and higher concentrations of large buoyant LDL), although the outcomes are not fully elucidated.[46,47]

PPARα and HDL Metabolism

PPARα agonists modulate the plasma levels of HDL-c through an increased HDL production rate, higher apoAI mRNA levels, stimulation of ATP-binding cassette transporter A1 (ABCA1)-mediated cholesterol efflux in macrophages, and reduced cholesteryl ester transfer protein (CETP) levels.[30,48] Interestingly, differential PPARα-dependent effects of gemfibrozil and fenofibrate on hepatic apoAI expression have been reported. Both raise HDL levels, while only fenofibrate (full PPARα agonist) increases apoAI concentrations.[49] Furthermore, it is not clear if an higher apoAI production observed upon fenofibrate treatment[50] affects the more mature (large) HDL



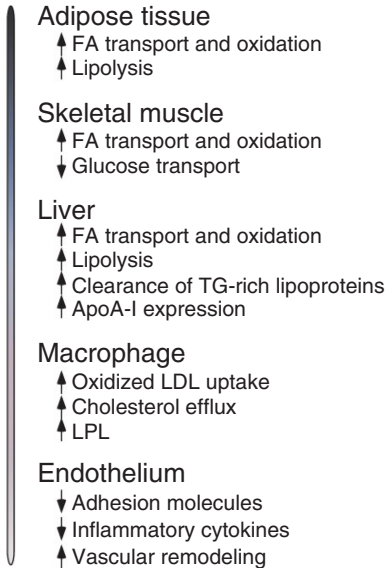


FIGURE 1 | Schematic representation of peroxisome proliferator-activated receptor (PPAR)α transcriptional activation (left): as a heterodimer of retinoid X receptors (RXR), PPARα binds the PPAR response elements (PPREs), containing direct repeats (DR) of hexameric sequences AGGTCA, interspaced only by a single nucleotide spacer (DR-1). Effects of PPARα activation in tissues involved in metabolic homeostasis (right): PPARα reduces plasma triglycerides (TG) by inducing fatty acid (FA) transport and oxidation in liver, adipose tissue, and muscle. In addition, PPARα induces lipolysis in adipocytes and hepatocytes. PPARα also modulates lipoprotein metabolism both in the liver, where PPARα induces ApoAI expression and clearance of TG-rich lipoproteins, and in macrophages, where PPARα promotes the efflux of cholesterol, the uptake of oxidized LDL, and the expression of lipoprotein lipase (LPL), thus promoting lipoprotein remodeling. In the endothelium, PPARα exerts athero-protective properties, inhibiting the release of adhesion molecules and inflammatory cytokines, while enhancing vascular remodeling.

that can promote the athero-protective process of the reverse cholesterol transport (RCT).

PPARα Clinical Usefulness

Although fibrates, both in monotherapy and in combination with statins (HMG-CoA-reductase inhibitors) or ezetimibe (cholesterol absorption inhibitor), are effective on both TG and HDL metabolism,[51–54] the currently available fibrates are weak ligands for PPARα and there is a quest for both highly selective PPARα agonists and SPPARMs (selective PPAR modulators). *Nissen et al.* provided the first description of the beneficial effects of a powerful (3000 times more potent than fenofibrate) and highly selective PPARα agonist (LY518674) that, when given to patients with hypercholesterolemia, lowered plasma TG and raised HDL-c and LDL-c.[55] More recently, LY518674 was given to subjects with MS for 8 weeks; along with a 23% reduction in plasma TG and no change in HDL concentration, LY compound was able to increase both apoAI and apoAII production rate by 31 and 71%, respectively, as well as the fractional catabolic rate of these two apolipoproteins.[56] Recently, *Chakravarthy et al.* identified a new endogenous ligand of PPARα, the phospholipid (PL) palmitoyl-2-oleoyl-sn-glycerol-3-phosphocholine (16:0/18:1-GPC), that exhibited a weak activation on PPARβ/δ but not on PPARγ *in vitro*. This ligand seems to be indispensable for hepatic PPARα activation and its function during fasting.[57] The clinical relevance of fibrate use in type 2 diabetes and MS is still under debate and requires further investigations. Although in clinical trials involving type 2 diabetes patients, such as Diabetes Atherosclerosis Intervention Study (DAIS) and Fenofibrate Intervention and Event Lowering in Diabetes (FIELD), fenofibrate appeared effective on the progression of diabetes-related microvascular disease, it is not clear whether fibrates alone may efficiently impact the lipid abnormalities associated with MS, or if a combination with other lipid-lowering drugs, such as statins or insulin-sensitizing agent metformin, should be always recommended in the management of the cardiovascular risk.[58] Future insights may come from ongoing trials such as Action to Control Cardiovascular Risk in Diabetes Trial or studies aiming to test more selective PPARα agonists in both *in vitro* and in animal models. From a translational point of view, a comment has to be made on the existence of species differences (mostly rodent vs human),[59,60] in apolipoprotein,[61] and glucose metabolism.[62,63] The difference is also evident in terms of carcinogenesis since a mouse-specific cancer susceptibility profile of PPARα activation is completely absent in the humanized model.[64] At present, the mechanisms responsible for PPARα species differences are not fully elucidated; however, it has been suggested that they may be due to differences in the level of PPARα expression or in the functional DNA-binding capacity or in PPREs found upstream of critical target genes.[59]

PPARβ/δ

Since its cloning in 1992,[65,66] PPARβ/δ has been the focus of far less research, compared with PPARα and PPARγ.[67] PPARβ/δ exhibits a broad expression pattern but is more abundantly expressed in lipid-metabolizing tissues such as skeletal muscle, heart, small intestine, and adipose tissue.[68] PPARβ/δ has a short N-terminal domain in which no *bona fide* ligand-independent activation domain has been identified so far, although a modulation of PPARβ/δ activity by cAMP-dependent phosphorylation may occur via this domain.[69] Among the PPAR subtypes, PPAR β/δ pocket is the smallest one and features a convoluted space where the hydrocarbon tail of unsaturated FA binds.[70] Polyunsaturated FA [eicosapentaenoic acid (EPA), arachidonic acid (ARA), and dihomo-γ-linoleic acid], prostaglandin A1, E2, and D2 bind to PPARβ/δ at low concentrations (micromolar range), whereas only prostacyclin (PGI) and its stable analog (carba-prostacyclin) activate PPARβ/δ-mediated transcription in a nanomolar range of concentrations.[71] Selective synthetic PPARβ/δ agonists have been recently developed (GW501516, GW0742), displaying both *in vitro* and *in vivo* a 1000-fold selectivity over the other PPAR isotypes.[72] The PPARβ/δ gene is conserved between species, and there are no studies of rare human genetic mutations; however, PPARβ/δ polymorphism (e.g., rs2016520) is associated with increased LDL-c and reduced HDL-c concentrations, suggesting a role for this transcription factor in human cholesterol metabolism.[73] In addition, the relationship between PPARβ/δ polymorphism rs2016520 and the risk of showing three or more components of the MS is influenced by dietary habits.[74] Finally, interaction between PPARβ/δ single polymorphism and fasting plasma glucose levels and body mass index has been shown as well.[75] The role of PPARβ/δ in adiposity, skeletal muscle physiology, and lipoprotein regulation identified this NR as promising candidate for therapeutic intervention in MS (Figure 2).

PPARβ/δ in Adipose Tissue

PPARβ/δ is expressed both in white adipose tissue (WAT) and BAT where it controls thermogenesis, FA

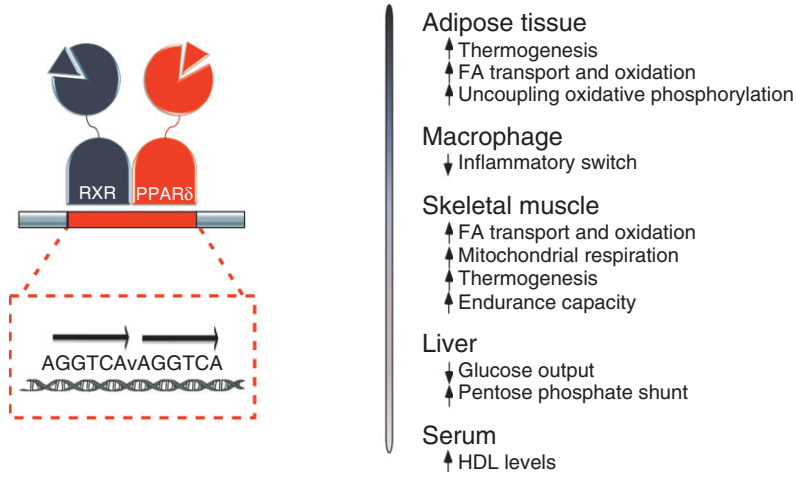


FIGURE 2 | Schematic representation of peroxisome proliferator-activated receptor (PPAR)β/δ transcriptional activation (left): as a heterodimer of retinoid X receptors (RXR), PPARβ/δ binds the PPAR response elements (PPREs), containing direct repeats (DR) of hexameric sequences AGGTCA, interspaced only by a single nucleotide spacer (DR-1). Effects of PPARβ/δ activation in tissues involved in metabolic homeostasis (right): in adipose tissue and muscle, PPARβ/δ induces fatty acid (FA) transport and oxidation, mitochondrial activity, and thermogenesis, thus increasing energy expenditure. In muscle, PPARβ/δ activation ameliorates the endurance capacity. In the liver, PPARβ/δ inhibits glucose output, thereby contributing to the peripheral glucose homeostasis. PPARβ/δ also induces increased serum high-density lipoprotein (HDL) levels, leading to enhanced reverse cholesterol transport (RCT), and reduced inflammation in the macrophages. These effects could further promote a PPARβ/δ-mediated cardiovascular prevention.

transport, and oxidation and uncoupling of oxidative phosphorylation through activation of its target genes such as long-chain acyl-CoA dehydrogenase, CPT-1, ACOX-1, long-chain acyl-CoA-synthetase, and uncoupling protein-1 (UCP-1).[67,76,77] In rodents, PPARβ/δ adipose tissue-specific over-expression protected from diet-induced obesity, triglyceride accumulation in adipocytes, hypertriglyceridemia, and hepatic steatosis.[76] Administration of PPARβ/δ agonist GW501516 was able to mimic the effects of a constitutively active PPARβ/δ transgene in obese murine models but not in rhesus monkeys,[78] leaving unanswered the question whether PPARβ/δ fat-specific activation may have a metabolic relevance on the whole-body homeostasis.

PPARβ/δ in Muscle

Skeletal muscle is a key metabolic tissue accounting for approximately 80% of insulin-stimulated glucose uptake and, compared with liver and adipose tissue, is subjected to an almost complete unidirectional flux of FA with all imported FA oxidized rather than stored and/or exported. Both obesity and insulin resistance are linked to a decrease in the proportion of oxidative slow twitch (type 1) fibers in skeletal muscle.[79,80] PPAR β/δ expression in skeletal muscle is 10–50 fold higher compared with that of PPARα or PPARγ and has been exclusively found in type 1 fibers that mainly use mitochondrial oxidative metabolism for energy production.[81,82] PPARβ/δ skeletal muscle-specific deletion was found associated with weight gain, insulin resistance, and reduced FA oxidation.[83] Conversely, muscle-specific over-expression protected mice from diet-induced obesity[81] and was accompanied by muscle fiber type remodeling that ameliorated insulin sensitization. Of note, the therapeutic potential of a reprogramming of muscle endurance by targeting the AMPK-PPARδ signaling axis via orally active PPARδ agonists (GW501516) has been recently reported. This approach may hold promise in regard to the management of clinical conditions where exercise is recommended, such as obesity and MS.[84]

PPARβ/δ and Lipoprotein Metabolism

Both animal and human studies have provided compelling evidence of a beneficial role of PPARβ/δ on lipoprotein metabolism. In obese as well as in hyper-cholesterolemic mouse models, PPARβ/δ agonist GW501516 increased HDL-c concentrations up to 50% along with a reduction of small dense LDL levels.[85–87] Obese rhesus monkeys have been used as model of human obesity and metabolic disorders and used in studies aimed to assess PPARβ/δ benefit on HDL metabolism.[78,88] PPARβ/δ agonist GW501516 given as single daily dose,[88] or twice a day,[78] raised HDL-c levels up to 43 or 79%, respectively; lowered LDL-c; and increased apoAI and apoAII levels and HDL particle size. PPARs activate human apoAI gene via a positive PPRE located in the apoAI promoter A

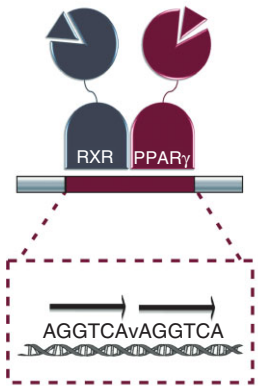
site; whereas a three nucleotide difference makes the positive PPRE nonfunctional in rodents, suggesting that primates, but not mice, are a good model for future investigations on the PPAR β/δ benefit on HDL-c and the RCT.[88,89] Although in some pilot studies in both normo-lipidemic and moderately obese subjects GW501516 treatment promoted a substantial increase in HDL-c,[90,91] the physiological relevance of PPAR β/δ-mediated HDL-raising effect remains still elusive in humans. Future insights may come from newer agonists, such as KD-3010 and MBX-8025, currently in the preclinical phases of research.[92]

PPARγ

First cloned in 1994,[93] PPARγ was later identified as the receptor for the TDZ class of insulin-sensitizing drugs in 1995.[94] The PPARγ gene is transcribed into three splice variants (PPARγ1, 2, and 3). PPARγ1 and 3 transcripts give rise to PPARγ1 protein that is highly produced in WAT, immune cells (macrophages and monocytes), intestine (colon), liver, and kidney, whereas the PPARγ2 transcript encodes for a protein exclusively expressed in WAT and BAT. Monounsaturated and polyunsaturated FA, eicosanoid FA-derivatives [13-hydroxyoctadecanoic acid (13-HODE) and 9-HODE; mainly found in oxidized LDL] and prostanoids, such as 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ2) bind to PPARγ with micromolar range affinity, as reported in radio-ligand binding assays.[95,96] The most highly studied of the PPARγ ligands are the TDZs, pioglitazone, and rosiglitazone, which have been approved for clinical use in type 2 diabetes since 1997 in US. (the physiological role of PPARγ activation is summarized in Figure 3).

PPARγ in Adipose Tissue

In vitro and *in vivo* studies have shown that PPARγ is the prime regulator of adipocyte differentiation.[97,98] PPARγ-null fibroblasts and embryonic stem cells are differentiation-incompetent *in vitro*[99] and germ-line deletion of PPARγ, as well as PPARγ hypomorphic mice demonstrate the essential requirement for this transcription factor in the formation of adipose tissue *in vivo*.[100] Naturally occurring PPARγ mutations in humans support the key role of this NR in adipose tissue development and distribution. Patients harboring dominant-negative mutations in PPARγ gene exhibit familial partial lipidystrophy type 3 (FPLD3), concomitant with severe insulin resistance, dyslipidemia (elevated serum TGs and low HDL-c), and early onset



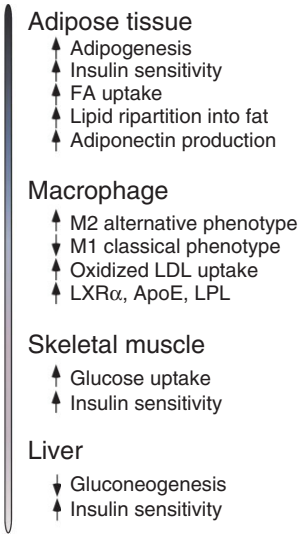


FIGURE 3 | Schematic representation of peroxisome proliferator-activated receptor (PPAR)γ transcriptional activation (left): as a heterodimer of retinoid X receptors (RXR), PPARγ binds the PPAR response elements (PPREs), containing direct repeats (DR) of hexameric sequences AGGTCA, interspaced only by a single nucleotide spacer (DR-1). Effects of PPARγ activation in tissues involved in metabolic homeostasis (right): PPARγ activation improves glucose metabolism, inducing insulin sensitivity (in the liver, skeletal muscle, and adipose tissue), increasing glucose uptake from the muscle and reducing hepatic gluconeogenesis. In the adipose tissue, PPARγ also induces fatty acid (FA) uptake, adipogenesis, increased fat storage, and a better lipid repartition into adipocytes, leading to the formation of small, newly formed, and active adipocytes. Nevertheless, PPARγ increases the production of adiponectin from adipocytes, which is negatively correlated to metabolic unbalances and MS. In macrophages, PPARγ also induces an increased M2/M1 ratio (thus inhibiting inflammation) and contributes to an enhanced uptake of oxidized low-density lipoprotein (LDL). In addition, PPARγ regulates the transcription of the liver X receptor (LXR), lipoprotein lipase (LPL), and apolipoprotein E (apoE), further preventing atherosclerosis.

hypertension.[101–103] Common (e.g., PPARγ Pro12Ala polymorphism) and rare (loss-of-function mutations) variants in the gene encoding PPARγ provide additional genetic evidence of the central role of PPARγ in the MS.[104–107] The finding that TDZs act as high affinity PPARγ agonists validated the efficacy of PPARγ modulation in the management of MS.[108] The TDZs are able to improve insulin sensitivity through increased lipid uptake and storage, decreased serum FFA and TG, reduced hepatic gluconeogenesis, increased peripheral glucose absorption and enhanced energy expenditure.[108] Studies *in vivo* had shown that WAT is the primary tissue responsible for the therapeutic effects of TDZs since mice lacking adipose tissue are refractory to the TDZ glucose-lowering effect. Additionally, PPARγ adipose-specific knockout mice are insulin-resistant and unresponsive to TDZs.[109–111] PPARγ activation in adipocytes stimulates a wide array of genes involved in FA uptake and storage including LPL, FATP-1, FA binding protein-4 (FABP-4), acyl-CoA synthase (ACS), perilipin, cell death-inducing DNA fragmentation factor subunit alpha (DFFA)-like effector A (CIDEA), CD36, and phosphoenolpyruvate-carboxykinase (PEPCK).[112–116] Adipocyte PPARγ activation leads to a lipid repartitioning from liver and skeletal muscle into fat, thus, reducing the circulating levels of FFA and eliminating the detrimental effects of lipid loading on insulin signaling (the so-called 'lipid steal phenomenon').[117] PPARγ activation promotes a fat redistribution with an expansion of adiposity in the subcutaneous adipose tissue at the expense of visceral fat,[118,119] contributing to the weight gain observed in patients treated with TDZs compared with those receiving sulphonylurea.[120] In addition, PPARγ activation in adipose tissue enhances the production of adiponectin,[121] promoting FFA oxidation and insulin sensitivity via stimulation of AMP-activated protein-kinase in skeletal muscle and through inhibition of PEPCK in the liver.

PPARγ and Inflammation

Many lines of evidence support the link between hepatic inflammation and insulin resistance[122] and a family of suppressors of cytokine signaling (SOCS) has been shown to be involved in both inflammation and insulin resistance. Chronic PPARγ activation by pioglitazone in high-cholesterol fructose diet-fed rats improved insulin sensitivity by decreasing the hepatic expression of SOCS3, tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6).[123] Along with the aforementioned effects in the liver and skeletal muscle, PPARγ activation enhances glucose transporter member 2 (GLUT2) expression in pancreatic β-cells, leading to an augmented insulin secretion.[124–126] Obesity and insulin resistance, key features of the MS, have been consistently associated with a state of low-grade inflammation which is caused likely by both adipocyte hypertrophy and macrophage infiltration into adipose tissue.[127] A polarization of adipose-resident macrophages from M2 (alternatively activated phenotype, less inflammatory) toward an M1 phenotype, characterized by enhanced secretion of pro-inflammatory mediators (TNF-α, IL-6) during diet-induced obesity, has been reported.[128] PPARγ is highly expressed in activated macrophages and is markedly induced upon IL-4 stimulation.[129,130] *Odegaard et al*. elegantly showed that macrophage PPARγ is required for maturation of alternatively activated macrophages and PPARγ disruption in myeloid cells impairs the alternative macrophage activation, predisposing mice to development of high fat diet-induced obesity, insulin resistance, glucose intolerance, and muscle mitochondria dysfunction.[131] A short-term treatment with the PPARγ agonist rosiglitazone promoted infiltration of M2 macrophages into adipose tissue in high fat-fed mice.[132] The inability to undergo differentiation into M2 phenotype reported, when macrophage PPARγ was genetically disrupted, was found not only in high fat diet-fed animals but also in normal low fat diet-fed animals.[133] This finding indicates that macrophage PPARγ expression might be necessary to maintain an anti-inflammatory M2 phenotype and important for the achievement of the full insulin-sensitizing effects of TDZ.[134] The intriguing link between insulin sensitivity, macrophage PPARγ, and adipose tissue has been seen in humans as well. TDZ treatment improved insulin sensitivity and was associated to a marked reduction of adipose tissue CD68, monocyte chemoattractant protein-1 (MCP-1) mRNA abundance, and circulating TNF-α levels.[135]

'Janus-face' of PPARγ Activation

Although extensively used in type 2 diabetes patients, TDZs are not completely devoid of side effects such as weight gain (~2–3 kg per 1% glycosylated hemoglobin that is lowered), anemia, pulmonary edema, congestive cardiac failure, and increased risk of myocardial infarction[120]; these outcomes will further limit the clinical usefulness of these drugs. To bypass the problem, it will be necessary to dissect the relative contributions of adipose versus non-adipose (macrophage, muscle, and liver) PPARγ activation to the systemic insulin sensitization; for this purpose, conditional PPARγ knockout mice have been used.[110,133,136,137] Unfortunately, these studies could neither address the molecular basis for PPARγ action in different cell types, nor how TDZs can increase

the ability of target cells to sense insulin. In addition, these studies did not circumvent all the complications caused by the loss-of-function of adipose PPARγ, such as the rebounded increase of PPARγ expression in liver and muscle.[109,138] Recent studies addressed some of the aforementioned gaps in our knowledge of PPARγ biology and physiology.[139–141] Selective PPARγ activation in adipocytes, but not in macrophages, is sufficient for whole-body insulin sensitization equivalent to systemic TDZ treatment in mice. These data offer an 'adipocentric' model, where fat is not only a contributor, but a central player in insulin sensitization,[140] providing evidence for the need of cell-restricted PPAR modulators. Intriguingly, selective PPARγ modulators (SPARMs) can improve insulin sensitivity without causing weight gain.[142–144] The recent work by *Lefterova et al.* provides new evidence for how cell-type-specific gene expression may be achieved by a single NR.[139]

Future efforts need to be directed toward the development of tissue-specific PPARγ agonists that can retain insulin-sensitizing properties without activating *de novo* adipogenesis and the consequent increased subcutaneous adiposity. In this regard, partial PPARγ/α agonists such as TDZ18 or small molecules such as harmine may hold this promise in a near future.[112,145]

LIVER X RECEPTORS

First identified in 1994 by screening a rat liver cDNA library, LXRs were originally classified 'orphans', as their natural ligands were unknown.[146,147] Subsequently 'adopted', when oxidized cholesterol derivatives, referred to as oxysterols, were shown to be endogenous ligands,[147,148] LXRs have emerged as keystones in lipid, carbohydrate, and inflammatory homeostasis.[148–151] In mammals, two LXR isoforms (LXRα and LXRβ) exist and both are highly expressed in the liver and the intestine, where their physiological roles have been best elucidated.[152] LXRα (NR1H3) is also expressed in WAT, macrophages, kidney, adrenal glands, lung, and spleen, whereas LXRβ (NR1H2) is ubiquitously expressed. LXRα and LXRβ not only exert overlapping but also separate metabolic functions, with the former being required for the control of hepatic cholesterol metabolism and the latter being the major regulator of glucose homeostasis and energy utilization in both muscle and WAT (Figure 4).[153] LXRs bind to cognate LXR-responsive elements (LXREs), consisting of DRs of the core sequence AGGTCA separated by four nucleotides (DR-4)[9,154] and to corepressors, such as silencing mediator of retinoic acid and thyroid hormone receptor[155] and NR corepressor (N-CoR).[156] As both LXRs form permissive heterodimers with the RXR, the complex can be activated either by oxysterols or retinoids. The binding of ligands results in conformational changes, allowing the interaction of the LXR–RXR complex with coactivators leading either to the transcription of target genes or the trans-repression of other genes not containing LXREs.[157,158] LXR natural ligands include 22(R)-, 24(S)-, 27-, and 24(S),25-hydroxy cholesterol at concentrations within the physiological range while synthetic LXR ligands (T0 901317, GW3965) show EC50 values for both isoforms in the low nanomolar range.[147,159]

LXR and Cholesterol Homeostasis

Liver X receptors function as whole-body 'cholesterol sensors' and most of their target genes in liver, intestine, and macrophages play a crucial role in the maintenance of cholesterol homeostasis and atherosclerosis. LXR is expressed in macrophages, being positively regulated by PPARγ, suggesting a link between the macrophage ability to remove cholesterol and the uptake of oxidized LDL.[9] LXRα may also induce its own transcript via an auto-regulatory loop in human but not in murine macrophages.[160,161] The identification of the role of LXR in cholesterol efflux from macrophages via ABCA1 and ABCG1 upregulation,[162] and in the removal of excess cholesterol from peripheral tissues via RCT, pointed to a possible anti-atherosclerotic effect of LXR activation.[151,162–167] ABCA1 and ABCG1 mediate cholesterol and PL efflux from macrophages to lipid-poor lipoproteins and mature HDL, respectively, leading to the formation of the lipid-enriched HDL (i.e., HDL-2 and HDL-3 particles).[168] ABCA1 mutations cause the Tangier disease, a rare genetic disorder characterized by the absence of HDL in plasma, the accumulation of cholesterol in macrophages and an increased incidence of CVD.[169–172] LXR activation cannot stimulate cholesterol efflux in fibroblasts from Tangier disease patients, demonstrating that ABCA1 is essential for the efflux pathway mediated by LXR.[166] Additionally, the activation of both LXR isoforms regulates apolipoprotein E (apoE) expression, both in the liver and macrophages. On the other hand, bone marrow transplantation from LXR-null mice into apoE-null mice results in a massive aortic lipid deposition.[150,173] ApoE is an extracellular cholesterol acceptor required for the hepatic uptake of chylomicron remnants, VLDL and HDL, and serves as extracellular acceptor for ABCA1-mediated cholesterol efflux.[150] The increase in HDL concentration found upon LXR activation is the result of a coordinate regulation of a series of proteins involved in

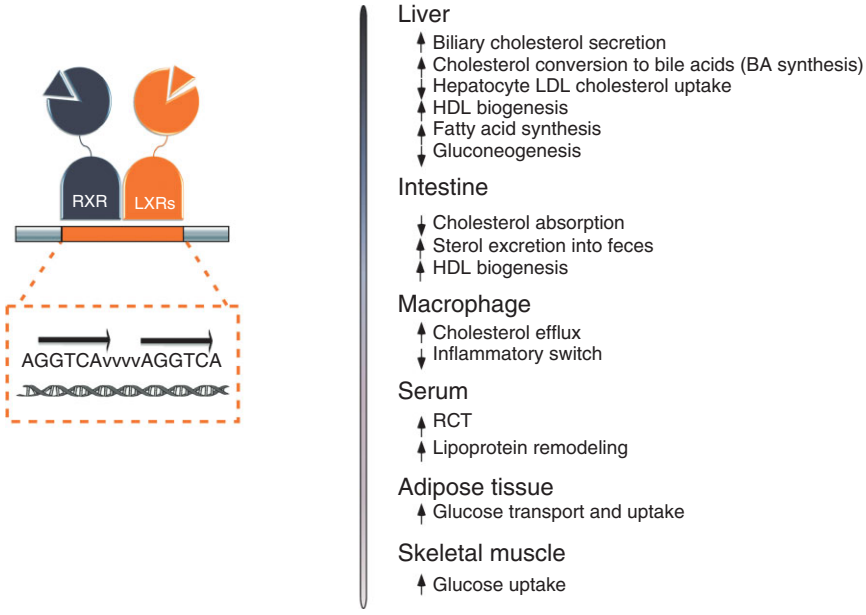


FIGURE 4 | Schematic representation of liver X receptors (LXRs) transcriptional activation (left): as a heterodimer of retinoid X receptors (RXR), LXRs bind the LXR response elements (LXREs), containing direct repeats (DR) of hexameric sequences AGGTCA, interspaced by four nucleotide spacers (DR-4). Effects of LXRs activation in tissues involved in metabolic homeostasis (right): LXRs are the master regulators of cholesterol metabolism. In the liver, LXRs promote cholesterol secretion in bile and its conversion to bile acids (BA), whereas inhibiting low-density lipoprotein (LDL) cholesterol uptake. In the intestine, LXRs inhibit cholesterol absorption while promoting its secretion. LXRs also promote reverse cholesterol transport (RCT) via an integrated mechanism involving the liver and intestine [increased high-density lipoprotein (HDL) biogenesis], macrophages (increased efflux of cholesterol), and serum (enhanced RCT and lipoprotein remodeling). The activation of LXRs also reduces glucose concentrations in blood (inhibiting hepatic gluconeogenesis, whereas increasing glucose uptake in muscle and adipose tissue), and enhances triglyceridemia [promoting hepatic fatty acid (FA) synthesis]. Finally, LXRs negatively modulate inflammatory pathways in macrophages.

HDL formation and lipoprotein remodeling, such as LPL, PL transfer protein (PLTP) and CETP, which all are LXR target genes.[150,152,173–175] Macrophage LXR has been reported to be crucial for protection from atherosclerosis.[163] LXR activation results in NF-κB signaling suppression,[176–178] and ultimately to the repression of inflammatory genes [inducible nitric oxide synthase (iNOS), cycloxygenase 2 (COX-2), IL-6 and IL-1β, and matrix metallo-peptidase 9 (MMP-9), tissue factor, and osteopontin], and chemokines (MCP-1 and MCP-3).[176–179] These are believed to be additional players in atherogenesis and CVD.[165]

In the intestine, LXRs inhibit cholesterol absorption due to a reduced Niemann-Pick C1 like 1 gene expression,[180] and promote both sterol excretion into feces via apical ABC transporter ABCG5 and ABCG8 stimulation, and increase in HDL-c levels, leading to reduced lipid deposition and atherosclerosis development.[181–183] Conversely, mutations in ABCG5/G8 are associated with β-sitosterolemia, which is characterized by increased sterol absorption and early onset atherosclerosis.[183–185] Hepatic LXR activation induces ABCA1,[151,162] ABCG1,[168] ABCG5, and ABCG8 thus enhancing biliary cholesterol secretion.[186–188] Accordingly, LXRα-null mice accumulate cholesterol esters in the liver and develop hepatomegaly when fed a cholesterol-enriched diet.[189,190] In rodent, but not in human liver, LXRα also induces the expression of cytochrome P450 7 α-hydroxylase (CYP7A1), the rate-limiting enzyme for cholesterol conversion to BAs.[191,192] Additionally, LXRs mediate an enhanced transcription of the E3-ubiquitin Inducible Degrader of the LDLR (Idol) 1, a protein able to induce LDL receptor (LDLR) degradation and that of other LDLR family members, such as the VLDL receptor (VLDLR) and apoE receptor 2 (ApoER2),[193,194] thereby further preventing hepatic lipid accumulation.

LXR and Glucose Homeostasis

The close interplay of lipid and carbohydrate metabolism as well as the identification of LXR as mediators of insulin action in the liver suggested a possible role of LXR in glucose homeostasis. Administration of LXR agonists to obese, insulin-resistant mice inhibited hepatic gluconeogenesis via a decreased expression of PEPCK, fructose-1,6-bisphosphatase (FBP-ase) and glucose-6-phosphatase (G6P), improving both glucose tolerance and insulin sensitivity.[195,196] Nevertheless, both

hepatic gluco-kinase and insulin-dependent glucose transporter (GLUT4) are direct targets of LXRs in adipose tissue, suggesting that an increased glucose uptake and catabolism in this tissue could also contribute to the improved insulin sensitivity in animals receiving LXR agonists.[195,196] LXRβ, and to a lesser extent LXRα, are expressed in both human and murine pancreatic islet cells where they enhance glucose-dependent insulin secretion,[197] an effect that is impaired in LXRβ knockout mice.[198] Collectively, the ability of LXRs to modulate glucose homeostasis results from a downregulation of hepatic gluconeogenesis, an increased insulin-stimulated glucose uptake from skeletal muscle and adipose tissue and an enhanced insulin secretion from the pancreas.

LXR and FA Metabolism

LXRs also regulate hepatic FA metabolism,[151] as they enhance the expression of the master transcriptional regulator of FA and TG biosynthesis, sterol regulatory element binding protein-1c (SREBP-1c),[199] leading to increased levels of FA synthase, ACS, and stearoyl-CoA desaturase 1 (SCD1) and repression of hepatic apoAV.[149,173,200,201] In addition, LXRs increase the expression of the glucose-sensitive transcription factor carbohydrate-responsive element binding protein (ChREBP).[202] By regulating both SREBP-1c and ChREBP, LXRs contribute to the induction of FA synthesis in response to glucose and insulin.[202,203] Accordingly, the LXR-driven fatty liver phenotype is completely abolished in LXR-null mice.[151] The hepatic steatosis developed upon LXR activation, along with the observation that LXR agonist administration to a diabetic mouse model (db/db) resulted in a severe lipogenic disease,[149,204] are the major pitfalls of pharmacological LXR activation. Several strategies have been proposed to avoid both the lipogenic effects and the LXR-mediated induction of CETP (correlating with increased LDL cholesterol levels) in humans but not in mice, which do not express CETP.[205]

The clinical relevance of LXR agonists for treatment of CVD remains to be established, as a direct extrapolation of evidences from the murine model to humans are limited by species-specific differences in lipoprotein metabolism and target genes.[163,206,207] Human studies addressing the cardioprotective potential of LXR activation are needed.

FARNESOID X RECEPTOR

The FXR (NR1H4), cloned in 1995,[208] was initially believed sensing farnesol and juvenile hormone III.[209] In 1999, cholic acid (CA) and chenodeoxycholic acid (CDCA) were shown to activate FXRα.[9,210–212] As the conversion of cholesterol to BAs is the major pathway for elimination of cholesterol,[191] FXR regulates both cholesterol and BA homeostasis. Bile acids are central mediators of the digestion and absorption of lipids, cholesterol, and lipid-soluble vitamins, and prevent the precipitation of cholesterol crystals in bile.[213,214] As 95% of BAs are reabsorbed by enterocytes and recirculate, and increased BA levels can be toxic, a feedback modulation of BA synthesis and absorption is essential for cholesterol and BA depletion.[191] Two FXR genes have been identified: FXRα and FXRβ. FXRβ is believed to be a lanosterol sensor in rodents, rabbits, and dogs, but constitutes a pseudogene in humans and primates.[215] FXRα is expressed in liver, intestine, kidney, adrenal gland, heart, and WAT,[209,216,217] and encodes four FXRα isoforms (FXRα1, FXRα2, FXRα3, and FXRα4), resulting from differential use of two promoters and alternative splicing.[216,217] FXR can bind to and activate or repress different FXR response elements (FXREs), as a classical heterodimer with RXR or as a monomer,[215,218] interacting with different coactivator complexes.[219–224] BAs can activate the transcription of FXR target genes at physiological concentrations acting as hormones.[210–212] Other FXRα ligands include natural agonists (Cafestol), semi-synthetic agonists [6α-ethyl-chenodeoxycholic acid (6α-ECDCA)], synthetic agonists (GW9047, GW4064, fexarine and fexaramine, 4-[(E)-2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propenyl]benzoic acid (TTNPB), AGN29, and AGN31), natural antagonists (guggulsterone), and synthetic antagonists (AGN34). Some of these compounds act as partial antagonists or gene-selective agonists/antagonists, depending on the target gene.[225–232]

FXR and Bile Acid Homeostasis

FXR controls the enterohepatic circulation of BAs by regulating the efflux of BAs from the liver, their intestinal absorption, and hepatic reuptake.[191] FXR regulates genes involved in BA transport, conjugation, and detoxification, as well as in BA (bile salt export pump and multidrug resistance protein 2) and PL (h-MDR3 or m-MDR2) biliary secretion.[162,213,214,233–240] In the liver, FXR represses the transcription of the gene encoding CYP7A1, through a bipartite mechanism involving coordinated actions in the intestine and the liver. In ileum, FXR induces the expression of fibroblast growth factor 15 (FGF15),[241,242] a hormone that plays an overarching role in regulating BA homeostasis. In liver, FXR induces the expression of small heterodimer partner (SHP),[243,244] an orphan NR that binds the CYP7A1 promoter through interactions with another NR, liver receptor homolog-1.[189,243–246]

Induction of both FGF15 and SHP is required for the FXR-mediated repression of CYP7A1 and BA synthesis. FXR also represses the hepatic expression of sterol 12α-hydroxylase (CYP8B1), an enzyme controlling the ratio of the primary BA cholate and β-muricholate in mice.[247]

FXR and Lipoprotein Homeostasis

Both FXR synthetic agonist and CDCA decrease serum total cholesterol levels (due to reduced cholesterol absorption and to a reduction in circulating HDL-c), reduce the expression of apoAI, and increase PLTP-mediated HDL remodeling.[218,248–254] Additionally, CDCA increases the expression of LDLR, leading to decreased LDL-C plasma levels.[255] In turn, the inhibition of CYP7A1 results in increased hepatocyte cholesterol and oxysterols levels, activating LXR with subsequent ubiquitation of LDLR.[149,194] In mice, FXR activation has been related to a net reduction in plasma LDL-C levels,[249,256,257] and these effects can be seen also in humans, but only upon long-term CDCA treatment.[258,259] On the other hand, the FXR antagonist guggulsterone has been shown to reduce total cholesterol and increase HDL serum levels.[227] The ability of BA to impact lipid metabolism has been first described in cholesterol gallstone patients receiving CDCA.[260] CDCA, but not ursodeoxycholic acid, that does not activate FXR, showed the ability of lowering plasma TG in patients both with cholesterol gallstones and hypertriglyceridemia.[253,260–262] In mice, FXR activation decreases plasma FFA and TG levels via SHP-mediated suppression of SREBP-1c and its target genes, and reduces VLDL-TG secretion, along with a increased TG clearance.[247–249,257,263–269] Additionally, FXR can increase FA oxidation and fat utilization via enhanced PPARα expression.[270,271] Conversely, FXR deficiency in rodents is associated with a massive cholesterol and TG deposition in the liver as well as elevated circulating FFA levels,[247,257,268,269] whereas in dyslipidemic patients BA-sequestrating agent treatment results in increased plasma TG and VLDL levels.[252,272,273]

FXR and Glucose Homeostasis

The link between FXR and glucose metabolism emerged when altered BA profile was found to be associated with type 2 diabetes in both humans and animal models,[274–276] suggesting that insulin may affect BA metabolism. At transcriptional level, FXR is positively regulated upon fasting and by glucose administration, although being negatively regulated by insulin and upon refeeding condition.[277,278] In fasted mice, BAs prevent gluconeogenesis, determining hypoglycemia.[277–281] Wild-type, obese, and insulin-resistant mice expressing an activated form of FXR, as well as those treated with FXR agonists (BAs or synthetic agonist), show reduced plasma glucose, hypoinsulinemia and improved insulin sensitivity, paralleled by raised hepatic glycogen levels, and increased hepatic signaling downstream of the insulin receptor.[256,257,268] BA or synthetic FXR agonist administration induces glycogen synthase kinase expression (promoting glycogen synthesis), and decreased gluconeogenesis by repressing PEPCK, G6P, and FBP-ase, via an FXR/SHP-dependent mechanism [involving hepatic nuclear factor 4α (HNF-4α), proliferator-activated receptor-gamma coactivator 1α (PGC-1α), and the Forkhead Transcription Factor].[279,281,282] This effect of BAs in physiological conditions could be mediated additionally by an FXR-independent mechanism, involving HNF-4α.[281] Paradoxically, FXR agonists, although being able to stimulate glucose output from primary hepatocytes, do not alter plasma glucose levels in fed wild-type mice.[280] All FXR effects seen in fasting mice upon FXR agonists are abrogated in FXR knockout mice, showing a lipo-atrophic phenotype, impaired glucose tolerance, and insulin resistance.[256,257,268] Loss of FXR disrupts glucose homeostasis and impairs insulin signaling in muscle and WAT where FXR is completely absent (muscle) or expressed at undetectable level (WAT) in normal animals.[256,268] These results could be related to unbalances in the FGF15/19 modulation of insulin signaling,[283,284] or to other changes in insulin or lipid signaling leading to elevated FFA and TG production, and finally to increased circulating FFA levels.[249,256,257,268] On the other hand, plasma glucose levels have been reported as unchanged,[257] increased[268] or reduced[256] in FXR knockout mice suggesting that other factors still need to be elucidated. These results seem to be discordant, probably due to differences in the genetic backgrounds of the mice or to specific experimental procedures. As FXR modulates the gluconeogenic program during the fasting period, FXR-knockout mice show an altered regulation during the shift from a glucose output regimen to a glucose utilization one.[277] Nevertheless, some of the BA beneficial effects on the modulation of energy expenditure and metabolism can also be driven through an FXR-independent pathway. CA-treated animals are protected from diet-induced obesity through the activation of G-protein-coupled BA receptor 1 (Gpbar1, also known as TGR5) and the induction of UCP-1 in BAT.[285] In this respect, the development of dual FXR and TGR5 ligands may hold promise in the management of metabolic disorders and obesity. Additionally, an increasing body of evidence supports a role for gut microbiota abnormalities in the perpetuation of the main features of MS, such as low grade

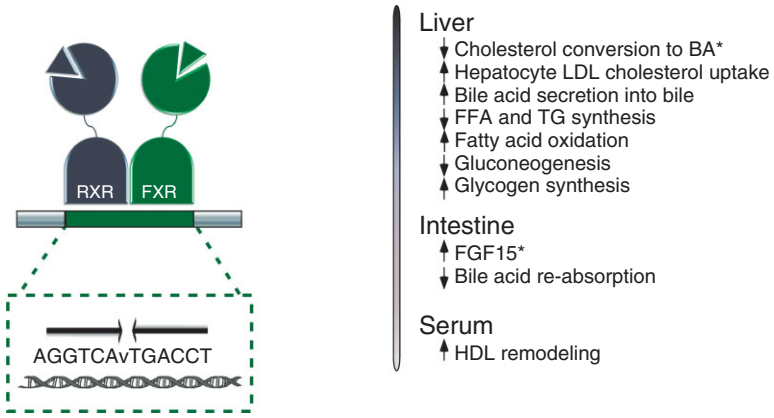


FIGURE 5 | Schematic representation of farnesoid X receptor (FXR) transcription activation (left): as a heterodimer of retinoid X receptors (RXR), FXR binds the FXR response elements (FXREs). The most common FXRE motif consists of inverted repeats (IR) of hexameric sequences AGGTCA, interspaced only by a single nucleotide spacer (IR-1). Effects of FXR activation in tissues involved in metabolic homeostasis (right): FXR plays the key role in bile acid (BA) metabolism, reducing hepatic BA synthesis, increasing BA secretion into bile, and inhibiting intestinal BA reabsorption. FXR additionally modulates cholesterol metabolism [increasing the hepatic uptake of low-density lipoprotein (LDL) cholesterol, reducing the conversion of cholesterol to BA, and promoting high-density lipoprotein (HDL) remodeling], reduces the circulating levels of glucose (promoting glycogen synthesis and inhibiting gluconeogenesis), free fatty acids (FFA), and triglycerides (TG; promoting FA oxidation while inhibiting FFA and TG synthesis). The reduction of the cholesterol conversion to BA can be achieved directly (activating FXR in the liver), or indirectly via an intestinal FXR mediated increase of FGF 15 (see asterisks).

of inflammation and insulin resistance.[286,287] *Inagaki et al.* found that FXR activation increases the expression of target genes exhibiting intestinal antibacterial properties (i.e., iNOS and IL-18), leading to a negative modulation of gut microflora.[288] Further investigation on the FXR-mediated modification of gut microbiota in a setting of MS and insulin resistance may disclose unknown mechanisms by which BA contribute to the whole-body energy homeostasis.

FXR and the Etiology of Atherosclerosis

FXR has also been implicated in the etiology of atherosclerosis not only due to the metabolic reasons we just discussed, but also via the modulation of inflammation.[289,290] Indeed, FXR is expressed in endothelium,[291] vascular smooth muscle cells,[292] and atherosclerotic lesions, but not in the macrophages.[269] The roles at this level are under debate, as BAs administration has been linked to responses that could be both beneficial,[291] or not,[293] for atherosclerosis. However, FXR knockout mice fed high-fat or high-cholesterol diet show a pro-atherogenic metabolic profile, which is not paralleled by an enhanced susceptibility to atherosclerosis.[269,294] As the murine model does not develop spontaneously atherosclerosis, FXR knockout mice have been crossed with genetic models of atherosclerosis. Unfortunately, the effects of loss of FXR in models of atherosclerosis were not disclosed, as these models showed contrasting and case-specific outcomes depending on the genetic background of the mice.[269,294,295]

Although FXR activation shows positive effects on lipid and glucose metabolism (Figure 5), pointing on the possibility of FXR as a possible target for MS treatment, these positive effects are not paralleled by sufficient data on the modulation of the atherosclerotic phenomenon by FXR agonists. The exact role of FXR on the atherogenic process is still poorly understood and should be clarified by further investigations before going into clinical confirmation in humans.

CONCLUSIONS

The rapid escalation of the incidence of obesity and MS calls for a careful appraisal of the effectiveness of the current therapies. Among the several therapeutic approaches used over the last decades, only lifestyle modifications and metformin are considered truly effective in the MS clinical picture. NRs, master transcriptional regulators of the metabolic homeostasis, are ideal targets for the pharmacological modulation of metabolic networks. Fibrates (PPARα agonists) and TZDs (PPARγ agonists) are well-established therapies for hypertriglyceridemia and diabetes, but their long-term benefits are still under discussion. Another promising option for hypertriglyceridemia could be the activation of FXR. In the treatment of MS-associated dyslipidemia, LXR ligands are also promising, as LXR activation is able to lower total cholesterol levels and increase nascent HDL levels, thus inducing RCT. A tissue-selective activation may prevent the

TABLE 1 | Relevance of Nuclear Receptors in Metabolic Syndrome

	PPARα	PPARβ/δ	PPARγ	LXR	FXR
Expression	Liver Kidney Heart Skeletal Muscle Small Intestine BAT Immune cells Endothelium	Heart Skeletal Muscle Adipose tissue Small Intestine Liver Immune cells Ubiquitous	**(γ1)** WAT Immune cells Colon Liver Kidney Skeletal Muscle **(γ2)** WAT BAT	**(α)** Intestine Liver Macrophages WAT Skeletal Muscle Kidney Lung Spleen Adrenal gland **(β)** Ubiquitous	**(α)** Liver Intestine Kidney Adrenal gland Heart WAT Endothelium Vascular SMC **(β)** Pseudogene in humans
Agonists					
Endogenous	Eicosanoids FAs 16:0/18:1-GPC	PGI carba-prostacyclin EPA ARA dihomo-γ-linoleic acid PG -A1, -E2, -D2	Fas Eicosanoids (13-HODE and 9 HODE) Prostanoids (15d-PGJ2)	Oxysterols: 22(R)-, 24(S)-, 27- and 24(S), 25-hydroxy cholesterol	CA CDCA
Approved	Clofibrate Fenofibrate	No	Pioglitazone Rosiglitazone	No	No
-Clinical indications	Hypertriglyceridemia Comb. dyslipidemia	/	Type II Diabetes	/	/
Experimental	LY518674 PPAR γ/α agonists (TDZ18, Harmine, etc.)	GW501516 GW0742 KD-3010 MBX-8025	SPARMs PPARγ/α agonists (TDZ18, Harmine, etc.)	T0 901317 GW3965	Cafestol 6α-ECDCA GW9047 GW4064 Fexarine Fexaramine TTNPB AGN29 AGN31
Main Metabolic Actions					
Weight	=/↓	↓↓	↑	=	=
Fatty Liver	↓	↓	↓	↑	↓
Plasma Glucose	=	↓	↓↓	↓	↓
Insulin Resistance	=	↓↓	↓↓	↓	↓
Plasma FFAs	↓↓	↓	↓	↑	↓
Plasma TGs	↓↓	↓	(Pioglitazone) ↓ (Rosiglitazone) =	↑↑	↓
Plasma LDL-c	=	↓	(Pioglitazone) = (Rosiglitazone) ↑	↓	↓
Plasma HDL-c	↑	↑↑	↑	↑↑	↓
RCT	↑	↑↑	↑	↑↑	↑
Inflammation	↓	↓	↓	↓	↓

The table provides an overview of nuclear receptors' tissue distribution, known agonists, and biological functions relevant for the pathophysiology and treatment of metabolic syndrome.

15d-PGJ2, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2; 16:0/18:1-GPC, palmitoyl-2-oleoyl-sn-glycerol-3-phosphocholine; ARA, arachidonic acid; BAT, brown adipose tissue; CA, cholic acid; CDCA, chenodeoxycholic acid; 6α-ECDCA, 6α-ethyl-chenodeoxycholic acid; EPA, eicosapentaenoic acid; FA, fatty acids; FFA, free FA; FXR, farnesoid X receptor; HODE, hydroxyoctadecanoic acid; LDL, low density lipoprotein; LXR, liver X receptors; HDL, high density lipoprotein; PG, prostaglandin; PGI, prostacyclin; RCT, reverse cholesterol transport; SMC, smooth muscle cells; SPARM, selective PPARγ modulator; TDZ, thiazolidinediones; TG, Triglycerides; TTNPB, 4-[(E)-2-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propenyl]benzoic acid; WAT, white adipose tissue.

List of symbols: '↑' means increase; '=' means no variation; '↓' means reduction.

well-documented LXR-mediated hepatic lipogenesis. Additionally, PPARβ/δ activation has been proposed as a mimetic of physical exercise by optimizing physical endurance, energy expenditure, and RCT. The subsequent beneficial effects on weight control and hepatic fitness could also implicate PPARβ/δ agonists as candidates for the treatment of metabolic disorders. We know that lipid-sensing NR pathways can explain the metabolic scenario that leads to MS and atherosclerosis. In the next few years, according to the NR activities summarized in Table 1, it is extremely important to test the potential of pharmacological strategies to target FXR, LXR, and PPARβ/δ for the treatment of MS.

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REFERENCES

1. Executive summary of the third report of The National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). *JAMA* 2001, 285:2486–2497.
2. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. *Lancet* 2005, 365:1415–1428.
3. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med* 1998, 15:539–553.
4. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith SC Jr, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation* 2005, 112: 2735–2752.
5. Alberti KG, Zimmet P, Shaw J. The metabolic syndrome—a new worldwide definition. *Lancet* 2005, 366:1059–1062.
6. Alberti KG, Zimmet P, Shaw J. Metabolic syndrome—a new world-wide definition. A Consensus Statement from the International Diabetes Federation. *Diabet Med* 2006, 23:469–480.
7. Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schutz G, Umesono K, Blumberg B, Kastner P, Mark M, Chambon P, et al. The nuclear receptor superfamily: the second decade. *Cell* 1995, 83: 835–839.
8. Bookout AL, Jeong Y, Downes M, Yu RT, Evans RM, Mangelsdorf DJ. Anatomical profiling of nuclear receptor expression reveals a hierarchical transcriptional network. *Cell* 2006, 126:789–799.
9. Chawla A, Repa JJ, Evans RM, Mangelsdorf DJ. Nuclear receptors and lipid physiology: opening the X-files. *Science* 2001, 294:1866–1870.
10. Mangelsdorf DJ, Evans RM. The RXR heterodimers and orphan receptors. *Cell* 1995, 83:841–850.
11. Lonard DM, O'Malley BW. Nuclear receptor coregulators: judges, juries, and executioners of cellular regulation. *Mol Cell* 2007, 27:691–700.
12. McKenna NJ, O'Malley BW. Nuclear receptors, coregulators, ligands, and selective receptor modulators: making sense of the patchwork quilt. *Ann N Y Acad Sci* 2001, 949:3–5.
13. McKenna NJ, O'Malley BW. Minireview: nuclear receptor coactivators—an update. *Endocrinology* 2002, 143:2461–2465.
14. Lanz RB, McKenna NJ, Onate SA, Albrecht U, Wong J, Tsai SY, Tsai MJ, O'Malley BW. A steroid receptor coactivator, SRA, functions as an RNA and is present in an SRC-1 complex. *Cell* 1999, 97:17–27.
15. Onate SA, Boonyaratanakornkit V, Spencer TE, Tsai SY, Tsai MJ, Edwards DP, O'Malley BW. The steroid receptor coactivator-1 contains multiple receptor interacting and activation domains that cooperatively enhance the activation function 1 (AF1) and AF2 domains of steroid receptors. *J Biol Chem* 1998, 273: 12101–12108.
16. Tontonoz P, Spiegelman BM. Fat and beyond: the diverse biology of PPARgamma. *Annu Rev Biochem* 2008, 77:289–312.
17. Nolte RT, Wisely GB, Westin S, Cobb JE, Lambert MH, Kurokawa R, Rosenfeld MG, Willson TM, Glass CK, Milburn MV. Ligand binding and co-activator assembly of the peroxisome proliferator-activated receptor-gamma. *Nature* 1998, 395: 137–143.

18. Spiegelman BM. PPAR-gamma: adipogenic regulator and thiazolidinedione receptor. *Diabetes* 1998, 47: 507–514.
19. Forman BM, Tontonoz P, Chen J, Brun RP, Spiegelman BM, Evans RM. 15-Deoxy-δ 12, 14-prostaglandin J2 is a ligand for the adipocyte determination factor PPAR gamma. *Cell* 1995, 83:803–812.
20. Kliewer SA, Forman BM, Blumberg B, Ong ES, Borgmeyer U, Mangelsdorf DJ, Umesono K, Evans RM. Differential expression and activation of a family of murine peroxisome proliferator-activated receptors. *Proc Natl Acad Sci U S A* 1994, 91:7355–7359.
21. Kliewer SA, Umesono K, Noonan DJ, Heyman RA, Evans RM. Convergence of 9-cis retinoic acid and peroxisome proliferator signalling pathways through heterodimer formation of their receptors. *Nature* 1992, 358:771–774.
22. Gottlicher M, Widmark E, Li Q, Gustafsson JA. Fatty acids activate a chimera of the clofibric acid-activated receptor and the glucocorticoid receptor. *Proc Natl Acad Sci U S A* 1992, 89:4653–4657.
23. Sohda T, Momose Y, Meguro K, Kawamatsu Y, Sugiyama Y, Ikeda H. Studies on antidiabetic agents. Synthesis and hypoglycemic activity of 5-[4-(pyridyl alkoxy)benzyl]-2,4-thiazolidinediones. *Arzneimittelforschung* 1990, 40:37–42.
24. Wang YX. PPARs: diverse regulators in energy metabolism and metabolic diseases. *Cell Res* 2010, 20:124–137.
25. Issemann I, Green S. Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. *Nature* 1990, 347:645–650.
26. Fruchart JC. Novel peroxisome proliferator activated receptor-α agonists. *Am J Cardiol* 2007, 100:n41–n46.
27. Tobin JF, Freedman LP. Nuclear receptors as drug targets in metabolic diseases: new approaches to therapy. *Trends Endocrinol Metab* 2006, 17:284–290.
28. Fruchart JC. Peroxisome proliferator-activated receptor-α (PPARα): at the crossroads of obesity, diabetes and cardiovascular disease. *Atherosclerosis* 2009, 205:1–8.
29. Staels B, Dallongeville J, Auwerx J, Schoonjans K, Leitersdorf E, Fruchart JC. Mechanism of action of fibrates on lipid and lipoprotein metabolism. *Circulation* 1998, 98:2088–2093.
30. Shah A, Rader DJ, Millar JS. The effect of PPAR-α agonism on apolipoprotein metabolism in humans. *Atherosclerosis* 2010, 210:35–40.
31. Tugwood JD, Issemann I, Anderson RG, Bundell KR, McPheat WL, Green S. The mouse peroxisome proliferator activated receptor recognizes a response element in the 5' flanking sequence of the rat acyl CoA oxidase gene. *EMBO J* 1992, 11:433–439.
32. Erol E, Kumar LS, Cline GW, Shulman GI, Kelly DP, Binas B. Liver fatty acid binding protein is required for high rates of hepatic fatty acid oxidation but not for the action of PPARα in fasting mice. *FASEB J* 2004, 18:347–349.
33. Hsu MH, Savas U, Griffin KJ, Johnson EF. Identification of peroxisome proliferator-responsive human genes by elevated expression of the peroxisome proliferator-activated receptor α in HepG2 cells. *J Biol Chem* 2001, 276:27950–27958.
34. Wolfrum C, Borrmann CM, Borchers T, Spener F. Fatty acids and hypolipidemic drugs regulate peroxisome proliferator-activated receptors α- and γ-mediated gene expression via liver fatty acid binding protein: a signaling path to the nucleus. *Proc Natl Acad Sci U S A* 2001, 98:2323–2328.
35. Motojima K, Passilly P, Peters JM, Gonzalez FJ, Latruffe N. Expression of putative fatty acid transporter genes are regulated by peroxisome proliferator-activated receptor α and gamma activators in a tissue- and inducer-specific manner. *J Biol Chem* 1998, 273:16710–16714.
36. Martin G, Schoonjans K, Lefebvre AM, Staels B, Auwerx J. Coordinate regulation of the expression of the fatty acid transport protein and acyl-CoA synthetase genes by PPARα and PPARgamma activators. *J Biol Chem* 1997, 272:28210–28217.
37. Costet P, Legendre C, More J, Edgar A, Galtier P, Pineau T. Peroxisome proliferator-activated receptor α-isoform deficiency leads to progressive dyslipidemia with sexually dimorphic obesity and steatosis. *J Biol Chem* 1998, 273:29577–29585.
38. Leone TC, Weinheimer CJ, Kelly DP. A critical role for the peroxisome proliferator-activated receptor α (PPARα) in the cellular fasting response: the PPARα-null mouse as a model of fatty acid oxidation disorders. *Proc Natl Acad Sci U S A* 1999, 96:7473–7478.
39. Peters JM, Hennuyer N, Staels B, Fruchart JC, Fievet C, Gonzalez FJ, Auwerx J. Alterations in lipoprotein metabolism in peroxisome proliferator-activated receptor α-deficient mice. *J Biol Chem* 1997, 272:27307–27312.
40. Kourimate S, Le MC, Langhi C, Jarnoux AL, Ouguerram K, Zair Y, Nguyen P, Krempf M, Cariou B, Costet P. Dual mechanisms for the fibrate-mediated repression of proprotein convertase subtilisin/kexin type 9. *J Biol Chem* 2008, 283:9666–9673.
41. Lambert G, Jarnoux AL, Pineau T, Pape O, Chetiveaux M, Laboisse C, Krempf M, Costet P. Fasting induces hyperlipidemia in mice overexpressing proprotein convertase subtilisin kexin type 9: lack of modulation of very-low-density lipoprotein hepatic output by the low-density lipoprotein receptor. *Endocrinology* 2006, 147:4985–4995.
42. Vu-Dac N, Gervois P, Jakel H, Nowak M, Bauge E, Dehondt H, Staels B, Pennacchio LA, Rubin EM,

Fruchart-Najib J, et al. Apolipoprotein A5, a crucial determinant of plasma triglyceride levels, is highly responsive to peroxisome proliferator-activated receptor α activators. *J Biol Chem* 2003, 278: 17982–17985.

43. Lavrentiadou SN, Hadzopoulou-Cladaras M, Kardassis D, Zannis VI. Binding specificity and modulation of the human ApoCIII promoter activity by heterodimers of ligand-dependent nuclear receptors. *Biochemistry* 1999, 38:964–975.
44. Schoonjans K, Peinado-Onsurbe J, Lefebvre AM, Heyman RA, Briggs M, Deeb S, Staels B, Auwerx J. PPARα and PPARgamma activators direct a distinct tissue-specific transcriptional response via a PPRE in the lipoprotein lipase gene. *EMBO J* 1996, 15:5336–5348.
45. Auwerx J, Schoonjans K, Fruchart JC, Staels B. Regulation of triglyceride metabolism by PPARs: fibrates and thiazolidinediones have distinct effects. *J Atheroscler Thromb* 1996, 3:81–89.
46. Chapman MJ. Fibrates in 2003: therapeutic action in atherogenic dyslipidaemia and future perspectives. *Atherosclerosis* 2003, 171:1–13.
47. Hiukka A, Fruchart-Najib J, Leinonen E, Hilden H, Fruchart JC, Taskinen MR. Alterations of lipids and apolipoprotein CIII in very low density lipoprotein subspecies in type 2 diabetes. *Diabetologia* 2005, 48:1207–1215.
48. Steiner G. Fenofibrate for cardiovascular disease prevention in metabolic syndrome and type 2 diabetes mellitus. *Am J Cardiol* 2008, 102:28L–33L.
49. Duez H, Lefebvre B, Poulain P, Torra IP, Percevault F, Luc G, Peters JM, Gonzalez FJ, Gineste R, Helleboid S, et al. Regulation of human apoA-I by gemfibrozil and fenofibrate through selective peroxisome proliferator-activated receptor α modulation. *Arterioscler Thromb Vasc Biol* 2005, 25:585–591.
50. Bilz S, Wagner S, Schmitz M, Bedynek A, Keller U, Demant T. Effects of atorvastatin versus fenofibrate on apoB-100 and apoA-I kinetics in mixed hyperlipidemia. *J Lipid Res* 2004, 45:174–185.
51. Tenenbaum A, Motro M, Fisman EZ, Tanne D, Boyko V, Behar S. Bezafibrate for the secondary prevention of myocardial infarction in patients with metabolic syndrome. *Arch Intern Med* 2005, 165: 1154–1160.
52. Secondary prevention by raising HDL cholesterol and reducing triglycerides in patients with coronary artery disease: the Bezafibrate Infarction Prevention (BIP) study. *Circulation* 2000, 102:21–27.
53. Keech A, Simes RJ, Barter P, Best J, Scott R, Taskinen MR, Forder P, Pillai A, Davis T, Glasziou P, et al. Effects of long-term fenofibrate therapy on cardiovascular events in 9795 people with type 2 diabetes mellitus (the FIELD study): randomised controlled trial. *Lancet* 2005, 366:1849–1861.
54. Robins SJ, Collins D, Wittes JT, Papademetriou V, Deedwania PC, Schaefer EJ, McNamara JR, Kashyap ML, Hershman JM, Wexler LF, et al. Relation of gemfibrozil treatment and lipid levels with major coronary events: VA-HIT: a randomized controlled trial. *JAMA* 2001, 285:1585–1591.
55. Nissen SE, Nicholls SJ, Wolski K, Howey DC, McErlean E, Wang MD, Gomez EV, Russo JM. Effects of a potent and selective PPAR-α agonist in patients with atherogenic dyslipidemia or hypercholesterolemia: two randomized controlled trials. *JAMA* 2007, 297:1362–1373.
56. Millar JS, Duffy D, Gadi R, Bloedon LT, Dunbar RL, Wolfe ML, Movva R, Shah A, Fuki IV, McCoy M, et al. Potent and selective PPAR-α agonist LY518674 upregulates both ApoA-I production and catabolism in human subjects with the metabolic syndrome. *Arterioscler Thromb Vasc Biol* 2009, 29:140–146.
57. Chakravarthy MV, Lodhi IJ, Yin L, Malapaka RR, Xu HE, Turk J, Semenkovich CF. Identification of a physiologically relevant endogenous ligand for PPARα in liver. *Cell* 2009, 138:476–488.
58. Sacks FM. After the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study: implications for fenofibrate. *Am J Cardiol* 2008, 102: 34L–40L.
59. Gonzalez FJ, Shah YM. PPARα: mechanism of species differences and hepatocarcinogenesis of peroxisome proliferators. *Toxicology* 2008, 246:2–8.
60. Palmer CN, Hsu MH, Griffin KJ, Raucy JL, Johnson EF. Peroxisome proliferator activated receptor-α expression in human liver. *Mol Pharmacol* 1998, 53:14–22.
61. Boyvat F, Harman A, Ozyer U, Aytekin C, Arat Z. Percutaneous sonographic guidance for TIPS in Budd-Chiari syndrome: direct simultaneous puncture of the portal vein and inferior vena cava. *AJR Am J Roentgenol* 2008, 191:560–564.
62. Kersten S, Seydoux J, Peters JM, Gonzalez FJ, Desvergne B, Wahli W. Peroxisome proliferator-activated receptor α mediates the adaptive response to fasting. *J Clin Invest* 1999, 103:1489–1498.
63. Patsouris D, Mandard S, Voshol PJ, Escher P, Tan NS, Havekes LM, Koenig W, Marz W, Tafuri S, Wahli W, et al. PPARα governs glycerol metabolism. *J Clin Invest* 2004, 114:94–103.
64. Gonzalez FJ, Peters JM, Cattley RC. Mechanism of action of the nongenotoxic peroxisome proliferators: role of the peroxisome proliferator-activator receptor α. *J Natl Cancer Inst* 1998, 90:1702–1709.
65. Schmidt A, Endo N, Rutledge SJ, Vogel R, Shinar D, Rodan GA. Identification of a new member of the steroid hormone receptor superfamily that is activated by a peroxisome proliferator and fatty acids. *Mol Endocrinol* 1992, 6:1634–1641.

66. Dreyer C, Krey G, Keller H, Givel F, Helftenbein G, Wahli W. Control of the peroxisomal β-oxidation pathway by a novel family of nuclear hormone receptors. *Cell* 1992, 68:879–887.

67. Bedu E, Wahli W, Desvergne B. Peroxisome proliferator-activated receptor β/δ as a therapeutic target for metabolic diseases. *Expert Opin Ther Targets* 2005, 9:861–873.

68. Barish GD, Narkar VA, Evans RM. PPAR δ: a dagger in the heart of the metabolic syndrome. *J Clin Invest* 2006, 116:590–597.

69. Krogsdam AM, Nielsen CA, Neve S, Holst D, Helledie T, Thomsen B, Bendixen C, Mandrup S, Kristiansen K. Nuclear receptor corepressor-dependent repression of peroxisome-proliferator-activated receptor δ-mediated transactivation. *Biochem J* 2002, 363(Pt 1):157–165.

70. Xu HE, Lambert MH, Montana VG, Parks DJ, Blanchard SG, Brown PJ, Sternbach DD, Lehmann JM, Wisely GB, Willson TM, et al. Molecular recognition of fatty acids by peroxisome proliferator-activated receptors. *Mol Cell* 1999, 3:397–403.

71. Grimaldi PA. Regulatory functions of PPARβ in metabolism: implications for the treatment of metabolic syndrome. *Biochim Biophys Acta* 2007, 1771: 983–990.

72. Berger J, Leibowitz MD, Doebber TW, Elbrecht A, Zhang B, Zhou G, Biswas C, Cullinan CA, Hayes NS, Li Y, et al. Novel peroxisome proliferator-activated receptor (PPAR) gamma and PPARδ ligands produce distinct biological effects. *J Biol Chem* 1999, 274:6718–6725.

73. Skogsberg J, Kannisto K, Cassel TN, Hamsten A, Eriksson P, Ehrenborg E. Evidence that peroxisome proliferator-activated receptor δ influences cholesterol metabolism in men. *Arterioscler Thromb Vasc Biol* 2003, 23:637–643.

74. Robitaille J, Gaudet D, Perusse L, Vohl MC. Features of the metabolic syndrome are modulated by an interaction between the peroxisome proliferator-activated receptor-δ -87T>C polymorphism and dietary fat in French-Canadians. *Int J Obes (Lond)* 2007, 31: 411–417.

75. Shin HD, Park BL, Kim LH, Jung HS, Cho YM, Moon MK, Park YJ, Lee HK, Park KS. Genetic polymorphisms in peroxisome proliferator-activated receptor δ associated with obesity. *Diabetes* 2004, 53:847–851.

76. Wang YX, Lee CH, Tiep S, Yu RT, Ham J, Kang H, Evans RM. Peroxisome-proliferator-activated receptor δ activates fat metabolism to prevent obesity. *Cell* 2003, 113:159–170.

77. Kang K, Hatano B, Lee CH. PPAR δ agonists and metabolic diseases. *Curr Atheroscler Rep* 2007, 9: 72–77.

78. Oliver WR Jr. Shenk JL, Snaith MR, Russell CS, Plunket KD, Bodkin NL, Lewis MC, Winegar DA, Sznaidman ML, Lambert MH, et al. A selective peroxisome proliferator-activated receptor δ agonist promotes reverse cholesterol transport. *Proc Natl Acad Sci U S A* 2001, 98:5306–5311.

79. Kriketos AD, Pan DA, Lillioja S, Cooney GJ, Baur LA, Milner MR, Sutton JR, Jenkins AB, Bogardus C, Storlien LH. Interrelationships between muscle morphology, insulin action, and adiposity. *Am J Physiol* 1996, 270(6 Pt 2):R1332–R1339.

80. Hickey MS, Carey JO, Azevedo JL, Houmard JA, Pories WJ, Israel RG, Dohm GL. Skeletal muscle fiber composition is related to adiposity and in vitro glucose transport rate in humans. *Am J Physiol* 1995, 268(3 Pt 1):E453–E457.

81. Wang YX, Zhang CL, Yu RT, Cho HK, Nelson MC, Bayuga-Ocampo CR, Ham J, Kang H, Evans RM. Regulation of muscle fiber type and running endurance by PPARδ. *PLoS Biol* 2004, 2:e294.

82. Braissant O, Foufelle F, Scotto C, Dauca M, Wahli W. Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-α, -β, and -gamma in the adult rat. *Endocrinology* 1996, 137:354–366.

83. Schuler M, Ali F, Chambon C, Duteil D, Bornert JM, Tardivel A, Desvergne B, Wahli W, Chambon P, Metzger D. PGC1α expression is controlled in skeletal muscles by PPARβ, whose ablation results in fiber-type switching, obesity, and type 2 diabetes. *Cell Metab* 2006, 4:407–414.

84. Narkar VA, Downes M, Yu RT, Embler E, Wang YX, Banayo E, Mihaylova MM, Nelson MC, Zou Y, Juguilon H, et al. AMPK and PPARδ agonists are exercise mimetics. *Cell* 2008, 134:405–415.

85. Takata Y, Liu J, Yin F, Collins AR, Lyon CJ, Lee CH, Atkins AR, Downes M, Barish GD, Evans RM, et al. PPARδ-mediated antiinflammatory mechanisms inhibit angiotensin II-accelerated atherosclerosis. *Proc Natl Acad Sci U S A* 2008, 105:4277–4282.

86. Tanaka T, Yamamoto J, Iwasaki S, Asaba H, Hamura H, Ikeda Y, Watanabe M, Magoori K, Ioka RX, Tachibana K, et al. Activation of peroxisome proliferator-activated receptor δ induces fatty acid β-oxidation in skeletal muscle and attenuates metabolic syndrome. *Proc Natl Acad Sci U S A* 2003, 100:15924–15929.

87. Leibowitz MD, Fievet C, Hennuyer N, Peinado-Onsurbe J, Duez H, Bergera J, Cullinan CA, Sparrow CP, Baffic J, Berger GD, et al. Activation of PPARδ alters lipid metabolism in db/db mice. *FEBS Lett* 2000, 473:333–336.

88. Wallace JM, Schwarz M, Coward P, Houze J, Sawyer JK, Kelley KL, Chai A, Rudel LL. Effects of peroxisome proliferator-activated receptor α/δ agonists on HDL-cholesterol in vervet monkeys. *J Lipid Res* 2005, 46:1009–1016.

89. Vu-Dac N, Chopin-Delannoy S, Gervois P, Bonnelye E, Martin G, Fruchart JC, Laudet V, Staels B. The nuclear receptors peroxisome proliferator-activated receptor α and Rev-erbα mediate the species-specific regulation of apolipoprotein A-I expression by fibrates. *J Biol Chem* 1998, 273:25713–25720.

90. Riserus U, Sprecher D, Johnson T, Olson E, Hirschberg S, Liu A, Fang Z, Hegde P, Richards D, Sarov-Blat L, et al. Activation of peroxisome proliferator-activated receptor (PPAR)δ promotes reversal of multiple metabolic abnormalities, reduces oxidative stress, and increases fatty acid oxidation in moderately obese men. *Diabetes* 2008, 57:332–339.

91. Sprecher DL, Massien C, Pearce G, Billin AN, Perlstein I, Willson TM, Hassall DG, Ancellin N, Patterson SD, Lobe DC, et al. Triglyceride:high-density lipoprotein cholesterol effects in healthy subjects administered a peroxisome proliferator activated receptor δ agonist. *Arterioscler Thromb Vasc Biol* 2007, 27:359–365.

92. Billin AN. PPAR-β/δ agonists for Type 2 diabetes and dyslipidemia: an adopted orphan still looking for a home. *Expert Opin Investig Drugs* 2008, 17:1465–1471.

93. Tontonoz P, Hu E, Graves RA, Budavari AI, Spiegelman BM. mPPAR gamma 2: tissue-specific regulator of an adipocyte enhancer. *Genes Dev* 1994, 8: 1224–1234.

94. Lehmann JM, Moore LB, Smith-Oliver TA, Wilkison WO, Willson TM, Kliewer SA. An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor gamma (PPAR gamma). *J Biol Chem* 1995, 270:12953–12956.

95. Kliewer SA, Lenhard JM, Willson TM, Patel I, Morris DC, Lehmann JM. A prostaglandin J2 metabolite binds peroxisome proliferator-activated receptor gamma and promotes adipocyte differentiation. *Cell* 1995, 83:813–819.

96. Krey G, Braissant O, L'Horset F, Kalkhoven E, Perroud M, Parker MG, Wahli W. Fatty acids, eicosanoids, and hypolipidemic agents identified as ligands of peroxisome proliferator-activated receptors by coactivator-dependent receptor ligand assay. *Mol Endocrinol* 1997, 11:779–791.

97. Farmer SR. Transcriptional control of adipocyte formation. *Cell Metab* 2006, 4:263–273.

98. Rosen ED, MacDougald OA. Adipocyte differentiation from the inside out. *Nat Rev Mol Cell Biol* 2006, 7:885–896.

99. Kubota N, Terauchi Y, Miki H, Tamemoto H, Yamauchi T, Komeda K, Satoh S, Nakano R, Ishii C, Sugiyama T, et al. PPAR gamma mediates high-fat diet-induced adipocyte hypertrophy and insulin resistance. *Mol Cell* 1999, 4:597–609.

100. Barak Y, Nelson MC, Ong ES, Jones YZ, Ruiz-Lozano P, Chien KR, Koder A, Evans RM. PPAR gamma is required for placental, cardiac, and adipose tissue development. *Mol Cell* 1999, 4:585–595.

101. Agostini M, Schoenmakers E, Mitchell C, Szatmari I, Savage D, Smith A, Rajanayagam O, Semple R, Luan J, Bath L, et al. Non-DNA binding, dominant-negative, human PPARgamma mutations cause lipodystrophic insulin resistance. *Cell Metab* 2006, 4:303–311.

102. Barroso I, Gurnell M, Crowley VE, Agostini M, Schwabe JW, Soos MA, Maslen GL, Williams TD, Lewis H, Schafer AJ, et al. Dominant negative mutations in human PPARgamma associated with severe insulin resistance, diabetes mellitus and hypertension. *Nature* 1999, 402:880–883.

103. Semple RK, Chatterjee VK, O'Rahilly S. PPAR gamma and human metabolic disease. *J Clin Invest* 2006, 116:581–589.

104. Jeninga EH, Gurnell M, Kalkhoven E. Functional implications of genetic variation in human PPARgamma. *Trends Endocrinol Metab* 2009, 20:380–387.

105. Ludtke A, Buettner J, Schmidt HH, Worman HJ. New PPARG mutation leads to lipodystrophy and loss of protein function that is partially restored by a synthetic ligand. *J Med Genet* 2007, 44:e88.

106. Agarwal AK, Garg A. A novel heterozygous mutation in peroxisome proliferator-activated receptor-gamma gene in a patient with familial partial lipodystrophy. *J Clin Endocrinol Metab* 2002, 87:408–411.

107. Hara K, Okada T, Tobe K, Yasuda K, Mori Y, Kadowaki H, Hagura R, Akanuma Y, Kimura S, Ito C, et al. The Pro12Ala polymorphism in PPAR gamma2 may confer resistance to type 2 diabetes. *Biochem Biophys Res Commun* 2000, 271:212–216.

108. Shulman AI, Mangelsdorf DJ. Retinoid x receptor heterodimers in the metabolic syndrome. *N Engl J Med* 2005, 353:604–615.

109. Chao L, Marcus-Samuels B, Mason MM, Moitra J, Vinson C, Arioglu E, Gavrilova O, Reitman ML. Adipose tissue is required for the antidiabetic, but not for the hypolipidemic, effect of thiazolidinediones. *J Clin Invest* 2000, 106: 1221–1228.

110. He W, Barak Y, Hevener A, Olson P, Liao D, Le J, Nelson M, Ong E, Olefsky JM, Evans RM. Adipose-specific peroxisome proliferator-activated receptor gamma knockout causes insulin resistance in fat and liver but not in muscle. *Proc Natl Acad Sci U S A* 2003, 100:15712–15717.

111. Jones JR, Barrick C, Kim KA, Lindner J, Blondeau B, Fujimoto Y, Shiota M, Kesterson RA, Kahn BB, Magnuson MA. Deletion of PPARgamma in adipose tissues of mice protects against high fat diet-induced obesity and insulin resistance. *Proc Natl Acad Sci U S A* 2005, 102:6207–6212.

112. Bermudez V, Finol F, Parra N, Parra M, Perez A, Penaranda L, Vilchez D, Rojas J, Arraiz N, Velasco M.

PPAR-gamma agonists and their role in type 2 diabetes mellitus management. *Am J Ther* 2010, 17:274–283.

113. Puri V, Ranjit S, Konda S, Nicoloro SM, Straubhaar J, Chawla A, Chouinard M, Lin C, Burkart A, Corvera S, et al. Cidea is associated with lipid droplets and insulin sensitivity in humans. *Proc Natl Acad Sci U S A* 2008, 105:7833–7838.
114. Matsusue K, Kusakabe T, Noguchi T, Takiguchi S, Suzuki T, Yamano S, Gonzalez FJ. Hepatic steatosis in leptin-deficient mice is promoted by the PPARgamma target gene Fsp27. *Cell Metab* 2008, 7:302–311.
115. Schoonjans K, Staels B, Auwerx J. The peroxisome proliferator activated receptors (PPARS) and their effects on lipid metabolism and adipocyte differentiation. *Biochim Biophys Acta* 1996, 1302:93–109.
116. Tontonoz P, Hu E, Devine J, Beale EG, Spiegelman BM. PPAR gamma 2 regulates adipose expression of the phosphoenolpyruvate carboxykinase gene. *Mol Cell Biol* 1995, 15:351–357.
117. Anghel SI, Wahli W. Fat poetry: a kingdom for PPAR gamma. *Cell Res* 2007, 17:486–511.
118. Mori Y, Murakawa Y, Okada K, Horikoshi H, Yokoyama J, Tajima N, Ikeda Y. Effect of troglitazone on body fat distribution in type 2 diabetic patients. *Diabetes Care* 1999, 22:908–912.
119. Kelly IE, Han TS, Walsh K, Lean ME. Effects of a thiazolidinedione compound on body fat and fat distribution of patients with type 2 diabetes. *Diabetes Care* 1999, 22:288–293.
120. Cheng AY, Fantus IG. Oral antihyperglycemic therapy for type 2 diabetes mellitus. *CMAJ* 2005, 172: 213–226.
121. Maeda N, Takahashi M, Funahashi T, Kihara S, Nishizawa H, Kishida K, Nagaretani H, Matsuda M, Komuro R, Ouchi N, et al. PPARgamma ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein. *Diabetes* 2001, 50:2094–2099.
122. Schenk S, Saberi M, Olefsky JM. Insulin sensitivity: modulation by nutrients and inflammation. *J Clin Invest* 2008, 118:2992–3002.
123. Collino M, Aragno M, Castiglia S, Miglio G, Tomasinelli C, Boccuzzi G, Thiemermann C, Fantozzi R. Pioglitazone improves lipid and insulin levels in overweight rats on a high cholesterol and fructose diet by decreasing hepatic inflammation. *Br J Pharmacol* 2010.
124. Xu ZK, Chen NG, Ma CY, Meng ZX, Sun YJ, Han X. Role of peroxisome proliferator-activated receptor gamma in glucose-induced insulin secretion *Acta Biochim Biophys Sin (Shanghai)* 2006, 38:1–7.
125. Im SS, Kim JW, Kim TH, Song XL, Kim SY, Kim HI, Ahn YH. Identification and characterization of peroxisome proliferator response element in the mouse GLUT2 promoter. *Exp Mol Med* 2005, 37:101–110.
126. Kim HI, Kim JW, Kim SH, Cha JY, Kim KS, Ahn YH. Identification and functional characterization of the peroxisomal proliferator response element in rat GLUT2 promoter. *Diabetes* 2000, 49:1517–1524.
127. Hotamisligil GS. Inflammation and metabolic disorders. *Nature* 2006, 444:860–867.
128. Lumeng CN, Bodzin JL, Saltiel AR. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. *J Clin Invest* 2007, 117:175–184.
129. Vats D, Mukundan L, Odegaard JI, Zhang L, Smith KL, Morel CR, Wagner RA, Greaves DR, Murray PJ, Chawla A. Oxidative metabolism and PGC-1β attenuate macrophage-mediated inflammation. *Cell Metab* 2006, 4:13–24.
130. Huang JT, Welch JS, Ricote M, Binder CJ, Willson TM, Kelly C, Witztum JL, Funk CD, Conrad D, Glass CK. Interleukin-4-dependent production of PPAR-gamma ligands in macrophages by 12/15-lipoxygenase. *Nature* 1999, 400:378–382.
131. Odegaard JI, Ricardo-Gonzalez RR, Goforth MH, Morel CR, Subramanian V, Mukundan L, Red EA, Vats D, Brombacher F, Ferrante AW, et al. Macrophage-specific PPARgamma controls alternative activation and improves insulin resistance. *Nature* 2007, 447:1116–1120.
132. Stienstra R, Duval C, Keshtkar S, van der Laak J, Kersten S, Muller M. Peroxisome proliferator-activated receptor gamma activation promotes infiltration of alternatively activated macrophages into adipose tissue. *J Biol Chem* 2008, 283:22620–22627.
133. Hevener AL, Olefsky JM, Reichart D, Nguyen MT, Bandyopadyhay G, Leung HY, Watt MJ, Benner C, Febbraio MA, Nguyen AK, et al. Macrophage PPAR gamma is required for normal skeletal muscle and hepatic insulin sensitivity and full antidiabetic effects of thiazolidinediones. *J Clin Invest* 2007, 117: 1658–1669.
134. Pascual G, Ricote M, Hevener AL. Macrophage peroxisome proliferator activated receptor gamma as a therapeutic target to combat Type 2 diabetes. *Expert Opin Ther Targets* 2007, 11:1503–1520.
135. Di Gregorio GB, Yao-Borengasser A, Rasouli N, Varma V, Lu T, Miles LM, Ranganathan G, Peterson CA, McGehee RE, Kern PA. Expression of CD68 and macrophage chemoattractant protein-1 genes in human adipose and muscle tissues: association with cytokine expression, insulin resistance, and reduction by pioglitazone. *Diabetes* 2005, 54:2305–2313.
136. Hevener AL, He W, Barak Y, Le J, Bandyopadhyay G, Olson P, Wilkes J, Evans RM, Olefsky J. Muscle-specific Pparg deletion causes insulin resistance. *Nat Med* 2003, 9:1491–1497.
137. Norris AW, Chen L, Fisher SJ, Szanto I, Ristow M, Jozsi AC, Hirshman MF, Rosen ED, Goodyear LJ, Gonzalez FJ, et al. Muscle-specific PPARgamma-deficient mice develop increased adiposity and insulin

resistance but respond to thiazolidinediones. *J Clin Invest* 2003, 112:608–618.

138. Koutnikova H, Cock TA, Watanabe M, Houten SM, Champy MF, Dierich A, Auwerx J. Compensation by the muscle limits the metabolic consequences of lipodystrophy in PPAR gamma hypomorphic mice. *Proc Natl Acad Sci U S A* 2003, 100:14457–14462.
139. Lefterova MI, Steger DJ, Zhuo D, Qatanani M, Mullican SE, Tuteja G, Manduchi E, Grant GR, Lazar MA. Cell-specific determinants of peroxisome proliferator-activated receptor gamma function in adipocytes and macrophages. *Mol Cell Biol* 2010, 30:2078–2089.
140. Sugii S, Olson P, Sears DD, Saberi M, Atkins AR, Barish GD, Hong SH, Castro GL, Yin YQ, Nelson MC, et al. PPARgamma activation in adipocytes is sufficient for systemic insulin sensitization. *Proc Natl Acad Sci U S A* 2009, 106:22504–22509.
141. Choi JH, Banks AS, Estall JL, Kajimura S, Bostrom P, Laznik D, Ruas JL, Chalmers MJ, Kamenecka TM, Bluher M, et al. Anti-diabetic drugs inhibit obesity-linked phosphorylation of PPARgamma by Cdk5. *Nature* 2010, 466:451–456.
142. Knouff C, Auwerx J. Peroxisome proliferator-activated receptor-gamma calls for activation in moderation: lessons from genetics and pharmacology. *Endocr Rev* 2004, 25:899–918.
143. Schupp M, Clemenz M, Gineste R, Witt H, Janke J, Helleboid S, Hennuyer N, Ruiz P, Unger T, Staels B, et al. Molecular characterization of new selective peroxisome proliferator-activated receptor gamma modulators with angiotensin receptor blocking activity. *Diabetes* 2005, 54:3442–3452.
144. Feldman PL, Lambert MH, Henke BR. PPAR modulators and PPAR pan agonists for metabolic diseases: the next generation of drugs targeting peroxisome proliferator-activated receptors? *Curr Top Med Chem* 2008, 8:728–749.
145. Waki H, Park KW, Mitro N, Pei L, Damoiseaux R, Wilpitz DC, Reue K, Saez E, Tontonoz P. The small molecule harmine is an antidiabetic cell-type-specific regulator of PPARgamma expression. *Cell Metab* 2007, 5: 357–370.
146. Apfel R, Benbrook D, Lernhardt E, Ortiz MA, Salbert G, Pfahl M. A novel orphan receptor specific for a subset of thyroid hormone-responsive elements and its interaction with the retinoid/thyroid hormone receptor subfamily. *Mol Cell Biol* 1994, 14:7025–7035.
147. Willy PJ, Umesono K, Ong ES, Evans RM, Heyman RA, Mangelsdorf DJ. LXR, a nuclear receptor that defines a distinct retinoid response pathway. *Genes Dev* 1995, 9:1033–1045.
148. Janowski BA, Willy PJ, Devi TR, Falck JR, Mangelsdorf DJ. An oxysterol signalling pathway mediated by the nuclear receptor LXR α. *Nature* 1996, 383: 728–731.
149. Tontonoz P, Mangelsdorf DJ. Liver X receptor signaling pathways in cardiovascular disease. *Mol Endocrinol* 2003, 17:985–993.
150. Laffitte BA, Repa JJ, Joseph SB, Wilpitz DC, Kast HR, Mangelsdorf DJ, Tontonoz P. LXRs control lipid-inducible expression of the apolipoprotein E gene in macrophages and adipocytes. *Proc Natl Acad Sci U S A* 2001, 98:507–512.
151. Repa JJ, Liang G, Ou J, Bashmakov Y, Lobaccaro JM, Shimomura I, Shan B, Brown MS, Goldstein JL, Mangelsdorf DJ. Regulation of mouse sterol regulatory element-binding protein-1c gene (SREBP-1c) by oxysterol receptors, LXRα and LXRβ. *Genes Dev* 2000, 14:2819–2830.
152. Zhang Y, Repa JJ, Gauthier K, Mangelsdorf DJ. Regulation of lipoprotein lipase by the oxysterol receptors, LXRα and LXRβ. *J Biol Chem* 2001, 276:43018–43024.
153. Korach-Andre M, Parini P, Larsson L, Arner A, Steffensen KR, Gustafsson JA. Separate and overlapping metabolic functions of LXR{α} and LXR{β} in C57Bl/6 female mice. *Am J Physiol Endocrinol Metab* 2010, 298:E167–E178.
154. Willy PJ, Mangelsdorf DJ. Unique requirements for retinoid-dependent transcriptional activation by the orphan receptor LXR. *Genes Dev* 1997, 11:289–298.
155. Chen JD, Evans RM. A transcriptional co-repressor that interacts with nuclear hormone receptors. *Nature* 1995, 377:454–457.
156. Horlein AJ, Naar AM, Heinzel T, Torchia J, Gloss B, Kurokawa R, Ryan A, Kamei Y, Soderstrom M, Glass CK, et al. Ligand-independent repression by the thyroid hormone receptor mediated by a nuclear receptor co-repressor. *Nature* 1995, 377:397–404.
157. Glass CK, Rosenfeld MG. The coregulator exchange in transcriptional functions of nuclear receptors. *Genes Dev* 2000, 14:121–141.
158. Zelcer N, Tontonoz P. Liver X receptors as integrators of metabolic and inflammatory signaling. *J Clin Invest* 2006, 116:607–614.
159. Aravindhan K, Webb CL, Jaye M, Ghosh A, Willette RN, DiNardo NJ, Jucker BM. Assessing the effects of LXR agonists on cellular cholesterol handling: a stable isotope tracer study. *J Lipid Res* 2006, 47:1250–1260.
160. Laffitte BA, Joseph SB, Walczak R, Pei L, Wilpitz DC, Collins JL, Tontonoz P. Autoregulation of the human liver X receptor α promoter. *Mol Cell Biol* 2001, 21:7558–7568.
161. Whitney KD, Watson MA, Goodwin B, Galardi CM, Maglich JM, Wilson JG, Willson TM, Collins JL, Kliewer SA. Liver X receptor (LXR) regulation of the LXRα gene in human macrophages. *J Biol Chem* 2001, 276:43509–43515.
162. Repa JJ, Turley SD, Lobaccaro JA, Medina J, Li L, Lustig K, Shan B, Heyman RA, Dietschy JM, Mangelsdorf DJ. Regulation of absorption and ABC1-mediated

efflux of cholesterol by RXR heterodimers. *Science* 2000, 289:1524–1529.

163. Tangirala RK, Bischoff ED, Joseph SB, Wagner BL, Walczak R, Laffitte BA, Daige CL, Thomas D, Heyman RA, Mangelsdorf DJ, et al. Identification of macrophage liver X receptors as inhibitors of atherosclerosis. *Proc Natl Acad Sci U S A* 2002, 99:11896–11901.
164. Schuster GU, Parini P, Wang L, Alberti S, Steffensen KR, Hansson GK, Angelin B, Gustafsson JA. Accumulation of foam cells in liver X receptor-deficient mice. *Circulation* 2002, 106:1147–1153.
165. Glass CK, Witztum JL. Atherosclerosis. The road ahead. *Cell* 2001, 104:503–516.
166. Venkateswaran A, Laffitte BA, Joseph SB, Mak PA, Wilpitz DC, Edwards PA, Tontonoz P. Control of cellular cholesterol efflux by the nuclear oxysterol receptor LXR α. *Proc Natl Acad Sci U S A* 2000, 97: 12097–12102.
167. Schwartz K, Lawn RM, Wade DP. ABC1 gene expression and ApoA-I-mediated cholesterol efflux are regulated by LXR. *Biochem Biophys Res Commun* 2000, 274:794–802.
168. Sabol SL, Brewer HB Jr, Santamarina-Fojo S. The human ABCG1 gene: identification of LXR response elements that modulate expression in macrophages and liver. *J Lipid Res* 2005, 46:2151–2167.
169. Bodzioch M, Orso E, Klucken J, Langmann T, Bottcher A, Diederich W, Drobnik W, Barlage S, Buchler C, Porsch-Ozcurumez M, et al. The gene encoding ATP-binding cassette transporter 1 is mutated in Tangier disease. *Nat Genet* 1999, 22:347–351.
170. Brooks-Wilson A, Marcil M, Clee SM, Zhang LH, Roomp K, van DM, Yu L, Brewer C, Collins JA, Molhuizen HO, et al. Mutations in ABC1 in Tangier disease and familial high-density lipoprotein deficiency. *Nat Genet* 1999, 22:336–345.
171. Rust S, Rosier M, Funke H, Real J, Amoura Z, Piette JC, Deleuze JF, Brewer HB, Duverger N, Denefle P, et al. Tangier disease is caused by mutations in the gene encoding ATP-binding cassette transporter 1. *Nat Genet* 1999, 22:352–355.
172. Singaraja RR, Brunham LR, Visscher H, Kastelein JJ, Hayden MR. Efflux and atherosclerosis: the clinical and biochemical impact of variations in the ABCA1 gene. *Arterioscler Thromb Vasc Biol* 2003, 23:1322–1332.
173. Jakel H, Nowak M, Moitrot E, Dehondt H, Hum DW, Pennacchio LA, Fruchart-Najib J, Fruchart JC. The liver X receptor ligand T0901317 down-regulates APOA5 gene expression through activation of SREBP-1c. *J Biol Chem* 2004, 279:45462–45469.
174. Costet P, Luo Y, Wang N, Tall AR. Sterol-dependent transactivation of the ABC1 promoter by the liver X receptor/retinoid X receptor. *J Biol Chem* 2000, 275: 28240–28245.
175. Curtiss LK, Boisvert WA. Apolipoprotein E and atherosclerosis. *Curr Opin Lipidol* 2000, 11:243–251.
176. Joseph SB, Castrillo A, Laffitte BA, Mangelsdorf DJ, Tontonoz P. Reciprocal regulation of inflammation and lipid metabolism by liver X receptors. *Nat Med* 2003, 9:213–219.
177. Terasaka N, Hiroshima A, Ariga A, Honzumi S, Koieyama T, Inaba T, Fujiwara T. Liver X receptor agonists inhibit tissue factor expression in macrophages. *FEBS J* 2005, 272:1546–1556.
178. Castrillo A, Joseph SB, Marathe C, Mangelsdorf DJ, Tontonoz P. Liver X receptor-dependent repression of matrix metalloproteinase-9 expression in macrophages. *J Biol Chem* 2003, 278:10443–10449.
179. Ogawa D, Stone JF, Takata Y, Blaschke F, Chu VH, Towler DA, Law RE, Hsueh WA, Bruemmer D. Liver x receptor agonists inhibit cytokine-induced osteopontin expression in macrophages through interference with activator protein-1 signaling pathways. *Circ Res* 2005, 96:e59–e67.
180. Duval C, Touche V, Tailleux A, Fruchart JC, Fievet C, Clavey V, Staels B, Lestavel S. Niemann-Pick C1 like 1 gene expression is down-regulated by LXR activators in the intestine. *Biochem Biophys Res Commun* 2006, 340:1259–1263.
181. Peng D, Hiipakka RA, Dai Q, Guo J, Reardon CA, Getz GS, Liao S. Antiatherosclerotic effects of a novel synthetic tissue-selective steroidal liver X receptor agonist in low-density lipoprotein receptor-deficient mice. *J Pharmacol Exp Ther* 2008, 327:332–342.
182. Yasuda T, Grillot D, Billheimer JT, Briand F, Delerive P, Huet S, Rader DJ. Tissue-specific liver X receptor activation promotes macrophage reverse cholesterol transport in vivo. *Arterioscler Thromb Vasc Biol* 2010, 30:781–786.
183. Berge KE, Tian H, Graf GA, Yu L, Grishin NV, Schultz J, Kwiterovich P, Shan B, Barnes R, Hobbs HH. Accumulation of dietary cholesterol in sitosterolemia caused by mutations in adjacent ABC transporters. *Science* 2000, 290:1771–1775.
184. Lee MH, Lu K, Patel SB. Genetic basis of sitosterolemia. *Curr Opin Lipidol* 2001, 12:141–149.
185. Lee MH, Lu K, Hazard S, Yu H, Shulenin S, Hidaka H, Hidaka H, Kojima H, Allikmets R, Sakuma N, et al. Identification of a gene, ABCG5, important in the regulation of dietary cholesterol absorption. *Nat Genet* 2001, 27:79–83.
186. Repa JJ, Berge KE, Pomajzl C, Richardson JA, Hobbs H, Mangelsdorf DJ. Regulation of ATP-binding cassette sterol transporters ABCG5 and ABCG8 by the liver X receptors α and β. *J Biol Chem* 2002, 277:18793–18800.
187. Graf GA, Li WP, Gerard RD, Gelissen I, White A, Cohen JC, Hobbs HH. Coexpression of ATP-binding cassette proteins ABCG5 and ABCG8 permits their

transport to the apical surface. *J Clin Invest* 2002, 110: 659–669.

188. Yu L, Hammer RE, Li-Hawkins J, Von BK, Lutjohann D, Cohen JC, Hobbs HH. Disruption of Abcg5 and Abcg8 in mice reveals their crucial role in biliary cholesterol secretion. *Proc Natl Acad Sci U S A* 2002, 99:16237–16242.
189. Peet DJ, Turley SD, Ma W, Janowski BA, Lobaccaro JM, Hammer RE, Mangelsdorf DJ. Cholesterol and bile acid metabolism are impaired in mice lacking the nuclear oxysterol receptor LXR α. *Cell* 1998, 93: 693–704.
190. Kalaany NY, Gauthier KC, Zavacki AM, Mammen PP, Kitazume T, Peterson JA, Horton JD, Garry DJ, Bianco AC, Mangelsdorf DJ. LXRs regulate the balance between fat storage and oxidation. *Cell Metab* 2005, 1:231–244.
191. Russell DW. The enzymes, regulation, and genetics of bile acid synthesis. *Annu Rev Biochem* 2003, 72: 137–174.
192. Chiang JY, Kimmel R, Stroup D. Regulation of cholesterol 7α-hydroxylase gene (CYP7A1) transcription by the liver orphan receptor (LXRα). *Gene* 2001, 262:257–265.
193. Hong C, Duit S, Jalonen P, Out R, Scheer L, Sorrentino V, Boyadjian R, Rodenburg KC, Foley E, Korhonen L, et al. The E3-ubiquitin ligase idol induces the degradation of the low-density lipoprotein receptor family members VLDLR and APOER2. *J Biol Chem* 2010.
194. Zelcer N, Hong C, Boyadjian R, Tontonoz P. LXR regulates cholesterol uptake through Idol-dependent ubiquitination of the LDL receptor *Science* 2009, 325: 100–104.
195. Cao G, Liang Y, Broderick CL, Oldham BA, Beyer TP, Schmidt RJ, Zhang Y, Stayrook KR, Suen C, Otto KA, et al. Antidiabetic action of a liver x receptor agonist mediated by inhibition of hepatic gluconeogenesis. *J Biol Chem* 2003, 278:1131–1136.
196. Laffitte BA, Chao LC, Li J, Walczak R, Hummasti S, Joseph SB, Castrillo A, Wilpitz DC, Mangelsdorf DJ, Collins JL, et al. Activation of liver X receptor improves glucose tolerance through coordinate regulation of glucose metabolism in liver and adipose tissue. *Proc Natl Acad Sci U S A* 2003, 100:5419–5424.
197. Chuang JC, Cha JY, Garmey JC, Mirmira RG, Repa JJ. Research resource: nuclear hormone receptor expression in the endocrine pancreas. *Mol Endocrinol* 2008, 22:2353–2363.
198. Gerin I, Dolinsky VW, Shackman JG, Kennedy RT, Chiang SH, Burant CF, Steffensen KR, Gustafsson JA, MacDougald OA. LXRβ is required for adipocyte growth, glucose homeostasis, and β cell function. *J Biol Chem* 2005, 280:23024–23031.
199. Horton JD, Goldstein JL, Brown MS. SREBPs: transcriptional mediators of lipid homeostasis. *Cold Spring Harb Symp Quant Biol* 2002, 67:491–498.
200. Pennacchio LA, Olivier M, Hubacek JA, Cohen JC, Cox DR, Fruchart JC, Krauss RM, Rubin EM. An apolipoprotein influencing triglycerides in humans and mice revealed by comparative sequencing. *Science* 2001, 294:169–173.
201. Joseph SB, Laffitte BA, Patel PH, Watson MA, Matsukuma KE, Walczak R, Collins JL, Osborne TF, Tontonoz P. Direct and indirect mechanisms for regulation of fatty acid synthase gene expression by liver X receptors. *J Biol Chem* 2002, 277:11019–11025.
202. Cha JY, Repa JJ. The liver X receptor (LXR) and hepatic lipogenesis. The carbohydrate-response element-binding protein is a target gene of LXR. *J Biol Chem* 2007, 282:743–751.
203. Denechaud PD, Bossard P, Lobaccaro JM, Millatt L, Staels B, Girard J, Postic C. ChREBP, but not LXRs, is required for the induction of glucose-regulated genes in mouse liver. *J Clin Invest* 2008, 118:956–964.
204. Chisholm JW, Hong J, Mills SA, Lawn RM. The LXR ligand T0901317 induces severe lipogenesis in the db/db diabetic mouse. *J Lipid Res* 2003, 44: 2039–2048.
205. Luo Y, Tall AR. Sterol upregulation of human CETP expression in vitro and in transgenic mice by an LXR element. *J Clin Invest* 2000, 105:513–520.
206. Joseph SB, McKilligin E, Pei L, Watson MA, Collins AR, Laffitte BA, Chen M, Noh G, Goodman J, Hagger GN, et al. Synthetic LXR ligand inhibits the development of atherosclerosis in mice. *Proc Natl Acad Sci U S A* 2002, 99:7604–7609.
207. Beaven SW, Tontonoz P. Nuclear receptors in lipid metabolism: targeting the heart of dyslipidemia. *Annu Rev Med* 2006, 57:313–329.
208. Seol W, Choi HS, Moore DD. Isolation of proteins that interact specifically with the retinoid X receptor: two novel orphan receptors. *Mol Endocrinol* 1995, 9: 72–85.
209. Forman BM, Goode E, Chen J, Oro AE, Bradley DJ, Perlmann T, Noonan DJ, Burka LT, McMorris T, Lamph WW, et al. Identification of a nuclear receptor that is activated by farnesol metabolites. *Cell* 1995, 81:687–693.
210. Makishima M, Okamoto AY, Repa JJ, Tu H, Learned RM, Luk A, Hull MV, Lustig KD, Mangelsdorf DJ, Shan B. Identification of a nuclear receptor for bile acids. *Science* 1999, 284:1362–1365.
211. Parks DJ, Blanchard SG, Bledsoe RK, Chandra G, Consler TG, Kliewer SA, Stimmel JB, Willson TM, Zavacki AM, Moore DD, et al. Bile acids: natural ligands for an orphan nuclear receptor. *Science* 1999, 284:1365–1368.

212. Wang H, Chen J, Hollister K, Sowers LC, Forman BM. Endogenous bile acids are ligands for the nuclear receptor FXR/BAR. *Mol Cell* 1999, 3:543–553.

213. Portincasa P, Moschetta A, Palasciano G. Cholesterol gallstone disease. *Lancet* 2006, 368:230–239.

214. Moschetta A, Bookout AL, Mangelsdorf DJ. Prevention of cholesterol gallstone disease by FXR agonists in a mouse model. *Nat Med* 2004, 10:1352–1358.

215. Otte K, Kranz H, Kober I, Thompson P, Hoefer M, Haubold B, Remmel B, Voss H, Kaiser C, Albers M, et al. Identification of farnesoid X receptor β as a novel mammalian nuclear receptor sensing lanosterol. *Mol Cell Biol* 2003, 23:864–872.

216. Huber RM, Murphy K, Miao B, Link JR, Cunningham MR, Rupar MJ, Gunyuzlu PL, Haws TF, Kassam A, Powell F, et al. Generation of multiple farnesoid-X-receptor isoforms through the use of alternative promoters. *Gene* 2002, 290:35–43.

217. Zhang Y, Kast-Woelbern HR, Edwards PA. Natural structural variants of the nuclear receptor farnesoid X receptor affect transcriptional activation. *J Biol Chem* 2003, 278:104–110.

218. Claudel T, Sturm E, Duez H, Torra IP, Sirvent A, Kosykh V, Fruchart JC, Dallongeville J, Hum DW, Kuipers F, et al. Bile acid-activated nuclear receptor FXR suppresses apolipoprotein A-I transcription via a negative FXR response element. *J Clin Invest* 2002, 109:961–971.

219. Savkur RS, Thomas JS, Bramlett KS, Gao Y, Michael LF, Burris TP. Ligand-dependent coactivation of the human bile acid receptor FXR by the peroxisome proliferator-activated receptor gamma coactivator-1α. *J Pharmacol Exp Ther* 2005, 312:170–178.

220. Rizzo G, Renga B, Antonelli E, Passeri D, Pellicciari R, Fiorucci S. The methyl transferase PRMT1 functions as co-activator of farnesoid X receptor (FXR)/9-cis retinoid X receptor and regulates transcription of FXR responsive genes. *Mol Pharmacol* 2005, 68:551–558.

221. Pineda Torra I, Freedman LP, Garabedian MJ. Identification of DRIP205 as a coactivator for the Farnesoid X receptor. *J Biol Chem* 2004, 279:36184–36191.

222. Bauer UM, Daujat S, Nielsen SJ, Nightingale K, Kouzarides T. Methylation at arginine 17 of histone H3 is linked to gene activation. *EMBO Rep* 2002, 3:39–44.

223. Ananthanarayanan M, Li S, Balasubramaniyan N, Suchy FJ, Walsh MJ. Ligand-dependent activation of the farnesoid X-receptor directs arginine methylation of histone H3 by CARM1. *J Biol Chem* 2004, 279: 54348–54357.

224. Zhang Y, Castellani LW, Sinal CJ, Gonzalez FJ, Edwards PA. Peroxisome proliferator-activated receptor-gamma coactivator 1α (PGC-1α) regulates triglyceride metabolism by activation of the nuclear receptor FXR. *Genes Dev* 2004, 18:157–169.

225. Maloney PR, Parks DJ, Haffner CD, Fivush AM, Chandra G, Plunket KD, Creech KL, Moore LB, Wilson JG, Lewis MC, et al. Identification of a chemical tool for the orphan nuclear receptor FXR. *J Med Chem* 2000, 43:2971–2974.

226. Ricketts ML, Boekschoten MV, Kreeft AJ, Hooiveld GJ, Moen CJ, Muller M, Frants RR, Kasanmoentalib S, Post SM, Princen HM, et al. The cholesterol-raising factor from coffee beans, cafestol, as an agonist ligand for the farnesoid and pregnane X receptors. *Mol Endocrinol* 2007, 21:1603–1616.

227. Urizar NL, Liverman AB, Dodds DT, Silva FV, Ordentlich P, Yan Y, Gonzalez FJ, Heyman RA, Mangelsdorf DJ, Moore DD. A natural product that lowers cholesterol as an antagonist ligand for FXR. *Science* 2002, 296:1703–1706.

228. Downes M, Verdecia MA, Roecker AJ, Hughes R, Hogenesch JB, Kast-Woelbern HR, Bowman ME, Ferrer JL, Anisfeld AM, Edwards PA, et al. A chemical, genetic, and structural analysis of the nuclear bile acid receptor FXR. *Mol Cell* 2003, 11:1079–1092.

229. Pellicciari R, Fiorucci S, Camaioni E, Clerici C, Costantino G, Maloney PR, Morelli A, Parks DJ, Willson TM. 6α-ethyl-chenodeoxycholic acid (6-ECDCA), a potent and selective FXR agonist endowed with anticholestatic activity. *J Med Chem* 2002, 45:3569–3572.

230. Pellicciari R, Costantino G, Camaioni E, Sadeghpour BM, Entrena A, Willson TM, Fiorucci S, Clerici C, Gioiello A. Bile acid derivatives as ligands of the farnesoid X receptor. Synthesis, evaluation, and structure-activity relationship of a series of body and side chain modified analogues of chenodeoxycholic acid. *J Med Chem* 2004, 47: 4559–4569.

231. Yu J, Lo JL, Huang L, Zhao A, Metzger E, Adams A, Meinke PT, Wright SD, Cui J. Lithocholic acid decreases expression of bile salt export pump through farnesoid X receptor antagonist activity. *J Biol Chem* 2002, 277:31441–31447.

232. Dussault I, Beard R, Lin M, Hollister K, Chen J, Xiao JH, Chandraratna R, Forman BM. Identification of gene-selective modulators of the bile acid receptor FXR. *J Biol Chem* 2003, 278:7027–7033.

233. Huang L, Zhao A, Lew JL, Zhang T, Hrywna Y, Thompson JR, De PN, Royo I, Blevins RA, Pelaez F, et al. Farnesoid X receptor activates transcription of the phospholipid pump MDR3. *J Biol Chem* 2003, 278:51085–51090.

234. Kast HR, Goodwin B, Tarr PT, Jones SA, Anisfeld AM, Stoltz CM, Tontonoz P, Kliewer S, Willson TM, Edwards PA. Regulation of multidrug resistance-associated protein 2 (ABCC2) by the nuclear receptors pregnane X receptor, farnesoid X-activated receptor, and constitutive androstane receptor. *J Biol Chem* 2002, 277:2908–2915.

235. Liu Y, Binz J, Numerick MJ, Dennis S, Luo G, Desai B, MacKenzie KI, Mansfield TA, Kliewer SA,

Goodwin B, et al. Hepatoprotection by the farnesoid X receptor agonist GW4064 in rat models of intra- and extrahepatic cholestasis. *J Clin Invest* 2003, 112: 1678–1687.

236. Lee H, Zhang Y, Lee FY, Nelson SF, Gonzalez FJ, Edwards PA. FXR regulates organic solute transporters α and β in the adrenal gland, kidney, and intestine. *J Lipid Res* 2006, 47:201–214.
237. Denson LA, Sturm E, Echevarria W, Zimmerman TL, Makishima M, Mangelsdorf DJ, Karpen SJ. The orphan nuclear receptor, shp, mediates bile acid-induced inhibition of the rat bile acid transporter, ntcp. *Gastroenterology* 2001, 121:140–147.
238. Jung D, Inagaki T, Gerard RD, Dawson PA, Kliewer SA, Mangelsdorf DJ, Moschetta A. FXR agonists and FGF15 reduce fecal bile acid excretion in a mouse model of bile acid malabsorption. *J Lipid Res* 2007, 48:2693–2700.
239. Pircher PC, Kitto JL, Petrowski ML, Tangirala RK, Bischoff ED, Schulman IG, Westin SK. Farnesoid X receptor regulates bile acid-amino acid conjugation. *J Biol Chem* 2003, 278:27703–27711.
240. Frankenberg T, Rao A, Chen F, Haywood J, Shneider BL, Dawson PA. Regulation of the mouse organic solute transporter α-β, Ostα-Ostβ, by bile acids. *Am J Physiol Gastrointest Liver Physiol* 2006, 290:G912–G922.
241. Inagaki T, Choi M, Moschetta A, Peng L, Cummins CL, McDonald JG, Luo G, Jones SA, Goodwin B, Richardson JA, et al. Fibroblast growth factor 15 functions as an enterohepatic signal to regulate bile acid homeostasis. *Cell Metab* 2005, 2:217–225.
242. Yu C, Wang F, Kan M, Jin C, Jones RB, Weinstein M, Deng CX, McKeehan WL. Elevated cholesterol metabolism and bile acid synthesis in mice lacking membrane tyrosine kinase receptor FGFR4. *J Biol Chem* 2000, 275: 15482–15489.
243. Goodwin B, Jones SA, Price RR, Watson MA, McKee DD, Moore LB, Galardi C, Wilson JG, Lewis MC, Roth ME, et al. A regulatory cascade of the nuclear receptors FXR, SHP-1, and LRH-1 represses bile acid biosynthesis. *Mol Cell* 2000, 6: 517–526.
244. Lu TT, Makishima M, Repa JJ, Schoonjans K, Kerr TA, Auwerx J, Mangelsdorf DJ. Molecular basis for feedback regulation of bile acid synthesis by nuclear receptors. *Mol Cell* 2000, 6:507–515.
245. Lehmann JM, Kliewer SA, Moore LB, Smith-Oliver TA, Oliver BB, Su JL, Sundseth SS, Winegar DA, Blanchard DE, Spencer TA, et al. Activation of the nuclear receptor LXR by oxysterols defines a new hormone response pathway. *J Biol Chem* 1997, 272: 3137–3140.
246. Brendel C, Schoonjans K, Botrugno OA, Treuter E, Auwerx J. The small heterodimer partner interacts with the liver X receptor α and represses its transcriptional activity. *Mol Endocrinol* 2002, 16: 2065–2076.
247. Sinal CJ, Tohkin M, Miyata M, Ward JM, Lambert G, Gonzalez FJ. Targeted disruption of the nuclear receptor FXR/BAR impairs bile acid and lipid homeostasis. *Cell* 2000, 102:731–744.
248. Lambert G, Amar MJ, Guo G, Brewer HB Jr, Gonzalez FJ, Sinal CJ. The farnesoid X-receptor is an essential regulator of cholesterol homeostasis. *J Biol Chem* 2003, 278:2563–2570.
249. Watanabe M, Houten SM, Wang L, Moschetta A, Mangelsdorf DJ, Heyman RA, Moore DD, Auwerx J. Bile acids lower triglyceride levels via a pathway involving FXR, SHP, and SREBP-1c. *J Clin Invest* 2004, 113:1408–1418.
250. Urizar NL, Dowhan DH, Moore DD. The farnesoid X-activated receptor mediates bile acid activation of phospholipid transfer protein gene expression. *J Biol Chem* 2000, 275:39313–39317.
251. Shepherd J, Packard CJ, Morgan HG, Third JL, Stewart JM, Lawrie TD. The effects of cholestyramine on high density lipoprotein metabolism. *Atherosclerosis* 1979, 33:433–444.
252. Angelin B, Einarsson K, Hellstrom K, Leijd B. Effects of cholestyramine and chenodeoxycholic acid on the metabolism of endogenous triglyceride in hyperlipoproteinemia. *J Lipid Res* 1978, 19:1017–1024.
253. Leiss O, Von BK. Different effects of chenodeoxycholic acid and ursodeoxycholic acid on serum lipoprotein concentrations in patients with radiolucent gallstones. *Scand J Gastroenterol* 1982, 17:587–592.
254. Bateson MC, Maclean D, Evans JR, Bouchier IA. Chenodeoxycholic acid therapy for hypertriglyceridaemia in men. *Br J Clin Pharmacol* 1978, 5:249–254.
255. Nakahara M, Fujii H, Maloney PR, Shimizu M, Sato R. Bile acids enhance low density lipoprotein receptor gene expression via a MAPK cascade-mediated stabilization of mRNA. *J Biol Chem* 2002, 277:37229–37234.
256. Cariou B, van HK, Duran-Sandoval D, van Dijk TH, Grefhorst A, Abdelkarim M, Caron S, Torpier G, Fruchart JC, Gonzalez FJ, et al. The farnesoid X receptor modulates adiposity and peripheral insulin sensitivity in mice. *J Biol Chem* 2006, 281: 11039–11049.
257. Zhang Y, Lee FY, Barrera G, Lee H, Vales C, Gonzalez FJ, Willson TM, Edwards PA. Activation of the nuclear receptor FXR improves hyperglycemia and hyperlipidemia in diabetic mice. *Proc Natl Acad Sci U S A* 2006, 103: 1006–1011.
258. Wang Y, Jones PJ, Woollett LA, Buckley DD, Yao L, Granholm NA, Tolley EA, Heubi JE. Effects of chenodeoxycholic acid and deoxycholic acid on cholesterol absorption and metabolism in humans. *Transl Res* 2006, 148:37–45.
259. Schoenfield LJ, Lachin JM. Chenodiol (chenodeoxycholic acid) for dissolution of gallstones: the National Cooperative Gallstone Study. A controlled trial of efficacy and safety. *Ann Intern Med* 1981, 95:257–282.

260. Bell GD, Lewis B, Petrie A, Dowling RH. Serum lipids in cholelithiasis: effect of chenodeoxycholic acid therapy. *Br Med J* 1973, 3:520–523.

261. Miller NE, Nestel PJ. Triglyceride-lowering effect of chenodeoxycholic acid in patients with endogenous hypertriglyceridaemia. *Lancet* 1974, 2:929–931.

262. Carulli N, Ponz de LM, Podda M, Zuin M, Strata A, Frigerio G, Digrisolo A. Chenodeoxycholic acid and ursodeoxycholic acid effects in endogenous hypertriglyceridemias. A controlled double-blind trial. *J Clin Pharmacol* 1981, 21:436–442.

263. Kast HR, Nguyen CM, Sinal CJ, Jones SA, Laffitte BA, Reue K, Gonzalez FJ, Willson TM, Edwards PA. Farnesoid X-activated receptor induces apolipoprotein C-II transcription: a molecular mechanism linking plasma triglyceride levels to bile acids. *Mol Endocrinol* 2001, 15:1720–1728.

264. Gutierrez A, Ratliff EP, Andres AM, Huang X, McKeehan WL, Davis RA. Bile acids decrease hepatic paraoxonase 1 expression and plasma high-density lipoprotein levels via FXR-mediated signaling of FGFR4. *Arterioscler Thromb Vasc Biol* 2006, 26: 301–306.

265. Shih DM, Kast-Woelbern HR, Wong J, Xia YR, Edwards PA, Lusis AJ. A role for FXR and human FGF-19 in the repression of paraoxonase-1 gene expression by bile acids. *J Lipid Res* 2006, 47: 384–392.

266. Bilz S, Samuel V, Morino K, Savage D, Choi CS, Shulman GI. Activation of the farnesoid X receptor improves lipid metabolism in combined hyperlipidemic hamsters. *Am J Physiol Endocrinol Metab* 2006, 290:E716–E722.

267. Hirokane H, Nakahara M, Tachibana S, Shimizu M, Sato R. Bile acid reduces the secretion of very low density lipoprotein by repressing microsomal triglyceride transfer protein gene expression mediated by hepatocyte nuclear factor-4. *J Biol Chem* 2004, 279:45685–45692.

268. Ma K, Saha PK, Chan L, Moore DD. Farnesoid X receptor is essential for normal glucose homeostasis. *J Clin Invest* 2006, 116:1102–1109.

269. Zhang Y, Wang X, Vales C, Lee FY, Lee H, Lusis AJ, Edwards PA. FXR deficiency causes reduced atherosclerosis in Ldlr-/- mice. *Arterioscler Thromb Vasc Biol* 2006, 26:2316–2321.

270. Pineda Torra I, Claudel T, Duval C, Kosykh V, Fruchart JC, Staels B. Bile acids induce the expression of the human peroxisome proliferator-activated receptor α gene via activation of the farnesoid X receptor. *Mol Endocrinol* 2003, 17:259–272.

271. Savkur RS, Bramlett KS, Michael LF, Burris TP. Regulation of pyruvate dehydrogenase kinase expression by the farnesoid X receptor. *Biochem Biophys Res Commun* 2005, 329:391–396.

272. Beil U, Crouse JR, Einarsson K, Grundy SM. Effects of interruption of the enterohepatic circulation of bile acids on the transport of very low density-lipoprotein triglycerides. *Metabolism* 1982, 31:438–444.

273. Crouse JR III. Hypertriglyceridemia: a contraindication to the use of bile acid binding resins. *Am J Med* 1987, 83:243–248.

274. Hassan AS, Ravi Subbiah MT, Thiebert P. Specific changes of bile acid metabolism in spontaneously diabetic Wistar rats. *Proc Soc Exp Biol Med* 1980, 164: 449–452.

275. Nervi FO, Severin CH, Valdivieso VD. Bile acid pool changes and regulation of cholate synthesis in experimental diabetes. *Biochim Biophys Acta* 1978, 529: 212–223.

276. Staels B, Kuipers F. Bile acid sequestrants and the treatment of type 2 diabetes mellitus. *Drugs* 2007, 67:1383–1392.

277. Duran-Sandoval D, Cariou B, Percevault F, Hennuyer N, Grefhorst A, van Dijk TH, Gonzalez FJ, Fruchart JC, Kuipers F, Staels B. The farnesoid X receptor modulates hepatic carbohydrate metabolism during the fasting-refeeding transition. *J Biol Chem* 2005, 280:29971–29979.

278. Duran-Sandoval D, Mautino G, Martin G, Percevault F, Barbier O, Fruchart JC, Kuipers F, Staels B. Glucose regulates the expression of the farnesoid X receptor in liver. *Diabetes* 2004, 53:890–898.

279. Yamagata K, Daitoku H, Shimamoto Y, Matsuzaki H, Hirota K, Ishida J, Fukamizu A. Bile acids regulate gluconeogenic gene expression via small heterodimer partner-mediated repression of hepatocyte nuclear factor 4 and Foxo1. *J Biol Chem* 2004, 279: 23158–23165.

280. Stayrook KR, Bramlett KS, Savkur RS, Ficorilli J, Cook T, Christe ME, Michael LF, Burris TP. Regulation of carbohydrate metabolism by the farnesoid X receptor. *Endocrinology* 2005, 146:984–991.

281. De FE, Mitro N, Gilardi F, Caruso D, Galli G, Crestani M. Coordinated control of cholesterol catabolism to bile acids and of gluconeogenesis via a novel mechanism of transcription regulation linked to the fasted-to-fed cycle. *J Biol Chem* 2003, 278: 39124–39132.

282. Borgius LJ, Steffensen KR, Gustafsson JA, Treuter E. Glucocorticoid signaling is perturbed by the atypical orphan receptor and corepressor SHP. *J Biol Chem* 2002, 277:49761–49766.

283. Kurosu H, Choi M, Ogawa Y, Dickson AS, Goetz R, Eliseenkova AV, Mohammadi M, Rosenblatt KP, Kliewer SA, Kuro-o M. Tissue-specific expression of βKlotho and fibroblast growth factor (FGF) receptor isoforms determines metabolic activity of FGF19 and FGF21. *J Biol Chem* 2007, 282:26687–26695.

284. Tomlinson E, Fu L, John L, Hultgren B, Huang X, Renz M, Stephan JP, Tsai SP, Powell-Braxton L,

French D, et al. Transgenic mice expressing human fibroblast growth factor-19 display increased metabolic rate and decreased adiposity. *Endocrinology* 2002, 143:1741–1747.

285. Watanabe M, Houten SM, Mataki C, Christoffolete MA, Kim BW, Sato H, Messaddeq N, Harney JW, Ezaki O, Kodama T, et al. Bile acids induce energy expenditure by promoting intracellular thyroid hormone activation. *Nature* 2006, 439:484–489.
286. Petruzzelli M, Moschetta A. Intestinal ecology in the metabolic syndrome. *Cell Metab* 2010, 11:345–346.
287. Vijay-Kumar M, Aitken JD, Carvalho FA, Cullender TC, Mwangi S, Srinivasan S, Sitaraman SV, Knight R, Ley RE, Gewirtz AT. Metabolic syndrome and altered gut microbiota in mice lacking Toll-like receptor 5. *Science* 2010, 328:228–231.
288. Inagaki T, Moschetta A, Lee YK, Peng L, Zhao G, Downes M, Yu RT, Shelton JM, Richardson JA, Repa JJ, et al. Regulation of antibacterial defense in the small intestine by the nuclear bile acid receptor. *Proc Natl Acad Sci U S A* 2006, 103:3920–3925.
289. Li J, Wilson A, Kuruba R, Zhang Q, Gao X, He F, Zhang LM, Pitt BR, Xie W, Li S. FXR-mediated regulation of eNOS expression in vascular endothelial cells. *Cardiovasc Res* 2008, 77:169–177.
290. Li YT, Swales KE, Thomas GJ, Warner TD, Bishop-Bailey D. Farnesoid x receptor ligands inhibit vascular smooth muscle cell inflammation and migration. *Arterioscler Thromb Vasc Biol* 2007, 27:2606–2611.
291. He F, Li J, Mu Y, Kuruba R, Ma Z, Wilson A, Alber S, Jiang Y, Stevens T, Watkins S, et al. Downregulation of endothelin-1 by farnesoid X receptor in vascular endothelial cells. *Circ Res* 2006, 98:192–199.
292. Bishop-Bailey D, Walsh DT, Warner TD. Expression and activation of the farnesoid X receptor in the vasculature. *Proc Natl Acad Sci U S A* 2004, 101:3668–3673.
293. Qin P, Tang X, Elloso MM, Harnish DC. Bile acids induce adhesion molecule expression in endothelial cells through activation of reactive oxygen species, NF-kappaB, and p38. *Am J Physiol Heart Circ Physiol* 2006, 291:H741–H747.
294. Hanniman EA, Lambert G, McCarthy TC, Sinal CJ. Loss of functional farnesoid X receptor increases atherosclerotic lesions in apolipoprotein E-deficient mice. *J Lipid Res* 2005, 46:2595–2604.
295. Guo GL, Santamarina-Fojo S, Akiyama TE, Amar MJ, Paigen BJ, Brewer B Jr, Gonzalez FJ. Effects of FXR in foam-cell formation and atherosclerosis development. *Biochim Biophys Acta* 2006, 1761:1401–1409.