Regulation of Metabolism by Nuclear Hormone Receptors

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The worldwide epidemic of metabolic disease indicates that a better understanding of the pathways contributing to the pathogenesis of this constellation of diseases need to be determined. Nuclear hormone receptors comprise a superfamily of ligand-activated transcription factors that control development, differentiation, and metabolism. Over the last 15 years a growing number of nuclear receptors have been identified that coordinate genetic networks regulating lipid metabolism and energy utilization. Several of these receptors directly sample the levels of metabolic intermediates and use this information to regulate the synthesis, transport, and breakdown of the metabolite of interest. In contrast, other family members sense metabolic activity via the presence or absence of interacting proteins. The ability of these nuclear receptors to impact metabolism and inflammation will be discussed and the potential of each receptor subfamily to serve as drug targets for metabolic disease will be highlighted.

1. Introduction

There is currently a worldwide epidemic of metabolic disease characterized by obesity, type II diabetes, hypertension, and cardiovascular disease. The factors behind this epidemic appear to be combination of genetic predisposition, high caloric diets, and our increasingly sedentary lifestyles. Indeed recent statistics from the American Heart Association indicate that almost 50% of American adults are at risk for cardiovascular disease and the Center for Disease Control reports that approximately 16 million have type II diabetes. Although a number of drugs are currently available to treat the constellation of metabolic ailments, the growing epidemic indicates that we still require a better understanding of the genetic networks and signal transduction systems that underlie the pathogenesis of these conditions. Further definition of the factors responsible for metabolic control may pave the way toward new drug targets with novel mechanisms of action for the treatment of human disease.

Nuclear receptors comprise a superfamily of ligand-dependent transcription factors that regulate genetic networks controlling cell growth, development, and metabolism. Consisting of 48 members in the human genome the superfamily includes the well-known receptors for steroids, thyroid hormones,
and vitamins. Members of the nuclear receptor superfamily are characterized by a conserved structural and functional organization consisting of a heterogeneous amino terminal domain, a highly conserved central DNA-binding domain (DBD), and a functionally complex carboxy terminal ligand-binding domain (LBD). The LBD mediates ligand binding, receptor homo- and heterodimerization, repression of transcription in the absence of ligand, and ligand-dependent activation of transcription when agonist ligands are bound.

Crystal structures of several LBDs support molecular and biochemical studies indicating that ligand binding promotes a conformational change in receptor structure. What appears to be a relatively flexible conserved helix near the carboxy terminus (helix 12) occupies unique positions when structures of unliganded, agonist-occupied, and antagonist-occupied LBDs are compared. Importantly, mutagenesis experiments indicate that helix 12, referred to as activation function 2 (AF-2), is necessary for ligand-dependent transactivation by nuclear receptors. The AF-2 helix contributes an essential surface to the formation of an agonist-dependent hydrophobic pocket that serves as a binding site for coactivators. The alternative positions occupied by the helix 12 in the unliganded or antagonist-occupied conformations preclude the formation of this binding pocket.

Classic experiments that defined the effects of glucocorticoids and thyroid hormone on metabolic control provided the foundation for the endocrine regulation of metabolism. Similar to these classical endocrine receptors, a number of orphan receptors first cloned based on homology to the well-conserved receptor DBD have subsequently been shown to regulate genetic networks that control important metabolic pathways. In many cases these same pathways are deranged in instances of metabolic disease and it is this class of metabolic sensing receptors that will be the focus of this review. Several of the receptors that will be discussed including the peroxisome proliferator activated receptors (PPARs), the liver X receptors (LXRs), and the farnesoid X receptor (FXR) and perhaps the retinoid-related orphan receptors (RORs) appear to function by directly sampling the levels of fatty acids and cholesterol derivatives via the receptor LBD and regulating genetic networks that control the synthesis, transport, and breakdown of the cognate ligand. Importantly, these fatty acid- and cholesterol-derived natural ligands bind to receptors with affinities close to the physiological concentrations known to exist for these metabolites. Thus, these receptors are poised to sense and respond to small changes in the flux through the metabolic pathways that they control. The estrogen receptor related receptors (ERRs) comprise an additional subfamily of nuclear receptors that also appear to play important roles in the regulation of metabolism. In contrast to the ligand-activated receptors mentioned earlier, the activity of the ERRz in particular appears to be controlled by the presence or
absence of interacting proteins instead of lipid-derived ligands. The focus of this review will be on the function of these “metabolic” nuclear receptors, the physiological pathways they regulate, and their potential as drug targets.

There are several reoccurring themes that appear throughout this review. First, the ability of individual nuclear receptors to regulate multiple genetic networks in different tissues has made drug discovery a challenging process for this class of potential drug targets. Second, in many cases the phenotype of a genetic knockout of a particular nuclear receptor does not accurately predict the physiological activity of a receptor-specific small molecule agonist or antagonist. Finally, there is an intimate connection between the regulation of metabolism and the control of inflammation.

II. The PPARs

Three distinct members of the PPAR subfamily each encoded by a distinct gene have been identified and well characterized. PPARα (NR1C1) is highly expressed in liver, kidney, and muscle. PPARγ (NR1C3) is enriched in adipose tissue and PPARβ/δ (NR1C2; referred to PPARδ in this review) appears to be ubiquitously expressed. All three PPARs bind to DNA as heterodimers with retinoid X receptors (RXR; NR2B subgroup) and prefer to bind to direct repeats of the nuclear receptor half-site AGGTCA separated by 1 nucleotide (DR1). Each subtype appears to have unique functions and PPARα and PPARγ are the targets of the fibrate and thiazolidinedione (TZD) classes of drugs, respectively.

A. PPARα

PPARα is the molecular target of the fibrate class of drugs used for the treatment of hypertriglyceridemia. Studies in vitro and in vivo demonstrate that PPARα directly regulates a network of genes encoding the proteins required for the uptake of fatty acids, enzymes required for the oxidation of fatty acids (β oxidation), and enzymes required for ketone body utilization by binding to control regions in the promoter of these genes. Thus, activation of PPARα promotes the utilization of fat as an energy source. Activation of PPARα also directly induces the genes encoding the apolipoproteins apoAI and apoII, which contribute to the protein core of high density lipoprotein (HDL) particles. Thus, fibrates have the added benefit of slightly raising HDL, the “good cholesterol.”

Given its role in controlling the utilization of fatty acids for energy production it is not surprising that PPARα is required for the normal response to fasting and starvation. Mice lacking PPARα accumulate triglycerides in the liver and become hypoketonic and hypoglycemic during fasting or starvation.
Recent studies indicate the fibroblast growth factor 21 (FGF21) functions as an endocrine hormone that mediates many of the effects of PPARα. The gene encoding FGF21 is directly induced by PPARα in response to fasting via a binding site in the promoter. FGF21 in turn stimulates lipolysis in adipose tissue and ketogenesis in the liver.\textsuperscript{24} Taken together PPARα appears to function as a sensor of the fed/starved state. The increase in fatty acids derived from adipose during fasting provides PPARα agonists that promote the utilization of fat as energy and further stimulates the release of fatty acids from the adipose by the endocrine action of FGF21\textsuperscript{24} (Fig. 1).

B. PPARγ

PPARγ is the master transcriptional regulator of adipogenesis and plays an important role in the process of lipid storage.\textsuperscript{25} Thus, PPARα and PPARγ have contrasting roles in the regulation of fat metabolism; PPARα promotes the utilization of fat in the liver and muscle while activation of PPARγ promotes storage in adipose. A number of naturally occurring fatty acids and prostanoids have been shown to act as PPARγ agonists; however, perhaps most importantly

\begin{figure}[h]
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\includegraphics[width=\textwidth]{fig1.png}
\caption{Regulation of fatty acid utilization by PPARα. Fatty acids directly bind to PPARα and act as agonists that increase transcriptional activity. Activated PPARα directly induces genes that encode the enzymes required for \(\beta\)-oxidation and ketogenesis. PPARα also increases expression of the gene encoding FGF21. FGF21 acts in an autocrine and paracrine fashion to further enhance \(\beta\)-oxidation and ketogenesis and FGF21 also stimulates lipolysis in adipose tissue to promote the release of fatty acids. See text for further details.}
\end{figure}
was the identification that the TZD class of insulin sensitizing drugs including rosiglitazone (Avandia) and pioglitazone (Actos) are PPARγ agonists. Although PPARγ was identified as the therapeutic target of TZDs in 1995 it is still fair to say that the mechanism underlying the insulin sensitizing activity of this class of drugs is still not completely defined. Fatty acid accumulation in insulin sensitive tissues such as liver and skeletal muscle has been shown to promote insulin resistance. Activation of PPARγ in adipose has been proposed to increase the number of adipocytes and promote the relocalization and storage of fat in adipose, protecting peripheral tissues from lipotoxicity. Consistent with this idea is the observation that selective knockout of PPARγ in adipose eliminates the therapeutic activity of TZDs in mice and that a common side effect of TZD treatment in humans is weight gain due to an increase in adipose mass. PPARγ is also expressed at relatively low levels in other tissues and selective knockout in skeletal muscle reduces the therapeutic activity of TZDs, suggesting that there are adipose-independent sites of PPARγ activity.

The TZDs have proven to be effective drugs for improving insulin sensitivity and treating type II diabetes. However, they are not without problems. Troglitazone, the first TZD in the clinic, was taken off the market because of cases of drug-induced liver damage. Additionally, recent meta-analyses have indicated that treatment with rosiglitazone is associated with increased risk of myocardial infarction and deaths due to cardiovascular events. Pioglitazone treatment was also shown to be associated with an increase in serious heart failure although a significantly lower risk of myocardial infarction and death was observed in this patient population. Finally, muraglitazar an investigational drug that is a dual agonist of PPARα and PPARγ was found to be associated with an increase in major cardiovascular events and increased incidence of death. The increases in cardiovascular events and mortality seen with these drugs are relatively small. Nevertheless, the wide scale use of PPARγ agonists in the type II diabetic population has raised serious concerns about the safety of these drugs for long term therapy.

The molecular basis underlying the increase in cardiovascular events is not clear. Changes in energy metabolism mediated by PPARs could significantly influence cardiac function. Additionally, several studies have identified edema as a side effect of TZD treatment that could impact heart function. Regulation of the epithelial sodium channel (ENaCγ) in the kidney by PPARγ has been suggested as potential mechanism behind the TZD-dependent edema but it remains to be seen if inhibiting this channel will decrease the cardiovascular events associated with TZD treatment. Based on the recent clinical data it is unlikely that additional PPARγ agonists will make it to the clinic unless a better understanding of the tissue-specific responses of this receptor is obtained.
C. PPAR\(\delta\)

Not surprisingly based on its ubiquitous expression pattern, genetic knock-out of PPAR\(\delta\) results in a number abnormalities including embryonic lethality secondary to placental defects, decreased adipose mass, mylination deficiencies, altered inflammatory responses, and impaired wound healing. More recent studies exploiting additional genetic models and synthetic agonists, however, have uncovered important functions for this receptor in the control of metabolism and inflammation.\(^{39,40}\)

X-ray crystallography, indicates that PPAR\(\delta\) has a relatively large ligand binding pocket and \textit{in vitro} studies indicate that fatty acids as well as eicosanoids including prostaglandin A1 and carbaprostacyclin function as agonists.\(^{39}\) Very low density lipoprotein (VLDL) particle associated fatty acids have also been demonstrated to induce PPAR\(\delta\) target genes in a receptor-dependent manner\(^{41}\) raising the possibility that PPAR\(\delta\) regulates the synthesis, transport, and catabolism of triglyceride-rich lipoprotein particles. Further support for a role of PPAR\(\delta\) in lipoprotein metabolism results from studies exploring the activity of the PPAR\(\delta\)-specific synthetic agonist GW501516. Treatment of animals, including, nonhuman primates with GW501516 significantly increases HDL particles, lowers triglycerides and low density lipoprotein (LDL) particles, and decreases fasting insulin levels.\(^{39,40}\) Mechanistic studies point to regulation of the gene encoding the ATP binding cassette transporter ABCA1 by PPAR\(\delta\) as an important step in the control of HDL levels.\(^{42}\) ABCA1 functions as a cholesterol transporter to transfer cholesterol out of cells to HDL particles\(^{43}\) and its function will be discussed further in Section III. PPAR\(\delta\) mediated downregulation of intestinal cholesterol absorption via regulation of the gene encoding Niemann-Pick C1-like protein 1 (NPCL-1), a cholesterol transporter that is the target of the cholesterol lowering drug ezetemide (Zetia), has also been suggested to play a role in the effect of PPAR\(\delta\) on lipid levels.\(^{44}\)

To examine the role of PPAR\(\delta\) in specific tissues, Wang \textit{et al.}\(^{45,46}\) fused the strong transcriptional activation domain of the viral transcription factor VP16 to the amino-terminus of PPAR\(\delta\) to create a “hyperactive” receptor that activates transcription even in the absence of agonists. Transgenic approaches were then used to express VP16–PPAR\(\delta\) in adipose (white and brown) and skeletal muscle. In both tissues VP16–PPAR\(\delta\) expression produced a dramatic increase in the \(\beta\)-oxidation of fatty acids. In adipose, the increase fat oxidation led to a decrease in adipose mass and protection from diet-induced obesity and insulin resistance. The protection against diet-induced obesity results, at least in part, from increased thermogenesis in brown fat secondary to the induction of genes involved in \(\beta\)-oxidation and the uncoupling of oxidative phosphorylation from ATP production.\(^{46}\) Uncoupling oxidative phosphorylation from ATP production by expression of uncoupling protein 1 (UCP-1) leads to a futile cycle that
generates heat when fat is metabolized. Weight loss was also observed in obese mice treated with the synthetic agonist GW501516, suggesting that the same PPARδ-dependent pathways can be activated pharmacologically. Nevertheless, a significant change in body weight was not detected when obese Rhesus monkeys were treated for 4 months with GW501516. As discussed earlier, however, significant agonist-dependent effects on lipid metabolism and insulin level was observed in these animals indicating that GW501516 is active in nonhuman primates. Perhaps species-dependent differences in the bioavailability, tissue distribution, and/or efficacy of GW501516 account the differences on adipose mass between rodents and primates.

In skeletal muscle expression of VP16–PPARδ induces genes involved in β-oxidation, mitochondrial respiration, and increases the proportion of slow twitch oxidative muscle fibers. In short the muscle of these animals become fat burning machines and interestingly these transgenic mice can run on a treadmill for significantly longer times than mice without VP16–PPARδ. Given the interest in "performance-enhancing" drugs, Narkar et al. tested the ability of GW501516 to increase endurance in mice (defined as running on a treadmill until exhaustion). In contrast to the result observed with the super-active VP16–PPARδ construct, activation of endogenous PPARδ with the synthetic agonist did not increase endurance. When agonist treatment was coupled with a minimal exercise regimen, however, the combination of drug with exercise produced a significantly larger increase in running time compared to exercise alone. Although pharmacological activation of PPARδ alone does not improve endurance, pharmacologic activation of AMP kinase, a kinase that is activated when energy levels are low, is sufficient by itself to improve endurance in sedentary mice. AMP kinase, like PPARδ, is known to play an important role in muscle fiber type specification and the endurance promoting activity of an AMP kinase activator is lost in Pparδ−/− mice. Thus, activation of PPARδ is necessary to improve endurance. Interestingly, activated AMP kinase increases the transcriptional activity of PPARδ at least in part by phosphorylation of the peroxisome proliferator activated receptor γ coactivator 1z (PGC-1z), a transcriptional coactivator that directly interacts with PPARδ. AMP kinase activity is also induced by exercise suggesting a simple model that exercise activated AMP kinase increases the transcriptional activity of PPARδ leading to expression of a genetic network involved in the specification slow twitch oxidative muscle fibers and improved endurance (Fig. 2A). If this linear pathway is correct, one must ask why neither exercise alone nor simply activating PPARδ with a synthetic agonist (bypassing AMP kinase) is sufficient to improve endurance? We would argue that there is a threshold level of PPARδ activity that must be achieved in order to increase endurance (shown schematically in Fig. 2B) and that the exercise regimen used and GW501516
individually do not achieve this level. Perhaps increasing the duration of the exercise or improving the efficacy and bioavailability of the synthetic agonist would allow these agents to function alone.

Synthetic PPARδ ligands have proven to be very effective in preclinical models of diabetes and GW501516 was taken into clinic for the treatment of dyslipidemia in 2006. It remains to be seen if synthetic ligands for PPARδ will prove to be effective for the treatment of human disease.

D. PPARs and Atherosclerosis

The important roles for the PPARs in the control of lipid metabolism prompted a number of studies investigating the activity of subtype selective agonists in mouse models of atherosclerosis. Generally, LDL receptor knockout (Ldlr<sup>−/−</sup>) mice or apoE knockout (apoE<sup>−/−</sup>) mice treated with synthetic ligand have been used as model systems. Based on these studies, one can conclude that activation of any of the 3 PPARs reduces atherosclerosis. One, however, could also conclude the opposite; that activation of the PPARs has little or no benefit for the treatment of atherosclerosis. It is clear that

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**Fig. 2.** PPARδ-dependent endurance pathway. (A) Exercise leads to activation of AMP kinase which phosphorylates PGC-1α. Phosphorylated PGC-1α acts as a transcriptional coactivator to increase the activity of PPARδ leading to the induction of endurance-promoting genes. (B) Graph illustrates the hypothetical activity of PPARδ under different conditions explored by Nakar et al. The black line denotes a hypothesized threshold level of PPARδ transcriptional activity needed for improved endurance.
differences in the genetic backgrounds of the mice, their sex, the particular agonist, the diet, and the environment can influence the outcomes of these experiments in ways that are not well understood.

Interestingly, treatment of hyperlipidemic \( Ldlr^{-/-} \) and \( apoE^{-/-} \) mice with PPAR ligands has relatively small effects on plasma lipid levels and little or no change in total plasma cholesterol is detected even in the studies that reported agonist-dependent decreases in atherosclerosis.\(^5^1\) Therefore, the predominant effect of the PPARs on atherosclerosis does appear to result from changes in plasma lipid levels. A critical event in the development of atherosclerosis is the recruitment of macrophages to the underlying epithelial layer of vessel walls and the uncontrolled uptake of oxidized cholesterol.\(^5^2\) Continued accumulation of oxidized cholesterol by macrophages and an associated inflammatory response leads to foam cell formation and the initiation of atherosclerosis.\(^5^2\) Importantly, all 3 PPAR subtypes are expressed in macrophages and \textit{in vitro} studies indicate that they can modulate cholesterol homeostasis in these cells.\(^3^9,5^1\) The ability of the PPARs to regulate macrophage cholesterol metabolism lies, at least in part, through their ability to control the expression of LXR\(_s\) and subsequent induction of reverse cholesterol transport,\(^5^3\) the process of transporting cholesterol out of cells to HDL (discussed more detail in Section III). PPAR-dependent/LXR-independent pathways that modulate macrophage cholesterol levels have also been identified.\(^5^4\)

E. PPARs and Inflammation

Along with the accumulation of oxidized LDL, inflammation plays a critical role in the development and progression of atherosclerosis.\(^5^2\) Additionally, recent findings indicate a close link between inflammation and insulin resistance.\(^5^5\) Pharmacologic or genetic inhibition of pathways that underlie inflammatory responses protect experimental animals from diet-induced insulin resistance as well as atherosclerosis,\(^5^2,5^6,5^7\) suggesting a direct role of inflammation in the pathology of these diseases. In both diseases, inflammatory responses mediated by macrophages appear to be crucial for disease progression. In atherosclerosis, cytokines secreted by macrophages at the vessel wall promote the recruitment of additional immune cells and the proliferation of smooth muscle cells that contribute atherosclerotic lesion development.\(^5^2\) Macrophages also accumulate in the adipose tissue of obese animals and humans where they produce inflammatory mediators that may contribute to the development of insulin resistance.\(^5^8,5^9\)

Anti-inflammatory activity is a property shared by many members of the nuclear hormone receptor superfamily including the 3 PPAR subtypes and occurs by inhibition of the transcriptional activity of the proinflammatory transcription factors activator protein 1 (AP-1) and nuclear factor kappa B (NF\(\kappa\)B).\(^6^0\) A number of mechanisms have been proposed for this process,
termed transrepression, including direct interactions between PPARs and the p65 subunit of NFκB, induction of the inhibitor of kappa B alpha (IκBα), regulation of c-Jun N-terminal kinase (JNK) activity, competition for limiting transcriptional coactivators and corepressors, and inhibition of corepressor clearance from NFκB regulated promoters. The contribution of chronic inflammation to metabolic disease has led to the idea that the anti-inflammatory activity of the PPARs, particularly in macrophages, may contribute to the beneficial effects of PPAR ligands in animal models of atherosclerosis and insulin resistance. To our knowledge this hypothesis has not yet been tested with either PPAR ligands or PPAR mutants that dissociate the process of activation of transcription from the process transrepression. Such dissociated ligands and receptor mutants have been identified for the glucocorticoid receptor, suggesting that such reagents could be identified for the PPARs. Nevertheless, recent studies discussed below have indicated that macrophages may be critical sites of action for the activity of PPARs.

Resident macrophages in tissues display significant heterogeneity. In obesity classically activated macrophages, also called M1 macrophages, accumulate in adipose and play role in mediating an inflammatory response that contributes to insulin resistance. In lean animals and people, most adipose associated macrophages display an alternatively activated or M2a phenotype. Alternatively, activated macrophages are less inflammatory and appear to play roles in tissue repair. Energy utilization also differs between these two populations of macrophages. Classically activated macrophages predominantly use glucose while a switch to oxidative metabolism is an integral component of alternative activation; linking metabolic control to macrophage phenotype and inflammation. Alternative activation is induced by IL-4 and IL-13 and studies indicating that both PPARγ and PPARδ are induced in IL-4/IL-13 treated macrophages promoted a number of investigators to examine the role of macrophage PPARs in models of diet-induced obesity.

Odegaard et al. and Hevener et al. used selective knockouts and bone marrow transplantations to delete PPARγ in macrophages and observed glucose intolerance and increased insulin resistance in mice exposed to a high fat diet. The Odegaard et al. study specifically explored macrophage phenotype and determined that alternative activation was impaired, suggesting that insulin resistance observed in the absence of PPARγ results from increased inflammation from M1 type macrophages. Hevener et al. additionally demonstrated that the therapeutic activity of rosiglitazone was compromised when macrophage PPARγ was selectively eliminated, indicating that the antiinflammatory activity of PPARγ contributes to the therapeutic activity of TZDs.

Using macrophage selective knockouts similar to those described for PPARγ, Kang et al. and Odegaard et al. demonstrated that PPARδ is required for turning on the gene expression program corresponding to
alternative activated (M2a) macrophages. The consequences of macrophage PPARδ deletion are impaired glucose tolerance and an exacerbation of insulin resistance in response to a high fat diet; once again supporting a role for macrophage inflammation in the pathology of insulin resistance. Based on these studies it appears that both PPARγ and PPARδ play important roles in establishing the alternative activated phenotype. It has been suggested that both receptors must have distinct roles since knockout of either subtype alone is sufficient to impair alternative activation. Nevertheless, the exact function of each receptor in the alternative activation pathway remains to be determined.

The macrophage-selective knockout experiments described earlier suggest that the anti-inflammatory activity of the PPARs may contribute to their therapeutic activity. Several investigators have gone a step further and suggested that specifically targeting PPARs in macrophages with tissue-selective small molecules may be a novel and effective method for treating metabolic disease. The enthusiasm for such approaches, however, must be tempered with the realization that other factors including genetic background, diet, and environment may contribute to the knockout phenotypes. In a separate study, Marathe et al. used bone marrow transplantation approaches to selectively knockout PPARγ and PPARδ in hematopoietic cells either individually or together. These authors concluded that in the C58BL/6 mice the two receptors have little or no impact on the development of diet-induced obesity and insulin resistance and that rosiglitazone is effective in the absence of macrophage PPARγ. The roles for PPARs as important regulators of metabolism are well documented and agonists for PPARα (fibrates) and PPARγ (TZDs) have been validated in humans for the treatment of metabolic disease. Nevertheless, the contribution of macrophage PPARs to the pathology of metabolic disease and the beneficial activity of PPAR agonists remains to be determined.

III. LXR

The LXR subgroup of the nuclear receptor superfamily is comprised of two subtypes, LXRα (NR1H3) and LXRβ (NR1H2) that are encoded by separate genes. The founding member of the subgroup LXRα, was originally cloned from a liver cDNA library, hence the name liver X receptor, and found to be highly expressed in the liver, kidney, and intestine. In contrast, LXRβ is more ubiquitously expressed. Both LXRs bind to DNA and regulate transcription as heterodimers with RXRs with preferred binding to direct repeats of the nuclear receptor half-site AGGTCA separated by four nucleotides (DR4).
A. Regulation of Hepatic Lipid Metabolism by LXR

The first link between LXR and lipid metabolism came from the identification of cholesterol derivatives including 22(R)-hydroxycholesterol, 24(S)-hydroxycholesterol, and 24(S),25-epoxycholesterol as ligands that directly bind to both LXRα and LXRβ and increase their transcriptional activity.\(^\text{16–18}\) More recent studies have also demonstrated that 27-hydroxycholesterol and cholestenoic acid are LXR ligands.\(^\text{76,77}\) The identification of hydroxycholest- ols as natural LXR ligands dovetailed nicely with the characterization of LXRα knockout mice. Apparently, normal under standard laboratory conditions, when challenged with a diet rich in cholesterol Lxrα\(^/-\) mice accumulate massive amounts of cholesterol in the liver. Molecular analysis uncovered aberrant regulation of several genes involved in lipid and cholesterol metabolism including Cyp7a1, which encodes cholesterol 7α hydroxylase, the rate-limiting enzyme in the conversion of cholesterol to bile acids.\(^\text{78}\) Subsequently, the ATP binding cassette transporters ABCG5 and ABCG8 which move cholesterol out of the liver and into the intestine were identified as LXR target genes.\(^\text{79,80}\) Thus, an increase in cholesterol levels is predicted to lead to an elevation in the concentration of cholesterol-derived LXR ligands resulting in the catabolism of cholesterol to bile acid and the excretion of cholesterol out of the liver (Fig. 3). Importantly, Cyp7a1, ABCG5, and ABCG8 all appear to be directly regulated by LXR\(^\text{18,79}\) although the binding site for LXR present in the murine Cyp7a1 gene is not conserved in the human gene.\(^\text{18}\)

Along with effects on cholesterol metabolism activation of LXR agonists also increases expression of genes involved in fatty acid metabolism including the master transcriptional regulator of fatty acid synthesis, the sterol response element binding protein 1c (SREBP1c)\(^\text{81,82}\) (Fig. 3). Additionally, several of the genes encoding the enzymes involved in fatty acid metabolism including fatty acid synthase (FAS) and stearoyl CoA desaturase 1 (SCD-1) are regulated directly or indirectly by LXR.\(^\text{83–85}\) The coordinate upregulation of fatty acid synthesis with reverse cholesterol transport is most likely to provide lipids for the transport and storage of cholesterol.

B. Regulation of Reverse Cholesterol Transport by LXR

Based on the defined role for LXR in hepatic cholesterol catabolism and excretion one might have expected that synthetic LXR agonists would lower plasma cholesterol levels. Quite surprisingly, however, treatment of animals with LXR agonists significantly elevates HDL cholesterol.\(^\text{82}\) Gene expression analysis in the livers and intestines of LXR agonist-treated mice identified ABCA1 as a direct LXR gene and this discovery stimulated great interest in the therapeutic potential of LXR agonists given the links between ABCA1, HDL metabolism, and atherosclerosis.\(^\text{86–88}\) ABCA1 is required for the process
of reverse cholesterol transport whereby cells efflux internal cholesterol to acceptor proteins on pre-β-HDL particles. Loss of functional ABCA1 results in Tangier disease, a condition in which patients have extremely low levels of circulating HDL and an increased risk for developing atherosclerosis. Examination of fibroblasts isolated from subjects with Tangier disease reveals that ABCA1 defective cells are unable to efflux cholesterol, suggesting that the low HDL levels and increased risk of atherosclerosis results from a loss of reverse cholesterol transport. Historically, Tangier disease patients present with large accumulations of cholesterol-laden macrophages in their lymph tissues, highlighting the role of ABCA1 and reverse cholesterol transport in macrophage cholesterol homeostasis.\textsuperscript{43,59,90} As described in Section II.D, accumulation of oxidized LDL cholesterol by macrophages in the arterial wall is an initiating step in the development of atherosclerotic lesions\textsuperscript{32} and recent studies with mouse knockouts of ABCA1 further support a link between reverse cholesterol transport and atherosclerosis.\textsuperscript{91-93} In support of the role of LXR as a direct regulator of ABCA1 expression and activity, treatment of primary macrophages
or cell lines with LXR agonists results in induction of the ABCA1 gene, increase levels of ABCA1 protein, and an increase in cholesterol efflux. A binding site for LXR–RXR heterodimers in the ABCA1 promoter has also been described. Subsequent studies identified other proteins involved in the reverse cholesterol transport including ABCG1, ABCG4, and apoE as direct LXR target genes. Thus, activation of LXR results in the mobilization of cholesterol in the periphery and stimulates the catabolism and excretion of cholesterol when it arrives in the liver (Fig. 3). Interestingly, genetic deletion of LXR activity in mice (Lxrα−/−/Lxrβ−/−) results in the accumulation of cholesterol-laden macrophages and splenomegaly similar to that observed in Tangier disease patients.

C. LXR and Atherosclerosis

The accumulation of oxidized LDL cholesterol by macrophages in blood vessel walls is an early event in the pathogenesis of atherosclerosis and it had long been suggested that reversing this process by pumping cholesterol out of macrophage foam cells would have an inhibitory effect on the progression of atherosclerosis. The ability of LXR to directly regulate reverse cholesterol transport in macrophages allowed two experiments to be carried out to test this hypothesis. First, transplantation of lethally irradiated apoE−/− and Ldlr−/− mice with bone marrow from wild type or Lxrα−/−/Lxrβ−/− mice demonstrated that genetic deletion of LXR leads to an increase in atherosclerosis is these well established mouse models. Second, treatment of apoE−/− and Ldlr−/− mice with synthetic LXR agonists leads to a reduction in atherosclerosis. Together the combination of genetic analysis and pharmacology clearly demonstrated the antiatherogenic activity of LXR. Not surprisingly, the mRNA levels for LXR target genes including ABCA1 and apoE are elevated in the atherosclerotic lesions of mice treated with LXR agonists. Subsequent studies combining bone marrow transplantation with the administration of synthetic LXR agonists have demonstrated that LXR activity in macrophages is necessary for the antiatherogenic effect of LXR ligands.

D. LXR and Inflammation

It is easy to assume that the ability of LXR to regulate lipid metabolism and reverse cholesterol transport provides the mechanistic basis for the antiatherogenic activity of LXR. Experiments using cultured macrophages, however, also demonstrate that LXR agonists can inhibit the expression of several proinflammatory genes including iNOS, COX-2, and MMP-9 and these compounds are effective in a murine model of irritant contact dermatitis.
Molecular studies indicate that activation of LXR decreases the transcriptional activity of NFκB using many of the same mechanisms described for the PPARs (see Section II.E).104

Since atherosclerosis is considered an inflammatory disease52 the question remains whether LXR mediates its antiatherogenic activity via control of reverse cholesterol transport, by limiting the inflammatory response, or both. Future studies that combine genetically altered macrophages (i.e., Abca1<sup>−/−</sup>) introduced by bone marrow transplantation along with the administration of LXR agonists can be used to define the individual contributions of reverse cholesterol transport and anti-inflammatory activity to therapeutic effects of LXR ligands. Additionally, studies with the glucocorticoid receptor have shown that it is possible to identify nuclear receptor ligands that repress inflammatory genes but do not activate positively regulated glucocorticoid receptor target genes.61,62 One expects that such dissociated ligands will also be identified for LXR.

A number of studies have suggested a link between viral or bacterial infections and atherosclerosis.105 In support of this hypothesis enhanced expression of Toll-like receptors (TLR), which mediate the innate immune response to invading pathogens via stimulation of proinflammatory pathways, has been detected in human atherosclerotic lesions.106 Interestingly, studies by Castrillo et al.107 indicate that activation of TLR4 inhibits the transcriptional activity of LXR and the ability of macrophages to efflux cholesterol. The cross talk between TLR4 signaling and LXR activity suggests one potential mechanistic basis for the impact of infectious agents on cardiovascular disease.

Along with effects in macrophages, recent studies have identified an important and specific role for LXRβ in T cell proliferation.108 T cell activation triggers induction of the oxysterol-metabolizing enzyme SULT2B1 and suppression of reverse cholesterol transport by decreasing the availability of endogenous LXR agonists. Consistent with a role for LXRβ in T cell proliferation is the observation that knockout of LXRβ confers a proliferative advantage while binding of agonists to LXRβ during T cell activation inhibits mitogen-driven expansion. LXRα is not expressed in lymphocytes indicating that this activity is subtype selective.108 In a coordinate fashion, the SREBP2 dependent pathway for cholesterol synthesis is activated108 (Fig. 4). SREBP2 is the major transcriptional regulator of proteins required for cholesterol uptake and synthesis including the LDL receptor and HMG-CoA reductase, the rate limiting enzyme in cholesterol biosynthesis.109 This coupling of decreased LXRβ activity with activation SREBP2 insures that intracellular cholesterol levels are sufficient to support cell growth and the rapid expansion of the T cell population upon stimulation. Interestingly, inactivation of the cholesterol transporter ABCG1 attenuates the ability of LXR agonists to inhibit proliferation, directly linking cholesterol homeostasis to the antiproliferative action
Mice lacking LXRβ also exhibit lymphoid hyperplasia and enhanced responses to antigenic challenge, indicating that proper regulation of LXR-dependent sterol metabolism is important for immune responses. These results implicate LXRβ signaling in a metabolic checkpoint that modulates cell proliferation and immunity.

**E. LXR and Diabetes**

Treatment of experimental animals with LXR agonists leads to increases in hepatic fatty acid synthesis and plasma triglyceride levels. Since elevations in fatty acids have been linked to insulin resistance and type II diabetes several investigators have examined the cross talk between LXR activity and glucose metabolism. Interestingly, along with upregulating fatty acid synthesis, activation of LXR also represses expression of the genes encoding the enzymes of gluconeogenesis in the liver including phosphoenolpyruvate carboxy kinase (PEPCK) and glucose 6-phosphatase and induces expression of GLUT4 in adipose tissue. Thus, in many ways activation of LXR mimics treatment with insulin. Perhaps not surprisingly in light of this “insulin-like” activity, LXR ligands decrease hepatic glucose output and lower blood glucose levels in animal models of type II diabetes. The observation, however, that LXR...
ligands can behave as insulin sensitizers even in face of relatively large increases in plasma triglyceride levels suggests the possibility of a broader role for LXR in regulating glucose homeostasis. Indeed, Mitro et al.\textsuperscript{112} have demonstrated that glucose can directly bind to LXR with relatively weak affinity (millimolar) and function as an agonist. Thus, LXR may directly sense glucose and regulate carbohydrate levels in manner similar to the control of cholesterol metabolism. The observation that LXR is also active in skeletal muscle\textsuperscript{113} and that $Lxr^{-/-}/Lxr^{-/-}$ mice are resistant to diet-induced obesity\textsuperscript{114} further supports a role for the LXR as important coordinators of energy metabolism.

\textbf{F. Therapeutic Potential of LXR Ligands}

The antiatherogenic, anti-inflammatory, and antidiabetic activities of LXR agonists in animal models have highlighted the therapeutic potential of LXR and LXR$\beta$. Nevertheless, the link between LXR activity and triglyceride metabolism has clearly dampened the enthusiasm surrounding this target class. Treatment of mice and hamsters with synthetic LXR agonists results in a significant increase in plasma triglyceride levels\textsuperscript{82} and elevations in LDL cholesterol have been observed in nonhuman primates.\textsuperscript{115} Treatment of patients with a drug that raises lipids is not a viable option for the treatment of metabolic diseases and approaches to separate the beneficial activities of LXR ligands from unwanted side effects need to be explored. Furthermore, studies in human cells have shown that LXR agonists also increase expression of the gene encoding the cholesterol ester transfer protein (CETP).\textsuperscript{116} CETP functions to transfer cholesterol esters from HDL to apolipoprotein B containing lipoprotein particles and CETP activity has been shown to inversely correlate with atherosclerosis.\textsuperscript{117–119} Indeed, CETP inhibitors are currently being explored for the treatment of atherosclerosis.\textsuperscript{118}

Interestingly, defects in hepatic cholesterol metabolism are detected in $Lxr^{-/-}$ single knockout mice indicating that LXR$\beta$ is not functionally redundant with LXRz.\textsuperscript{78} In contrast, cholesterol and triglyceride levels appear normal in $Lxr^{-/-}$ mice suggesting that LXRz mediates most, if not all, of the effects of LXR ligands on triglyceride metabolism.\textsuperscript{120} The relatively low level of LXR$\beta$ in the liver most likely accounts for lack of functional redundancy in this tissue. Nevertheless, in macrophages either LXRz or LXR$\beta$ alone appears to be sufficient to mediate the effects of LXR ligands on reverse cholesterol transport and inflammatory gene expression. Additionally, a recent study by Bradley et al.\textsuperscript{121} indicates that the antiatherogenic activity of LXR agonists are maintained in $apoE^{-/-}/Lxr^{-/-}$ double knockout mice, suggesting that LXR$\beta$ alone is sufficient to limit atherosclerosis. Taken together these observations have led several investigators to suggest that LXR$\beta$-selective ligands may provide a
mechanistic basis for identification of LXR ligands with improved therapeutic profiles. The enthusiasm for LXR\textsubscript{a}\textemdash\textminus\textsubscript{selective} ligands must be tempered with the realization that the spectrum of activities measured in the complete absence of LXR\textsubscript{a} activity may differ when a subtype selective synthetic ligand is used. Additionally, the observation that the ligand binding pockets of LXR\textsubscript{a} and LXR\textsubscript{b} defined by crystallography\textsuperscript{123\textendash126} differ by only one amino acid suggests that identification of selective ligands may not be simple.

While the therapeutic activity of LXR\textsubscript{b}\textemdash\textminus\textsubscript{selective} ligands is still an open question, it has been possible to identify ligands for other nuclear receptors that exhibit a restricted set of activities and therefore allow the separation of beneficial therapeutic activities from unwanted side effects. Perhaps the best examples of such compounds are the selective estrogen receptor modulators such as roloxifene that function as estrogen receptor agonists in some tissues and estrogen receptor antagonists in others. More recently, synthetic ligands for PPAR\textsubscript{\gamma} have been identified that appear to separate the insulin sensitizing activity of PPAR\textsubscript{\gamma} from unwanted effects on weight gain. A common feature of all these selective receptor modulators is that they appear to function as partial or weak agonists when characterized \textit{in vitro}. When bound to receptors selective modulators produce unique conformational changes that cannot be achieved by more typical agonists. The outcome of these unique conformations is an alteration in interactions between receptors and the down-stream coregulator proteins that mediate the transcriptional response leading to ligand\textemdash specific effects on gene expression. Since the LXRs function in multiple tissues to mediate effects on lipid metabolism, glucose homeostasis, and inflammation we expect that the identification of selective LXR modulators will yield compounds with beneficial therapeutic activities.

IV. FXR

FXR\textsubscript{a} (NR1H4), like the PPARs and LXRs, was originally identified as an orphan member of the nuclear hormone receptor superfamily and subsequently shown be activated by the direct binding of farnesol. FXR binds to DNA as an obligate heterodimer with RXR and prefers to bind to inverted repeats of the nuclear receptor half-site AGGTCA separated by four basepairs (IR4). A second FXR subtype, FXR\textsubscript{b} (NR1H5), has been identified in rodents, rabbits, and dogs but is a pseudogene in humans and primates. FXR\textsubscript{b} will not be discussed in this review and we will refer to FXR\textsubscript{a} as simply FXR. The expression pattern of FXR is relatively restricted to the liver, intestine, kidney, and adrenal gland. Low levels of expression of the FXR mRNA have also been reported in adipose tissue, heart, and smooth muscle cells, however, it is not clear if FXR is functionally active in the latter three
locations. Hepatic mRNA levels of FXR are elevated by prolonged fasting and by overexpression of the transcriptional coactivator PGC-1α. PGC-1α is an important transcriptional regulator of hepatic gluconeogenesis and energy metabolism suggesting that FXR may play a role in the response to nutritional status.

A. FXR and the Control of Bile Metabolism

A number of studies have demonstrated that bile acids such as chenodeoxycholic acid (CDCA), deoxycholic acid (DCA), and cholic acid (CA) bind directly to the FXR and function as activators of FXR-regulated genes. Importantly, FXR activation by bile acids leads to downregulation of Cyp7a1, the gene encoding the rate limiting enzyme (cholesterol 7α-hydroxylase) in the conversion of cholesterol to bile acids. Inhibition of Cyp7a1 by FXR occurs indirectly via the FXR-dependent upregulation of the small heterodimeric partner (SHP, NR0B2) a transcriptional repressor (Fig. 5). SHP lacks the canonical DBD found in most other members of the nuclear receptor superfamily but can dimerize with other receptors via a LBD. SHP interacts strongly with corepressors proteins so dimerization with SHP generally leads to

![Diagram of FXR and bile acid synthesis regulation](image)

**Fig. 5.** Regulation of bile acid synthesis by FXR. In the liver, bile acids act as FXR agonists leading to the induction of SHP, a transcriptional repressor. SHP strongly represses the transcription of CYP8B1 and relatively weakly represses CYP7A1. The net result of activating FXR in the liver is a change in bile acid composition toward the more hydrophobic bile acids MCA (mice) or CDCA (humans). MCA and CDCA poorly promote the absorption of cholesterol in the intestine relative to CA. In the intestine, activation of FXR increases the expression of FGF19 which acts in a paracrine fashion to strongly inhibit CYP7A1 in the liver and an overall decrease in bile acid synthesis. Bile acids resins sequester bile acids in the intestine, decreasing FXR activity, reducing the levels of FGF19, and consequently upregulating the levels of CYP7A1. See text for details.
an inhibition of transcription.\textsuperscript{140} In the context of Cyp7a1, SHP dimerizes with and inhibits the liver receptor homolog 1 (LRH1, NR5A2), a third nuclear hormone receptor that functions as a constitutive activator of Cyp7a1.\textsuperscript{139} SHP also inhibits expression of the Cyp8b1 gene encoding sterol 12α-hydroxylase.\textsuperscript{141,142} Sterol 12α-hydroxylase sits at a branch point in the bile acid synthetic pathway that determines the polarity of primary bile acids (Fig. 5). When Cyp8b1 is active CA is the primary product; when Cyp8b1 is inactive CDCA (humans) and muricholic acid (MCA) (mice) are the predominant species.\textsuperscript{143}

Along with regulation of bile acid synthetic enzymes via control of SHP expression, FXR induces the gene encoding FGF19 (FGF15 is the mouse ortholog) which also functions to inhibit expression of Cyp7a1\textsuperscript{144,145} (Fig. 5). FXR19 is expressed in the intestine, not the liver, suggesting that intestinal FXR senses the level of bile acids at this site and regulates hepatic bile acid synthesis in an endocrine fashion via the production of FGF19.\textsuperscript{144,145} FGF19 binds to FGF receptor 4 on the surface of hepatocytes and appears to inhibit Cyp7a1 by activating a JNK-dependent\textsuperscript{145} or mitogen activated kinase (MAP)-dependent\textsuperscript{146} phosphorylation cascade. FGF19 also stimulates the normal refilling of the gallbladder with bile acids after cholecystokinin-dependent emptying.\textsuperscript{147} Taken together these results suggest that when bile acid levels are high in the intestine that FXR functions to inhibit further synthesis and stimulate gallbladder filling. Interestingly, tissue-specific knockouts of FXR indicate that the intestine is the dominant site for FXR-dependent regulation of Cyp7a1 while the liver is the dominant site for regulation of Cyp8b1.\textsuperscript{148} Thus, activation of FXR in the intestine leads to an inhibition of bile acid synthesis while in the liver activation of FXR would primarily impact the composition of bile acids produced (Fig. 5). The consequence of altering bile acid composition on lipid metabolism will be discussed further below.

B. Gallstones, Cholestasis, and Bacterial Growth

Bile comprised of bile acids, phospholipids, and cholesterol is stored in the gallbladder and released upon feeding. An excess of cholesterol to bile acids in the bile leads to the precipitation of cholesterol and formation of cholesterol gallstones. According to the American Gastroenterological Association, almost 24 million Americans suffer from gallstones and the condition can be quite painful. Along with controlling bile acid synthesis, FXR also regulates genes required for the hepatic uptake, conjugation, and excretion of bile acids.\textsuperscript{149–152} The bile salt export pump (BSEP), multidrug resistant-associated protein 2 (MRP2, ABCC2), and the multidrug resistance P-glycoprotein 3 (MDR3, ABCB4) are localized on the canalicular membrane of hepatocytes and secrete bile acids from hepatocytes into the bile canaliculi.\textsuperscript{133} These three transporters are regulated directly by FXR and induced by FXR agonists.\textsuperscript{132,133} Moschetta et al.\textsuperscript{153} demonstrated that treatment with the synthetic FXR agonist
GW4064 reduces the number of gallstones in a gallstone susceptible strain of mice. The beneficial effect of the FXR agonist most likely arises from increased expression of the canalicular bile acid transporters and increased transport of bile acids into the gallbladder. In contrast, FXR knockout mice are more susceptible to developing gallstones than FXR\textsuperscript{+/+} mice when placed on a lithogenic diet.\textsuperscript{153} The current treatment for gallstones is surgical removal of the gallbladder (cholecystectomy) raising the possibility that FXR agonists could provide a novel nonsurgical treatment for this disease.

Cholestasis is defined as any condition that impairs the flow of bile acids out of the liver and is many times associated with liver damage. Generally, cholestatic diseases are defined as obstructive (the flow of bile is physically blocked) or nonobstructive.\textsuperscript{154,155} Not surprisingly, GW4064 and 6-ethyl CDCA, a derivative of CDCA that is a potent FXR agonist, has proven effective in several models of drug induced nonobstructive cholestasis.\textsuperscript{156–158} The activity of FXR agonists in these models most likely arises from their ability to induce the transport of bile acids out of the liver and to reduce further bile acid synthesis. Inflammation and fibrosis are commonly associated with liver damage and FXR agonists also reduce the expression of markers of fibrosis and inflammation perhaps via induction of the transcriptional repressor SHP as described for Cyp7a1.\textsuperscript{159–161} As we have seen with the PPARs and LXR\textsubscript{s}, regulation of metabolism by FXR appears to be intimately linked with the control of inflammation.

Obstruction of bile flow is also associated with intestinal bacterial growth and injury to the intestinal mucosa. Fxr\textsuperscript{-/-} mice have bacterial overgrowth in the ileum and compromised epithelial barrier most likely resulting from impaired bile flow. On the other hand, FXR activation induces a number of genes in the intestine involved in enteroprotection including angiogenin, nitric oxide synthase, and IL-18.\textsuperscript{162}

C. FXR and Lipid Metabolism

Clinical trials examining the utility of bile acids for the treatment of cholesterol gallstones first demonstrated that increasing bile acid levels leads to a corresponding decrease in plasma triglycerides.\textsuperscript{163–165} The observation that Fxr\textsuperscript{-/-} mice have elevated triglyceride levels\textsuperscript{166} and that a synthetic FXR agonist lowers triglycerides in rats\textsuperscript{167} supports an essential role for FXR in mediating the effects of bile acids on triglyceride metabolism. Indeed, FXR has been identified as a direct regulator of a number of genes encoding proteins involved in triglyceride synthesis and catabolism including apoCII, apoCIII, apoAV, FGF19, SREBP1c, syndecan 1, the VLDL receptor, complement C3/acylation stimulating protein, Insig-2, and PPAR\textsubscript{z}.\textsuperscript{144,150,151,168–172} The contribution of these different mechanisms to FXR-dependent control of triglycerides remains to be determined.
While a role for FXR in the control of triglyceride metabolism appears to be well established, the effect of FXR on cholesterol homeostasis is less clear. Repression of Cyp7a1 by FXR should inhibit the conversion of cholesterol to bile acids resulting in an increase in cholesterol levels (Fig. 5). In support of this prediction, humans with a genetic deficiency in CYP7A1 present with elevated cholesterol levels. Nevertheless, no evidence for increased cholesterol was observed in humans treated with bile acids and genetic knockout of FXR results in an increase in cholesterol levels; not the expected decrease predicted if feedback inhibition of Cyp7a1 is eliminated. Similarly, studies in animals and humans have shown that bile acids repress the production of VLDL. As described earlier, activation of FXR in the liver will strongly inhibit Cyp8b1 expression. Sterol 12α-hydroxylase, the enzyme encoded by Cyp8b1, sits at a branch point in the bile acid synthetic pathway and its enzymatic activity is required for the synthesis of CA. The parallel arm in the pathway leads to synthesis of MCA in mice and CDCA in humans. Thus, modulating expression of Cyp8b1 expression alters bile composition (Fig. 5). Importantly, individual bile acids differ in their ability to promote intestinal cholesterol absorption and MCA, of all bile acids tested, promotes the lowest amount of cholesterol absorption while CA promotes the greatest amount. CDCA is in between CA and MCA. Thus, activating FXR in the liver should decrease cholesterol absorption in the intestine. On the other hand, eliminating FXR activity in the liver with antagonists or by genetic knockout should increase cholesterol absorption by the intestine (Fig. 5). Thus, we would argue that many of the effects of modulating FXR activity on lipid levels may result from influencing the absorption of fat and cholesterol in the intestine.

D. FXR and Atherosclerosis

The ability of FXR to control triglyceride and cholesterol metabolism suggests that activating or inhibiting this receptor should have significant effects on the development and progression of atherosclerosis. Indeed, treatment of apoE−/− mice with 6-ethyl CDCA, a derivative of CDCA that functions as an FXR agonist, reduces atherosclerosis. Based on this data and on studies demonstrating elevated triglyceride and cholesterol levels in Fxr−/− mice, one would predict that eliminating FXR activity in a proatherosclerotic mouse background would increase atherosclerosis. The predicted result of increased atherosclerosis in apoE−/−/Fxr−/− mice was observed in one published study. Two other studies, however, one using apoE−/−/Fxr−/− mice and the other using Ldlr−/−/Fxr−/− mice detected decreased atherosclerosis compared to apoE−/− and Ldlr−/− controls. Interestingly, both of the studies that measured decreased atherosclerosis detected changes in plasma lipid levels that one would expect to actually promote atherosclerosis. Both studies also detected decreased expression of CD36 in macrophages isolated
from double knockout mice. CD36 is a scavenger receptor responsible for the uptake of oxidized cholesterol by macrophages and decreased expression would be expected to reduce atherosclerosis by limiting macrophage foam cell development. FXR is not expressed in macrophages and both studies suggest that alterations in lipid profiles that result from deleting FXR may influence the expression of CD36 in peripheral cells. In the study using the \( Ldlr^{-/-} \) background, the authors also detected a significant increase in VLDL cholesterol and decreases in LDL and HDL. This change in lipoprotein particle profile, particularly the decrease in LDL, may also contribute to the decrease in atherosclerosis observed in this study. The different results seen among the various atherosclerosis studies again highlight the care that needs to be taken in interpreting such experiments. Furthermore, these studies clearly illustrate that it can be difficult to predict the activity of a nuclear receptor agonist or antagonist from the phenotype of a knockout.

E. FXR and Diabetes

Analysis of \( Fxr^{-/-} \) mice has demonstrated impaired glucose tolerance and insulin resistance in the liver and in peripheral tissues (muscle and fat). The effects on insulin resistance most likely arise from the elevated fatty acid levels observed in these mice. Similarly, the synthetic FXR agonist GW4064 improves insulin sensitivity in several diabetic models including \( db/db \), \( ob/ob \), and KK-A(\( y \)) mice. Infection of these mice with an adenovirus expressing a “super-active” VP16–FXR construct also improves insulin sensitivity suggesting that the liver is the major site of FXR activity with regards to diabetes. Examination of these diabetic models suggests that activation of FXR reduces hepatic gluconeogenesis and increases glycogen synthesis thus reducing plasma glucose levels. The effects of FXR on gluconeogenesis and glycogen synthesis are somewhat surprising given the report that FXR is induced by prolonged fasting, a condition when gluconeogenesis would be expected to be high and glycogen synthesis low. The mechanistic basis for these effects remains to be determined.

F. Control of Liver Regeneration and Tumorigenesis by FXR

Recent studies indicate that deletion of FXR in mice impairs the process the liver regeneration after hepatectomy and increases the incidence of hepatocellular adenoma, carcinoma, and hepatoblastocellular carcinoma in older (12 months) animals. Opposite results are observed when wild-type mice are fed diets enriched in bile acids; however, to our knowledge...
synthetic agonists have not been examined in these models. It appears that the ability of FXR to control genes involved in inflammation and cell cycle control accounts for these observations. \(^{186-188}\)

**G. Therapeutic Potential of FXR Ligands**

Studies in humans with bile acid resins such as cholestyramine that absorb bile acids indicate that depletion of bile acids decreases cholesterol levels. \(^{189}\) The activity of bile acid resins most likely results from the upregulation of \(\text{CYP7A1}\) and increased catabolism of cholesterol to restore the bile acids that have been removed (Fig. 5). While these resins are an effective means to lower cholesterol compliance is generally poor. Bile acid resin powders must be mixed with water or fruit juice and taken once or twice daily with meals. Tablets must be taken with large amounts of fluids to avoid gastrointestinal symptoms. Resin therapy may also produce a variety of side effects including constipation, bloating, nausea, and gas. The observation that FXR controls the bile acid-dependent repression of \(\text{CYP7A1}\) suggested to many that an FXR antagonist would block bile acid-dependent repression of \(\text{CYP7A1}\) and produce the benefits of a bile acid resin without unwanted side effects. \(^{132}\) Guggulsterone, the active ingredient of a naturally occurring medicinal cholesterol lowering agent isolated from the guggul tree, has been reported to be an FXR antagonist. \(^{190}\) The putative cholesterol-lowering activity of guggulsterone is consistent with the predicted activity of an FXR antagonist. Guggulsterone, however, appears to have multiple biological targets and it is not clear if any of the physiological effects of this compound are derived from inhibiting FXR. \(^{190-192}\) In contrast, all the activities associated with FXR agonists including lowering lipid levels, decreasing atherosclerosis, improving insulin sensitivity, and hepatoprotection appear to be beneficial. We would suggest that the apparent disconnect between the activities of bile acid resins (upregulate \(\text{CYP7A1}\), lower cholesterol) and FXR agonists (downregulate \(\text{CYP7A1}\), lower cholesterol) lies in the site of the action of these two agents. Bile acid resins function in the intestine and remain there for extended periods. Depleting bile acids in the intestine will decrease the expression of \(\text{FGF19}\), relieve the feedback inhibition on \(\text{CYP7A1}\), and promote the breakdown of cholesterol (Fig. 5). In contrast, the synthetic agonists tested are relatively rapidly absorbed into the bloodstream and accumulate in the liver. In the liver, activation of FXR will induce SHP and strongly inhibit expression of \(\text{CYP8B1}\) shifting bile acid composition toward MCA/CDCA and limiting cholesterol absorption in the intestine (Fig. 5). Activation of FXR in the liver has the added benefits of promoting bile flow, and protecting against tissue damage. Thus, all the preclinical studies suggest that FXR agonists have the ideal therapeutic profile for the treatment of human disease. Currently, 6-ethyl CDCA is in phase II clinical trial for the
treatment of cholestasis and Wyeth has completed a Phase I clinical study of a synthetic FXR agonist (information on ClinicalTrials.gov). The data from these studies are not currently available.

V. RORα

The RAR-related orphan receptor (ROR) family consists of three members (RORα, NR1F1; RORβ, NR1F2; and RORγ, NR1F3) that bind to DNA as monomers to regulate transcription. ROR response elements (ROREs) consist of a six basepair A/T rich region immediately preceding a half site AGGTCA motif. RORs are constitutively active transcription factors which recruit coactivators in the absence of exogenous ligand. Nevertheless, crystal structures suggest that these orphan receptors can bind and be regulated by ligands. All-trans retinoic acid was crystallized with LBD of RORβ and can inhibit RORβ transcriptional activity in cells, suggesting a possible role of RORβ in regulating retinoid action. Interestingly, a structure of RORz obtained using baculovirus-expressed protein unexpectedly identified cholesterol as a ligand for RORz. This suggests that cholesterol or derivatives of cholesterol might function as physiological ligands for RORz. Based on this structure, point mutations of RORz that prevent cholesterol binding were generated and the ability of these mutants to activate a reporter gene with an RORE was impaired. Further confirmation of cholesterol as a RORz ligand was provided by decreased RORz activity in cells treated with lovastatin or hydroxypropyl-B-cyclodextrin to lower intracellular cholesterol. Under these cholesterol-depleted conditions, adding back cholesterol restores RORz activity. Although it cannot be ruled out that the effect of cholesterol is indirect, these results demonstrate that RORz activity can be modulated by cholesterol status in cells. Thus, RORz may act as a lipid sensor of the intracellular cholesterol levels and play an important role in the regulation of cholesterol homeostasis.

A. Regulation of Lipid Metabolism by RORs

RORz is highly expressed not only in blood, brain, skin, intestine but also in metabolically important tissues such as liver, muscle, and adipose tissue. The physiological function of RORz in vivo has been revealed using staggerer mice (RORz<sup>sg/sg</sup>). These mice contain a 122-bp deletion in the coding sequence of RORz that prevents translation of a functional LBD. The most obvious defect in RORz<sup>sg/sg</sup> mice is their balance deficit due to massive neurodegeneration in the cerebellum, caused by a developmental defect in Purkinje cells.
This cerebellar atrophic phenotype was also found in RORz knockout (RORz<sup>−/−</sup>) mice, providing validation that the RORz<sup>−/−</sup> defects are due to the functional disruption of RORz gene.<sup>205</sup>

The role of RORz in lipid homeostasis and atherosclerosis/obesity was uncovered by the observation of metabolic abnormalities in RORz<sup>−/−</sup> mice. RORz<sup>−/−</sup> mice are smaller than wild type despite hyperphagia and have lower total and HDL cholesterol levels. The decrease in HDL is thought to arise from reduced plasma ApoA-I and ApoA-II, both major apolipoproteins of HDL, in these mice and from decreased expression of ApoA-I in the intestine, previously shown to be a direct target gene for RORz.<sup>206</sup> The reverse cholesterol transporters ABCA1 and ABCG1 genes are also downregulated in the liver from RORz<sup>−/−</sup> mice.<sup>207</sup> Not surprisingly given the low levels of HDL, when fed with an atherogenic diet RORz<sup>−/−</sup> mice are more susceptible to develop atherosclerosis compared to wild-type mice.<sup>208</sup>

Plasma triglycerides levels from RORz<sup>−/−</sup> mice are reduced and this is correlated with decreased hepatic and intestinal expression of ApoC-III, a component of HDL and VLDL that regulates triglyceride levels and is also a RORz target gene.<sup>209</sup> Interestingly, a recent genome-wide association study indicated that individuals who are heterozygous for a null mutation in ApoC-III have low plasma triglycerides, a favorable cholesterol profile and decreased risk for cardiovascular disease.<sup>210</sup> Consistent with the lowering of plasma triglycerides, liver triglycerides are also found to be decreased in RORz<sup>−/−</sup> mice.<sup>207</sup> Examination of genes involved in lipogenesis in the liver show that SREBP-1c and FAS expression are reduced with little change in LXR expression, suggesting factors other than LXR may be involved in regulating expression of these genes. Using transfection and chromatin immunoprecipitation assays, RORz was shown to bind to the SREBP-1c promoter and modulate its activity. Thus, the decreased triglyceride levels detected in staggerer mice may be a direct result of RORz deficiency.

Perhaps related to the defect in triglyceride metabolism, RORz<sup>−/−</sup> mice have reduced adiposity associated with decreased fat pad mass and adipocyte size<sup>207</sup> and are resistant to diet-induced weight obesity. Compared to wild-type mice, there is a lack of fat accumulation in the gonadal and inguinal white adipose and interscapular brown adipose of RORz<sup>−/−</sup> mice on high fat diet.<sup>207</sup> These results further demonstrate the importance of RORz in lipogenesis in the adipose tissue and liver.

In muscle, overexpression of a dominant negative RORz that lacks the entire LBD and part of the hinge region results in a reduced expression of SREBP-1c and other lipogenic genes (FAS, SCD-1, SCD-2). Similarly, genes involved in lipid and cholesterol efflux and homeostasis (ABCA1, apoE, CAV3) are repressed in these cells versus the wild-type cells. Other genes involved in lipid absorption (CD36, FABP3), lipid catabolism (LPL, CPT1, ACS4, adipor1, REGULATION OF METABOLISM BY NUCLEAR RECEPTORS 27
adipoR2, PGC-1), lipid storage (ADRP) are also downregulated. Collectively, these in vivo and in vitro studies demonstrate that RORα is an important modulator of lipid homeostasis in the intestine, liver, adipose tissue, and muscle.

The involvement of the other two ROR family members, RORβ and RORγ, in lipid metabolism is less clear. RORβ expression is restricted mostly to brain and retina and expressed poorly in other tissues such as liver, muscle, and adipose tissue. RORγ is highly expressed in muscle and its function in lipid and glucose metabolism has been examined using the in vitro muscle cell model. In these studies, although expression of a dominant negative RORγ that lacks AF-2 domain significantly represses RORγ-dependent gene expression, genes involved in lipid metabolism are not affected. Similarly, RORγ-deficient mice have normal triglycerides and cholesterol levels. Interestingly, serum glucose levels are significantly lower in RORγ-deficient mice but normal in staggerer mice, suggesting that RORs have different roles in lipid and glucose homeostasis.

Rev-erbα is an orphan nuclear receptor which can bind ROREs and act as a dominant-negative repressor of RORs by competing for the same DNA binding sites. Consistent with the idea that RORs and Rev-erbα compete for binding sites is the observation that Rev-erbα can repress ApoA-I and ApoC-III gene expression in HepG2 cells and primary hepatocytes. In Rev-erbα-deficient mice, serum triglycerides are elevated and this is associated with the increases in serum apoC-III concentration and liver apoC-III RNA levels. Since RORs and Rev-erbs are coexpressed in many tissues, the effects of these receptors on lipid metabolism are likely to be determined by the ratio of their expression levels, which can be altered by diet or circadian rhythm (see below).

B. Role of RORs in Circadian Rhythm Control and Links to Metabolism

There is increasing evidence suggesting that regulation of the biological clock is linked to energy metabolism. Mutation of the CLOCK (Circadian locomotor output cycles kaput) gene, a key transcription factor of the molecular circadian clock, results in a phenotype of metabolic disease in mice. Homozygous Clock mutant mice lose the diurnal rhythm in food intake and their metabolic rates are decreased. They gain more weight than wild-type mice and have higher triglycerides, cholesterol, and glucose in the serum and accumulation of fat in the liver, all hallmarks of metabolic syndrome. Conversely, a high fat diet which induces the same metabolic phenotypes also alters the cyclic expression of genes important for maintaining circadian rhythm, emphasizing the interrelationship of metabolic homeostasis and molecular circadian clock.

Genomic profiling of suprachiasmatic nuclei (SCN) and liver has identified an important role of the RORE in gene expression during circadian cycle. This implies that RORs and Rev-erbα may be important transcriptional circadian
Indeed, RORs and Rev-erbs display circadian expression in the SCN and peripheral tissues important for metabolism (liver, muscle, WAT, BAT).\textsuperscript{202,220} In these tissues, Rev-erbs and Rev-erb\(\beta\) show similar circadian pattern with highest level at CT (circadian time) 4–8 and lowest at CT16–20. Expression of different ROR isoforms is more tissue-specific. In the SCN, ROR\(\gamma\) is not detected and ROR\(\beta\) expression is high but shows little oscillation whereas ROR\(\alpha\) expression is cyclic with highest level at CT4–12. In the WAT, ROR\(\alpha\) expression peaks at CT12, right after the peaks of Rev-erbs expression. In the liver and BAT, ROR\(\gamma\) expression reaches its maximal level at CT16 and minimal level at CT4, showing an antiphase pattern to Rev-erbs expression. Interestingly, expression of Bmal1 (Brain and muscle ARNT like protein 1), another important gene involved in regulating circadian clock,\textsuperscript{221} generally reaches the lowest level and highest level in both SCN and metabolically important tissues (liver, muscle, fat) at the peak and trough of Rev-erbs expression, respectively (Fig. 6).\textsuperscript{202,220} Bmal1 gene contains ROREs in its promoter region and is likely be regulated by RORs and Rev-erbs. Indeed, Bmal1 has been shown to be a target gene of ROR\(\alpha\) and ROR\(\gamma\) and its transcription is activated by RORs and repressed by Rev-erbs through competing for the same ROREs (Fig. 6).\textsuperscript{222–224}

The importance of Rev-erbs and ROR\(\alpha\) in Bmal1 transcription and circadian rhythm is further demonstrated in mice deficient in these orphan nuclear receptors. In wild-type mice the amplitudes of Bmal1 RNA oscillations in the

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{regulation_of_metabolism_by_nuclear_receptors.png}
\caption{Schematic representation of circadian Bmal1 expression and its regulation by RORs and Rev-erbs. RORs and Rev-erbs bind to the ROREs in the promoter of Bmal1 gene and activate or repress its transcription depending on the relative expression levels of these two classes of orphan nuclear receptors. Both Rev-erbs and Rev-erb\(\beta\) cycle in an antiphase to Bmal1 in all tissues (SCN, liver, muscle, adipose) whereas three ROR isoforms exhibit different circadian patterns in different tissues. Decrease of Rev-erbs expression in cells may allow the RORs to function as an activator which is inhibited when Rev-erbs levels are high.}
\end{figure}
liver are at least 20-fold with lowest point at CT8. This cyclic change in Bmal1 expression is reduced to less than 2-fold in Rev-erbα−/− mice. Similarly, the dramatic decrease of Bmal1 expression in the SCN at CT4 compared to that at CT16 is also lost in these mice. The Rev-erbα−/− mice have shortened period lengths of their locomotor activity rhythm in constant darkness or light, and the distribution of period lengths is much more variable than in wild-type mice. Interestingly, the more variable and shortened period of locomotor activity rhythm is also seen in the RORαsg/sg mice. In the SCN of the RORαsg/sg mice, the peak Bmal1 expression at CT15–18 is much reduced compared to wild-type mice. These studies suggest that RORα and Rev-erbα are required for maintaining normal cyclic Bmal1 expression and circadian clock function.

The RORαsg/sg and Rev-erbα−/− mice, as discussed earlier, have abnormal triglycerides levels. Plasma triglycerides in mice show circadian rhythm with highest level at CT4 and lowest level at CT16. This cyclic change in triglycerides is lost in Bmal1−/− mice, with similar levels at CT4 and CT16. It is possible that Bmal1 expression, regulated by RORs and Rev-erbs, may be required for lipid homeostasis. Mouse embryonic fibroblasts (MEFs) isolated from Bmal1−/− mice and Bmal1-knockdown 3T3-L1 cells are resistant to adipocyte differentiation and accumulate less lipids. This is correlated with a significantly reduced induction of adipocyte-related genes such as PPARγ and aP2. Reexpression of Bmal1 in Bmal1−/− MEFs can restore the adipocyte gene expression and lipid accumulation. In mature 3T3-L1 adipocytes, overexpression of Bmal1 increases lipid synthesis activity which is associated with an increase in the RNA levels of genes involved in lipid metabolism such as SREBP-1a, FAS, and aP2. Interestingly, the RORαsg/sg mice have reduced adiposity and decreased expression of lipogenic genes. The similarity of these lipogenic deficiency phenotypes in Bmal1−/− and ROR−/− cells provides another link between circadian rhythm and lipid metabolism.

C. RORs and Inflammation

As we have observed with the other metabolic sensing nuclear receptors, RORα also appears to play an important role in regulating inflammation. In the immune system, RORα is expressed in both lymphoid and myeloid cells with highest expression in macrophages. Peritoneal macrophages isolated from the RORαsg/sg mice express higher RNA levels of IL-1β, TNFα, and IL-1α than wild-type mice upon LPS stimulation. In RORα−/− mice, bone marrow derived macrophages also produce more IL-6 and TNFα than those obtained from wild-type mice. Since inflammation is also a key element in atherosclerosis, the activity of RORα was examined in primary human aortic smooth muscle cells. RORα is expressed in these cells and overexpression of RORα inhibits TNFα-induced IL-6, IL-8, and COX-2 expression. In addition, RORα upregulates
gene transcription of IκBα, the major inhibitory protein of the NF-κB signaling pathway, through a RORE in the IκBα promoter in these primary muscle cells. The role for RORα in regulating IκBα was confirmed in vivo by the significantly lower basal levels of IκBα mRNA in aortas from the RORα<sup>−/−</sup> mice than the wild-type mice. These studies suggest that RORα is anti-inflammatory and may have a protective role during inflammation and metabolic disease.

D. Therapeutic Potential of RORα Ligands

Studies in vivo and in vitro have demonstrated the importance of RORα in regulating expression of genes that control cholesterol and triglyceride levels as well as circadian rhythm. Recent studies linking the circadian rhythm to energy metabolism and demonstrating the essential role of ROREs in circadian gene expression suggest that RORs and their dominant negative regulators Rev-erbs are likely to be important players in integrating the circadian clock with lipid metabolism. Identification of cholesterol and ATRA as RORα and RORβ ligands suggests that it may be possible to develop small molecules that can bind RORs and modulate their activities. Based on the atherosclerotic and inflammatory phenotypes of RORα<sup>−/−</sup> mice, a RORα agonist will likely be beneficial in improving cardiovascular disease. Nevertheless, an RORα agonist may also potentially increase lipid synthesis in the adipose and liver. As we have observed with the other receptors discussed in the review, the challenge for drug discovery targeting RORα will be to identify small molecules that function in a tissue-selective manner in order to maximize the therapeutic benefit relative to unwanted side effects.

VI. ERRα

Estrogen receptor related receptorα (ERRα; NR1B1) is one of the three members of the ERR family (ERRα, ERRβ, NR1B2; ERRγ, NR1B3) that bind to the ERR responses element (ERRE) composed of a nuclear receptor half-site preceded by three nucleotides with the consensus sequence TNAAGGTCA. ERRα is most related to estrogen receptor α (ERα), sharing a high degree of homology within their DBD (68% identity). Not surprisingly, ERRα also bind estrogen receptor response elements (ERE) consisting of an inverted repeat of the core AGGTCA separated by three nucleotide. Indeed, several ERE-containing gene promoters can be regulated by both ERα and ERRα, suggesting potential cross talk between the signaling pathways mediated by these two receptors.

ERRα is constitutively active, assuming a LBD conformation that resembles that of agonist-bound nuclear receptors in the absence of ligand. A crystal structure of ERRα complexed with an LXXLL peptide from PGC-1α
indicates that the LBD of ERRα is almost completely filled with hydrophobic side chains, resulting in a small ligand binding pocket which can only be occupied by compounds with no more than the equivalent of four or five carbon atoms. Based on the sequence similarity between ERRα and ERα, a series of estrogen-like compounds were screened for their ability to modulate ERRα transcriptional activity. The phytoestrogens genistein, daidzein, and biochanin as well as 6,3′,4′-trihydroxyflavone were identified as agonists of ERRα by a virtual ligand screening using the agonist-bound ERα as the template. The activity of these phytoestrogens on ERRα was confirmed in cell transfection assays but they also activate ERs and other ERRs. Using a yeast one-hybrid assay with an ERRα-binding DNA region from the human aromatase gene, two organochlorine (pesticides toxaphene and chlordane) were identified to interact with ERRα. These compounds exhibit weak agonist activity on ERα but inhibit transcriptional activity of ERRα in mammalian cells. Diethylstilbestrol (DES), a potent ER agonist, was also identified as an antagonist of ERRα with an IC₅₀ of ~1 μM using an in vitro coactivator interaction assay. In cells, DES disrupts the ERRα-coactivator interaction and inhibits the constitutive transcriptional activity of ERRα. The first synthetic ERRα ligand XCT790, and inverse agonist, was derived from high throughput screening using an in vitro coactivator interaction assay. This compound is a potent inhibitor of ERRα transcriptional activity in cells (IC₅₀: 300–500 nM) with no significant activity on related nuclear receptors such as ERRγ and ERα at concentrations below 10 μM, providing a pharmacological tool for understanding the functions of ERRα. Recently, another ERRα inverse agonist was derived from high throughput screening and crystallized with ERRα LBD. This compound inhibits the interaction of ERRα and PGC-1α peptide in a biochemical assay with an IC₅₀ of 190 nM. Binding of this antagonist positions Helix 12 in the coactivator binding groove where the PGC-1α peptide is located in the apo-ERRα crystal structure, providing an explanation for the molecular mechanism of an ERRα inverse agonist. The identification of numerous ERRα inverse antagonists and few agonists suggest that the small ligand binding pocket is likely to accommodate only tightly packed molecules and most compounds that bind will result in a disruption of the ERRα transcriptional activity.

A. ERRα and the PGC-1α Pathway

ERRα is ubiquitously expressed with higher levels in the tissues (muscle, kidney, heart, and BAT) that have high metabolic needs. This expression pattern of ERRα is very similar to that of PGC-1α (which has been shown to induce ERRα gene expression through the ERRE in the promoter region and also function as an ERRα coactivator). Physiological stimuli such as
starvation, exposure to cold temperatures, and exercise increase both PGC-1α and ERRα gene expression, suggesting that these two proteins may mediate similar cellular functions.

PGC-1α plays an important role in regulating energy metabolism and mitochondrial biogenesis in muscle and BAT. In the course of identifying transcription factors and their cis-regulatory elements that are important in mediating the activities of PGC-1α, ERR binding sites (ERREs) were found to be one of the key motifs enriched in the genes upregulated by PGC-1α in muscle cells. This suggests that an important function of PGC-1α in muscle is to modulate transcriptional activity of ERRs. The ERRE motif is highly enriched in the promoters of genes involved in oxidative phosphorylation which are also targeted by PGC-1α. The role of ERRα in the regulation of PGC-1α-mediated mitochondrial functions was demonstrated by using the ERRα-specific antagonist XCT790. In C2C12 myotube cells, PGC-1α increases expression of ERRα and genes involved in oxidative phosphorylation but XCT790 diminishes this induction. XCT790 also significantly reduces the PGC-1α-increased total mitochondrial respiration. The requirement of ERRα in the PGC-1α mediated mitochondrial biogenesis is demonstrated in an osteosarcoma cell line SAOS2. Overexpression of PGC-1α in these cells induces genes encoding mitochondrial proteins such as mitochondrial DNA replication and transcription (mtTFA), cytochrome c, somatic (Cyt c), and ATP synthase β (ATPsynβ), and mitochondrial DNA contents are also increased. Inhibition of ERRα expression using an siRNA approach results in the decrease in expression of these mitochondrial proteins and functions mediated by PGC-1α. In these studies, ERRα is also shown to bind to the ERREs in the promoters of ATPsynβ and Cyt c genes.

PGC-1α also plays a role in regulating glucose and fatty acid metabolism in an ERRα-dependent manner. Overexpression of PGC-1 in C2C12 myotube cells induces gene expression of pyruvate dehydrogenase kinase 4 (PDK4), which contains an ERRE in the promoter region. PGC-1α-mediated activation of the PDK4 promoter requires ERRα since this activity is not seen when using MEFs from Errα−/− mice compared to those from the wild-type mice. Stimulation of PDK expression suggests that glucose oxidation is inhibited and energy utilization is switched from glucose to fatty acids. Indeed, PGC-1α expression in C2C12 myotube cells decreases the glucose oxidation rate and is accompanied by an increase in the expression of median-chain acylcoenzyme A dehydrogenase (MCAD), an enzyme that mediates the first step in the mitochondrial β-oxidation of fatty acids. MCAD is a target gene of ERRα and coexpressed with ERRα and PGC-1 in tissues that preferentially utilize fatty acids as energy substrates. The PGC-1-induced MCAD expression can be inhibited using the ERRα-specific inhibitor XCT790, further demonstrating the requirement of ERRα in PGC-1-mediated fatty acid oxidation.
Another metabolic function of PGC-1α affected by ERRα is its activity on gluconeogenesis in the liver. Overexpression of PGC-1α in primary hepatocytes increases expression of gluconeogenic enzymes phosphoenolpyruvate carboxy-kinase (PEPCK) and glucose-6-phosphatase (G6Pase) and results in increased glucose production. Injection of an adenovirus expressing PGC-1α into rats also increases blood glucose levels, associated with increases in PEPCK and G6Pase expression. Similarly, PGC-1α also increases PECK gene expression in HepG2 cells and this is accompanied by an increase in ERRα expression as predicted. However, coexpression of ERRα decreases this PGC-1α-mediated induction of PEPCK which contains an ERRE in its promoter region, suggesting ERRα functions as a transcriptional repressor of PGC-1α in this context. Indeed, inhibition of ERRα expression using a siRNA increases PGC-1α-mediated gluconeogenesis in HepG2 cells. The inhibitory activity of ERRα on PGC-1α-mediated gluconeogenesis is further confirmed by the significantly increased expression of PEPCK and glycerol kinase genes in livers from the ERRα−/− mice compared to the wild-type mice. Interestingly, ERRα still functions as an activator of PGC-1α genes involved in the oxidative phosphorylation in the liver since the PGC-1α-induced mitochondrial gene expression in HepG2 cells is not activated but inhibited by ERRα siRNA. This suggests that ERRα represses gene expression in a promoter-specific manner and is not a general inhibitor in the liver.

B. ERRα and Diabetes

PGC-1α and the genes involved in the oxidative phosphorylation are decreased in human diabetic muscles, suggesting that muscle mitochondrial dysfunction is linked to diabetes. PGC-1α is also increased in the livers of diabetic mouse models, supporting the hypothesis that increased glucose production stimulated by PGC-1α has a negative impact on insulin sensitivity. The ability of PGC-1α to increase expression of genes involved in both mitochondrial oxidative phosphorylation in muscle and gluconeogenesis in liver suggest that tissue-specific modulation of PGC-1α will be needed to achieve a net beneficial effect for the treatment of diabetes. However, the ability of ERRα to activate PGC-1α-induced oxidative phosphorylation in muscle and repress PGC-1α-mediated gluconeogenesis in liver (as discussed earlier) presents an opportunity to increase mitochondrial function without the detrimental effect on glucose production by targeting ERRα.

C. ERRα in Adipose and Intestine

ERRα−/− mice have a 50–60% reduction in the weight of the inguinal, epididymal, and peritoneal fat pads with decreased adipocytes size. These mice are also resistant to high fat diet-induced obesity. Microarray analysis of
the adipose tissues from ERRz−/− mice show downregulation of the genes involved in fatty acid synthesis such as FAS, and SREBP1. Lipogenesis in the Errα−/− mice assessed by treating with 3H2O and measuring the amount of radioactive label incorporated into triacylglycerol show that there is a 30–50% decrease in 3H incorporation into lipids in the inguinal, epidymal, and perirenal fat. These results indicate that ERRz is important for normal lipid metabolism in the white adipose tissue.

In the brown adipose tissue (BAT) of Errα−/− mice, expression of mitochondrial genes important for oxidative phosphorylation and fatty acid oxidation is decreased, demonstrating the importance of ERRz in mediating mitochondrial energy metabolism in this tissue. Mitochondrial density and DNA contents are also decreased in the BAT of Errα−/− mice compared to the wild-type mice, confirming the in vivo function of ERRz in mitochondrial biogenesis. The physiological significance of the decreased mitochondrial function and mass is shown by the inability of Errα−/− mice to maintain proper body temperature when exposed to cold, suggesting a defect in adaptive thermogenesis.

The lean phenotype of Errα−/− mice arises without decreased food consumption or increased energy expenditure. One possible explanation for the phenotype of these mice is a defect in intestinal adsorption of dietary nutrients including fat. ERRz is expressed in epithelia cells throughout the small intestine and fat malabsorption is observed in the Errα−/− pups. Microarray analysis of intestinal RNA from Errα−/− mice show that genes involved in oxidative phosphorylation are downregulated and intestinal β-oxidation activity is also reduced, confirming the in vivo role of ERRz in mitochondrial metabolism in this tissue. Additionally, expression of genes involved in dietary lipid digestion and absorption such as pancreatic lipase-related protein 2 (PLRP2), fatty acid-binding protein 1 and 2 (L-FABP and I-FABP), and apolipoprotein A-IV (apoA-IV) are also downregulated in the intestine of Errα−/− mice.

D. ERRα and Cancer

The observation that ERRs and ERs can bind to the same DNA sequences suggests that ERRs may influence well characterized estrogen-dependent pathways. Interestingly, ERRα has been identified as a negative prognostic factor for breast cancers. ERRα is expressed in many breast cancer cell lines including the ER-positive MCF-7 and ER-negative MDA-MB-231 cells. Treatment of these cell lines with high concentrations of DES, which antagonizes ERR activity, inhibits cell proliferation. Knockdown of ERRα expression in the MDA-MB-231 cells using siRNA also results in a reduction in cell migration in vitro and tumor growth when implanted as xenografts into athymic
nude mice. Recently, the angiogenic vascular endothelial growth factor (VEGF) was identified as a direct target gene for ERRα, providing another evidence linking ERRα to tumor progression and metastasis. These results suggest that pharmacological manipulation of ERRα activity may be of therapeutic use in treating breast cancer patients.

E. Therapeutic Potential of ERRα Ligands

Although Errα−/− mice exhibit a relatively mild metabolic phenotype this could be due to the upregulation of compensatory pathways. Indeed, expression of PGC-1α is increased in the heart, liver, and muscle of Errα−/− mice and ERRγ is also upregulated in the heart of these mice. Several studies indicate mitochondrial oxidative phosphorylation is reduced in patients with type II diabetes and ability of ERRα to induce mitochondrial biogenesis and oxidative phosphorylation in conjunction with PGC-1α suggests the straightforward hypothesis that an ERRα agonist would be useful for the treatment of this disease (Fig. 7). Nevertheless, this simple hypothesis is confounded by the recent observation that over expression of PGC-1α in muscle actually promotes insulin resistance. Additionally, to our knowledge none of the compounds reported to have ERR agonist activity in vitro and in cell culture systems have been shown to act as ERR agonists in animals. Hydrophobic amino acid residues in the LBD of ERRα appear to function as an intramolecular agonist favoring a transcriptionally active conformation and we feel it is unlikely that synthetic agonists with the efficacy to significantly influence gene expression in animals will be identified. On the contrary, ERRα antagonists that disrupt the active confirmation have been identified and we anticipate that the further development of such inhibitors with sufficient systemic exposure will help elucidate the in vivo function of ERRα. Clearly, such inhibitors may have therapeutic value for the treatment of cancer and one wonders about the metabolic impact of such ligands. Will inhibiting ERRα function to decrease

\[
\text{↓ Gluconeogenesis (e.g., PEPCK)} \\
\text{↑ Fatty acid oxidation (e.g., MCAD)} \\
\text{↓ Glucose oxidation (e.g., PDK4)} \\
\text{↑ Lipid transport (e.g., ApoA-IV)} \\
\text{↑ Oxidative phosphorylation (e.g., Cyt c)} \\
\text{↓ Tumor growth (e.g., pS2)} \\
\text{↓ Angiogenesis (e.g., VEGF)}
\]

Fig. 7. ERRα activity and its target genes. ERRα binds to the ERREs in the promoters of its target genes and activates or represses their expression. The mitochondrial functions and other activities of ERRα have been characterized using a combination of the Errα−/− mice, siRNA, and ERRα-specific antagonists.
lipid absorption perhaps having a beneficial effect on plasma lipids or will decreases in oxidative phosphorylation speed the progression of diabetes (Fig. 7)?

VII. Summary

Over the last 15 years a growing number of nuclear hormone receptors have been demonstrated to play important roles in the control of metabolism at the level of gene expression. Nature has seized on the ability of nuclear receptors to function as ligand-controlled transcription factors and exploited this ability to evolve a family of metabolic sensors that coordinate metabolism in response to changes in the levels of important end-products and intermediates. Recent studies have also defined an intimate link between the regulation of metabolism and the control of inflammation. Excitingly, many of the receptors highlighted in this review appear to function at this interface. Although the biological necessity for linking metabolism with inflammation is not yet clear, the best evidence suggests that control of energy utilization plays an important role in determining inflammatory status. Since many of the pathways regulated by the PPARs, LXR, FXR, ROR, and ERR are deranged in states of metabolic disease these receptors have become important drug targets for development of novel drugs for human disease. Indeed, preclinical and clinical studies have uncovered many beneficial activities for small molecules that regulate these receptors. Nevertheless, in many cases there also appear to be unwanted activities of many of these small molecules that can or may limit their therapeutic use. Future studies that better define the tissue-specific activities of the metabolic nuclear receptors and the trans-acting factors that interact with receptors to impart tissue-selective gene expression patterns will pave the way toward the identification of effective drugs for metabolic disease.

References

76. Song C, Liao S. Cholestenoic acid is a naturally occurring ligand for liver X receptor alpha. Endocrinology 2000;141:4180–4.


232. Vanacker JM, Pettersson K, Gustafsson JA, Laudet V. Transcriptional targets shared by estrogen receptor-related receptors (ERRs) and estrogen receptor (ER) alpha, but not by ERbeta. *EMBO J* 1999;18:4270–9.


