Orphan Nuclear Receptors and the Regulation of Nutrient Metabolism: Understanding Obesity

Nuclear hormone receptors (NRs) are a superfamily of eukaryotic ligand-dependent transcription factors that translate endocrine, metabolic, nutritional, developmental, and pathophysiological signals into gene regulation. Members of the NR superfamily (on the basis of sequence homology) that lack identified natural and/or synthetic ligands are/were classified as “orphan” NRs. These members of the NR superfamily are abundantly expressed in tissues associated with major metabolic activity, such as skeletal muscle, adipose, and liver. Subsequently, in vivo genetic studies on these orphan NRs and exploitation of novel natural and synthetic agonists has revealed that orphan NRs regulate 1) carbohydrate, lipid, and energy homeostasis in a tissue-specific manner, and 2) the pathophysiology of dyslipidemia, obesity, Type 2 diabetes, and cardiovascular disease. This review discusses key studies that have implicated the orphan NRs as organ-specific regulators of metabolism and mediators of adverse pathophysiological effects. The emerging discovery of novel endogenous orphan NR ligands and synthetic agonists has provided the foundation for therapeutic exploitation of the orphans in the treatment of metabolic disease.

Nuclear Hormone Receptors

Nuclear hormone receptors (NRs) belong to a superfamily of structurally related transcription factors that translate endocrine, metabolic, nutritional, developmental, and pathophysiological signals into gene regulation. This unique DNA-binding factor superfamily is comprised of 48 structurally conserved members in humans and function as ligand-modulated and constitutively active transcription factors. NRs typically consist of 1) a variable amino-terminal regulatory domain (region A/B) that contains the activation function 1 (AF-1) domain that modulates NR function, independent of ligand interactions (120); 2) a highly conserved DNA binding domain (DBD; region C) that mediates high-affinity binding of the receptor to specific DNA sequences (hormone response elements); 3) a hinge region (region D); and 4) a conserved carboxy-terminal ligand binding domain (LBD; region E) that mediates ligand binding and receptor dimerization (28).

The NR superfamily includes classical steroid (endocrine) NRs with high-affinity lipid hormones as ligands (examples include the androgen, estrogen, thyroid, and glucocorticoid receptors). Following transcript sequencing of these classical steroid receptors, other expressed gene products were identified as NRs on the basis of sequence homology/structural organization. At the time of discovery, these expressed gene products were denoted as “orphan NRs” since the endogenous natural ligands that modulated their activity were unknown (35). Subsequently, low-affinity endogenous ligands have been identified for approximately one-third of these receptors designated as “adopted orphans.” Table 1 lists all human NRs by ligand classifications. Synthetic and/or biological ligands (not thought to be endogenous ligands) have also been discovered for some orphan NRs, highlighting that these receptors are potential drug targets. This review specifically focuses on the orphan and adopted orphan NRs and summarizes key studies that have implicated these NRs as key mediators of metabolism and obesity in humans and animals. In subsections, adopted orphan NRs will be discussed first followed by discussion on orphan NRs.

Orphan NRs and Metabolism

The majority of orphan and adopted orphan NRs are abundantly expressed in tissues involved in lipid, carbohydrate, and energy homeostasis (9); this therefore provides an association between these receptors and metabolic function. Genetic
and pharmacological studies have identified that a number of orphan (and/or adopted) NRs regulate nutrient metabolism, obesity, and Type 2 diabetes in an organ-/tissue-specific manner. Some key examples include PPARγ, a regulator of lipid storage, development (113), and insulin sensitivity (61).
adipose tissue. Furthermore, the related receptor PPARγ regulates oxidative metabolism in skeletal muscle (24, 66, 118), whereas PPARα regulates hepatic lipid and carbohydrate metabolism (2, 125). Other key examples include ERRα/γ, which are of oxidative metabolism (42, 78, 103) in multiple tissues, RORα (55, 56) (a regulator of insulin sensitivity and glucose/lipid metabolism in skeletal muscle), NUR77 (90, 94), FXR (106), and LXRα (107) (regulators of hepatic lipid and carbohydrate metabolism).

**Obesity and Diabetes in the 21st Century**

“Overweight and obesity are defined as abnormal or excessive fat accumulation that presents a risk to health” (124). Overweight and obesity are significant risk factors for several chronic diseases including 1) cardiovascular disease, 2) diabetes, 3) musculoskeletal disorders, and 4) cancers. The underlying central cause for obesity is an imbalance between caloric intake vs. expenditure. The global worldwide surge in overweight and obesity are attributable to 1) diet, 2) lifestyle, and 3) genetics and environment.

The World Health Organization (WHO) has described the problem of obesity as a “worldwide epidemic” (123). The latest projections by the WHO suggest that, by 2015, ~3 billion adults will be overweight and obese. Twenty million children under the age of 5 yr are overweight globally in 2005. In the US, for adults (2007–2008), the age-adjusted prevalence of obesity was 33.8%; correspondingly, the estimate for overweight and obesity combined (BMI ≥ 25) was 68.0% (30). The direct medical care costs of obesity in the United States were estimated at $147 billion in 2008 or almost 10% of all medical spending (29). In 2007, Type 2 diabetes was estimated to cost the United States $153 billion in higher medical costs and $65 billion in reduced productivity (19).

The increasing prevalence of Type 2 diabetes in the latter part of the 20th century and the 21st century has been driven by changes in diet, lifestyle, and the worldwide epidemic of overweight and obesity. An article in 1997 entitled “Science, medicine, and the future noninsulin dependent diabetes mellitus: the gathering storm” predicted “a massive increase in the global prevalence of type 2 diabetes” (81). This “gathering storm” is no longer on the horizon, and Type 2 diabetes has arrived in the new millennium. Diabetes currently affects 346 million people worldwide, and the WHO estimates this number is likely to double by 2030 (28, 122).

**Orphan NRs and the Pathophysiology of Obesity/Insulin Resistance**

Gain and loss-of-function analysis of several orphan (and adopted) NRs by genetic and/or pharmacological manipulation modulates the susceptibility to weight gain, insulin sensitivity, and glucose tolerance. For example, loss of the adopted NR PPARγ in mice (5) and overexpression/agonist treatment of PPARγ also leads to resistance to weight gain (117). In terms of human mutations, loss-of-function mutations of PPARγ result in lipodystrophy and insulin resistance (6), which is consistent with the mouse model.

In terms of orphan NRs, animal models with loss of ERRα (64) or functional RORα (56) are resistant to high-fat, diet-induced obesity and insulin resistance, whereas, conversely, loss of NUR77 leads to greater susceptibility to diet-induced weight gain (15). Inflammation has also been implicated in the pathogenesis of obesity/diabetes (105), and recently RORα has also been associated with this process (46). Interestingly, although excessive weight gain generally correlates with insulin resistance in humans and animals, modulation of susceptibility to weight gain by orphan NRs does not always correlate with insulin resistance/diabetes due to differing mechanisms of action (these are outlined in subsequent sections of this review). For example, SHP knockout mice are resistant to diet-induced obesity with a paradoxical loss of insulin sensitivity (83).

In summary, genetic and pharmacological approaches in rodent models have demonstrated that orphan NRs play a significant role in the modulation of weight gain and insulin resistance/diabetes.

**Orphan NRs and Oxidative Metabolism: Susceptibility to Diet-Induced Obesity**

**Diet-Dependent Weight Gain and Mitochondrial Efficiency**

Mitochondrial efficiency and oxidative metabolism are key drivers of resistance to diet-induced obesity. Adopted orphan NRs in the PPAR subgroup have been implicated in the regulation of diet-induced obesity in mouse models. Transgenic overexpression of activated PPARγ or PPARγ agonist treatment results in resistance to diet-induced weight gain via enhanced oxidation (117). In particular, PPARγ also controls oxidative gene expression in skeletal muscle cells (24, 74), and overexpression of PPARγ results in a shift toward oxidative skeletal muscle fibers (118). Similarly,
PPARα agonist treatment enhances hepatic oxidative metabolism (21, 22).

Multiple orphan NRs also regulate oxidative metabolism and resistance to diet-induced obesity. Similar to PPARα, overexpression of activated NOR1 in murine skeletal muscle results in a shift toward oxidative (type IIa/X) skeletal muscle fibers, increased mitochondrial density, increased oxidative gene expression, and increased metabolic rate (86). Furthermore, loss of the related NR4A member, NUR77, reduces metabolic rate and leads to greater susceptibility to diet-induced weight gain (15); however, the mechanisms behind this phenotype remain unclear.

ERRα regulates genes implicated in fatty acid metabolism (42) and mitochondrial biogenesis (103), and knockout mice have reduced body and fat mass conductive with defective oxidative metabolism (64). Treatment of skeletal muscle cells with a synthetic ERRα agonist impeded mitochondrial respiration in these cells (72). Furthermore, ERRα appears to regulate MCAD and PPARα in skeletal muscle tissue and myoblasts, respectively (42, 72). Both MCAD and PPARα enhanced fatty acid/mitochondrial oxidation, which is consistent with the attenuated oxidative metabolism observed in knockout mice (64). The related estrogen-related receptor γ (ERRγ) also induces oxidative metabolism in skeletal muscle (78) and maintains postdevelopmental oxidative metabolism in heart (1).

Homozygous SHP knockout mice are resistant to diet-induced obesity (83, 114). In mixed background mice, this phenotype is associated with increased PGC1α expression in brown adipose tissue (114); however, on a C57B16 background, homozygous SHP knockout mice have normal brown adipose PGC1α levels, and the phenotype appears due to hepatic de-repression of PPARα and enhanced β-oxidation (83). Similarly, heterozygous COUP-TFI knockout mice display reduced adiposity, resistance to diet-induced obesity, increased metabolic rate, and enhanced mitochondrial biogenesis in white adipose tissue (63). Attenuation of both COUP-TFIs in cultured skeletal muscle cells altered the expression of genes involved in energy expenditure (76). Loss of RORα function in “staggerer” mice leads to decreased adiposity, resistance to diet-induced weight gain, and enhanced oxygen consumption (46, 56). The mechanism has not been completely resolved; however, the mice display elevated expression of PGC1α and Lipin1 and reduced SREBP1c expression (56).

**Ophan NRs and Adipogenesis**

In the context of adipogenesis, the adopted orphan NRs have critical roles. For example, PPARγ is a potent regulator and mediator of mammalian adipogenesis (112, 113). This receptor plays a central role in the control of adipocyte gene expression and differentiation and has been extensively reviewed. Other PPAR receptors are not key regulators of adipogenesis, for example, ectopically expressed PPARα weakly promotes adipogenesis and PPARδ has no effect (11). The bile acid-dependent NR, FXR, promotes adipocyte differentiation. FXR agonists and antagonists, respectively, enhance and repress adipocyte differentiation in vitro (101). Furthermore, FXR agonist induces genes associated with adipogenesis in vivo.

Independent of PPARs, many current orphan NRs play important roles in adipogenesis and lipid storage. ERRα and ERRγ regulate adipogenesis. ERRα-null mice and ERRα knockdown exhibit reduced adiposity and alterations in the expression of genes regulating adipogenesis (20, 64). ERRγ also induces adipogenesis in vitro (51); however, adipogenesis related to ERRγ has not been examined in vivo.

The orphan NR COUP-TFI also regulates adipogenesis. Heterozygous COUP-TFI mice exhibit less adipose tissue and decreased expression of key regulators for white adipose tissue development (63). Contrary to this, in vitro studies have shown overexpression of COUP-TFI prevents adipogenesis, whereas shRNA-mediated reduction of COUP-TFI promotes differentiation (126). This difference is thought to be due to temporal differences in COUP-TFI expression during adipogenesis (63). Similar to COUP-TFI, the role of the NR4A family in adipogenesis is unclear. Using in vivo models of adipogenesis, two studies have implicated the NR4A subgroup as regulators of adipogenesis (14, 33), however, conversely, one study suggested the NR4A subgroup had no involvement (4). In contrast, although chronic overexpression of NUR77 inhibited adipogenesis, transient overexpression promoted adipogenesis, thus suggesting that transient induction of NUR77 may be required for adipogenesis (33).

Overexpression RORα in 3T3-L1 cells impairs adipogenesis, whereas, conversely, mouse embryonic fibroblasts from staggerer loss-of-RORα function mice differentiate more efficiently into adipocytes (25). Interestingly, staggerer mice display reduced adiposity (46), which is not consistent with enhanced adipogenesis. Overexpression of REV-ERBα in 3T3-L1 preadipocytes results in increased expression of adipogenic markers along with an increase in lipid accumulation (31). In an in vitro model of adipogenesis, the synthetic REV-ERBα ligand SR6452 resulted in induction of adipocyte differentiation with synergetic effects with thiazolidinediones (54).
Orphan NRs, Carbohydrate Metabolism, and Metabolic Disease

Insulin Sensitivity and Glucose Tolerance

Aberrant insulin sensitivity and glucose uptake (and disposal) underlie the pathophysiology of Type 2 diabetes. The vast majority of insulin-stimulated glucose uptake is performed by skeletal muscle, the liver, and adipose tissue. All members of the adopted orphan PPAR subgroup have been implicated in the regulation of insulin-stimulated glucose uptake. This is underlined by the utility of PPARγ agonists (thiazolidinediones) in the treatment of human Type 2 diabetes (61). The regulation of insulin sensitivity by PPARγ agonists appears to be predominately mediated by the action of PPARγ in adipose tissue (38, 129); however, PPARγ may also have local effects on insulin sensitivity in both skeletal muscle (41) and liver (69).

In mouse models, activation/overexpression of the related receptors PPARα (18, 110) and PPARδ (66, 117, 118) improves insulin sensitivity in genetically obese/high-fat diet mice. PPARα acts predominately via transcriptional changes in the liver (18, 110), whereas PPARδ acts via modulation of skeletal muscle gene expression and the contractile protein remodeling (24, 66, 118). Supporting this, PPARδ knockout mice display glucose intolerance and insulin resistance (58). Also similar to PPARs, a synthetic activator of the adopted orphan CAR reduced serum glucose levels and improved glucose tolerance/insulin sensitivity in mice (23).

Similarly, several current orphan receptors have been implicated in the regulation of insulin-stimulated glucose uptake. SHP−/− mice are glucose intolerant and display hepatic insulin resistance; however, interestingly, these mice are resistant to diet-induced obesity (83). A synthetic agonist of LRH-1 (1,2-dilauroyl-sn-glycero-3-phosphocholine) improved glucose tolerance in mouse models of insulin resistance, and this appears to be specifically mediated by LRH-1 since this effect is not observed in LRH-1 knockout mice (59).

The role of NUR77 in terms of insulin sensitivity is currently unclear. Following a high-fat diet, NUR77 knockout mice exhibited marked reduction of insulin-stimulated glucose in skeletal muscle (15). However, local overexpression of NUR77 in skeletal muscle did not alter insulin-stimulated glucose uptake in skeletal muscle (47). NUR77 expression in human muscle biopsies closely correlated with body-fat content and insulin sensitivity (47).

Although the function of the above orphan receptors positively correlate with insulin sensitivity, loss of COUP-TFII, and RORα expression/function enhances insulin sensitivity in mouse models. Loss of RORα function in “staggerer” mice enhances insulin sensitivity and glucose uptake in skeletal muscle (independent of adiposity and body mass) (55). The mechanism involves increased AKT and phospho-AKT expression. In a similar manner, COUP-TFII knockout mice display increased insulin sensitivity and glucose tolerance, with these effects partially mediated by skeletal muscle (63).

Glycogen Metabolism

Glycogen is used to store glucose in both liver and skeletal muscle, with hepatic glycogen exclusively used for blood glucose homeostasis. The adopted orphan NRs HNF4α, FXR, and PPARα appear to be required for normal glycogen synthesis. In late-stage mouse embryos with hepatic loss of HNF4α, significantly smaller hepatic glycogen stores are observed (84). Glycogen synthase and glucose-6-phosphatase expression were also ablated in these late-stage mice embryos, suggesting that glycogen synthesis is controlled by HNF4α; however, since hepatic loss of HNF4α also caused multiple changes to hepatic development, other developmental effects may be disrupting hepatic glycogen storage (84). Knockout of FXR decreases hepatic glycogen storage/production (13, 26), whereas activation (by synthetic agonist GW4064) or hepatic overexpression increases hepatic glycogen storage (130). Similarly, PPARα knockout mice also exhibit decreased hepatic glycogen (3); however, glycogen levels/flux have not been examined in response to synthetic agonists.

The orphan NR NUR77 controls hepatic glucose production (90) and is also associated with gene expression that controls glycogen metabolism in both skeletal muscle (e.g., glycogen phosphorylase) (16) and liver (e.g., glucose-6-phosphatase) (90). Supporting this, glycogen synthesis is increased in skeletal muscle and cultured myoblasts that overexpress NUR77 (47); however, the effects of NUR77 directly on hepatic glycogen have not yet been investigated.

Gluconeogenesis

In humans, gluconeogenesis occurs in the liver and to a lesser extent the kidney; in the context of orphan NRs, only liver gluconeogenesis has been examined. The adopted orphan NR LXR is known to suppress hepatic gluconeogenesis and key genes (PEPCK, etc.) associated with gluconeogenesis (12, 107). The anti-diabetic action of an LXR agonist is mediated by inhibition of hepatic gluconeogenesis (12). The anti-diabetic action of LXR agonists is thought to result predominantly from suppression of hepatic gluconeogenesis (12). Conversely, PPARα and HNF4α appear to be pro-gluconeogenic. Synthetic PPARα agonists induce hepatic glucose production and gluconeogenesis (85), whereas PPARα knockout mice display suppressed gluconeogenesis.
(85, 110), and HNF4α expression transcribes genes involved in gluconeogenesis (127).

The orphan NR ERRα also appears to suppress hepatic gluconeogenesis via suppression of the key gluconeogenic enzyme phosphoenolpyruvatecarboxykinase (PEPCK) in animal models (40). Interestingly, both SHP (127) and DAX1 (79) appear to repress HNF4α and, therefore, repress gluconeogenesis. The ectopic adenoviral overexpression of NUR77 stimulated glucose production both in vitro and in vivo, raised blood glucose levels in vivo, and induced genes involved in gluconeogenesis (90). Expression of an inhibitory mutant NUR77 receptor reduced hepatic glucose production and lowered blood glucose levels in vivo (90). Conversely, treatment of mice with the NUR77 agonist cytosporone B increased fasting blood glucose levels (128).

The synthetic and selective RORα inverse agonist SR3335 has also been shown to suppress pyruvate-induced blood glucose levels consistent with suppressed gluconeogenesis in a mouse model of diet-induced obesity and suppressed the expression of genes involved in gluconeogenesis in vitro (52). Genes involved in gluconeogenesis in vitro were also repressed by the pan RORα/γ inverse agonist T0901317 (53). Conversely, oxysterols and the synthetic RORα/γ agonist SR1078 induced glucose-6-phosphase and PEPCK expression in liver cells, which are key genes involved in gluconeogenesis (115, 116).

Orphan NRs and Lipid Homeostasis

The activity of several adopted orphan NRs is regulated by dietary lipids including fatty acids (PPARα/β/γ) (32, 50), oxygenated sterols (ROα/γ and LXα/β) (43, 60, 116), and lipid metabolites (including bile acids, etc) (FXR, HNF4α, and LRH-1) (39, 59, 67). In concordance, adopted orphan NRs are key regulators of fatty acid, triglyceride, and cholesterol metabolism.

Fatty Acid/Triglyceride Catabolism and Transport

The majority of fatty acids are stored as triglycerides, which are broken down to free fatty acids for oxidation (lipolysis). In vitro, knockdown of the adopted orphan NR LXα attenuated isoprenaline-induced lipolysis in adipose cells (108). However, in animals, CAR activation enhances hepatic β-oxidation (23). PPARα also modulates gene expression associated with fatty acid transport (73).

Knockdown of the orphan NR NUR77 attenuated isoprenaline-induced lipolysis in skeletal muscle cells (70). Also in cultured skeletal muscle cells, attenuation of both NOR1 and COUP-TF(I and II) receptors reduced fatty acid oxidation (76, 87). Several orphan NRs modulate gene expression associated with fatty acid transport including NUR77 (70), NOR1 (88), REV-ERBβ (96), HNF4α (37), both COUP-TF receptors (76), and RORα/γ (45).

Lipogenesis and the Pentose Phosphate Pathway

The adopted orphan receptors LXRα/β regulate genes that enhance/activate lipogenesis (100, 104). Mice lacking LXRα were also observed to be deficient in expression of the major regulators of lipogenesis: sterol regulatory element binding protein-1 (SREBP1c), fatty acid synthase (FAS), steroyl-coA desaturase 1 (SCD1), and acyl-coA carboxylase (ACC). This hypothesis was supported by the subsequent demonstration that the synthetic LXR agonist T1317 induces expression of lipogenic genes and raises plasma triglyceride levels in mice (89, 104). Like LXR, PPARα agonists activate lipogenesis and induce the expression of similar key genes associated with lipogenesis in the liver (e.g., FAS, SCD1, ACC) (58). Along with increased lipogenesis, this study also showed that PPARα agonist treatment induces the expression of 6-phosphogluconate dehydrogenase (PGD), the rate-limiting step of the pentose phosphate pathway that supplies reducing equivalents required for lipogenesis. Conversely, several studies have demonstrated that a synthetic CAR agonist represses hepatic lipogenesis and plasma/hepatic triglyceride levels (7, 23, 34). CAR also repressed PGD (23).

In terms of orphan NRs, suppression of RORα function in skeletal muscle cells has been associated with the attenuation of genes involved in lipogenesis (e.g., SCD1/SREBP1c/FAS) (57), and, supporting this, decreased serum/hepatic triglyceride levels and SREBP1c expression were also observed in the RORα “staggerer” mouse model (56, 98). Furthermore, expression of a truncated RORα loss-of-function mutant in mouse skeletal muscle led to decreased SREBP1c expression (95).

Similarly, a synthetic LRH-1 agonist lowers hepatic triglyceride levels by repressing lipogenesis, and this appeared to be mediated by the repression of SREBP1c and downstream target genes (59). Similarly, hepatic NUR77 overexpression also inhibits SREBP1c expression and downstream SREBP1c target genes and results in a reduction in hepatic triglyceride (94). Attenuation of REV-ERBβ also induces the expression of key genes involved in lipogenesis (e.g., SCD1/SREBP1c) in skeletal muscle cells (96, 97); however, this has not been examined in vivo.

Cholesterol Homeostasis

A few adopted orphan NRs are regulated by cholesterol and cholesterol-related compounds. These
include LXRα/β, which is regulated by some oxygenated sterols (43, 60), FXR, which is regulated by bile acids (67), and possibly the current orphan NR RORα/γ, which is reported as inversely regulated by oxygenated sterols (116). These NRs (along with others) regulate cholesterol homeostasis.

Treatment of wild-type mice and cultured skeletal muscle cells with a synthetic LXR agonist induced the expression of genes associated with cholesterol synthesis and cellular transport in skeletal muscle, and this induction was ameliorated in LXRα/β knockout mice treated with the agonist (75). LXRα/β also regulates the expression of hepatic cholesterol ester transfer protein and lipoprotein lipase expression (65, 131). Mice carrying a targeted disruption of the LXRα gene fail to induce transcription of the gene encoding cholesterol 7α-hydroxylase (CYP7A1) in response to dietary cholesterol, implicating LXRs in the control of bile acid synthesis (89). In terms of cholesterol transport, both LXRs and LXRα are regulators of reverse cholesterol transport in macrophages (17) and in skeletal muscle cells (75). Obesity and metabolic syndrome are associated with low HDL cholesterol levels, and this is one pathogenic factor involved in atherosclerosis. Activation of LXRs and PPARs increases HDL levels by enhancing reverse cholesterol transport. In particular, the activation of reverse cholesterol transport in macrophages by LXRs is likely to be responsible for the potent anti-atherogenic activity of LXR agonists (44, 62). Agonists of PPARα (71) and PPARγ (82) increase HDL cholesterol levels in humans, and these receptors have also been shown to regulate reverse cholesterol transport (10, 77). Supporting this, PPARα knockout mice show increased plasma HDL cholesterol levels (92). The regulation of reverse cholesterol transport in macrophages by PPARα is mediated by LXR signaling (77). PPARα appears to be a key regulator of plasma lipoproteins by induction of genes (e.g., HDL apolipoproteins in humans) that decrease hepatic VLDL production and the induction of genes that promote lipoprotein lipolysis (27).

A synthetic CAR agonist decreased plasma apolipoprotein B-containing lipoproteins and the rate of atherosclerosis in low-density lipoprotein receptor-deficient mice (102). Likewise, REV-ERBα also appears to regulate lipid transport as deficient mice displayed elevated serum and hepatic levels of apoC-III together with increased serum VLDL (99). Hepatic expression of NUR77 via adenoviral overexpression vectors in mice significantly increases plasma LDL levels compared with control animals (94).

The adopted orphan NR FXR appears to function as a bile acid sensor that controls the hepatic regulation of genes involved in bile acid production [such as cholesterol 7alpha-hydroxylase (CYP7A1)] (36), cholesterol synthesis (121), cholesterol transport (48),

![Diagram](http://physiologyonline.physiology.org/)

**FIGURE 1. Overview of the tissue-level metabolic functions and pathophysiology associated with orphan (and adopted orphan) nuclear receptors**
and triglyceride synthesis (121). Initial studies on the orphan NR RORα using the “staggerer” mouse model displayed hypochondriacal proteinemia, which is associated with decreased plasma levels of the major HDL proteins and accelerated development of atherosclerosis on atherogenic diets (68). Later studies on RORα in skeletal muscle cells and staggerer mice revealed decreased serum ApoCIII (98) and reduced expression of genes associated with cholesterol efflux (e.g., ABCA1 and APOA1) in skeletal muscle (56). Similarly, attenuation of both COUP-TFs in cultured skeletal muscle cells altered the expression of genes involved in cholesterol efflux (ABCA1, etc.) (76). Like FXR, COUP-TFII also regulates cholesterol bile acid production via regulation of CYP7A1 (109). The orphan NR LRH-1 regulates bile acid synthesis as the LRH-1 agonist 1,2-dilauroyl-sn-glycero-3-phosphocholine (DLPC) induces biosynthetic enzymes in mouse liver and bile acid levels (59). Mice lacking hepatic HNF4α expression exhibited greatly reduced serum cholesterol and increased serum bile acid concentrations (37).

Conclusions

The orphan (and adopted) NRs have emerged as regulators of metabolism and pathologies such as obesity and Type 2 diabetes (see FIGURE 1 for overview). Currently, the only approved ligands for orphan NRs for human therapeutics are fibrates (agonists of PPARα used as hypolipidemic agents), thiazolidinediones (agonists of PPARγ used as anti-diabetic drugs), and the NUR77/NOR1 agonist, 6-mercaptopurine (119) (predominately used as an anti-cancer drug). Currently, concerns about increased risk of coronary heart disease has reduced the utility of the PPARγ agonists such as the thiazolidinedione, rosiglitazone (80). This, along with evolving molecular studies on PPARs, has led to an increased focus on agonists of PPARδ. Interestingly, despite positive results in phase II clinical trials (reduced fasting serum triglycerides, total cholesterol, and LDL and reduced liver fat), the PPARδ agonist GW501516 has not yet entered phase III trials (8). Further refined PPARδ agonists such as MBX-8025 and KD3010 are also under human clinical trials. A proof-of-concept study conducted at 30 US research sites concluded that, in overweight patients with mixed dyslipidemia, MBX-8025 resulted in favorable changes to serum lipids (significantly reduced LDL/triglyceride levels and raised HDL levels) and also reduced the number of patients with metabolic syndrome (111). The PPARδ agonist KD3010 is currently ready for phase II trials with potential for treating in Type 2-dependent diabetes mellitus and nonalcoholic steatohepatitis (Kalypsys). A number of other synthetic orphan nuclear receptor agonists/activators are currently in animal and human clinical trials. Some key examples include 1) FXR agonists that have entered clinical trials for chronic cholestatic conditions such as INT-747 (6α-ethyl-CDCA) for primary biliary cirrhosis and fexaramine for hypercholesterolemia (91); 2) LXR agonists that have entered clinical trials to enhance reverse cholesterol transport via upregulation of liver cholesterol transport (49); 3) finally, recently the NR4A subgroup have been implicated in oxidative metabolism (86) and glucose homeostasis (15, 16, 90), suggesting therapeutic uses for NR4A agonists. Local administration of the NUR77/NOR1 activator 6-mercaptopurine has been successfully used in rodent models of atherosclerosis (93). In conclusion, orphan (and adopted orphan) NRs regulate various aspects of metabolism, and these receptors provide an excellent platform for the discovery of new therapies for the treatment of metabolic pathologies.

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