REVIEW ARTICLE

MECHANISMS OF DISEASE

Retinoid X Receptor Heterodimers in the Metabolic Syndrome

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N Engl J Med 2005;353:604-15.

The metabolic syndrome, also known as syndrome X, is characterized by abdominal obesity, atherogenic dyslipidemia, hypertension, insulin resistance, inflammation, and prothrombotic states.[1] Diagnostic of the metabolic syndrome are abnormalities in three or more of the clinical criteria of the Adult Treatment Panel III of the National Cholesterol Education Program, which include the following: a waist circumference of more than 102 cm in men and more than 88 cm in women; a triglyceride level of 150 mg per deciliter or more; a level of high-density lipoprotein (HDL) cholesterol of less than 40 mg per deciliter in men and less than 50 mg per deciliter in women; a blood pressure of 130/85 mm Hg or more; and a fasting glucose level of 110 mg per deciliter or more.[2] The age-adjusted prevalence of this syndrome in the United States from 1988 to 1994 was estimated to be 23.7 percent, and the scope of the public health challenge it poses is likely to increase.[3] The major sequelae are cardiovascular disease and type 2 diabetes mellitus, but the syndrome also increases the risk of polycystic ovary syndrome, fatty liver, cholesterol gallstones, asthma, sleep disturbances, and some forms of cancer. A prospective study of Finnish men reported a connection between the metabolic syndrome and an increased risk of death associated with cardiovascular disease and all-cause mortality[4] — a finding that underscores the severity of this disease.

The pathogenesis of the metabolic syndrome is thought to involve a complex interaction of multiple factors, which include obesity and abnormal fat distribution; insulin resistance; hepatic, vascular, and immunologic factors; and lifestyle and genetic contributions.[1] In addition to behavioral therapies that promote weight reduction through exercise and dietary modification, management of the metabolic syndrome includes a combination of medical therapies targeted to reduce specific metabolic risk factors.[5] Statins and fibric acid derivatives (fibrates) are effective first-line treatments for atherogenic dyslipidemia and have been shown to reduce the risk of cardiovascular disease.[2] Combination therapy with a statin and a fibrate prevents the lowering of HDL cholesterol that is observed with the use of a statin alone and can improve abnormal serum lipoprotein profiles. The ability of statins and fibrates to induce severe myopathy, a toxic effect that is more frequent in combination treatment, limits their use in some patients.[6] Metformin and thiazolidinediones improve insulin sensitivity, but it is unknown if they reduce the risk of cardiovascular disease, and there are dose-limiting toxic effects. Experience with medical therapies highlights the potential of restoration of individual metabolic abnormalities in the treatment of the metabolic syndrome. Although numerous treatment options are available, the syndrome and its long-term sequelae often prove refractory to these interventions.

Intense interest in the development of drugs with new mechanisms of action for the metabolic syndrome has focused attention on nuclear receptors. Nuclear receptors are transcription factors that serve as intracellular receptors for endocrine hormones and

dietary lipids. In contrast to extracellular receptors, which bind to peptide ligands (e.g., growth factors and insulin) and activate cytoplasmic kinase cascades, nuclear receptors interact directly with lipophilic ligands and regulate expression of target genes. The retinoid X receptor (RXR), a member of the nuclear-receptor superfamily, is a common binding partner for a subgroup of other nuclear receptors. The resulting functional complex of one RXR molecule with one distinct nuclear-receptor molecule is known as a heterodimer. Drugs that target RXR and its heterodimerization partners are already in clinical use for the treatment of cancer, dermatologic diseases, endocrine disorders, and the metabolic syndrome (Table 1).

Like the lipid abnormalities in familial combined hyperlipidemia, moderately elevated levels of plasma triglycerides and cholesterol occur in the metabolic syndrome. Homeostatic regulation of lipid metabolism requires cellular sensors that can monitor the concentration of bioactive lipids and coordinate the enzymatic cascades that regulate lipid synthesis and catalysis. Abnormal function of the lipid-sensing system not only underlies dyslipidemia but also contributes to deficiencies in carbohydrate metabolism and other integrated physiologic processes. Recent work has shown that RXR and its heterodimerization partners bind to a variety of ligands derived from cholesterol, fatty acids, and fat-soluble vitamins and regulate target genes that mediate transport and catalysis of dietary lipids. The focus of this review is on new advances in understanding the function of RXR heterodimers in normal intermediary metabolism and in the pathophysiology of the metabolic syndrome. We also consider the promising findings about how drugs that target RXR heterodimers may be used in the management of the metabolic syndrome.

REVERSE ENDOCRINOLOGY OF RXR HETERODIMERS

Nuclear receptors that function as RXR heterodimers were cloned on the basis of their homology to the steroid hormone receptors and were characterized before their ligands were known. All members of the nuclear-receptor superfamily share a canonical domain structure (a structure shared by several proteins) that includes an N-terminal activation domain and conserved DNA and ligand-

Table 1. Approved Drugs Targeting RXR Heterodimers.*

Target Receptor	Drug	Compound Class	Brand Name	Indication
RXRα, RXRβ, and RXRγ	Bexarotene	Rexinoid (e.g., LG1069)	Targretin	Refractory cutaneous T-cell lymphoma
RARα, RARβ, and RARγ; RXRα, RXRβ, and RXRγ	Alitretinoin	Retinoid (e.g., 9-*cis*-retinoic acid)	Panretin	Kaposi's sarcoma (topical only)
RARα, RARβ, and RARγ	Tretinoin	Retinoid (e.g., all-*trans*-retinoic acid)	Retin-A, Renova, Vesanoid	Acute promyelocytic leukemia, acne
RARα, RARβ, and RARγ	Isotretinoin	Retinoid (e.g., 13-*cis*-retinoic acid)	Accutane	Severe nodular acne
Vitamin D receptor	Calcitriol	1,25-dihydroxyvitamin D_3	Rocaltrol, Calcijex	Hypocalcemia due to chronic renal failure and hypoparathyroidism
Vitamin D receptor	Ergocalciferol	Vitamin D_2	Calciferol	Vitamin D–resistant rickets, hypoparathyroidism, familial hypophosphatemia
TRα and TRβ	Levothyroxine	Thyroid hormone (e.g., L-thyroxine or T_4)	Levo-T, Unithroid, Levothyroid, Levoxyl, Synthroid	Hypothyroidism, euthyroid goiters, Hashimoto's thyroiditis
PPARγ	Pioglitazone	Thiazolidinedione	Actos	Type 2 diabetes mellitus (monotherapy or combination therapy)
PPARγ	Rosiglitazone	Thiazolidinedione	Avandia	Type 2 diabetes mellitus (monotherapy or combination therapy)
PPARα	Fenofibrate	Fibrate	Tricor	Types IIa, IIb, IV, and V hyperlipidemia
PPARα	Gemfibrozil	Fibrate	Gemfibrozil, Gemcor, Lopid	Types IIb, IV, and V hyperlipidemia

* Drugs approved by the Food and Drug Administration that target RXR or its heterodimeric partners, and their clinical indications, are listed.

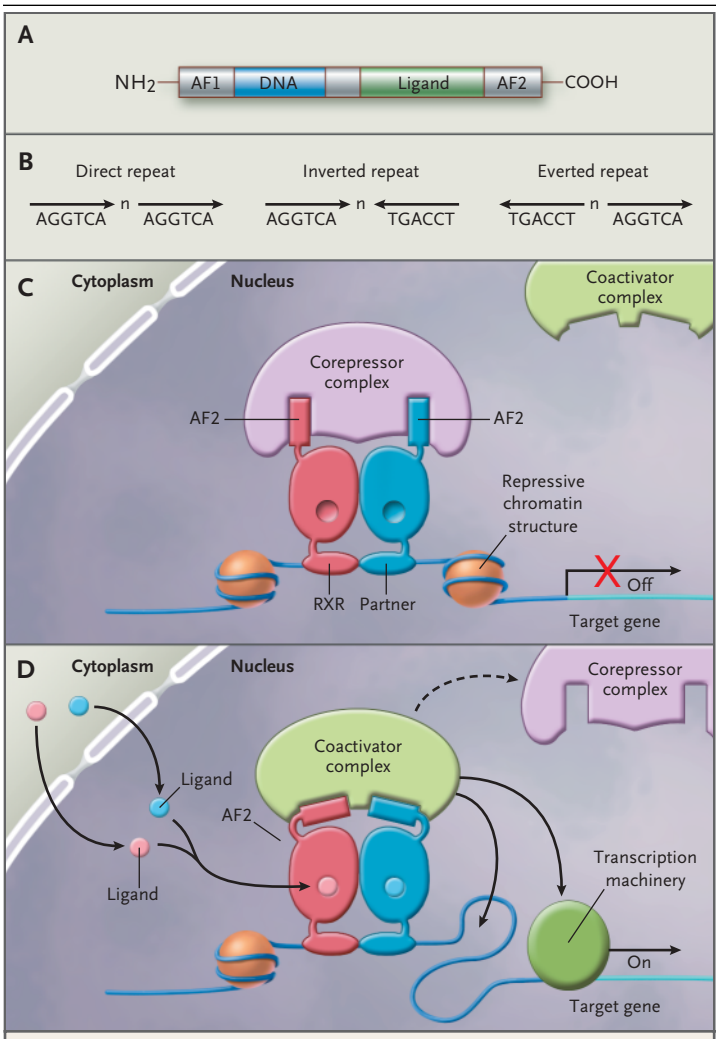

Figure 1. Nuclear Receptors as Ligand-Dependent Transcription Factors.

Panel A shows the canonical structure of the nuclear receptor, which includes N-terminal activation function 1 (AF1), DNA binding, ligand binding, and C-terminal AF2 domains. Response elements in the nuclear receptor can be configured as either direct, inverted, or everted repeats of the hexad core sequence AGGTCA (Panel B). The number of nucleotides between the two core elements (n) confers additional specificity. As shown in Panels C and D, RXR heterodimers constitutively bind to response elements in the promoter regions of target genes. In Panel C, in the absence of agonist ligand, the AF2-domain conformation promotes receptor interaction with corepressors. The multiple-subunit corepressor complex stabilizes repressive local chromatin structure and blocks access of the transcription machinery (red X) to the promoter. As shown in Panel D, ligands diffuse across the nuclear membrane and bind to receptor ligand-binding domains. Ligand binding triggers a conformational change in the AF2 domain, which destabilizes corepressor interaction and promotes coactivator binding. Multiple-subunit coactivator complexes activate local chromatin structure and recruit the transcription machinery to the promoter, where target-gene transcription commences.

binding domains (Fig. 1A). Nuclear receptors function as ligand-dependent transcription factors by binding to specific DNA sequences called response elements within the regulatory regions of target gene promoters. Each response element consists of a consensus sequence (AGGTCA) that is configured as a single element or as two tandem elements in a direct, everted, or inverted repeat, which permits binding of nuclear receptors as monomers, homodimers, or heterodimers (Fig. 1B).[7] A number of nuclear receptors must interact with RXR to form heterodimers that can bind to DNA response elements and activate target gene expression.[8,9] Structural studies of various nuclear-receptor ligand-binding domains have revealed a scaffold composed of 12 alpha helixes with a central hydrophobic pocket that directly binds a number of hormonal, lipid, and synthetic ligands. Analysis of structure–activity relationships for many agonist-bound nuclear receptors shows that helix 12, the AF2 helix, adopts a strikingly similar active conformation in all nuclear receptors.[10] Nuclear receptors activate or repress target gene expression through ligand-dependent interactions with accessory proteins, known as coactivators and corepressors. These cofactors form multiple-subunit complexes that modify local chromatin structure and recruit the transcription machinery to target gene promoters.[11] The coactivators and corepressors sense the ligand-binding status of nuclear receptors by recognizing alternative AF2 conformations (Fig. 1C and 1D). In addition to providing a mechanism for ligand-dependent transcriptional regulation, cofactors allow coordinated regulation of nuclear-receptor signaling. For example, specific cofactors, such as the peroxisome-proliferator–activated receptor (PPAR) γ coactivator 1, appear to have important roles in metabolic control by nuclear receptors.[12]

A 15-year effort to identify the ligands and physiologic roles of the RXR heterodimers has revealed a central role for these receptors as the body's lipid sensors. This ongoing research effort is known as "reverse endocrinology" because it originated with the characterization of cloned-receptor sequences as opposed to classic endocrine bioassays.[13] Together, the concerted application of the numerous methods can be described as a nuclear-receptor "discovery cycle," in which each step of the cycle may be used autonomously to lead to important discoveries (Fig. 2).

Initially, RXR heterodimer receptors are fed into the cycle by identifying their lipophilic, small-molecule agonists (i.e., ligands). Agonists resulting from such screens can then be used to identify target genes whose expression is regulated by the receptor. Once the physiologic role of a receptor has been implicated by its tissue distribution, ligand identity, and target genes, the receptor can be further tested with the use of genetic studies of loss and gain of function in animal models. Such experiments provide the rationale for translational research in human disease and, ultimately, for the development of nuclear-receptor ligands as therapeutic drugs. Efforts to create RXR heterodimer agonists with reduced adverse effects are analogous to the successful development of tissue-selective estrogen-receptor modulators (e.g., tamoxifen and raloxifene).[14] Continued refinement of lead pharmacologic compounds through the discovery cycle can provide new biologic insights and candidate drugs.

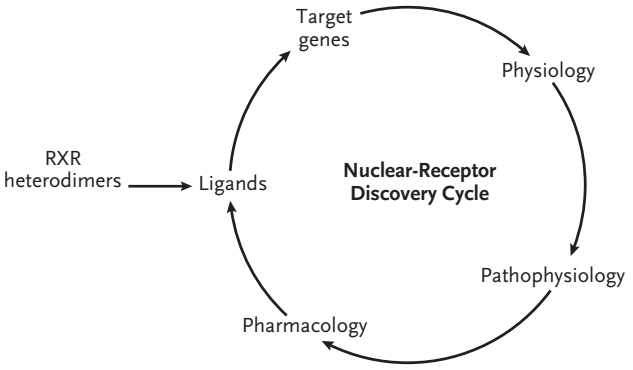

Figure 2. Nuclear-Receptor Discovery Cycle.
Concerted application of numerous approaches has clarified the physiologic and pathologic roles of nuclear receptors and has created new pharmacologic opportunities.

This discovery cycle has already revealed a central role for RXR heterodimers as cellular lipid sensors that might participate in the pathogenesis of metabolic disease (Table 2 and Fig. 3).[15] It is important to note that although nuclear receptors link lipid binding to the regulation of genes involved in the maintenance of metabolic homeostasis, the potentially protective roles of these receptors in disease are a consequence of pathologic conditions (e.g., a lipid-rich diet). As a result, pharmacologic manipulation of receptor activity can be expected to be associated with both beneficial and adverse metabolic effects in various contexts. We will use the discovery cycle as a framework to discuss the potential role of selected, individual RXR heterodimers in the metabolic syndrome. Although other RXR heterodimers (e.g., retinoic acid receptors and vitamin D receptor) are therapeutically important (Table 1), their involvement in the metabolic syndrome has not been shown, and they will not be discussed further.

RXRs: PARTNERS IN SIGNALING

The discovery that RXRs can be activated by 9-*cis* retinoic acid, an endogenous vitamin A derivative that is now in clinical use (Table 1), represents the first successful implementation of the discovery cycle and validates the reverse endocrinology concept.[8] Nuclear receptors that partner with RXR to form a heterodimer can be divided into functionally distinct permissive and nonpermissive groups (Table 3). RXR heterodimers that are formed by RXR and a permissive binding partner (e.g., PPARs, liver X receptors, and farnesoid X receptor [FXR]) can be activated by agonists for both RXR and the partner receptor.[16] For example, an RXR–PPAR heterodimer can be activated by both RXR and PPAR agonists independently or together to cause a synergistic activation. In contrast, RXR heterodimers that contain nonpermissive partners (e.g., vitamin D receptor and thyroid hormone receptor) can be activated only by the partner receptor's agonist but not by an RXR agonist. Permissive partners serve as receptors for dietary lipids and may allow RXR activation in order to establish steady-state expression levels for metabolic enzymes and transporters. In contrast, nonpermissive partners function primarily as hormone receptors and may inhibit RXR activation in order to place target genes under tight hormonal control. In this way, a small change in hormone concentration substantially alters the level of target gene expression, a property that meets the requirements of endocrine physiology.

The ability of RXR agonists to regulate target genes of multiple permissive partners implies that in vivo such compounds may have pharmacologic use as panagonists of several metabolically important pathways.[17] The observation that liver-specific deletion of RXR in mice results in abnormalities in all metabolic pathways regulated by RXR hetero-

Table 2. Nuclear Receptor Regulation of Lipid, Cholesterol, and Bile-Acid Metabolism.*

Nuclear Receptor	Tissue Distribution	Ligand	Physiologic Function	Associated Disease Process†
Retinoid X receptors				
RXRα, RXRβ, and RXRγ	Ubiquitous	9-*cis*-retinoic acid, DHA, rexinoids‡	Common heterodimer partner	Same as for heterodimeric partners
Peroxisome-proliferator–activated receptors				
PPARα	Liver, heart, muscle, kidney	Fatty acids, fibrates‡	Fatty-acid oxidation	Dyslipidemia, diabetic cardiomyopathy
PPARγ	Adipose, macrophage, muscle	Fatty acids, eicosanoids, thiazolidinediones‡	Adipogenesis, lipid storage	Insulin resistance, obesity, metabolic syndrome
PPARδ	Ubiquitous	Fatty acids	Fatty-acid oxidation, energy expenditure	Dyslipidemia, obesity
Liver X receptors				
LXRα and LXRβ	LXRα: liver, adipose tissue, kidney, intestine; LXRβ: ubiquitous	Oxysterols	Cholesterol homeostasis, fatty-acid synthesis	Atherosclerosis, dyslipidemia
Farnesoid X receptor				
FXR	Liver, intestine, kidney	Bile acids	Bile-acid homeostasis	Cholestasis, gallstone disease, dyslipidemia
Thyroid hormone receptors				
TRα and TRβ	Ubiquitous	Thyroid hormone‡	Metabolic rate, neural development, cholesterol metabolism	Cardiac dysfunction, dyslipidemia, obesity

* Tissue distribution, ligands, physiologic functions, and pathologic roles of RXRs and their heterodimeric partners involved in metabolic regulation are shown. DHA denotes docosahexaenoic acid.
† Increased or decreased activity of the indicated receptor contributes to the pathophysiology of the disease listed. In some cases, a receptor agonist or antagonist might be an effective therapy for the disease process.
‡ Indicated are ligands currently used in the clinic — rexinoids for cancer, fibrates for dyslipidemia, thiazolidinediones for type 2 diabetes mellitus and the metabolic syndrome, and thyroid hormone for deficiency states.

dimers underscores the central, pleiotropic role of RXR.[18] Although RXR agonists have therapeutic value (Table 1) and might offer enhanced potency through panactivation of permissive heterodimers, this advantage is likely to be offset by poor selectivity. In addition, the propensity of RXR agonists to induce hypertriglyceridemia in animals and humans[19] indicates that targeting the heterodimeric partners of RXRs is likely to result in more suitable candidates for drug therapy.

PPARs: FATTY-ACID SENSORS

PPARα

The PPARs are nuclear receptors that bind to fatty-acid–derived ligands and activate the transcription of genes that govern lipid metabolism. The primary sites of action of PPARα, which recognizes monounsaturated and polyunsaturated fatty acids and eicosanoids, are liver, heart, muscle, and kidney.[20,21] Consistent with its role in regulating fatty-acid metabolism, PPARα activates a program of target gene expression involved in fatty-acid uptake (fatty-acid–binding protein), beta oxidation (medium-chain acyl-CoA dehydrogenase, carnitine palmitoyltransferase I, and acyl-CoA oxidase), transport into peroxisomes (ATP-binding cassette transporters D2 and D3), and omega oxidation of unsaturated fatty acids (cytochrome P-450 4A1 and 4A3).[22-25]

In the fasting state, PPARα is activated by adipose-derived fatty acids, thereby enhancing the generation of ketone bodies through hepatic fatty-acid oxidation. Fasting PPARα-deficient mice have severe hypoglycemia and hypoketonemia, fatty liver, and elevated plasma nonesterified fatty acids, revealing the important role of this receptor in the hypoglycemic response.[24,26] PPARα-deficient mice that are fed a high-fat diet are unable to up-regulate fatty-acid catalysis and develop hepatic steatosis in the absence of obesity.[27] In cardiac muscle, PPARα activation decreases glucose uptake and causes a shift from glucose use to fatty-acid oxidation.[28] For

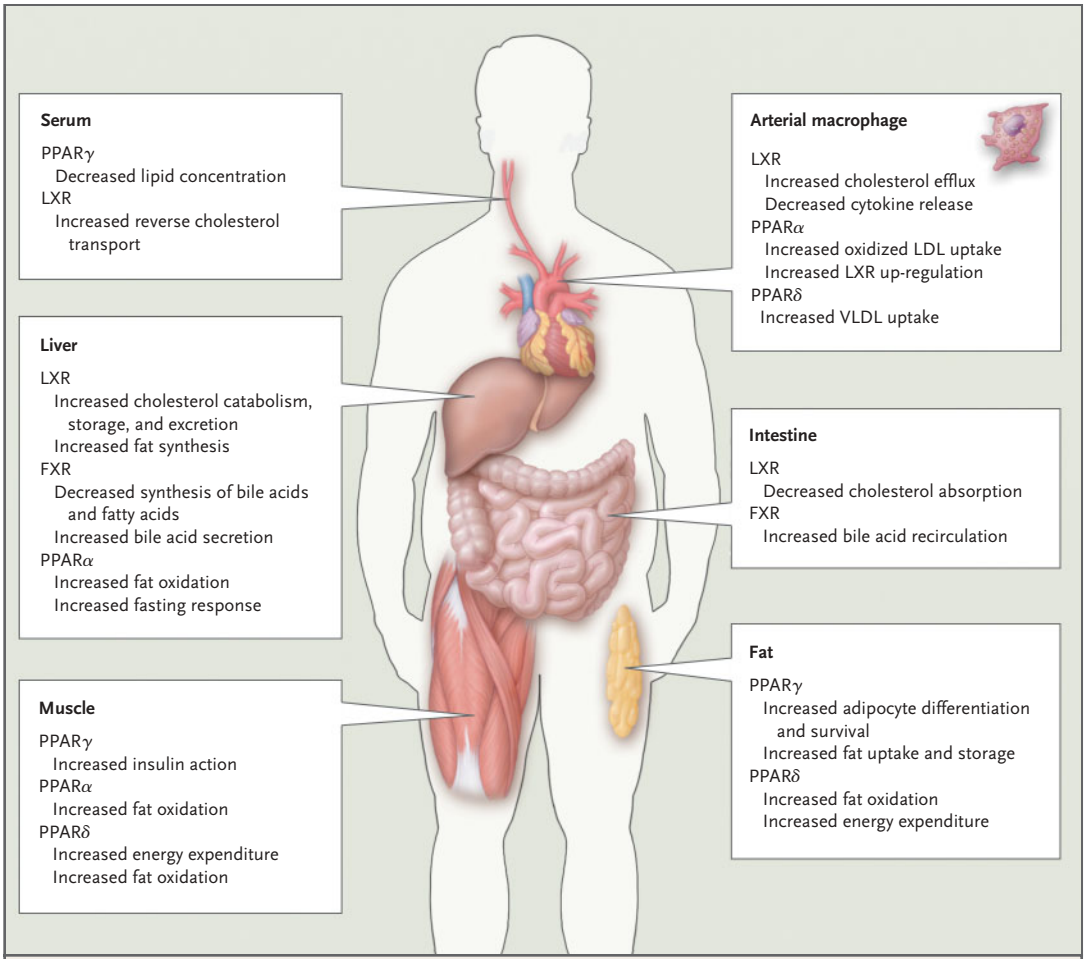

Figure 3. Regulation of Cholesterol and Lipid Handling in Metabolic Organ Systems by Nuclear Receptors.

The major actions of RXR heterodimer partners at sites that participate in intermediary metabolism, absorption, energy storage, energy expenditure, and lipid transport are indicated. Dysfunction in these organ systems contributes to the pathogenesis of the metabolic syndrome. LDL denotes low-density lipoprotein cholesterol, and VLDL very-low-density lipoprotein cholesterol.

this reason, supraphysiologic activation of PPARα in the heart brings about lipid accumulation, ventricular hypertrophy, and systolic dysfunction — a phenotype that resembles diabetic cardiomyopathy. Taken together, mouse models suggest that PPARα functions to increase fatty-acid use in the fasting state and that in the pathophysiologic context of a high-fat diet, PPARα-induced fatty-acid catabolism might prevent hypertriglyceridemia. Consistent with this prediction, an activated variant of PPARα (Leu162Val) is associated with low serum triglyceride levels and reduced adiposity.[29]

The finding that fibrate drugs, such as fenofibrate and gemfibrozil, act as PPARα agonists makes this receptor an attractive target in the treatment of atherogenic dyslipidemia.[30,31] Fibrates, which reduce the risk of cardiovascular disease in patients with hypertriglyceridemia and a low-to-normal level of serum HDL cholesterol, most likely decrease serum triglyceride levels and cause slight increases in levels of serum HDL cholesterol by PPARα-mediated activation of fatty-acid beta oxidation.[32] A well-known side effect of synthetic PPARα agonists in rodents is hepatomegaly due to proliferation of peroxisomes, specialized organelles for fatty-acid beta oxidation.[27,33] Fortunately, these effects are rodent-specific and are not observed in humans. Selective PPARα agonists that increase fatty-acid catabolism without causing lipid accumulation in the heart might be effective treatments for dyslipidemia.

Table 3. Ligand Permissivity Associated with Function of RXR Heterodimer.*

Variable	Permissive	Nonpermissive
Receptors	PPARs, LXRs, FXR	VDR, TRs
Ligand	Dietary lipids	Endocrine hormones
Ligand affinity	Micromolar to nanomolar	Nanomolar to picomolar
Physiologic range of ligand	Broad	Narrow
Ligand regulation	Feed-forward	Feedback

* Permissive RXR heterodimer partners bind to dietary lipids with low affinity and activate feed-forward enzymatic cascades that regulate ligand catabolism. Nonpermissive RXR partners are high-affinity endocrine receptors that regulate ligand concentration by negative feedback of hormone synthesis. Although pathologic states result when the concentration of hormonal ligands occurs outside of narrow limits, the physiologic range of dietary lipid concentration is broader. VDR denotes vitamin D receptor and TR thyroid hormone receptor.

PPARγ

PPARγ is expressed in adipocytes, macrophages, and muscle, where it regulates development, lipid homeostasis, and glucose metabolism. Endogenous PPARγ agonists include fatty acids and eicosanoids.[20,34,35] The PPARγ genetic program includes target genes involved in the uptake of glucose in muscle (c-Cbl associated protein and glucose transporter 4), lipid metabolism (scavenger receptor, adipocyte-fatty-acid–binding protein, lipoprotein lipase, fatty-acid–binding protein, acyl-CoA synthetase, and CYP4B1), and energy expenditure (glycerol kinase and uncoupling proteins 2 and 3).[36-44] Mice lacking PPARγ in the germ line are embryonic lethal because of a placental defect,[45-47] but creation of conditional PPARγ knockouts and the use of in vitro fibroblast-differentiation assays have confirmed the essential role of PPARγ in adipocyte differentiation and survival.[45,47,48] In addition, specific deletion of the *PPARγ* gene in fat and muscle causes insulin resistance, demonstrating the importance of this receptor in peripheral insulin sensitivity.[48,49]

It is interesting to note that heterozygous PPARγ knockout mice have improved insulin sensitivity and are not susceptible to the insulin resistance and obesity associated with a high-fat diet.[46] This finding is consistent with the therapeutic action of PPARγ partial agonists, such as the thiazolidinediones, and confirms the notion that partial activation of PPARγ is required to promote nominal, but not excessive, adipose storage depots and thereby maintain a proper insulin response. Possible mechanisms of PPARγ-induced insulin sensitivity include increased lipid uptake and storage, leading to decreased free fatty acids and serum triglycerides, suppression of hepatic gluconeogenesis, and a small contribution toward increased uptake of glucose by adipose tissues. PPARγ activation also increases energy expenditure by inducing a futile cycle of triglyceride synthesis from free fatty acids and increasing uncoupled respiration through uncoupling proteins.[44]

In addition to regulating glucose and lipid metabolism, PPARγ is a potential modifier of atherogenesis. Signaling through PPARγ, components of oxidized low-density lipoprotein (LDL) increase expression of the scavenger receptor CD36, resulting in lipid accumulation in macrophages.[50,51] PPARγ also activates the macrophage LXR-ABCA1 cholesterol efflux pathway,[52] which may explain the finding that PPARγ ligands inhibit the formation of atherosclerotic lesions in LDL-receptor–deficient mice.[53]

Human genetics has provided independent corroboration of the central role of PPARγ in the metabolic syndrome.[54] Dominant negative mutations in PPARγ are the cause of monogenic disease with features of the metabolic syndrome, including severe insulin resistance, type 2 diabetes mellitus, and hypertension.[55] The PPARγ Pro12Ala variant is associated with a low body-mass index and insulin sensitivity, and it appears to protect against the metabolic syndrome.[56]

The landmark finding that the thiazolidinedione class of insulin sensitizers, including rosiglitazone and pioglitazone (Table 1), function as high-affinity PPARγ agonists has validated the efficacy of PPARγ modulation in treating the metabolic syndrome.[57] Although thiazolidinediones have become important first-line agents for increasing insulin sensitivity, adverse effects including weight gain, adipogenesis, and toxic effects in the liver have limited their use. In addition, recent data indicating that PPARγ agonists have carcinogenic potential in rodents have prompted the Food and Drug Administration to require two-year carcinogenicity studies in rodents in its consideration of new drugs in this class. The effort to design safe and selective PPARγ modulators that retain an insulin-sensitizing function without activating adipocyte differentiation and lipid accumulation is ongoing.[58] Second-generation PPARγ agonists have the promise to improve multiple metabolic measures and reduce the risk of cardiovascular disease in patients with the metabolic syndrome.

PPARδ

PPARδ is expressed ubiquitously and is activated by fatty acids and components of very-low-density lipoprotein (VLDL).[59,60] PPARδ target genes control beta oxidation in murine brown fat (long-chain and very-long-chain acyl-CoA synthetase, long-chain and very-long-chain acyl-CoA dehydrogenase, and acyl-CoA oxidase), energy expenditure (uncoupling proteins 1 and 3), and lipid storage (macrophage adipose differentiation–related protein).[61,62] Similar to conventional targeting of PPARγ, most PPARδ knockout mice die in midgestation as a result of defects related to the placenta. Surviving mice show markedly decreased adipose tissue, a finding that is not recapitulated in adipose-specific knockout mice and suggests a requirement for PPARδ in peripheral tissues.[63] Genetic activation of PPARδ in adipocytes and treatment with a synthetic PPARδ agonist result in increased beta oxidation of fatty acids, energy expenditure, and resistance to diet-induced obesity.[61] PPARδ also mediates transcriptional responses to VLDL-derived triglycerides in macrophages.[60]

In the pathophysiological context of a high-fat diet, PPARδ could function to increase adipose fatty-acid catabolism and may play a role in VLDL-induced lipid accumulation in atherosclerotic foam cells. A high-affinity synthetic PPARδ agonist has been shown to increase HDL and decrease LDL, triglycerides, and fasting insulin in obese rhesus monkeys.[64] These studies suggest that therapeutic activation of PPARδ has the potential to decrease diet-induced obesity without activating the PPARγ-dependent adipogenic program.

LXRs: STEROL SENSORS

The LXRs are nuclear receptors that bind oxidized cholesterol derivatives (oxysterols) such as 24(S),25-epoxycholesterol.[65] LXRα is expressed primarily in liver, adipose tissue, intestine, macrophage, and kidney, whereas LXRβ is ubiquitous. In response to an increased concentration of cellular oxysterols, LXRs activate genes involved in "reverse cholesterol transport" from peripheral tissues to the liver and hepatic cholesterol metabolism.[66] LXRs induce the expression of proteins that stimulate cholesterol efflux from macrophages (ABCA1 and ABCG1), promote cholesterol transport in serum and uptake into liver (apolipoprotein E, phospholipid transfer protein, lipoprotein lipase, and cholesterol ester transfer protein), increase cholesterol catabolism into bile acids (CYP7A1), increase biliary secretion of cholesterol (ABCG5 and ABCG8), and inhibit absorption of cholesterol in the intestine (ABCG5, ABCG8, and ABCA1).[17,67-73] LXRs also increase the synthesis of fatty acids and triglycerides by up-regulating sterol regulatory element-binding protein 1c (SREBP-1c), the master regulator of fatty-acid synthesis.[74] Activation of LXR represses lipopolysaccharide induction of inflammatory mediators in macrophages, a mechanism with potential significance in atherosclerosis.[75]

Studies in animals have confirmed the physiologic role of LXRs as mediators of cholesterol metabolism and have suggested protective functions in the pathological contexts of atherosclerosis and hypercholesterolemia. In *Lxrα*-knockout mice, abnormal uptake and elimination of dietary cholesterol results in hepatic failure because of a profound accumulation of cholesterol esters.[72] High-affinity synthetic LXR agonists have been shown to increase hepatobiliary cholesterol secretion, decrease cholesterol absorption, and increase HDL levels in animal models.[17,76] In atherosclerosis-prone mouse models, LXR agonist treatment leads to increased HDL levels and decreased formation of atherogenic lesions.[77] Transplantation of bone marrow cells that are deficient in both LXRα and LXRβ into susceptible animals results in increased atherogenesis.[78] The propensity of LXR agonists to induce hepatic and serum hypertriglyceridemia, most likely via SREBP-1c up-regulation, is a potential barrier to the development of LXR agonists as cholesterol-lowering and antiatherogenic agents.[74,76]

Several approaches have the potential to lead to the development of selective LXR modulators that could decrease cholesterol accumulation and inhibit atherosclerosis without adversely affecting other serum lipid measures. For example, of the two LXR subtypes, LXRα is a more potent activator of SREBP-1c, suggesting that LXRβ-specific agonists might preferentially decrease cholesterol without causing substantial hypertriglyceridemia. Coactivator-specific LXR ligands might also have desirable effects on serum lipid profiles owing to distinct coactivator requirements at the SREBP-1c and ABCA1 promoters. Finally, certain derivatives of plant sterols, which are not absorbed but can activate LXR in enterocytes, would be expected to inhibit intestinal cholesterol absorption without inducing serum hypertriglyceridemia.[79] Creative attempts to maximize the therapeutic properties of LXR ligands are a promising example of the ap-

plication of biologic insight to receptor pharmacology.

FXR: BILE ACID SENSOR

Expressed in the enterohepatic system, kidney, and adrenals, FXR functions as a nuclear receptor for bile acids such as chenodeoxycholic acid and cholic acid.[80-82] FXR target genes regulate the secretion of bile acids and phospholipids into bile (bile salt efflux pump and multidrug-resistance proteins 2 and 3), the intestinal reabsorption of bile acid (ileal bile acid–binding protein), and hepatic cholesterol uptake from serum HDL (phospholipid transfer protein).[80,83-86] FXR indirectly mediates negative feedback repression of bile-acid synthesis by inducing a transcriptional repressor that decreases expression of CYP7A1, the rate-limiting enzyme in bile-acid synthesis.[87,88] FXR-deficient mice have increased serum levels of bile acids, total bile-acid pool size, and fecal bile-acid excretion — findings consistent with altered bile-acid homeostasis due to defective feedback inhibition of hepatic synthesis.[89] These defects lead to increased levels of serum total cholesterol, HDL, and triglycerides and to decreased HDL clearance.[90] Thus, it is perhaps not surprising that FXR agonists have a marked ability to reduce levels of hepatic and serum triglycerides and may be useful in treating hypertriglyceridemia.[91]

A recent finding suggests that FXR agonists may be effective in treating cholesterol gallstone disease, a condition that results from increased hydrophobicity of bile salts and supersaturation of biliary cholesterol.[86] Pharmacologic support for this idea comes from the finding that a potent synthetic FXR agonist can prevent all the sequelae of cholesterol gallstone disease in a murine model that mimics the human disease.[86] The clinical relevance of this finding may be particularly applicable in treating patients who have undergone cholecystectomy and are readmitted for recurring symptoms and acute pancreatitis associated with microlithiasis.

THYROID HORMONE RECEPTORS

The thyroid hormone receptors (TRs) are expressed throughout the body and regulate numerous metabolic functions such as lipid and carbohydrate metabolism, blood pressure, and body mass in response to thyroid hormone. Although TR activation could increase metabolism and promote weight loss, TR agonists have not been useful in the metabolic syndrome because of cardiac side effects and other adverse effects. Recent evidence, which suggests that TRα plays an important role in cardiac function and that TRβ preferentially regulates energy consumption and cholesterol metabolism, offers the possibility that isoform-specific TR agonists might safely increase energy expenditure.[92] To that end, a selective TRβ agonist has been demonstrated to reduce serum cholesterol, LDL, and body weight without increasing heart rate in primates.[93] Further studies will be required to determine if TRβ-specific activation can ameliorate aspects of the metabolic syndrome in humans.

PERSPECTIVES

The discovery cycle involving nuclear receptors has elucidated the molecular and physiological basis for a new class of pharmacophores that show promise for treating the metabolic syndrome. The availability of numerous approaches — including focused chemical-library screening, structure-based ligand design, and an enhanced understanding of nuclear-receptor regulation — should clearly aid this drug-discovery process. In addition, high-throughput efforts to catalogue nuclear-receptor expression and function, such as the Nuclear Receptor Signaling Atlas (www.nursa.org), are helping to establish a comprehensive database of the physiologic and pathologic features of nuclear-receptor systems. Given the disappointing number of new drugs being developed at most pharmaceutical companies, continued research into the RXR heterodimer discovery cycle for the improved treatment of the metabolic syndrome is a promising strategy whose time has come.

Supported by the Howard Hughes Medical Institute; the Robert A. Welch Foundation; the National Institutes of Health Pharmacological Sciences Training Program and Medical Scientist Training Program; and a Nuclear Receptor Signaling Atlas grant (U19DK62434) from the National Institutes of Health. Dr. Mangelsdorf is an investigator for the Howard Hughes Medical Institute.

Dr. Mangelsdorf reports having been a coinventor of several intellectual properties licensed to Ligand Pharmaceuticals and Exelixis and having received lecture and consulting fees from X-Ceptor Therapeutics, Merck Frosst, Ligand Pharmaceuticals, Boehringer Ingelheim, Pfizer, Sumitomo, and Chugai-Roche.

We are indebted to members of the Mango laboratory at the University of Texas Southwestern Medical Center for their helpful discussions.

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