Diet, Fatty Acids, and Regulation of Genes Important for Heart Disease

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Diets rich in omega-3 polyunsaturated fatty acids (n-3 PUFAs), such as alpha-linoleic acid, eicosapentaenoic acid, and docosahexaenoic acid, are associated with decreased incidence and severity of coronary heart disease. Similarly, conjugated linoleic acids (CLAs), which are found in meat and dairy products, have beneficial effects against atherosclerosis, diabetes, and obesity. The effects of n3-PUFAs and CLAs are in contrast to fatty acids with virtually identical structures, such as linoleic acid and arachidonic acid (*ie,* n-6 PUFAs). This article discusses the possibility that cognate receptors exist for fatty acids or their metabolites that are able to regulate gene expression and coordinately affect metabolic or signaling pathways associated with coronary heart disease. Three nuclear receptors are emphasized as fatty acid receptors that respond to dietary and endogenous ligands: peroxisome proliferator activated receptors, retinoid X receptors, and liver X receptors.

Introduction

Coronary heart disease (CHD) is the leading cause of death in industrialized countries and is of rising concern worldwide. The relationship between CHD and diet has been studied for nearly 100 years, essentially since the first observation of high-fat and high-cholesterol diets producing atherosclerosis in rabbits [1••,2•]. Epidemiologic studies have demonstrated that diets high in saturated fatty acids and/or cholesterol increase serum cholesterol and risk of developing CHD. Correlations between diet and incidence of CHD across geographic boundaries and among emigrants have also been noted. These discoveries have lead to the diet-heart hypothesis, which suggests that dietary saturated fat and cholesterol are the major cause of CHD and atherosclerosis in humans [2•]. Although dietary fat has dominated the diet-heart hypothesis, there are many other foodstuffs and nutrients that may be involved in the etiology of this disease. Fiber, antioxidants, folic acid, calcium, and carbohydrate content of food have an impact on heart disease and atherosclerosis as well [1••].

Not All Fats Created Equal

The type of fat in the diet, in particular the saturation of the fatty acid component, dramatically impacts CHD. For example, all three major classes of fatty acids (saturated, monounsaturated, and polyunsaturated) increase high-density lipoprotein (HDL) cholesterol in humans; however, saturated fatty acids increase and polyunsaturated fatty acids (PUFAs) decrease low-density lipoprotein (LDL) cholesterol. The increased ratio of LDL to HDL in the case of saturated fats is associated with increased risk of developing CHD. Saturated fatty acids are generally considered atherogenic and increase thrombosis [1••]. *Trans* fatty acids, found in vegetable shortenings and deep-fried food, raise LDL to HDL ratios to a much greater degree than saturated fat [1••]. One potential mechanism by which *trans* fats adversely affect insulin resistance, diabetes, and CHD is by inhibiting essential fatty acid metabolism.

Two PUFAs that cannot be made in the body (and both of which are essential fatty acids) are linoleic acid (LA, an n-3 fatty acid) and alpha-linolenic acid (ALA, an n-6 fatty acid). In conditions of LA deficiency, arachidonic acid (AA) may also be considered essential. Once in the body, LA and ALA may be converted to others PUFAs such as AA, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) (Fig. 1). Although many fats have been associated with increasing the risk of CHD (*eg*, saturated and *trans* fatty acids), EPA and DHA have been associated with a variety of beneficial health effects. For this reason, diets that are high in ALA, EPA, and DHA have been sought, and these diets include fish oils, flaxseed, mustard seeds, soy beans, walnut oil, and green leafy vegetables.

Polyunsaturated fatty acids are important for maintaining membrane integrity and as precursors to bioactive prostaglandins, which regulate inflammation, blood clotting, and lipid metabolism. Thus, it is necessary to have diets sufficient in PUFAs (n-3 and n-6) to maintain a variety of biologic processes. Positive effects of diets high in n-3 fatty acids include reducing abdominal fat, preventing cardiac arrhythmia, lowering serum triacylglycerol levels, decreasing thrombosis, and improving endothelial function. As noted by Hu and Willett [2•], several studies

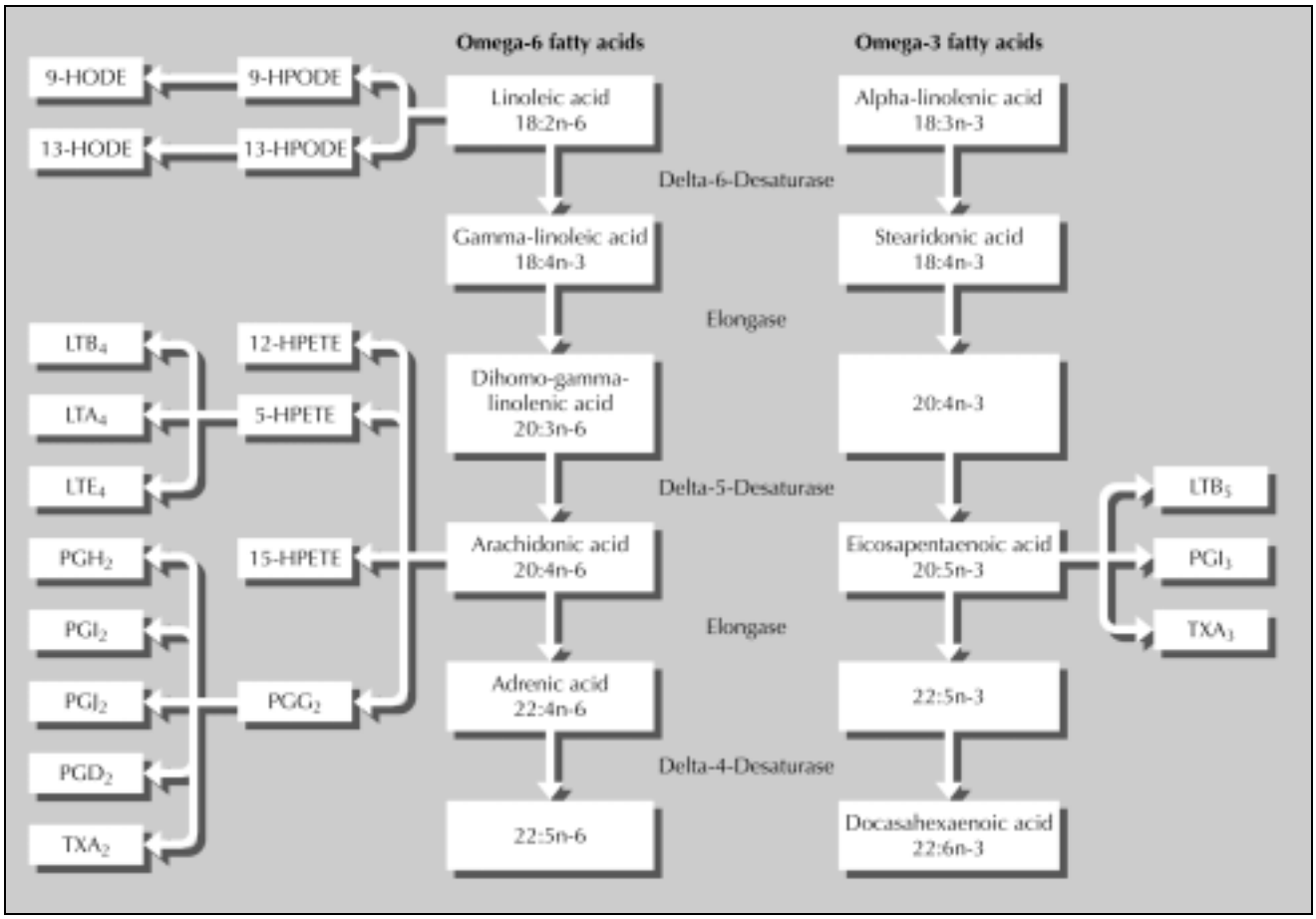


Figure 1. Metabolism of linoleic acid (omega-6 polyunsaturated fatty acid) and alpha-linolenic acid (omega-3 polyunsaturated fatty acid). These fatty acids are thought to be important regulators of coronary heart disease. The fatty acids or their metabolites may be ligands for nuclear receptors such as peroxisome proliferator activated receptor, liver X receptor, and retinoid X receptor, and they may control gene expression. (HODE—hydroxyoctadecadienoic acid; HPETE—hydroperoxyeicosatetraenoic acid; HPODE— hydroxyperoxyoctadecadienoic acid; LT—leukotriene; PG—prostaglandin; TX—thromboxane.)

have shown association of fish intake and/or flaxseed oil (high in ALA) with decreased fatalities from CHD. Importantly, blood levels of EPA and DHA are strongly associated with decreased risk of death, myocardial infarction, and stroke.

Conjugated linoleic acid (CLA) collectively refers to a group of LA derivatives with several positional (double bonds in carbon 9 and 11 or 10 and 12) and geometric (*cis*, Z and *trans*, E) isomers. CLAs are relatively abundant in ruminant meat and heat-processed dairy products. They are also formed from LA in the intestine of livestock by bacterial flora and are deposited in tissues and milk. CLA has received widespread attention due to its anticancer [3-5], antiatherosclerotic [6], and antidiabetic effects [7] in laboratory animals. Whether CLA is metabolized to bioactive molecules such as those noted for ALA and LA has not been determined. However, it is evident from animal studies that CLA has effects on CHD that resemble those of n-3 PUFAs.

The question that remains is why some PUFAs, in particular n-3 PUFAs (ALA, EPA, and DHA) and CLAs, are associated with reduced risk of CHD whereas closely related n-6 PUFAs (LA and AA), and monosaturated (oleic acid) and saturated (palmitic acid) fatty acids are either not as effective or are detrimental to heart health. One explanation may be inhibition of n-6 metabolism by these other structurally similar compounds. This would increase the production of metabolites associated with platelet aggregation, inflammation, and vasoconstriction (leukotriene B_4, prostaglandin [PG] I_2), and thromboxane [TX] A_2) at the expense of those metabolites that have antiaggregation, anti-inflammation, and antivasoconstriction properties (leukotriene B_5, PGI_3, and TXA_3). Another explanation, and the option explored herein, is that cognate receptors exist that preferentially respond to a particular structure of fatty acid. These specific "lipid sensors" would affect gene expression in a tissue-specific, sex-specific, and developmentally specific manner and thereby affect the development of CHD, perhaps by altering enzymes and proteins involved in the transport or metabolism of cholesterol and fatty acids. Also, in order for these receptors to be involved in the beneficial effects of dietary fatty acids, they must be able to

distinguish subtle changes in physical structure of the "good lipids" from "bad lipids," such as n-3 versus n-6 PUFAs, CLA versus LA, and PGI_3 versus PGI_2.

Nuclear Receptors As Sensors of Dietary Lipids

A likely family of proteins that may act as lipid sensors that meet the criteria stated here are the nuclear receptors (NR). Members of the NR superfamily act as intracellular transcription factors that directly regulate gene expression in response to lipophilic molecules [8–13,14•]. They affect a wide variety of functions, including fatty acid metabolism, reproductive development, and detoxification of foreign substances. To date, over 300 NRs have been cloned, many with unknown endogenous ligands (orphan receptors). Phylogenic analysis has shown six subfamilies (NR1 to NR6) with various groups and individual genes [15]. Several NRs have evolved to respond to dietary lipids (Fig. 2) and include the fatty acid receptors peroxisome proliferator activated receptor (PPAR), retinoid X receptor (RXR), liver X receptor (LXR), and hepatocyte nuclear factor-4α (HNF4α) [14•,16]. The receptors shown in Figure 2 may be considered constituents of a large group of NRs known as the "metabolic nuclear receptors," which act as overall sensors of metabolic intermediates, xenobiotics, and compounds in the diet and allow cells to respond to environmental changes by inducing the appropriate metabolic genes and pathways [17••].

Most NRs regulate gene expression in predominantly the same fashion (Fig. 2B). Prior to activation, NRs often exist in multiprotein complexes that vary depending on the family of receptor under question. When a ligand binds to its cognate receptor, a conformational change occurs ("activation") that changes the protein-protein interfaces of the molecule. As a result, the activated receptor interacts with a NR response element (NRE) within the regulatory region of a target gene; upon recruitment of various transcriptional coactivators and subsequently RNA polymerase II (polII), initiation of transcription of the target gene occurs.

In the following sections, the three likely candidates for NRs that respond to dietary fatty acids (*ie*, PPAR, RXR and LXR) are described. The dietary and metabolic intermediates that activate these receptors (Table 1) as well as the genes regulated by these NRs that contribute to prevention of CHD (Fig. 2) are emphasized.

Peroxisome proliferator activated receptors

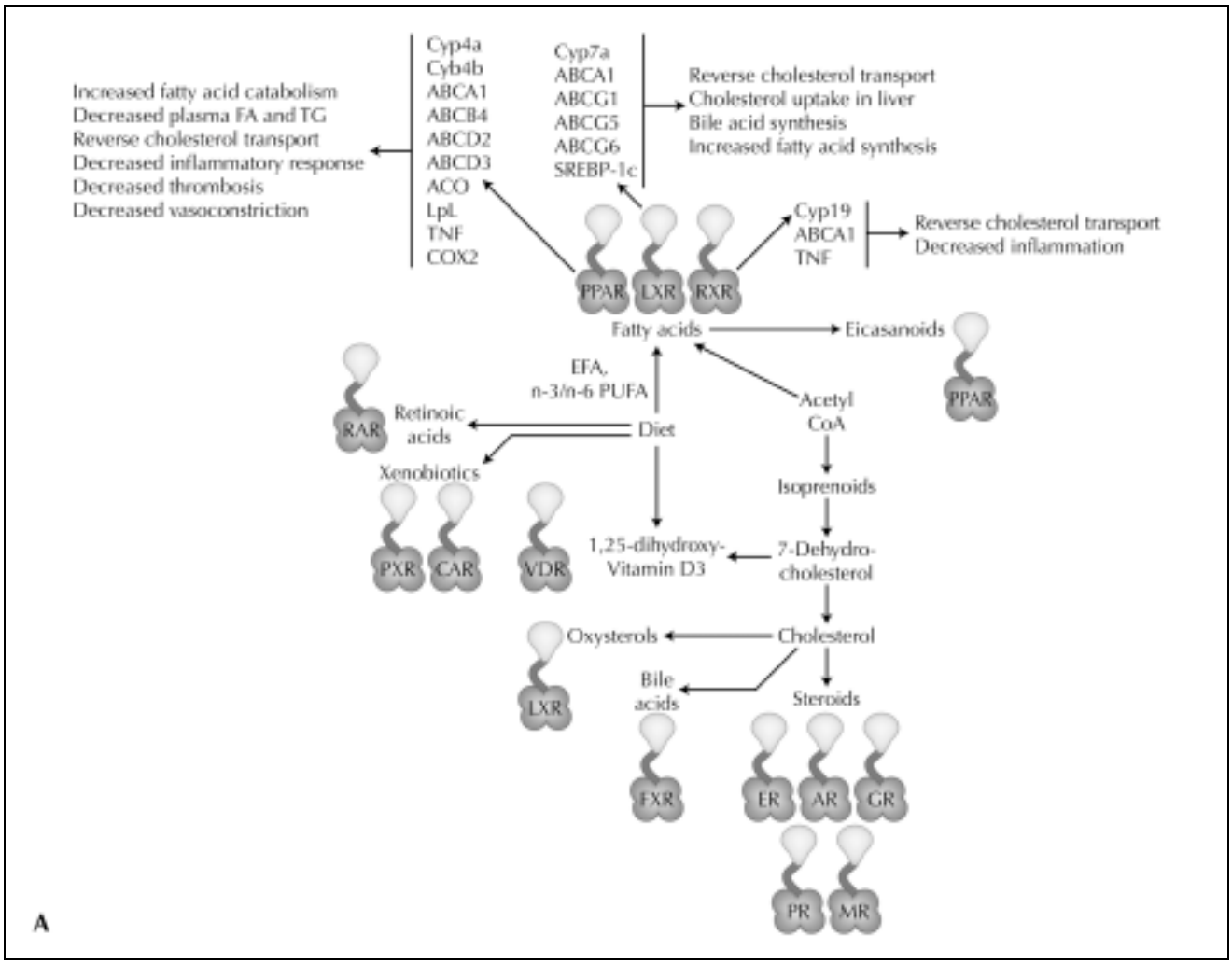
Of the several identified fatty acid receptors, perhaps the family that can best explain the effects of n-3 PUFAs and the CLAs are the PPARs. The PPAR receptors were originally named based on their ability to respond to xenobiotics (peroxisome proliferators); however, they were also the first to be examined as a fatty acid receptor. It has now been well established that PPAR is a ligand-activated transcription factor involved in gene expression in a tissue-, sex-, and species-dependent manner [14•,17••,18,19•]. The PPARs exist as three subtypes (α, β, and γ) that vary in expression, ligand recognition, and biologic function.

Peroxisome proliferator activated receptor α was the first transcription factor identified as a prospective fatty acid receptor [20–22]. Based on numerous studies from the PPARα knockout ($PPAR\alpha^{-/-}$), this receptor plays a role in the regulation of an extensive network of genes involved in glucose and lipid metabolism. In particular, PPARα regulates fatty acid transport; fatty acid binding proteins; fatty acyl-coenzyme A (CoA) synthesis; microsomal, peroxisomal, and mitochondrial fatty acid oxidation; ketogenesis; and fatty acid desaturation.

Several groups have implicated saturated and unsaturated fatty acids as natural ligands for PPARα [23]. Natural PPARα ligands in human serum include palmitic acid, oleic acid, LA, and AA. Notably, PPARα is the only PPAR subtype that binds to a wide range of saturated fatty acids. The 9z 11e CLA isomer is a potent PPARα ligand with a dissociation constant (K_d) in the low nanomolar range [24], and it affects PPAR-responsive enzymes including acyl-CoA oxidase (ACO), liver fatty acid binding protein (L-FABP), and cytochrome P450 4A1 (CYP4A1) [25]. Similar to other PUFAs, the effects of CLA on body composition are seen in the PPARα-null mouse [26], suggesting that this NR is not the key target for this response.

Triglyceride-rich lipoproteins, including very low-density lipoproteins (VLDL) and LDL, contain PPARα ligands [27,28]. Activation of PPARα is seen when lipoprotein lipase (LPL) is added to VLDL, showing that the endogenous ligands are probably fatty acids or their metabolites esterified into triacylglycerols. Metabolism of AA by CYP4A results in a variety of PPARα ligands, including 5,6, epoxyeicosatrienoic acids (EET); 8,9 EET; 11,12 EET; 14,14 EET; 20-hydroperoxyeicosatetraenoic acid (20-HETE); and 20-, 14-, and 15-hydroxyepoxyeicosatrienoic acids (HEET) [29]. Leukotriene B_4 has also been reported to be a selective PPARα ligand [30]. PGD_2 and PGD_1 activate PPARα in transient transfection reporter assay systems [31]. The lipoxygenase metabolite 8(S)-HETE is a high-affinity PPARα ligand, although it is not found at sufficient concentrations in the correct tissues to be characterized as a natural ligand. Because no single high-affinity natural ligand has been identified, Willson *et al.* [23] have proposed that one physiologic role of PPARα may be to sense the total flux of fatty acids in metabolically active tissues.

Peroxisome proliferator activated receptor γ is expressed in many tissues, including adipose, muscle, vascular cells, macrophages, and epithelial cells of the mammary gland, prostate, and colon [32]. Activated PPARγ induces LPL and fatty acid transporters (CD36) and enhances adipocyte differentiation, as well as inhibiting cytokine and cyclooxygenase-2 (COX-2) expression, perhaps by modulating nuclear factor-κB (NFκB) function. The PPARγ-null mouse is nonviable, implicating an important role for this protein in ontogeny [33] and also making the examination of a role for this receptor in gene expression difficult.



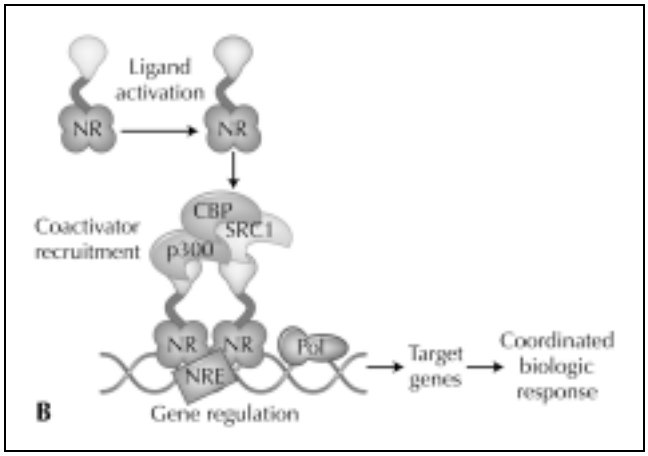


Figure 2. Dietary control of gene expression by nuclear receptors. **A,** Nuclear receptors involved in responding to dietary components and intermediary metabolism. The genes and coordinated biologic responses regulated by the fatty acid receptors PPAR, LX, and RXR are shown. **B,** Mechanism of action of nuclear receptors in regulation of gene transcription. (ABC—ATP binding cassette transporter; ACO—acyl-coenzyme A oxidase; AR—androgen receptor; CAR—constitutive androstane receptor; COX-2—cyclooxygenase 2; CPB—CREB-binding protein; CYP—cytochrome P450; EFA—essential fatty acid; ER—estrogen receptor; FA—fatty acid; FXR—farnesoid X receptor; GR—glucocorticoid receptor; LPL—lipoprotein lipase; LXR—liver X receptor; MR—mineralocorticoid receptor; NR—nuclear receptor; NRE—NR response element; Pol—RNA polymerase; PPAR—peroxisome proliferator activated receptor; PR—progesterone receptor; PUFA—polyunsaturated fatty acid; PXR—pregnane X receptor; RAR—retinoic acid receptor; RXR—retinoid X receptor; SRC1—steroid receptor coactivator-1; SREBP—sterol regulatory element binding protein; TG—triacylglycerol; TNF—tumor necrosis factor; VDR—vitamin D receptor.)

Clinically relevant antidiabetic agents such as pioglitazone and rosiglitazone are potent PPARγ agonists (K_d in low nanomolar range). A number of fatty acids and eicosanoid derivatives bind and activate PPARγ in the micromolar range [30]. Unlike the PPARα subtype, PPARγ has a clear preference for PUFAs. The fatty acids LA, AA, and EPA bind PPARγ within the range of concentrations of free fatty acids found in human serum [34]. Although fatty acids are not particularly efficacious activators of PPARγ, intracellular conversion of fatty acids to eicosanoids through enhanced expression of 15-lipoxygenase greatly increased PPARγ-mediated transactivation [34]. CLA isomers, in particular 9Z11Z and 10E12Z CLA, are ligands for PPARγ [35]. In macrophages, CLA decreased expression

Table 1. Endogenous and dietary ligands for fatty acid receptors PPAR, LXR, and RXR

Nuclear receptor	Ligand
PPARα	Saturated and unsaturated fatty acids
	Omega-3 fatty acids
	Conjugated linoleic acids
	LPL-treated VLDL
	VLDL
	5,6, EET; 8,9 EET; 11,12 EET; 14,14 EET; 20,14,15-HEET
	2-arachidonylglycerol; 15-S-HETE-G
	Long chain alkylamines
	8-S-HETE
	PGD2, PGD1
	Leukotriene B4
PPARγ	Saturated and unsaturated fatty acids
	Mono- and polyunsaturated fatty acids from triglycerides
	Conjugated linoleic acids
	LPL-treated VLDL
	VLDL
	PGA1, PGD2, PGD1
	OxLDL, 9-S-HODE, 13-HODE
	15-S-HETE
PPARβ	Polyunsaturated acids including linoleic acid, linolenic acid, arachidonic acid, and eicosapentaenoic acid
	Conjugated linoleic acid
	Lysophosphatidic acid
	Hexadecyl azelaic phosphatidylcholine
	13-S-HODE, 15-S-HETE, 5-S-HETE, 12-S-HETE
	PGD1, PGD2, PGA1
LXR	Unsaturated fatty acids (antagonists)
	Polyunsaturated fatty acids have little effect on LXR activity
	22(R) hydroxycholesterol, 20(S)-hydroxycholesterol, 24(S), 25-epoxycholesterol
	6a-Hydroxy bile acids
	Cholestenoic acid
	Oxysterol 5,6-24(S),25-diepoxycholesterol
RXR	Saturated and mono-unsaturated fatty acids
	Polyunsaturated fatty acids, including docosahexaenoic acid
	Conjugated linoleic acids
	9-*cis* retinoic acid
	Phytol metabolites

EET—epoxyeicosatrienoic acids; HEET—hydroxyepoxyeicosatrienoic acid; HETE—hydroperoxyeicosatetraenoic acid; HETE-G—hydroxyeicosatetraenoic-glycerol ester; HODE—hydroxyoctadecadienoic acid; LPL—lipoprotein lipase; LXR—liver X receptor; OxLDL—oxidized low-density lipoprotein; PG—prostaglandin; PPAR—peroxisome proliferator activated receptor; RXR—retinoid X receptor; VLDL—very low-density lipoprotein.

of proinflammatory signals including COX-2, tumor necrosis factor-α (TNF-α), and inducible nitric oxide synthase (iNOS) in a PPARγ-dependent manner [36].

Similar to PPARα, incubation of triglyceride-rich lipoproteins with LPL results in the production of PPARγ ligands [27,28]. In particular, oxidized LDL (oxLDL) products such as 9-S-hydroxyoctadecadienoic acid (9-S-HODE) and 13-S-HODE are good PPARγ activators. Phospholipids are also potent PPARγ ligands, including lysophosphatidic acid (LPA) [37] and hexadecyl azelaic phosphatidylcholine (AzPC) [38].

Peroxisome proliferator activated receptor β (FAAR, NUC1, or PPARδ) is the least understood of the three subtypes in many respects, including the identification of target genes as well as endogenous and dietary ligands. This receptor is ubiquitously expressed and is often found in higher abundance than PPARα or γ. Examination of the PPARβ-null mice has shown a role for PPARβ in development, myelination of the corpus callosum, lipid metabolism, and epidermal cell proliferation [39]. There has been some indication that PPARβ is involved in adipogenesis [39], although this has been refuted [40]. Few high-affinity ligands for PPARβ are known, either xenobiotic or endogenous. However, fatty acids are weak activators of this receptor, with roughly the same preference as PPARα [23]. CLA isomers, in particular a putative furan metabolite of CLA, activate PPARβ in COS-1 cell transfection experiments [25]. Similar to PPARα and γ, incubation of triglyceride-rich lipoproteins with LPL results in the production of PPARβ ligands [27,28]. PGA_1, PGD_2, and PGD_1 can activate PPARβ in reporter assays [31].

Role of PPAR in coronary heart disease

The potential of highly potent PPAR activators in the treatment of atherosclerosis has been noted by other investigators [17••,18,41,42•,43,44]. Both PPARα and PPARγ play key roles in regulating fatty acid metabolism, albeit in seemingly opposite directions [45,46]. The result of PPARα activation in rodent hepatocytes and certain other tissues is a dramatic increase in the peroxisomal enzymes with a modest increase in mitochondrial oxidation of fatty acids. In addition, lipid transport proteins such as FABP and acyl-CoA binding protein (ACBP), as well as genes involved in fatty acid and cholesterol export, are under the control of PPARα. The targeted disruption of PPARα results in aberrant lipid metabolism, with fat droplets accumulating in liver cells. Not only is peroxisomal metabolism affected, but also the constitutive levels of mitochondrial β-oxidation are less in the PPARα-null mouse, showing the importance of this protein in overall fatty acid homeostasis.

The array of genes regulated by PPARγ in adipocytes is indicative of fatty acid accumulation. This regulation of gene expression is concomitant with increased differentiation of immature adipocytes into mature fat-storing cells [47]. These genes include LPL [48], adipocyte fatty acid binding protein (aP2) [49], and CD36 [50]. Adipocyte-secreted cytokines and hormones such as TNF-α and leptin are also PPARγ target genes [51,52]. The genes regulated by PPARγ in macrophages are similar to those in the adipocyte and include LPL and CD36. Treatment of macrophages with PPARγ synthetic agonists inhibits the production of several cytokines such as interleukin 1-β and TNF-α and may result in an anti-inflammatory response [53]. Another link between PPARγ and inflammation is the fact that 15-deoxy PGJ2 (a product of the cyclooxygenase pathway) and nonsteroidal anti-inflammatory drugs are potent activators of PPARγ [54]. It is unclear what role PPARβ may play in regulating genes involved in CHD at this time.

Retinoid X receptors

Retinoid X receptors are involved in the transduction of retinoid signaling pathway, although their role in regulation of gene expression induced by n-3 PUFAs has garnered increasing attention. RXRs (α, β, or γ) can form homodimers or they may serve as a dimerization partner for other NRs, including retinoic acid receptors (RAR), thyroid hormone receptor, vitamin D_3 receptor, and PPARs. As a heterodimerization partner, RXR is involved in regulation of multiple cellular pathways. RXRα and β have ubiquitous distribution, whereas RXRγ is expressed in certain organs such as heart, skeletal muscle, and central nervous system structures.

Although intensely studied for synthetic ligands, little is known of the natural activators of this receptor [55•]. RXR is activated in vitro by the vitamin A metabolite 9-*cis* retinoic acid (9-*cis* RA), but the levels of this molecule in vivo are extremely low. Through reporter assays it was observed that DHA is an RXR ligand [55•]. Docosatetraenoic acid, a structurally related compound, activates RXR with a much higher concentration [55•]. DHA's effect was not observed in other nuclear receptors such as RAR, thyroid hormone receptor, and vitamin D receptor, although as stated previously, this fatty acid activates PPARα. Recently, several fatty acids including unsaturated, mono-unsaturated, and PUFAs such as AA and DHA have been identified as ligands of RXR, thus confirming the activation observed in reporter assays [56]. The 9E11E CLA isomer was by far the most potent of the CLA isomers at activating RXRα and was comparable to the efficacy seen with 9-*cis* RA [14•]. Phytanic acid, a branched chain fatty acid derived from chlorophyll, has also been reported to activate RXR, albeit weakly [57]. Phytanic acid is capable of adipocyte differentiation and induces aP2 mRNA in 3T3-L1 preadipocytes and may act as a natural rexinoid in 3T3-L1 cells [57].

Role of RXR in coronary heart disease

Retinoid X receptor α agonists are capable of reducing atherosclerosis in apolipoprotein E knockout mice, an established experimental model of atherosclerosis [58]. Retinoids are capable of increasing the expression of ABCA1, a gene associated with reverse transport of cholesterol. Cholesterol efflux from peritoneal macrophages was significantly increased in an RXR-dependent fashion [58]. RXR-selective agonists counteract diabetes by decreasing hyperglycemia, hypertriglyceridemia, and hyperinsulinemia [58]. Null mutation of RXRα gene resulted in developmental lethality in mice; they died in utero and demonstrated severe myocardial and ocular malformations [59]. The malformations resembled severe vitamin A syndrome, suggesting a physiologic role of RXRα in retinol responses [59].

Liver X receptors

Liver X receptors (LXRα and LXRβ) are transcription factors commonly known as cholesterol sensors [17••,60,61•]. Although they are important regulators of transport and metabolism of sterols and fatty acids, whether they are direct sensors of n-3 PUFAs has been questioned. Expression of LXRα is restricted, whereas LXRβ is ubiquitously present. LXRα is present in certain organs, namely liver, kidney, intestine, adipose tissue, and adrenals. LXRα and β share a high degree of amino acid similarity (80%) and are considered paralogues; as a result there are very few subtype-specific agonists. Oxysterols, including 24(S), 25-epoxycholesterol, 22R-hydroxycholesterol, and 24(S)-hydroxycholesterol, are natural ligands of LXRs. Unsaturated fatty acids as well as AA and other PUFAs competitively block activation of LXR by oxysterols [62]. This offers a potential mechanism for the ability of dietary PUFAs to decrease the synthesis and secretion of fatty acids and triglycerides in liver [62]. This suppressive effect can be eliminated by

deletion and mutation of LXR responsive elements (LXREs) that are located in the promoter region of SREBP-1c. However, others have shown that the unsaturated fatty acid suppression of SREBP-1 and its targeted lipogenic genes is independent of LXRα [63]. Perhaps the effects of fatty acids on LXR-mediated events are being affected by a direct interaction between PPARα and LXRα [64]. In fact, several xenobiotic PPARα ligands antagonize LXR's transcriptional activity [65].

Role of LXR in coronary heart disease

There is increasing interest in LXR agonists, whether dietary or pharmaceutical, in the prevention of CHD [60,61•, 66,67]. The nonsteroidal LXR agonist GW3965 significantly reduced atherosclerosis in murine models of hyperlipidemia [68]. LXR-mediated genes include those associated with cholesterol and bile acid metabolism (*eg*, ABCA1, ABCG1, APOE, and CYP7A), as well as those with fatty acid synthesis and regulation (SREBP1c, LPL, FAS). Previous studies showed that activation of PPARγ induced the expression of LXRα and ABCA1 and removed cholesterol from macrophages [69]. Hence, LXR was considered further downstream than PPARγ in reducing atherosclerosis.

Liver X receptor α knockout mice were unable to respond to dietary cholesterol and failed to induce cholesterol 7-hydroxylase (Cyp7A), the rate limiting enzyme for bile acid synthesis [70]. This resulted in excessive cholesterol accumulation in the liver followed by impairment of functions. LXRα knockout animals also have altered expression of genes associated with lipid metabolism. Interestingly, LXRβ knockout mice were unaffected when challenged with dietary cholesterol [71]. Selective bone marrow knockouts of macrophage LXRs increase atherosclerotic lesions in $ApoE^{-/-}$ and $LDLR^{-/-}$ mice, suggesting a role as an endogenous inhibitor of atherosclerosis [68].

Conclusions

Diets high in n-3 fatty acids have long been associated with decreased risk of CHD. ALA and its metabolites EPA and DHA are found in high concentrations in flaxseed and fish oils and are thought to improve heart health through decreasing thrombosis, inflammation, and plaque formation in arteries. The mechanism of these effects may be the result of regulation of gene expression via NRs, several of which are known to be "fatty acid receptors". PPARα and PPARβ are receptors for unsaturated, mono-unsaturated, and polyunsaturated fatty acids, as well as for several AA metabolites. Activation of PPARα is associated with increased fatty acid catabolism, decreasing inflammation, and stimulating the reverse cholesterol pathway. PPARγ has a clear preference for PUFAs and is also the target of AA metabolites. This receptor is involved in storage of lipids in adipocytes as well as in decreasing inflammation and stimulating the reverse cholesterol pathway. RXR is an important heterodimerization partner for NRs and can affect numerous metabolic pathways. DHA and several other PUFAs bind to and activate this central NR. LXR's role as a sensor of fatty acids is somewhat controversial, although it is clearly an oxysterol receptor. Several studies have shown that fatty acids (unsaturated and saturated) antagonize LXR activity. This receptor is involved in fatty acid synthesis, bile acid synthesis, and reverse cholesterol transport; synthetic agonists are being touted as antiatherosclerosis agents. Taken together, these NRs represent potential targets for n-3 PUFAs that can help explain their mechanism of action in preventing CHD. In particular, the profile of beneficial effects of ALA, EPA, DHA, and CLA most resemble those seen for synthetic PPARγ ligands such as rosiglitazone. This connection warrants further critical examination and may ultimately result in modifying diet recommendations to maximize PPARγ activation, and hence decrease the incidence and severity of CHD.

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

1.•• Renaud S, Lanzmann-Petithory D: **Coronary heart disease: dietary links and pathogenesis.** *Pub Health Nutr* 2001, **4:**459–474.

The diet-heart hypothesis states that environmental factors predispose individuals to CHD. This review article examines the results from several recent epidemiology studies involving fatty acids and CHD. An optimal diet is suggested that contains a 5:1 ratio of omega-3 fatty acids to non-omega-3 fatty acids is suggested.

2.• Hu FB, Willett WC: **Optimal diets for prevention of coronary heart disease.** *JAMA* 2002, **288:**2569–2578.

This review article discusses the results of several large epidemiology studies on dietary fats and CHD.

3. Belury MA, Nickel KP, Bird CE, Wu Y: **Dietary conjugated linoleic acid modulation of phorbol ester skin tumor promotion.** *Nutr Cancer* 1996, **26:**149–157.
4. Ip C, Chin SF, Scimeca JA, Pariza MW: **Mammary cancer prevention by conjugated dienoic derivative of linoleic acid.** *Cancer Res* 1991, **51:**6118–6124.
5. Yang H, Glickman BW, de Boer JG: **Sex-specific induction of mutations by PhIP in the kidney of male and female rats and its modulation by conjugated linoleic acid.** *Environ Mol Mutagen* 2002, **40:**116–121.
6. Lee KN, Kritchevsky D, Pariza MW: **Conjugated linoleic acid and atherosclerosis in rabbits.** *Atherosclerosis* 1994, **108:**19–25.
7. Houseknecht KL, Vanden Heuvel JP, Moya-Camarena SY, *et al.*: **Dietary conjugated linoleic acid normalizes impaired glucose tolerance in the Zucker diabetic fatty fa/fa rat.** *Biochem Biophys Res Commun* 1998, **244:**678–682.
8. Honkakoski P, Negishi M: **Regulation of cytochrome P450 (CYP) genes by nuclear receptors.** *Biochem J* 2000, **347:**321–337.
9. Weatherman RV, Fletterick RJ, Scanlan TS: **Nuclear-receptor ligands and ligand-binding domains.** *Annu Rev Biochem* 1999, **68:**559–581.
10. Kumar R, Thompson EB: **The structure of the nuclear hormone receptors.** *Steroids* 1999, **64:**310–319.
11. Di Croce L, Okret S, Kersten S, *et al.*: **Steroid and nuclear receptors. Villefranche-sur-Mer, France, May 25-27, 1999.** *EMBO J* 1999, **18:**6201–6210.
12. McDonnell DP, Vegeto E, Gleeson MA: **Nuclear hormone receptors as targets for new drug discovery.** *Biotechnology (N Y)* 1993, **11:**1256–1261.

13. Wahli W, Martinez E: **Superfamily of steroid nuclear receptors: positive and negative regulators of gene expression.** *FASEB J* 1991, **5:**2243–2249.

14.• Khan SA, Vanden Heuvel JP: **Role of nuclear receptors in the regulation of gene expression by dietary fatty acids (review).** *J Nutr Biochem* 2003, **14:**554–567.

Nuclear receptors such as PPAR, LXR, and RXR are known fatty acid receptors and share a structurally conserved ligand binding domain. In addition to a variety of endogenous fatty acids, these receptors are also the target of CLA, a component of meat and dairy products with a wide variety of beneficial health effects.

15. Committee NRN: **A unified nomenclature system for the nuclear receptor superfamily.** *Cell* 1999, **97:**161–163.

16. Clarke SD, Jump DB: **Polyunsaturated fatty acid regulation of hepatic gene transcription.** *J Nutr* 1996, **126:**1105S–1109S.

17.•• Francis GA, Fayard E, Picard F, Auwerx J: **Nuclear receptors and the control of metabolism.** *Annu Rev Physiol* 2003, **65:**261–311.

Although not specifically addressing fatty acids or diet on CHD, this review article is a great resource on the biology of a wide variety of nuclear receptors including PPAR, RXR, and LXR. Emphasis is placed on receptors involved in energy, glucose, fatty acid, and cholesterol metabolism and the coordination of gene expression involved in diseases such as diabetes and obesity.

18. Wahli W: **Peroxisome proliferator-activated receptors (PPARs): from metabolic control to epidermal wound healing.** *Swiss Med Wkly* 2002, **132:**83–91.

19.• Hihi AK, Michalik L, Wahli W: **PPARs: transcriptional effectors of fatty acids and their derivatives.** *Cell Mol Life Sci* 2002, **59:**790–798.

PPARs were the first proteins identified as fatty acid receptors. The mechanism of action of PPARs is emphasized in this review article.

20. Vanden Heuvel JP: **Peroxisome proliferator-activated receptors: a critical link among fatty acids, gene expression and carcinogenesis.** *J Nutr* 1999, **129:**575S–580S.

21. Latruffe N, Vamecq J: **Peroxisome proliferators and peroxisome proliferator activated receptors (PPARs) as regulators of lipid metabolism.** *Biochimie* 1997, **79:**81–94.

22. Gelman L, Fruchart JC, Auwerx J: **An update on the mechanisms of action of the peroxisome proliferator- activated receptors (PPARs) and their roles in inflammation and cancer.** *Cell Mol Life Sci* 1999, **55:**932–943.

23. Willson TM, Brown PJ, Sternbach DD, Henke BR: **The PPARs: from orphan receptors to drug discovery.** *J Med Chem* 2000, **43:**527–550.

24. Moya-Camarena SY, Vanden Heuvel JP, Blanchard SG, *et al.*: **Conjugated linoleic acid is a potent naturally occurring ligand and activator of PPARalpha.** *J Lipid Res* 1999, **40:**1426–1433.

25. Moya-Camarena SY, Vanden Heuvel JP, Belury MA: **Conjugated linoleic acid activates peroxisome proliferator-activated receptor alpha and beta subtypes but does not induce hepatic peroxisome proliferation in Sprague-Dawley rats.** *Biochim Biophys Acta* 1999, **1436:**331–342.

26. Peters JM, Park Y, Gonzalez FJ, Pariza MW: **Influence of conjugated linoleic acid on body composition and target gene expression in peroxisome proliferator-activated receptor alpha- null mice.** *Biochim Biophys Acta* 2001, **1533:**233–242.

27. Ziouzenkova O, Perrey S, Asatryan L, *et al.*: **Lipolysis of triglyceride-rich lipoproteins generates PPAR ligands: evidence for an antiinflammatory role for lipoprotein lipase.** *Proc Natl Acad Sci U S A* 2003, **100:**2730–2735.

28. Chawla A, Lee CH, Barak Y, *et al.*: **PPARdelta is a very low-density lipoprotein sensor in macrophages.** *Proc Natl Acad Sci U S A* 2003, **100:**1268–1273.

29. Cowart LA, Wei S, Hsu MH, *et al.*: **The CYP4A isoforms hydroxylate epoxyeicosatrienoic acids to form high affinity peroxisome proliferator-activated receptor ligands.** *J Biol Chem* 2002, **277:**35105–35112.

30. Krey G, Braissant O, Fu LH, *et al.*: **Fatty acids, eicosanoids, and hypolipidemic agents identified as ligands of peroxisome proliferator-activated receptors by coactivator- dependent receptor ligand assay.** *Mol Endocrinol* 1997, **11:**779–791.

31. Yu K, Bayona W, Kallen CB, *et al.*: **Differential activation of peroxisome proliferator-activated receptors by eicosanoids.** *J Biol Chem* 1995, **270:**23975–23983.

32. Spiegelman BM: **PPAR-gamma: adipogenic regulator and thiazolidinedione receptor.** *Diabetes* 1998, **47:**507–514.

33. Barak Y, Nelson MC, Ong ES, *et al.*: **PPAR gamma is required for placental, cardiac, and adipose tissue development.** *Mol Cell* 1999, **4:**585–595.

34. Willson TM, Wahli W: **Peroxisome proliferator-activated receptor agonists.** *Curr Opin Chem Biol* 1997, **1:**235–241.

35. DeGrazia MJ, Thompson J, Vanden Heuvel JP, Peterson BR: **Synthesis of a high-affinity fluorescent ligand PPARg ligand for high-throughput fluorescence polarization assays.** *Bioorgan Medicinal Chem* 2003, **11:**4325–4332.

36. Yu Y, Correll PH, Vanden Heuvel JP: **Conjugated linoleic acid decreases production of pro-inflammatory products in macrophages: evidence for a PPARgamma-dependent mechanism.** *Biochim Biophys Acta* 2002, **1581:**89–99.

37. McIntyre TM, Pontsler AV, Silva AR, *et al.*: **Identification of an intracellular receptor for lysophosphatidic acid (LPA): LPA is a transcellular PPARgamma agonist.** *Proc Natl Acad Sci U S A* 2003, **100:**131–136.

38. Davies SS, Pontsler AV, Marathe GK, *et al.*: **Oxidized alkyl phospholipids are specific, high affinity peroxisome proliferator-activated receptor gamma ligands and agonists.** *J Biol Chem* 2001, **276:**16015–16023.

39. Peters JM, Lee SS, Li W, *et al.*: **Growth, adipose, brain, and skin alterations resulting from targeted disruption of the mouse peroxisome proliferator-activated receptor beta(delta).** *Mol Cell Biol* 2000, **20:**5119–5128.

40. Brun RP, Tontonoz P, Forman BM, *et al.*: **Differential activation of adipogenesis by multiple PPAR isoforms.** *Genes Dev* 1996, **10:**974–984.

41. Barbier O, Torra IP, Duguay Y, *et al.*: **Pleiotropic actions of peroxisome proliferator-activated receptors in lipid metabolism and atherosclerosis.** *Arterioscler Thromb Vasc Biol* 2002, **22:**717–726.

42.• Duval C, Chinetti G, Trottein F, *et al.*: **The role of PPARs in atherosclerosis.** *Trends Mol Med* 2002, **8:**422–430.

This articles explains the mechanism by which PPAR ligands affect cardiovascular disease. PPARs can affect endothelial as well as immune cells involved in atherosclerosis.

43. Fruchart JC, Staels B, Duriez P: **PPARS, metabolic disease and atherosclerosis.** *Pharmacol Res* 2001, **44:**345–352.

44. Vosper H, Khoudoli GA, Graham TL, Palmer CN: **Peroxisome proliferator-activated receptor agonists, hyperlipidaemia, and atherosclerosis.** *Pharmacol Ther* 2002, **95:**47–62.

45. Kersten S, Desvergne B, Wahli W: **Roles of PPARs in health and disease.** *Nature* 2000, **405:**421–424.

46. Escher P, Wahli W: **Peroxisome proliferator-activated receptors: insight into multiple cellular functions.** *Mutat Res* 2000, **448:**121–138.

47. Rocchi S, Auwerx J: **Peroxisome proliferator-activated receptor-gamma: a versatile metabolic regulator.** *Ann Med* 1999, **31:**342–351.

48. Schoonjans K, Staels B, Auwerx J: **The peroxisome proliferator activated receptors (PPARS) and their effects on lipid metabolism and adipocyte differentiation.** *Biochim Biophys Acta* 1996, **1302:**93–109.

49. Tontonoz P, Hu E, Graves RA, *et al.*: **mPpar gamma 2: tissue-specific regulator of an adipocyte enhancer.** *Genes Dev* 1994, **8:**1224–1234.

50. Tontonoz P, Nagy L, Alvarez JG, *et al.*: **PPARgamma promotes monocyte/macrophage differentiation and uptake of oxidized LDL.** *Cell* 1998, **93:**241–252.

51. Zhang B, Berger J, Hu E, *et al.*: **Negative regulation of peroxisome proliferator-activated receptor-gamma gene expression contributes to the antiadipogenic effects of tumor necrosis factor-alpha.** *Mol Endocrinol* 1996, **10:**1457–1466.

52. Kubota N, Terauchi Y, Miki H, *et al.*: **PPAR gamma mediates high-fat diet-induced adipocyte hypertrophy and insulin resistance.** *Mol Cell* 1999, **4:**597–609.

53. Ricote M, Huang JT, Welch JS, Glass CK: **The peroxisome proliferator-activated receptor (PPARgamma) as a regulator of monocyte/macrophage function.** *J Leukocyte Biol* 1999, **66:**733–739.
54. Lehmann JM, Lenhard JM, Oliver BB, *et al.*: **Peroxisome proliferator-activated receptors alpha and gamma are activated by indomethacin and other non-steroidal anti-inflammatory drugs.** *J Biol Chem* 1997, **272:**3406–3410.
55.• de Urquiza AM, Liu S, Sjoberg M, *et al.*: **Docosahexaenoic acid, a ligand for the retinoid X receptor in mouse brain.** *Science* 2000, **290:**2140–2144.

DHA has for years been associated with improved cognitive functions, although the mechanism by which this fatty acid affected gene expression in the brain was unclear. Through the discovery of DHA binding to RXR in the brain, this article proposes a mechanism by which DHA affects cognition and ushered in the era of RXR being appreciated as a fatty acid receptor.

56. Lengqvist J, De Urquiza AM, Bergman AC, *et al.*: **Polyunsaturated fatty acids including docosahexaenoic and arachidonic acid bind to the retinoid X receptor alpha ligand binding domain.** *Mol Cell Proteomics* 2004, In press.
57. Lemotte PK, Keidel S, Apfel CM: **Phytanic acid is a retinoid X receptor ligand.** *Eur J Biochem* 1996, **236:**328–333.
58. Claudel T, Leibowitz MD, Fievet C, *et al.*: **Reduction of atherosclerosis in apolipoprotein E knockout mice by activation of the retinoid X receptor.** *Proc Natl Acad Sci U S A* 2001, **98:**2610–2615.
59. Kastner P, Grondona JM, Mark M, *et al.*: **Genetic analysis of RXR alpha developmental function: convergence of RXR and RAR signaling pathways in heart and eye morphogenesis.** *Cell* 1994, **78:**987–1003.
60. Lund EG, Menke JG, Sparrow CP: **Liver X receptor agonists as potential therapeutic agents for dyslipidemia and atherosclerosis.** *Arterioscler Thromb Vasc Biol* 2003, **23:**1169–1177.
61.• Millatt LJ, Bocher V, Fruchart JC, Staels B: **Liver X receptors and the control of cholesterol homeostasis: potential therapeutic targets for the treatment of atherosclerosis.** *Biochim Biophys Acta* 2003, **1631:**107–118.

LXR is a fatty acid receptor that plays a significant role in atherosclerosis. This article shows that LXR is a sensor of hydroxylated cholesterol molecules and controls many aspects of lipid homeostasis.

62. Ou J, Tu H, Shan B, *et al.*: **Unsaturated fatty acids inhibit transcription of the sterol regulatory element-binding protein-1c (SREBP-1c) gene by antagonizing ligand- dependent activation of the LXR.** *Proc Natl Acad Sci U S A* 2001, **98:**6027–6032.
63. Pawar A, Botolin D, Mangelsdorf DJ, Jump DB: **The role of liver X receptor-alpha in the fatty acid regulation of hepatic gene expression.** *J Biol Chem* 2003, **278:**40736–40743.
64. Miyata KS, McCaw SE, Patel HV, *et al.*: **The orphan nuclear hormone receptor LXR alpha interacts with the peroxisome proliferator-activated receptor and inhibits peroxisome proliferator signaling.** *J Biol Chem* 1996, **271:**9189–9192.
65. Laffitte BA, Repa JJ, Joseph SB, *et al.*: **LXRs control lipid-inducible expression of the apolipoprotein E gene in macrophages and adipocytes.** *Proc Natl Acad Sci U S A* 2001, **98:**507–512.
66. Joseph SB, Tontonoz P: **LXRs: new therapeutic targets in atherosclerosis?** *Curr Opin Pharmacol* 2003, **3:**192–197.
67. Jaye M: **LXR agonists for the treatment of atherosclerosis.** *Curr Opin Investig Drugs* 2003, **4:**1053–1058.
68. Joseph SB, McKilligin E, Pei L, *et al.*: **Synthetic LXR ligand inhibits the development of atherosclerosis in mice.** *Proc Natl Acad Sci U S A* 2002, **99:**7604–7609.
69. Chawla A, Barak Y, Nagy L, *et al.*: **PPAR-gamma dependent and independent effects on macrophage-gene expression in lipid metabolism and inflammation.** *Nat Med* 2001, **7:**48–52.
70. Peet DJ, Turley SD, Ma W, *et al.*: **Cholesterol and bile acid metabolism are impaired in mice lacking the nuclear oxysterol receptor LXR alpha.** *Cell* 1998, **93:**693–704.
71. Alberti S, Schuster G, Parini P, *et al.*: **Hepatic cholesterol metabolism and resistance to dietary cholesterol in LXRbeta-deficient mice.** *J Clin Invest* 2001, **107:**565–573.
72. Gottlicher M, Demoz A, Svensson D, *et al.*: **Structural and metabolic requirements for activators of the peroxisome proliferator-activated receptor.** *Biochem Pharmacol* 1993, **46:**2177–2184.
73. Moya-Camarena SY, Van den Heuvel JP, Belury MA: **Conjugated linoleic acid activates peroxisome proliferator-activated receptor alpha and beta subtypes but does not induce hepatic peroxisome proliferation in Sprague-Dawley rats.** *Biochim Biophys Acta* 1999, **1436:**331–342.
74. Kozak KR, Gupta RA, Moody JS, *et al.*: **15-Lipoxygenase metabolism of 2-arachidonylglycerol. Generation of a peroxisome proliferator-activated receptor alpha agonist.** *J Biol Chem* 2002, **277:**23278–23286.
75. Van Veldhoven PP, Mannaerts GP, Declercq P, Baes M: **Do sphingoid bases interact with the peroxisome proliferator activated receptor alpha (PPAR-alpha)?.** *Cell Signal* 2000, **12:**475–479.
76. Muga SJ, Thuillier P, Pavone A, *et al.*: **8S-lipoxygenase products activate peroxisome proliferator-activated receptor alpha and induce differentiation in murine keratinocytes.** *Cell Growth Differ* 2000, **11:**447–454.
77. Murakami K, Ide T, Suzuki M, *et al.*: **Evidence for direct binding of fatty acids and eicosanoids to human peroxisome proliferators-activated receptor alpha.** *Biochem Biophys Res Commun* 1999, **260:**609–613.
78. Houseknecht KL, Vanden Heuvel JP, Moya-Camarena SY, *et al.*: **Dietary conjugated linoleic acid normalizes impaired glucose tolerance in the Zucker diabetic fatty fa/fa rat [published erratum appears in Biochem Biophys Res Commun 1998 Jun 29;247(3):911].** *Biochem Biophys Res Commun* 1998, **244:**678–682.
79. Nagy L, Tontonoz P, Alvarez JG, *et al.*: **Oxidized LDL regulates macrophage gene expression through ligand activation of PPARgamma.** *Cell* 1998, **93:**229–240.
80. Shappell SB, Gupta RA, Manning S, *et al.*: **15S-Hydroxyeicosatetraenoic acid activates peroxisome proliferator-activated receptor gamma and inhibits proliferation in PC3 prostate carcinoma cells.** *Cancer Res* 2001, **61:**497–503.
81. Wigren J, Surapureddi S, Olsson AG, *et al.*: **Differential recruitment of the coactivator proteins CREB-binding protein and steroid receptor coactivator-1 to peroxisome proliferator-activated receptor gamma/9-cis-retinoic acid receptor heterodimers by ligands present in oxidized low-density lipoprotein.** *J Endocrinol* 2003, **177:**207–214.
82. Song C, Hiipakka RA, Liao S: **Selective activation of liver X receptor alpha by 6alpha-hydroxy bile acids and analogs.** *Steroids* 2000, **65:**423–427.
83. Song C, Liao S: **Cholestenoic acid is a naturally occurring ligand for liver X receptor alpha.** *Endocrinology* 2000, **141:**4180–4184.
84. Janowski BA, Grogan MJ, Jones SA, *et al.*: **Structural requirements of ligands for the oxysterol liver X receptors LXRalpha and LXRbeta.** *Proc Natl Acad Sci U S A* 1999, **96:**266–271.
85. Kitareewan S, Burka LT, Tomer KB, *et al.*: **Phytol metabolites are circulating dietary factors that activate the nuclear receptor RXR.** *Mol Biol Cell* 1996, **7:**1153–1166.