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# Reviews: Current Topics
# Role of nuclear receptors in the regulation of gene expression by dietary fatty acids (Review)

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**Abstract**

Long chain fatty acids, derived either from endogenous metabolism or by nutritional sources play significant roles in important biological processes of membrane structure, production of biologically active compounds, and participation in cellular signaling processes. Recently, the structure of dietary fatty acids has become an important issue in human health because ingestion of saturated fats (containing triglycerides composed of saturated fatty acids) is considered harmful, while unsaturated fats are viewed as beneficial. It is important to note that the molecular reason for this dichotomy still remains elusive. Since fatty acids are important players in development of pathology of cardiovascular and endocrine system, understanding the key molecular targets of fatty acids, in particular those that discriminate between saturated and unsaturated fats, is much needed. Recently, insights have been gained on several fatty acid-activated nuclear receptors involved in gene expression. In other words, we can now envision long chain fatty acids as regulators of signal transduction processes and gene regulation, which in turn will dictate their roles in health and disease. In this review, we will discuss fatty acid-mediated regulation of nuclear receptors. We will focus on peroxisome proliferators-activated receptors (PPARs), liver X receptors (LXR), retinoid X receptors (RXRs), and Hepatocyte Nuclear Factor alpha (HNF-4$\alpha$), all of which play pivotal roles in dietary fatty acid-mediated effects. Also, the regulation of gene expression by Conjugated Linoleic Acids (CLA), a family of dienoic fatty acids with a variety of beneficial effects, will be discussed.

*Keywords*: Fatty acid; Nuclear receptor; Gene; Transcription 

## 1. Dietary fatty acids and health

Well-established biological functions of lipids include regulation of thermogenesis, products of intermediary metabolism, synthesis of biologically active macromolecules, and regulation of gene expression associated with lipid and carbohydrate metabolism. These physiological functions are carried out by triglycerides, fatty acids and other lipids, synthesized endogenously as well as from fat sources of the diet. The fatty acid component of the triglycerides, or free fatty acids (FFA) can be saturated or unsaturated depending on the presence of double bonds in their chemical structures. Epidemiologically, ingestion of food enriched in saturated fat has been related to the development of several important diseases including coronary artery disease (CAD), obesity, diabetes and cancer. Interestingly, diet containing polyunsaturated fatty acids (PUFA) such as fish oil can counteract atherosclerosis and prevent heart disease [1–3]. Dietary PUFAs are also beneficial against cancer [4–6], hyperlipidemia [7] and diabetes [8]. The mechanism for these opposing effects is not yet properly understood.

The term Conjugated Linoleic Acid (CLA) collectively refers to a group of linoleic acid (18:2, c9, c12) 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, foods that are causative factors in several human diseases. These conjugated fatty acids are formed from linoleic acid in the intestine of livestock by bacterial flora and are deposited in tissues and milk. CLA has received widespread attention due to its anti-cancer [9–11], anti-atherosclerotic [12] and anti-diabetic effects [8] in laboratory animals. It is important to note that although CLA has been extensively examined for its thera-

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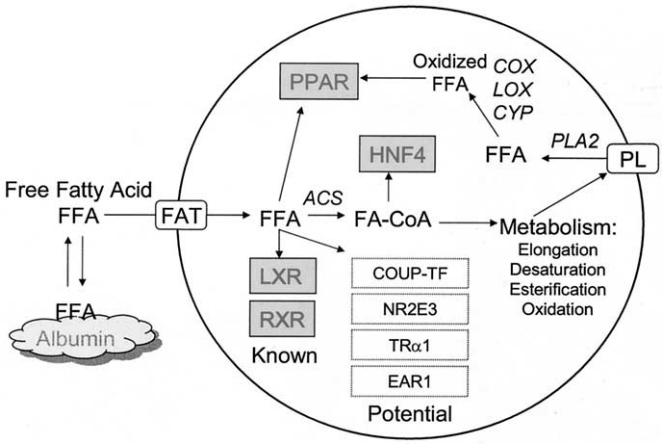


Fig. 1. Nuclear receptors that act as fatty acid sensors. NRs with a definite role in regulation of gene expression via fatty acids are shown in gray boxes. Members of the NR superfamily that have sequence similarity to other fatty acid-regulated receptors, but unknown regulation, are shown in white boxes.ABREVIATIONS: FFA, free fatty acid; FAT, fatty acid transporter; PPAR, peroxisome proliferator-activated receptor; LXR, liver-X-receptor; RXR, retinoid X receptor; HNF-4, hepatocyte nuclear factor 4; COUP-TF, chicken ovalbumin upstream promoter transcription factor; NR2E3, nuclear receptor family 2, member3; TR$\alpha$1, thyroid receptor 1$\alpha$; EAR1, similar to orphan nuclear receptor NR1D1 (V-erbA related protein EAR-1) (Rev-erbA-alpha); PL, phospholipid; ACS, acyl-CoA synthetase; COX, cyclooxygenase; LOX, lipoxygenase; CYP, Cytochrome P450.

peutic effects, a clear defined mechanism by which this fatty acid exerts its effects has not emerged. However, in the present review, we will shade some light on molecular mechanisms of CLA biology.

The fact that distinct structure-activity relationships exists is suggestive of a cognate receptor for fatty acids. Several members of the nuclear receptor (NR) superfamily have been studied as fatty acid receptors include the peroxisome proliferators-activated receptors (PPARs), retinoid X receptors (RXRs), liver X receptors (LXRs) and more recently hepatocyte nuclear factor (HNF-4$\alpha$). These NRs regulate gene expression in response to FFA, CoA thioesters or oxidation products of fatty acids released from phospholipids or triglycerides (Fig. 1).

## 2. Nuclear Receptors

Unlike receptors found on the cell surface, members of the nuclear hormone receptor (NR) superfamily are restricted to metazoan organisms such as nematodes, insects, and vertebrates (There are several excellent reviews on steroid hormone receptors, including references [13–28]). These proteins are intracellular transcription factors that directly regulate gene expression in response to lipophilic molecules. They affect a wide variety of functions, including fatty acid metabolism, reproductive development, and detoxification of foreign substances. As will be discussed, many of the NRs act as ligand-inducible transcription factors, responding to endogenous and exogenous chemicals. However, the majority of known NRs do not have an identifiable physiologically relevant ligand, and are deemed *orphan receptors*. To date, over 300 NRs have been cloned. Early classification of these receptors was based on ligands, DNA binding properties or other functional characterization. Recently a more systematic classification has been proposed, based on sequence similarity. Phylogenic analysis has shown six subfamilies (NR1-6) with various groups and individual genes [15]. As discussed below, most NRs including those that respond to dietary fatty acids have the same basic structure.

### 2.1. Functional Domains

NRs generally follow a standard blueprint, with distinct functional domains. The N terminus of the NR, sometimes called the modulator, hypervariable or A/B domain, has transactivation activity, termed activation function 1 (AF-1). This acidic activation domain is ligand-independent, or constitutively functional. The A/B domain's sequence and length are highly variable between receptors (i.e., GR vs. RXR) and among receptor subtypes (RXR$\alpha$ vs. $\beta$). In addition, this region is the most frequent site of alternative splicing and secondary start sites, and contains a variety of kinase recognition sequences. For these reasons, it is thought that the variable N- terminal sequences may be responsible for the receptor-, species-, and cell type-specific effects as well as promoter context-dependent properties of NR transactivation [29].

NRs bind to response elements (NREs) in their target promoters through the DNA binding domain (DBD) or C domain. Composed of two zinc fingers, the DBD is the most conserved region within the NHR superfamily. The first zinc finger contains the proximal-or P-box region, an alpha helix that is responsible for high-affinity recognition of the "core half-site" of the response element. Located within the second zinc finger is the distal or D-box, an $\alpha$-helix which lies perpendicular to P-box helix, and is a site that mediates receptor dimerization. NRs bind to DNA as heterodimers, homodimers, or monomers, depending on the class of receptor. The steroid hormone receptors GR, PR, ER, AR and MR (receptors for glucocorticoid, progesterone, estrogen, androgen and mineralocorticoids, respectively) bind to DNA as homodimers and recognize a palindromic response element [30]. However, thyroid, retinoid, vitamin D and peroxisome proliferator receptors (TR, RAR, VDR and PPAR), liver X receptor and hepatocyte nuclear factor (LXR and HNF) as well as most orphan receptors, bind to DNA as a heterodimer with retinoid-x-receptor (RXR). However, the three dimensional structure of the RXR heterodimer complex produces different DNA binding affinities. Response elements may be direct repeats ($DR_x$, AGGTCA-$N_x$-AGGTCA, where N is any nucleotide and x is any number of residues from 0 to 10), everted repeats ($ER_x$, ACTGGA-$N_x$-AGGTCA) or inverted repeats ($IR_x$, AGGTCA-Nx-ACTGGA).

Immediately adjacent to the DNA binding domain is the D or hinge domain. This particular region has an ill-defined function. The hinge domain contains the carboxy-terminal extension (CTE) of the DBD, which may be involved in recognizing the extended 5′ end of the NRE. The D-domain appears to allow for conformational changes in the protein structure following ligand binding. Also, this region may contain nuclear localization signals and protein-protein interaction sites.

The sequence of the ligand binding domain (LBD) or E/F domain varies substantially between NRs, but they all share a common structure of 10 to 13 $\alpha$-helices organized around a hydrophobic binding pocket. Residues within the binding pocket confer specificity, determining whether the LBD will accept steroid hormones, retinoid compounds or the host of xenobiotic ligands that affect receptor function. Ligand-dependent activation requires the presence of activation function 2 (AF-2), located at the extreme C terminus of the NR. LBDs also contain nuclear localization signals, protein interaction with dimerization motifs for heat shock proteins, coregulators and other transcription factors.

### *2.2. Basic Mechanism of Action*

The mechanism of action of nuclear hormone receptors can take one of two basic forms, that of steroid hormone receptors (SHRs) or that of retinoid/thyroid/Vitamin D receptors [31]. In the absence of ligand, the transcriptionally inactive SHRs MR, PR, GR, AR and ER are sequestered in a large complex comprising the receptor, heat shock protein-90 (HSP90), Hsp70, FKBP52/51 and possibly other proteins [31]. The cellular localization of this inactive complex is somewhat controversial and cytoplasmic or nuclear localization may be observed depending on the cell type and the conditions examined; however, the central dogma is that SHRs are cytosolic in the un-liganded form. One consequence of hormone binding to receptor is a distinct conformational change in receptor structure (discussed below). This conformational change marks the beginning of the signal transduction process. In the case of the SHRs subfamily (GR, AR, MR, PR), hormone binding elicits a dissociation of hsps and the release of a monomeric receptor from the complex. Genetic analysis and in vitro protease digestion experiments indicate that the conformational changes in receptor structure induced by agonists are similar but distinct from those produced by antagonists. However, both conformations appear to be incompatible with hsp binding.

The TR, RAR and VDR subfamily of NRs (which includes PPAR, LXR, RXR) do not avidly interact with hsps and are localized predominantly in the nucleus in the absence of ligand. A possible exclusion to this rule may be PPAR$\alpha$, which interacts with hsp70 [32] and hsp90 [33] and is found both in the cytosol and nucleus in its unactivated form. Some unliganded NRs of this class may interact with DNA and act as transcription repressors. This may be the result of interaction with co-repressor proteins. An interesting exception to this observation is the constitutively active receptor (CAR) that is transcriptionally active in the absence of its ligand. Hormone induced conformational changes also occur upon activation of this class of NR, suggesting that alteration of receptor shape by ligands is a key step in the activation pathway.

Evidence suggests that receptors of the GR subfamily (which includes ER, AR and PR) cooperatively bind to DNA as homodimers. The TR, RAR, VDR, PPAR and most of the orphan receptors form heterodimers with other members of the intracellular receptor superfamily. TR, RAR, PPAR and VDR can utilize RXRs as partners for heterodimer formation. The DNA site of contact depends on certain sequences within the C-domain, namely the proximal (P-box) and distal (D-box) zinc finger motifs (see description of the C-domain above). The P-box determines the half-site recognized, while the D-box determines the spacing between half-sites. Following activation, the SHRs receptors are capable of interacting with DNA, and both classes of NRs (SHRs and TR/RAR) can now recruit co-activators. The DNA bound NR complex is now a substrate for the general transcription apparatus and the initiation of transcription commences.

### *2.3. Ligands and Activators*

Ligands for NRs are as varied as the proteins themselves; however, a few generalized comments can be made. All ligands are lipophilic and can easily transverse the plasma membrane as well as the nuclear membrane, if required. Obviously, this holds true for dietary fatty acids that can be expected to dissolve into membrane structures relatively easily. The affinity ($K_d$) of the ligand-receptor complex is generally in the nM range, but can vary from pM to $\mu$M. It should be noted however, that the concentrations of each natural ligand should approach their $K_d$ to be considered a physiologically-relevant ligand. This is of particular importance when considering reclassifying (or *adopting*) an orphan receptor in the process of reverse endocrinology. Some receptors, such as PPAR$\gamma$, have a large ligand-binding cavity that allows for the association of a variety of endogenous ligands [34].

Structural studies of empty and ligand-bound LBDs have led to the “mousetrap” model of NR activation [25, 35]. The ligand is attracted to the *trap*, the receptor’s electrostatic potential, and a conformational change takes place, preventing the ligand’s exit. In the same way that the sprung mousetrap is more stable than the primed trap, ligand binding to the NRs ligand-binding domains stabilizes their structures relative to the unliganded receptor. The ligand forms an integral part of the hydrophobic core of the liganded LBD. This structural change is different for ligands that are full agonists versus those that are partial agonists or antagonists. Much attention is focused on the accessibility of the AF-2 domain to accessory proteins. The AF-2 domain can

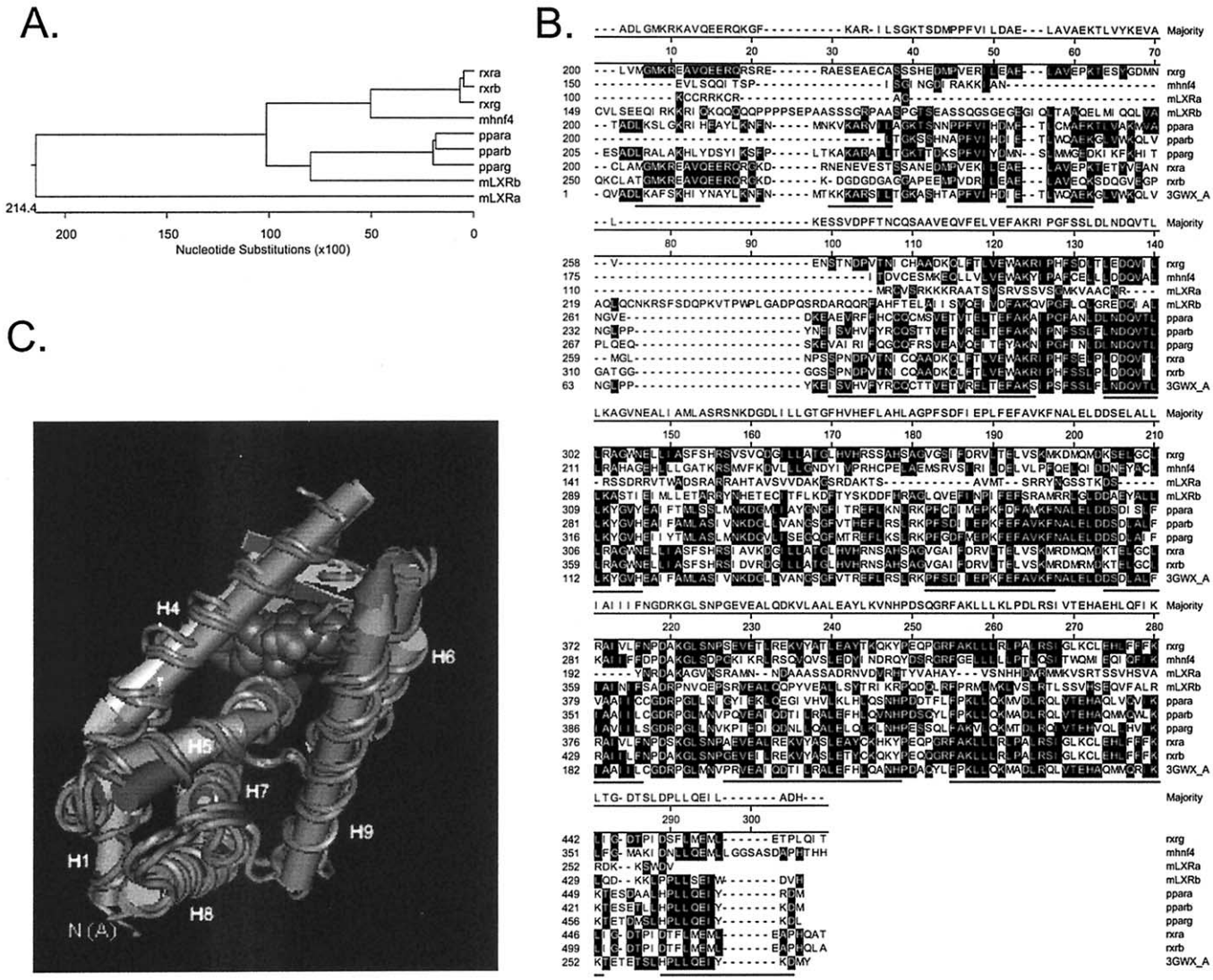


Fig. 2. Ligand binding domain of fatty acid-binding nuclear receptors. The ligand-binding domain of the fatty acid receptors PPAR$\alpha$, $\beta$, $\gamma$, RXR$\alpha$, $\beta$, LXR$\alpha$, $\beta$ and HNF4$\alpha$ are compared using Panel A, Phylogenic comparison of the LBD of mouse fatty acid receptors (ClustalW in DNAStar (Madison , WI)). The sequences utilized were: PPAR$\alpha$, Accession number 1350914, residues 281 to 467; PPAR$\beta$, 548577, 253 to 439; PPAR$\gamma$, 1346767, 288 to 474; RXR$\alpha$, 133702, 278 to 467; RXR$\beta$, 1350912, 331 to 520; RXR$\gamma$, 1350914, 274 to 463; LXR$\alpha$, AAD16050, 154 to 260; LXR$\beta$, Q60644, 261 to 445; HNF4$\alpha$, P49698, 183 to 465. Panel B, Comparison of the primary amino acid sequence of the mouse LBDs as well as human PPAR$\beta$ (3WGX_A, 1 to 271). Residues that are identical to the majority are boxed. The ten alpha helices in mouse PPAR$\alpha$ are indicated with lines below 3WGX_A; Panel C, The crystal structure fatty acid bound to PPARs (NCBI structure database, accession 3WGX_A), PPAR$\gamma$ (1PRG_A) and RXR$\alpha$ (1DFF_A) are aligned with the NCBI conserved domain database and visualized with Cn3D. A fatty acid bound to PPAR is shown as a space fill model. Structures that are conserved are depicted. H, $\alpha$-helix; N, amino terminus.

serve as an activator of transcription when excised from the rest of the protein and linked to a heterologous DNA-binding domain. In this model, binding of the ligand molecule induces a conformational change in the LBD, whereby the AF-2 sequences fold back against the binding pocket, obstructing the opening and causing rearrangements in adjacent helices. In the process, a new surface is revealed that recruits specific transcriptional coactivators. This model may explain why receptor antagonists block transactivation; these compounds do not induce the proper conformational rearrangements in the LBD, interfering with the formation of the transcriptional activation complex.

The LBD of fatty acid receptors have many common characteristics, both at the primary and tertiary structural level, as shown in Fig. 2. Phylogenic analysis of the LBDs (Fig. 2A) is very similar to that reported for the full length proteins [15] with the RXRs and HNF4$\alpha$ (NR2B1, 2, 3 and NR2A1) clustering together whereas the PPARs and LXR$\beta$ show more similarity to each other (NR1C1, 2, 3 and NR1H2). The LBD of LXR$\alpha$ (NR1H3) showed the least percent identity with any of the other fatty acids receptors. The structure of several NR LBDs have been elucidated by X-ray crystallography, including all three PPARs and RXR$\alpha$. As with all NRs, the fatty acid receptors form a

hydrophobic pocket for the binding of ligand (Fig. 2C) utilizing a series of alpha helices.

The majority sequence of the aligned fatty acid receptors (Fig. 2B) was used to search the protein database and the conserved domain database at the National Center for Biotechnology Institute (NCBI). As expected, the majority sequence was identified as the NR LBD. In addition to showing significant homology with the known fatty acid receptors (PPAR, RXR, LXR, HNF4$\alpha$, similarity scores ranging from 250 to 114 bits or 73 to 36% identical), several other NRs that have not been examined for their ability to bind fatty acids were observed. Chicken ovalbumin upstream transcription factor 1 and 2 (COUPTF1 and 2, NR2F1 and 2, score 124 bits), COUP-TF3 (NRF3, 118 bits), NR2E3 (114 bits), thyroid hormone receptor$\alpha$ (TR$\alpha$, NR1A1, 107 bits), and EAR1 (NR1D1, 99 bits) showed a significant amount of similarity. To date, these NRs have not been characterized as fatty acid receptors.

### *2.4. Reverse endocrinology*

The increasing use of bioinformatics and the spread of genome projects has lead to the discovery of hundreds of proteins which share structural characteristics with NRs. By some estimation, the Human Genome Project alone will reveal that there are as many as 2,000 NRs [36]. When a NHR is discovered without any knowledge of its natural ligand, it was dubbed an orphan nuclear receptor (ONR). Efforts to understand ONR function and identify their physiological ligands (a process known as *reverse endocrinology*) [21] have led to the discovery of novel metabolic pathways involving the PPAR, LXR, and farnesoid X receptor (FXR), new developmental systems involving the benzoate X receptor (BXR), novel classes of ligands (benzoates, terpenoids), and alternative mechanisms for NR receptor regulation and function [37]. Thus, it is entirely possible that new fatty acid-activated NRs will be discovered or an existing NR will be adopted.

## 3. Peroxisome proliferator-activated receptors (PPARs)

The PPAR family of receptors were originally named based on their ability to respond to exogenous chemicals (peroxisome proliferators), however they were also the first to be examined as a fatty acid receptor. Peroxisome proliferators represent a group of functionally diverse chemicals which when administered to rodents cause an increase in the number and size of hepatic peroxisomes. The peroxisome is a subcellular organelle primarily involved in lipid metabolism, although other biological functions including hydrogen peroxide degradation and metabolism of protein and purine have also been well documented. Peroxisome proliferators include a wide variety of exo- and endogenous chemicals such as hypolipidemic agents (e.g., clofibric acid, gemfibrozil), industrial chemicals, herbicides, polyunsaturated fatty acids and their metabolites, and leukotrienes. Structurally, most of these chemicals resemble naturally occurring fatty acids in containing a large hydrophobic region and an acidic functional group. Pleiotropic effects of peroxisome proliferators observed in laboratory animals include: decreased body weight, hepatomegaly, hepatic peroxisome proliferation, induction of enzymes associated with lipid metabolism and upon prolonged administration, development of hepatocarcinogenesis. A nuclear hormone receptor was discovered that was activated by these chemicals [38] hence the name peroxisome proliferator-activated receptor (PPAR). It was not until later that the endogenous activators of PPAR were realized, namely fatty acids. 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 [38–41].

### *3.1. PPAR subtypes and expression*

To date, three subtypes of PPAR ($\alpha$, $\beta$ and $\gamma$) have been identified in several species including human [42]. Activation of PPAR$\alpha$ which is predominantly expressed in hepatic tissue, results in peroxisome proliferation, hypolipidemia and liver tumors in rodents. It has been demonstrated that the responsiveness of rodent hepatocytes to peroxisome proliferators is mediated by PPAR$\alpha$, i.e., if PPAR$\alpha$ is ablated the prototypical enzyme induction and peroxisome proliferation is not observed [43]. PPAR$\beta$ is expressed ubiquitously and often at higher levels than PPAR$\alpha$ and $\gamma$, although the function of this particular subtype remains unclear. PPAR$\gamma$ is essential for adipocyte and macrophage differentiation. PPAR$\gamma$ has the additional complexity of transcripts $\gamma$1, $\gamma$2 and $\gamma$3, with tissue and differentiation specific expression. All three mRNAs share the six 3′ exons [29]. PPAR$\gamma$1 mRNA comprises two 5′ non-coding exons (A1 and A2), PPAR$\gamma$2 has a specific 5′ exon (B1) while PPAR$\gamma$3 utilizes a third promoter upstream of exon A2. The proteins produced by PPAR$\gamma$1 and PPAR$\gamma$3 are identical, while PPAR$\gamma$2 is 30 amino acids larger. PPAR$\gamma$2 is found almost exclusively in the adipocyte, whereas PPAR$\gamma$1 is found in several tissues [44].

### *3.2. Endogenous PPAR ligands*

Many mono- and polyunsaturated fatty acids bind directly to PPAR$\alpha$ at physiological concentrations and cause transcriptional activation. Long-chain unsaturated fatty acids such as linoleic acid, polyunsaturated fatty acids (PUFAs) including arachidonic, eicosapentaenoic, and linolenic acids, as well as the branched chained fatty acid phytanic acid, bind to PPAR$\alpha$ with reasonable affinity ($\mu$M range, [45]). 19- and 20-hydroxylates of epoxyeicosatrienoic acid (an arachidonate metabolites) are capable of activating PPAR$\alpha$; 20, 14, 15-HEET, and the mixture of 20, 8,9-, and 20,11,12-HEETs are potent ligand of this receptor subtype [46]. Eicosanoid metabo-

lites of the linoleic acid cascade have a higher affinity for PPAR$\alpha$ than does the parent compound. The eicosanoids 8(S)-hydroxyeicosatetraenoic acid (8(S)-HETE) and leukotriene B4 (LTB4) are relatively potent PPAR$\alpha$ ligands [47,48]. Despite the higher affinity of these compounds *in vitro*, it is unclear whether the concentration of 8S-HETE or LTB4 is sufficient to cause activation of PPAR$\alpha$ *in vivo*. This has lead some to speculate that PPAR$\alpha$ has evolved to respond to the cumulative amount of fatty acids within the cell [45].

In contrast to PPAR$\alpha$, PPAR$\gamma$ has a preference for PUFAs over mono- or unsaturated fatty acids, although even the PUFAs are not considered potent activators. Lipoxygenase products of arachidonic acid, 9-HODE and 13-HODE are slightly more efficacious than PUFAs [49]. The most widely studied endogenous PPAR$\gamma$ activator is 15-deoxy-$\Delta^{12,14}$-prostaglandin $J_2$ (PGJ2) [50]. This prostaglandin acts similarly to the potent synthetic PPAR$\gamma$ ligands, the thiazolidindiones, in inducing adipocyte differentiation. In addition, the eicasanoid 15-S-HETE is a potent activator of PPAR$\gamma$ [49]. On the other hand, glyceryl eicosanoid 15-HETE-G, resulting from oxidation of 2-arachidonyl glycerol by 15-lipoxygenase acts as a PPAR$\alpha$ activator [51].

The PPAR$\beta$ subtype has a fatty acid preference that is similar to that of PPAR$\alpha$, although the amount of activation is much less. PUFAs, methyl palmitate and dihomo-$\gamma$-linolic acid are known fatty acid ligands of PPAR$\beta$; Eicosapentaenoic acid may be the most potent activator of this chemical class [52]. The eicosanoids $PGA_1$ and $PGD_2$ activate PPAR$\beta$ in the low $\mu$M concentration range. A synthetic molecule similar in structure to the prostaglandin $PGI_2$, carbaprostacyclin, is one of the most efficacious PPAR$\beta$ ligands yet described.

In essence, these studies suggest that PPARs respond to fatty acids and their metabolites. Each subtype has preferences for certain branches of the linoleic and arachidonic acid metabolic cascades, although there is a fair amount of overlap among the receptor subtypes. In addition, the apparent affinity constants ($K_d$) for most of these chemicals are within the range known to exist in cells or in serum.

### 3.3. Target genes

PPARs function in a manner very similar to that of the vitamin D, retinoic acid and thyroid hormone receptors [53]. The activated PPAR binds to DNA as a heterodimeric complex with retinoid-X-receptor (RXR; NR2B) [54]. As mentioned above, the PPAR/RXR complex controls gene expression by interacting with specific DNA response elements (peroxisome proliferator response elements, PPREs) located upstream of target genes [55]. Genes containing PPRE motifs include acyl-CoA oxidase (ACO) [55], peroxisomal bifunctional enzyme (PBE or BIF [56], liver fatty acid-binding protein (L-FABP) [57], and microsomal CYP4A [58], although several others have been discovered [59]. Members of the RXR-interacting subgroup of NRs typically bind to DNA elements containing two copies of direct repeat arrays spaced by 1 to 6 nucleotides (DR1-DR6). The idealized consensus binding site (AGGTCA) is similar for most members of this class with the specificity dictated by the number of nucleotides between half-sites as well as the 5' flanking elements [59]. In the case of PPAR, a DR1 motif is preferred with PPAR interacting with the 5' repeat and RXR ($\alpha$, $\beta$ or $\gamma$) binding to the 3' motif [60]. The PPRE is similar for all three PPAR subtypes [59–61]. Interestingly, the consensus PPRE is also recognized by transcription factors HNF [62], COUP-TF [63, 64], ARP-1 [65], RAR [65], RZR [66], and TAK-1 [67];the interaction of these transcription factors with the PPRE may inhibit PPAR's ability to activate gene expression.

### 3.4. Physiological role

The ultimate response of a cell to PPAR activators is the sum total of the genes being regulated in that cell. Both PPAR$\alpha$ and PPAR$\gamma$ are playing key roles in regulating fatty acid metabolism, albeit in seemingly opposite direction (reviewed in, [29,68]. The result of PPAR$\alpha$ 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 PPAR$\alpha$. The targeted disruption of PPAR$\alpha$ 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 $\beta$-oxidation is less in the PPAR$\alpha$ null mouse, showing the importance of this protein in overall fatty acid homeostasis.

The array of genes regulated by PPAR$\gamma$ 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 [69]. These genes include lipoprotein lipase [61], aP2 [70], and CD36 [71]. The key genes regulated by PPAR$\gamma$ that are associated with diabetes have not been conclusively demonstrated but may involve adipocyte-secreted cytokines and hormones such as TNF$\alpha$ and leptin [72,73]. The genes regulated by PPAR$\gamma$ in macrophages are similar to those in the adipocyte and include lipoprotein lipase and CD36. Treatment of macrophages with PPAR$\gamma$ synthetic agonists inhibits the production of several cytokines such as interleukin 1-$\beta$ and TNF-$\alpha$ and may result in an anti-inflammatory response [44]. Another link between PPAR$\gamma$ and inflammation is the fact that 15-deoxy $PGJ_2$, a product of the cyclooxygenase pathway, and non-steroidal anti-inflammatory drugs (NSAIDS) are potent activators of PPAR$\gamma$ [74]. More recently, PPAR$\gamma$ has shown to be beneficially involved in different types of cancer [75–77]. More studies

are needed to understand the exact role of PPAR$\gamma$ in neoplastic processes.

Genes that are targeted specifically by PPAR$\beta$ have not yet been described. This is partially attributed to the lack of potent and specific activators of this NR and the fact that a PPAR$\beta$ null model has only recently become available. Using a binding and selection assay, a PPAR$\beta$-specific PPRE has been described [78], therefore it is possible that genes under exclusive regulation of this subtype exist.

### 3.5. LXR

Liver X receptors (LXR$\alpha$ and LXR$\beta$) are transcription factors commonly known as cholesterol sensors [79, 80]. They are important regulators of transport and metabolism of sterols and fatty acids. Expression of LXR$\alpha$ is restricted, whereas LXR$\beta$ is ubiquitously present [81,82]. LXR$\alpha$ is present in certain organs namely liver, kidney, intestine, adipose tissue and adrenals. LXR alpha and beta share a high degree of amino acid similarity ($\sim$80%) and are considered paralogues. Oxysterols including 24(S), 25-epoxycholesterol, 22(R)-hydroxycholesterol, and 24(S)-hydroxycholesterol, are natural ligands of LXRs. Several LXR-mediated genes include those associated with cholesterol and bile acid metabolism (for example ABC1, ABCG1, Apo-E, and CYP7A) as well as those with fatty acid synthesis and regulation (SREBP1c, LPL, FAS). Interestingly, unsaturated fatty acids are positive regulators of LXR alpha but not of LXR beta in hepatocytes [83]. These fatty acids inhibit lipogenesis by suppressing SREBP-1c expression, by competing with proteins binding to response elements in the SREBP-1c promoter [84]. This suppressive effect can be eliminated by deletion and mutation of LXR responsive element (LXREs) located in the promoter region of SREBP-1c [84].

PPAR$\alpha$ and LXR$\alpha$ physically interact and antagonize each others action [85]. Xenobiotic PPAR$\alpha$ ligands antagonize LXR's transcriptional activity [86]. Another level of antagonism may also exist as unsaturated fatty acids inhibit oxysterol binding to LXR (reviewed in [87]). For example, in vitro, arachidonate and other unsaturated fatty acids competitively blocked activation of LXR by oxysterols [88]. This offers a potential mechanism for ability of dietary PUFAs to decrease the synthesis and secretion of fatty acids and triglycerides in liver [88].

### 3.6. Physiological roles

#### 3.6.1. Central nervous system

LXR has recently been shown to be involved with the proper functioning of rodent brain [89]. Using double-knockout mice lacking the expression of both LXR alpha and beta, it was shown that the receptor is critical for lipid homeostasis in the brain, particularly that of cholesterol, and their deficit results in serious neurogenerative changes along with alteration in gene expression. However, it is also important to note that LXR agonists are capable of increasing the secretion of A$\beta$ molecules linked with Alzheimer's disease, where development of site-specific LXR antagonist will be beneficial [89].

#### 3.6.2. Diabetes

LXR is involved in ameliorating type-II diabetes in rodent model. An LXR agonist was found to decrease blood glucose level, inhibit hepatic glucose output, and improve glucose tolerance in diabetic model of rodents [90]. In these rodents, expression of glucose-6-phosphatase and PEPCK, both involved in gluconeogensis, were also markedly reduced. Insulin treatment to rodents significantly increase LXR$\alpha$ mRNA [83]. Deletion of both LXRs resulted in suppression of insulin-mediated enzymes associated with fatty acid and cholesterol metabolism.

#### 3.6.3. Skeletal muscle

Activation of LXR in skeletal muscle, resulted in induction of ABCA1, SREBP-1c and Apo-E, genes involved in fatty acid and cholesterol metabolism [91]. Furthermore, in differentiated myotubes genes involved in reverse cholesterol transport including ABCA1 and ABCG1 are induced, whereas PPAR$\gamma$ activators remained virtually ineffective. Skeletal muscle is an important site of cholesterol efflux, and LXR$\alpha$ and other LXR-regulated genes are markedly increased during myogenesis [91].

#### 3.6.4. Atherosclerosis

The nonsteroidal LXR agonist, GW3965 significantly reduced atherosclerosis in two murine models of hyperlipidemia [92], suggesting protective role of the receptor in atherosclerosis. The compound also increased expression of ABCA1 and ABCG1, associated with cholesterol transport. Previous studies showed that activation of PPAR$\gamma$ induced the expression of LXR$\alpha$ and ABCA1, and removed cholesterol from macrophages [93]. Hence, LXR was considered further downstream than PPAR$\gamma$ in reducing atherosclerosis.

Animals without LXR$\alpha$ gene (knockout mice) were unable to respond to dietary cholesterol and failed to induce cholesterol 7-hydroxylase, the rate limiting enzyme for bile acid synthesis [94]. This resulted in excessive cholesterol accumulation in the liver followed by impairment of functions. LXR$\alpha$ knockout animals also have altered expression of genes associated with lipid metabolism. Interestingly, LXR$\beta$ knockout mice were unaffected when challenged with dietary cholesterol [95]. Selective bone marrow knockouts of macrophage LXRs increase atherosclerotic lesions in ApoE$^{-/-}$ and LDLR$^{-/-}$ mice, suggesting the role of the transcription factor as an endogenous inhibitor of atherosclerosis [92]. In addition, LXRs are involved in Apo E regulation of adipocytes [86].

### 3.7. Hepatocyte nuclear factor (HNF-4α)

Hepatocyte nuclear factor-4α (HNF-4α) is a nuclear receptor that is considered an "orphan receptor" since a natural ligand has not yet been identified. Although HNF-α displays ~40% sequence homology with mammalian RXRα, it exists as oligomeric-dimers in solution and does not participate in heterodimerization [96]. The receptor is expressed in liver, kidney, intestine and pancreas. HNF-4α is involved in lipid, carbohydrate, protein and drug metabolism, as well as hematopoiesis and blood coagulation (reviewed in [97]).

Hertz *et al.* showed that long-chain fatty acids directly modulate the transcriptional activity of HNF-4α by binding as their acyl-CoA thioesters to the ligand-binding domain of the receptor [98, 99]. Activation or inhibition of HNF4α transcriptional activity may be a function of chain length and the degree of saturation of the fatty acyl-CoA ligands. For example, HNF-4α is activated by fatty acid [C-16:0] but strongly inhibited by others [C-18:0] and C-18:3 [98]. These findings were confirmed by increasing the sensitivity of experimental procedures [100]. Specific binding of fatty acyl-CoAs with HNF-4α LBD and alteration of its secondary structure following fatty acyl-CoA binding was observed. In addition, the alteration of secondary structure by saturated and unsaturated fatty acyl-CoA was opposite in nature and similar to their functional effects on the receptor. Apart from direct binding, PUFA can also modulate HNF-4α in an indirect manner as observed in the regulation of glucose 6-phosphatase. Glucose 6-phosphatase (G6Pase), an important enzyme of intermediary metabolism, releases endogenous glucose into blood from gluconeogenic tissues in conditions like diabetes and fasting. PUFAs have been shown to suppress promoter activity of this enzyme; this effect being produced by interfering with HNF-4α binding to its cognate sites in the gene, where it produces enhancing effects [101].

MODY (maturity-onset diabetes of the young) represents an endocrine disorder that results from mutation of transcription factors involved in pancreatic beta cell-mediated insulin secretion [102,103]. MODY1, a subtype of the disease occurs due to mutation in HNF-4α. In this disease, glucose-stimulated insulin secretion is markedly altered without causing insulin resistance, an important condition observed in NIDDM. It is important to note that HNF-4α can activate the insulin gene in direct and indirect manners, in the latter case by activating/regulating HNF1α, another transcription factor involved in insulin gene regulation [102,103].

The PPARα agonist Wy-14,643 significantly reduces the availability of HNF-4α binding to the DR-1 sequence and prevents transactivation of CYP7A1, the rate limiting gene associated with bile acid synthesis [104]. However, i*n vitro* PPARα/RXRα did not show binding to the DR-1 sequence of the gene, and PPARα and Wy-14,643 did not inhibit HNF-4α binding to the DR-1 sequence. However, the activated PPAR/RXR heterodimer decreased HNF-4α expression in HepG2 cells. Co-expression of HNF-4α, significantly decreases the inhibitory effect of PPARα [105]. On the other hand, CoA thioesters of certain peroxisome proliferators demonstrate high affinity for HNF-4α (in nM range) and can serve as pharmacological ligands [100].

Physiologically, HNF-4α plays an important role in hepatocyte differentiation, ureagenesis and in regulation of genes associated with lipid metabolism. Liver specific HNF-4α null mice demonstrated elevated lipid accumulation in the liver. In addition, serum cholesterol and triglyceride levels were significantly reduced whereas serum bile acid concentration was markedly elevated in these animals [106]. The liver specific HNF4α knockout mice also have increased serum ammonia and decreased serum urea levels [107], this effect resulting from the lack of regulation of ornithine transcarbamylase by HNF-4α.

### 3.8. RXR

Retinoid X receptors are involved in the transduction of retinoid signaling pathway. Although RXRs (α, β or γ) can form homodimers, they 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 [108–111]. Although intensely studied for synthetic ligands, little is known of the natural activators of this receptor [112]. 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 docosahexaenoic acid (DHA), a long-chain polyunsaturated fatty acid that is highly enriched in the adult mammalian brain, is an RXR ligand [112]. It is noteworthy to report that DHA constitutes a major portion of fatty acid of the mammalian brain. Docosatetraenoic acid, a structurally related compound, activates RXR with a much higher concentration [112]. DHA's effect was not observed in other nuclear receptors such as RAR, thyroid hormone receptor and Vitamin D receptor, although the fatty acid activates PPARα [113]. Previous work has shown that DHA is essential for brain maturation, and deficiency of DHA in both rodents and humans leads to impaired spatial learning and other neurological abnormalities. These data suggest that DHA may influence neural function through activation of an RXR signaling pathway [112]. Phytanic acid, a branched chain fatty acid derived from chlorophyll has also been reported to activate RXR *albeit* weakly [114]. Phytanic acid is capable of adipocyte differentiation and induces aP2 mRNA in 3T3-L1 preadipocytes [115] and may act as a natural rexinoid in 3T3-L1 cells.

RXR alpha agonists are capable of reducing atherosclerosis in apolipoprotein E knockout mouse, an established

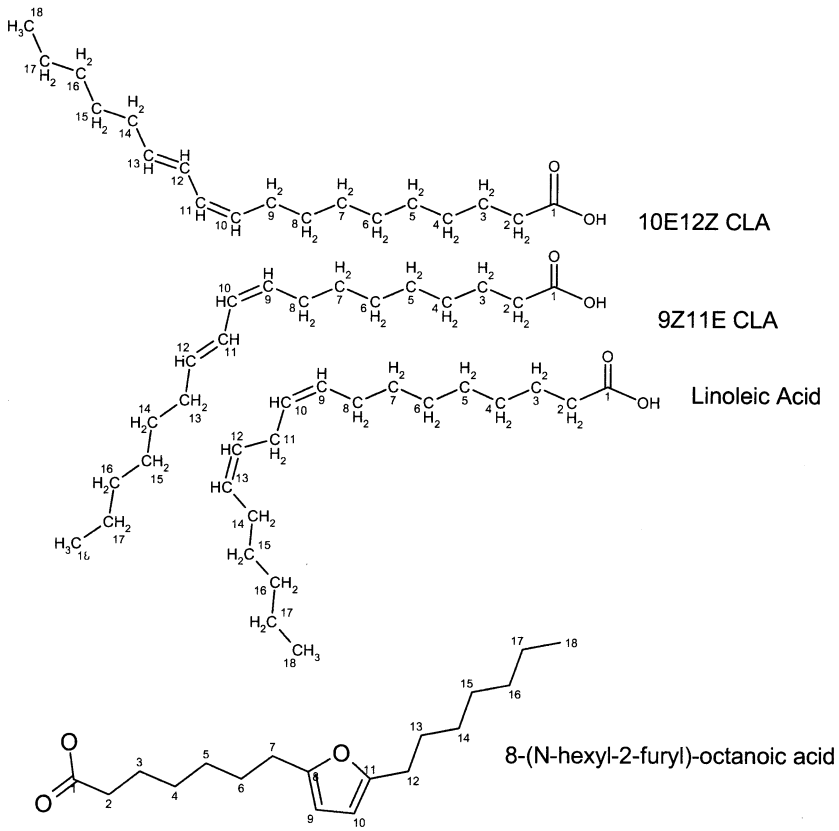


Fig. 3. Structure of conjugated linoleic acids

experimental model of atherosclerosis [116]. Retinoids are capable of increasing the expression of ABC-1, a gene associated with reverse transport of cholesterol. Cholesterol efflux from peritoneal macrophages was significantly increased in a RXR-dependent fashion [116]. RXR-selective agonists are capable of counteracting NIDDM by decreasing hyperglycemia, hypertriglyceridemia and hyperinsulinaemia [117]. These agents however, produce hypothyroidism in humans and rodents [118]. Null mutation of RXR$\alpha$ gene resulted in developmental lethality in mice;they died *in utero* and demonstrated severe myocardial and ocular malformations [119]. The malformations resembled severe Vitamin A syndrome, suggesting a physiological role of RXR$\alpha$ [119]. The AF-2 domain of the receptor has been shown to be important in the development of placental barrier [120]. RXR$\alpha$ is also involved in hair cycling and in epidermal keratinocyte proliferation and differentiation [121].

### *3.9. Conjugated linoleic acids as ligands for NRs*

The term conjugated linoleic acid (CLA) refers to a group of linoleate (c9, c12-octadecienoate) derivatives which exhibit a possibility of 8 positional (double bonds in positions 9 and 11 or 10 and 12 on the carbon chain) and geometric isomers (*cis* and *trans*, *Z* and *E*). (See Fig. 3). Virtually all *cis*- and *trans*-isomeric combinations of CLA have been identified in food; however, the 9Z11E CLA and the 10E12Z CLA isomers predominate in these mixtures (approximately 85 to 90%) with ten other minor CLA isomers representing the remaining percentage. Since it appears that different isomers might have different activities in vivo, one must be cognizant of whether a mixture of CLA isomers or the individual chemical is being examined.

CLAs have been associated with a variety of beneficial effects in laboratory animals (reviewed in [122–124]). Since these fatty acids are produced in cows and other livestock, but not humans, emphasis has been placed on dietary intervention to increase CLA consumption and promote human health. In addition, CLAs are currently being widely used as dietary supplements in humans and feed additives in livestock, mainly due to their ability to alter body composition [125]. Despite the accumulating evidence of the positive health effects of CLA, and their escalating use, little is know about how these fatty acids exert their activity.

As mentioned above, CLA is a mixture of a variety of possible geometric and positional isomers. The concentration and ratio of each of these isomers varies depending on many factors including livestock feeding practices and food production processes [126–130]. The CLA isomers may have different properties as their incorporation into triacylglycerols and phospholipids varies slightly [128,131, 132]. It has been a relatively recent event in which the individual CLA isomers have become available in sufficient

quantity for analysis of biochemical effects; this has resulted in the speculation and some support for the premise that CLA-specific isomer effects exist [133–134]. For example, Pariza *et al*. have described the 10E12Z isomer as being the predominant adipogenic CLA isomer [134], while 9Z11E is the more potent anti-carcinogenic CLA [135]. The slight physical differences between the CLA isomers resulting in different biological effects are highly suggestive of the existence of cognate receptor(s) for these molecules.

Due to the similarity between the effects of CLAs and that of hypolipidemic drugs and TZDs, we hypothesized that these dietary fatty acids served as PPAR ligands. Evidence supports that all three PPAR subtypes are affected by various CLA isomers. The 9z 11e CLA isomer has been identified as a potent PPAR$\alpha$ ligand with a $K_D$ in the low nM range [136]. Further evidence to support CLA as a member of the peroxisome proliferator and PPAR$\alpha$ activator include induction of mRNA and protein of PPAR-responsive enzymes including ACO, L-FABP and CYP4A1 [136]. Interestingly, the effects of CLA on body composition are seen in the PPAR$\alpha$ null mouse [137], suggesting that this NR is not the key target for this response.

Several studies suggest that CLA is a weak activator of PPAR$\beta$. A mixture of CLA isomers as well as 9z 11z- and 9z 11e-CLA can significantly activate PPAR$\beta$ in preadipocytes (unpublished observations). In addition, a putative CLA metabolite (furan-CLA) has also previously been reported to activate PPAR$\beta$ in COS-1 cell transfection experiments [138–139].

The biological effects of CLA are most reminiscent of PPAR$\gamma$ ligands such as ciglitazone. For example, CLA ameliorates the symptoms of Type II Diabetes Mellitus in the Zucker fa/fa diabetic (ZDF) rats and affects differentiation of adipose tissue [8]. CLA treatment improved the hyperinsulinemia and hyperleptinemia characteristics of ZDF rats and potently reduced circulating triglycerides and free fatty acids [8]. In addition, this dietary fatty acid is capable of decreasing pro-inflammatory signals including COX-2, TNF$\alpha$, iNOS in macrophages [140] and may be beneficial in immunological disorders. The regulation of iNOS transcription by CLA requires PPAR$\gamma$, as demonstrated using a dominant negative construct. CLAs demonstrate isomer-specificity in activating the receptor. For example, 9Z11Z-, and 10E12Z CLA can markedly activate PPAR$\gamma$ in 3T3-L1 preadipocytes in transfection and ligand binding assays (unpublished observations), whereas 9Z 11Z-, 9Z 11E-, 10E 12Z-, and 9E,11E-, and furan -CLA served as potent activators of this receptor subtype in RAW 267.4 cells [140].

Despite the convincing data supporting a role of PPAR$\gamma$ in eliciting some of the effects of CLA, other NRs may also contribute. For example, we observed that 9E11E CLA markedly activated RXR$\alpha$ in COS-1 cells, suggesting its putative role in signal transduction of CLAs (Fig. 4). Similar research is currently underway with the remaining fatty acids receptors.

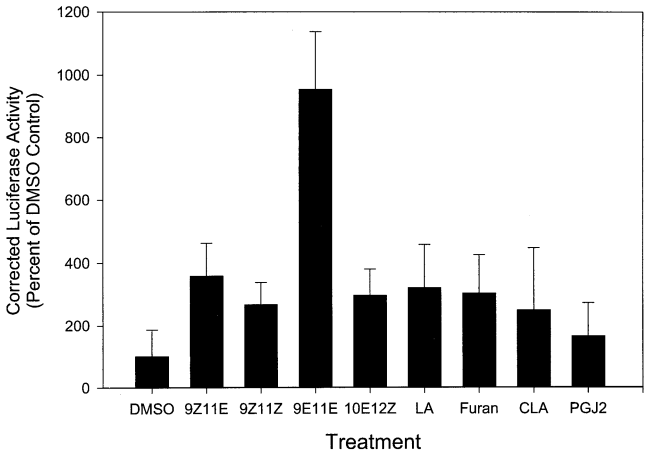


Fig. 4. CLA isomers activate hRXR$\alpha$. The RXR$\alpha$-GAL4 reporter system was used in COS-1 cells. All isomers tested at 100 $\mu$M, except $PGJ_2$ (10 $\mu$M). Mean ± SEM, *n* 3. Shown is one experiment representative of three independent experiments.

## 4. Conclusions

The structure of fatty acids is an important facet of their health effects with diets rich in saturated fats being associated with diabetes, heart disease and cancer. Ingestion of PUFAs and CLAs, on the other hand, are often considered inversely related to these pathophysiological conditions. Thus, there are important cellular mechanisms that are able to differentiate between these subtle structural changes. In addition, fatty acids have distinct tissue-, sex- and species-specific responses (reviewed in [87, 99]). These observations are consistent with the existence of specific receptors that recognize distinct fatty acid structures and regulate gene expression accordingly. There are several nuclear receptors that are able to bind to dietary fatty acids and regulate gene expression and the cellular phenotype, as summarized in Fig. 1. PUFAs have rapid effects on gene expression resulting in changes in mRNAs encoding several lipogenic enzymes within hours (reviewed in [99]) Some of the biological effects of PUFAs may be attributed to their ability to bind to and regulate activity of PPARs, LXRs and RXRs ([87,99]. Similarly, there is increasing data to suggest that CLAs are activators of the PPAR family of nuclear receptors [139]. The activation of PPAR$\gamma$ in particular intriguing since the biological effects of CLAs are similar to that of potent PPAR$\gamma$ ligands, the thiazolidinediones, including amelioration of diabetes, inflammation and cancer in animal models [123,124]. Although the nuclear receptors have been the focus of the present review, the readers should bear in mind that there are other ways in which fatty acids may regulate gene expression. For example, protein kinase C (PKC) [141] and NF$\kappa$B [99,142] have been proposed to be targets of CLA and PUFAs, respectively, and alteration of the lipid component of the cellular membrane would be expected to result in myriad of signaling events. However, the ability of nuclear receptors to regulate gene

expression resulting from specific ligand-macromolecule interactions, and the association of several of these proteins with treating diseases such as diabetes and cancer, makes them important keys to the puzzle in determining the dichotomous effects of diets high in saturated versus unsaturated fatty acids on human health.

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