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POLYUNSATURATED FATTY ACID REGULATION OF GENES OF LIPID METABOLISM

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■ **Abstract** Apart from being an important macronutrient, dietary fat has recently gained much prominence for its role in regulating gene expression. Polyunsaturated fatty acids (PUFAs) affect gene expression through various mechanisms including, but not limited to, changes in membrane composition, intracellular calcium levels, and eicosanoid production. Furthermore, PUFAs and their various metabolites can act at the level of the nucleus, in conjunction with nuclear receptors and transcription factors, to affect the transcription of a variety of genes. Several of these transcription mediators have been identified and include the nuclear receptors peroxisome proliferator-activated receptor (PPAR), hepatocyte nuclear factor (HNF)-4α, and liver X receptor (LXR) and the transcription factors sterol-regulatory element binding protein (SREBP) and nuclear factor-κB (NFκB). Their interaction with PUFAs has been shown to be critical to the regulation of several key genes of lipid metabolism. Working out the mechanisms by which these interactions and consequent effects occur is proving to be complicated but is invaluable to our understanding of the role that dietary fat can play in disease management and prevention.

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INTRODUCTION

Due to the recent spike in lipid-related disorders such as obesity and diabetes, dietary fat has been relegated to the darkest depths of our collective fears. Despite this, as our understanding of molecular mechanisms and biochemical pathways increases, we find that fatty acids are no longer just sources of energy, but also fine modulators of cellular signaling and metabolism.

The first and most obvious role of fat is as an essential nutrient; the well-characterized effects of essential fatty acid deficiency include flaky skin, diarrhea, and ultimately, death. Also, due to its energy density, it is the ideal storage form of excess energy. At the cellular level, fatty acids form an essential part of the phospholipid bilayer of membranes and serve as the precursors to signaling molecules such as steroids and prostaglandins. Apart from these established roles for fatty acids, it has now become increasingly clear that polyunsaturated fatty acids can specifically and rapidly effect changes in cellular metabolism, differentiation, and growth through alterations in gene expression patterns (96). This review sorts through some of the recent evidence implicating a role for polyunsaturated fatty acids (PUFAs) in affecting gene expression, with a special focus on genes of lipid metabolism.

Linoleic (n-6) and linolenic (n-3) acids cannot be synthesized de novo by mammals and are hence essential in the diet. The n-3 fatty acids, especially eicosapentanoic acid (20:5) and docosahexanoic acid (22:6) can be obtained from high-fat fish and marine mammals, while the n-6 fatty acids are concentrated in organ meats and vegetable oils. n-3 and n-6 PUFAs have been known to confer various health benefits, including increased insulin signaling (110), enhanced immune response (48, 80), decreased plasma lipid levels (40, 88), and decreased incidence of lung disease (100) and coronary heart disease (106, 107, 117). In addition, consumption of PUFAs is beneficial in certain cancers, including breast, prostate, and colorectal (24, 93). PUFAs have also been shown to be essential to the developing brain (1). At the same time, there is some concern over the consumption of n-6 fatty acids because of their known proinflammatory and proaggregatory effects. Thus, further understanding of the cellular processes affected by these fatty acids will be invaluable in increasing our present nutrition knowledge (96).

CELLULAR FATTY ACID METABOLISM

The fatty acid composition of a meal directly parallels the plasma fatty acid composition in the postabsorptive state. Following a meal, more than 95% of dietary fat (85%–90% in infants) is absorbed through the small intestine and channeled through the portal (fatty acids with less than 10 carbons) or lymphatic systems

to form chylomicrons (CMs), the largest of the circulating lipoprotein classes. Along with chylomicrons, very-low-density lipoproteins (VLDLs) packaged in the liver also contribute to the plasma fatty acid profile. Apolipoproteins on the surface of CMs and VLDLs allow for their recognition by two different lipases (hepatic and lipoprotein) so that complex lipids carried in the lipoprotein can be hydrolyzed to free fatty acids (FFAs). Although there is still some discussion about whether subsequent cellular uptake of FFA occurs solely through diffusion or via transporter-mediated uptake, considerable evidence exists for a saturable transporter for long-chain fatty acids (LCFAs) in several tissues, such as intestine, heart, adipose, and liver (45a, 59). Several proteins have been identified as candidates for mediating fatty acid uptake in these tissues, including CD36 fatty acid transporter, caveolin, and fatty acid transport proteins (59).

Once within the cell, LCFAs are rapidly converted to fatty acyl-CoA thioesters by acyl-CoA synthetases (ACSs), six of which have been characterized (21). Fatty acyl-CoAs can then be sequestered through binding to acyl-CoA binding proteins (35). Alternately, fatty acyl-CoAs are channeled to various fates (Figure 1); they can be incorporated into complex lipids such as triglycerides (TGs) and phospholipids (PLs) or further oxidized into metabolites such as epoxy- and hydroxy-fatty acids. These metabolites, in turn, have profound effects on gene regulation and cell-signaling pathways (Figure 1). For example, phosphatidylinositols, a class of phospholipids, serve as substrates for phospholipase-C (PLC) and are cleaved to inositol-triphosphate (IP_3) and diacylglycerol (DAG) in response to hormones such as vasopressin. The water-soluble IP_3 then translocates to the endoplasmic reticulum (ER) membrane where it promotes Ca^{2+} release from the ER, thereby activating various signaling pathways (Figure 1). Sphingolipids such as sphingomyelin and ceramide are yet other examples of complex lipids that can serve as cellular signaling molecules (76).

Another pathway for fatty acid metabolism is through their oxidation via the cyclooxygenase (COX) or lipooxygenase (LOX) pathways (76). Arachidonic acid is metabolized through these pathways to give rise to prostaglandins and thromboxanes (COX pathway) or to leukotrienes (LOX pathway) that can then act locally through G-protein mechanisms to increase cellular Ca^{2+} and cAMP levels (Figure 1). Changes in concentrations of these intracellular second messengers have been linked to changes in gene expression patterns (23, 34).

FATTY ACID REGULATION OF GENE EXPRESSION: BACTERIA AND YEAST

Regulation of genes of lipid metabolism by fatty acids has been documented in unicellular as well as more complex organisms. In *Escherichia coli*, the bacterial enzyme FadD, which is analogous to mammalian ACS, catalyzes the conversion of free fatty acids to fatty acyl-CoAs (8). Once activated, these CoAs have been shown to bind to the transcription factor FaDR and sequester it from its response

element on various genes including fabA, fadL, fadD, fadE, fabB, and fadH. This results in downregulation of fatty acid synthesis via fabA and upregulation of fatty acid transport and metabolism (8).

Yeasts are capable of thriving entirely on LCFAs as their sole carbon source. When grown on LCFAs, there is an increase in peroxisomal proliferation, which indicates that fatty acids are capable of altering structural components in yeast (8). Faa1p and Faa4p, the yeast acyl-CoA synthetases, are responsible for the activation of LCFAs to acyl-CoAs. These acyl-CoAs can then be used for energy or stored as complex lipids. Furthermore, they can affect the transcription of various genes apart from those involved in peroxisome proliferation. Regulation of genes in the yeast seems to occur through the specific transcription factors Oaf1p/Pip1p and Oaf2p/Pip2p (8). Oaf1p and Oaf2p have been shown to form a heterodimer in response to exogenous fatty acids and bind to a cis element in the DNA promoter, termed the oleate responsive element (ORE), with the sequence CGGNNNTNA(N9–12)CCG. More than 40 yeast genes have been shown to contain this ORE in their promoter regions and are therefore good candidates for Oaf1p/Oaf2p-mediated regulation by fatty acids.

One key gene involved in lipid metabolism in the yeast is the OLE1 yeast Δ-9 desaturase, analogous to the mammalian stearoyl CoA desaturase (SCD) gene (8). The OLE1 gene has been shown to be repressed by both mono- and polyunsaturated fatty acids; however, this repression seems to be independent of Oaf1p/Oaf2p. Recently, an ER membrane protein, Mga2p, has been identified as a key player in the transcriptional and post-transcriptional mechanisms regulating the destabilization of OLE1 mRNA upon exposure to unsaturated fatty acids (16).

FATTY ACID REGULATION OF MAMMALIAN GENE EXPRESSION

The role of polyunsaturated fatty acids in the regulation of mammalian gene expression has been well characterized and is the focus of the remainder of this review. Some of the first reports of direct regulation of lipogenesis by fatty acids came from Allman & Gibson (2), who described the upregulation of fatty acid–synthesizing enzymes in the liver and epididymal fat pads of mice on high-carbohydrate diets. The authors showed that supplementation of a fat-free diet with either saturated fatty acids or cholesterol had no effect, but supplementation with linoleic acid greatly reduced ^{14}C-acetate incorporation into LCFAs within four days of feeding (2). Enzyme activities of fatty acid synthase (FAS), malic enzyme, and glucose-6-phosphate dehydrogenase (G6PD) were decreased, demonstrating rapid, direct, and specific regulation of enzymes of lipid metabolism by a particular fatty acid (2). These results were independently confirmed by various groups that showed decreases in hepatic lipogenic capacity upon linoleic acid feeding in wild-type (52) as well as genetically obese fa/fa rats (118). However, it was not until 1982

that a specific mechanism of action for this effect was suggested. Schwartz & Abraham (101a) used a specific antibody assay to show that corn oil supplementation of a high-carboyhydrate diet reduced the rate of synthesis of FAS protein in mouse liver. The question of whether the changes observed were effected directly by the fatty acid or by one of its metabolites was settled by the demonstration that columbinic acid, a PUFA that is not further metabolized to a prostaglandin, was equally capable of reducing FAS protein synthesis as linoleic acid (101a). However, while these authors suggested that the effect of linoleic acid on FAS protein synthesis was due to its desaturated nature, it has since been shown that several fatty acids as well as their various metabolites are capable of specifically effecting changes in gene expression patterns in liver, adipose, and other tissues.

While dietary fat could certainly affect cell signaling and gene expression by affecting membrane phospholipid content or through production of eicosanoids (Figure 1), it was the discovery of nuclear receptors capable of binding fatty acids that established a direct role for fatty acids in gene regulation (36). Within hours of feeding animals diets rich in PUFAs, there is a rapid and sustained activation of genes of lipid oxidation and a decrease in genes encoding enzymes of lipid synthesis (20, 55, 56, 62, 122). The result of this twofold action of PUFAs is a negative fat balance, thereby making PUFAs a good candidate for the dietary management of hyperlipidemia. Although the nuclear actions of PUFAs were first studied in hepatic cells, their actions have since been confirmed in 3T3-L1 cultured adipocytes (68, 103, 112) as well as in various tissues, including small intestine (77), pancreas (12), the immune system (111), and neonatal mouse brain (26).

Repression of Gene Expression by PUFAs

The inhibition of lipogenic genes in the liver by PUFAs is a very strong signal that overrides even the prolipogenic capacity of insulin and carbohydrates, which also tend to be highest postprandially. PUFA-rich diets in rodents have been shown to repress a variety of genes, including stearoyl CoA desaturase 1(SCD1), acetyl CoA carboxylase (ACC), FAS, L-type pyruvate kinase (L-PK), Δ-5 and Δ-6 desaturases, G6PD, and insulin-sensitive glucose transporter (GLUT)-4 in the liver (17–19, 54, 78, 95, 103, 112, 119). SCD1, L-PK, ACC, and FAS repression by PUFAs occurs at the level of transcription, whereas G6PD is controlled via post-transcriptional mechanisms (54, 95). Many of these genes are repressed by both n-3 and n-6 PUFAs. This seems to be a direct effect of the fatty acids themselves since addition of prostaglandin (PG) inhibitors does not abolish the effects of PUFAs on these genes (28, 119).

The findings in liver are quite different from what has been seen in adipose tissue (119). The suppression of genes such as FAS in adipose tissue of rat seems to be site-specific. PUFA-rich diets elicit this response in retroperitoneal but not in subcutaneous fat depots (86), perhaps due to known differences in site-specific

metabolism of fatty acids (102). Also, unlike in liver, many adipose tissue genes seem to respond specifically to n-3 PUFAs (86). An exception to this generality is the regulation of SCD1 in adipose tissue by n-6 PUFAs (102). Obese animals show dramatically higher levels of SCD1 in both liver and adipose tissue. However, feeding both genetically obese (fa/fa) and lean Zucker rats a high n-6 fatty acid diet results in suppression of lipid synthesis (53, 118). This is consistent with the findings of Sessler & Ntambi (102) that addition of arachidonic acid, linoleic acid (n-6), and eicosapentanoic acid (n-3) to 3T3-L1 adipocytes all result in repression of SCD1 mRNA in a dose-dependent manner through alterations in mRNA stability.

Also unlike in liver, PUFA-mediated repression of lipogenic genes in adipose tissue involves eicosanoid production (54). As described above, eicosanoids are oxidative derivatives of PUFAs released from the phospholipids of plasma membranes that are capable of eliciting both acute changes in proteins as well as long-term changes in mRNA abundance (54). It has been shown, for example, that arachidonate suppresses FAS and S14 mRNA in 3T3-adipocytes. Inhibition of the COX pathway in these cells abolished the effect of arachidonate (68). In this study, the prostaglandins PGE2 and PGF2α were shown to function via a pertussis-sensitive G-protein coupled receptor (68), but G-protein-independent mechanisms for prostanoid inhibition of gene expression have also been described (54).

More recently, one of the key examples of PUFA regulation of lipogenesis comes from its effects on leptin, an adipose-derived hormone. Leptin has gained much attention due to its role in the prevention of obesity; high plasma leptin levels are closely associated with increased adiposity and obesity, and weight loss results in attenuation of these high plasma leptin levels (32). Although a high-fat diet in humans has been associated with high plasma leptin levels, substitution of PUFAs for saturated fat in the diet results in decreased leptin levels independent from changes in body mass (91). Raclot et al. (86) showed that n-3 PUFA [especially docosahexanoic acid (DHA)] feeding in rats resulted in decreased leptin mRNA in retroperitoneal fat, but not in subcutaneous fat, independent of plasma insulin levels. Reseland et al. (92) showed that addition of eicosapentanoic acid (EPA) and DHA but not saturated or monounsaturated fatty acids to human trophoblasts (BeWo) in culture resulted in decreased transcription of a transfected human leptin promoter. This was accompanied by decreased levels of peroxisome-proliferator receptor (PPAR)-γ and sterol-regulatory element-binding protein (SREBP)-1 mRNA levels, providing insight into the mechanisms by which n-3 PUFAs exert their effects on leptin (92). However, given the role of leptin in appetite suppression and repression of lipogenic genes such as SCD1, the physiological significance of PUFA-mediated repression of leptin remains to be determined.

Another adipose-derived hormone, resistin, has recently been reported to be inhibited by PUFAs. Resistin belongs to the resistin-like molecules (RELM) gene family. In the mouse, resistin is mainly expressed in adipose tissue, whereas RELMα, β, and γ are expressed in adipose tissue and lung, intestine, and

hematopoietic tissues, respectively. The human homologs of resistin and RELMβ are expressed in macrophages and small intestine, respectively. There is evidence that resistin functions to inhibit glucose uptake in 3T3-L1 adipocytes (108) as well as L6 rat skeletal muscle cells (74). Thiazolidinediones, ligands for the nuclear receptor PPARγ discussed below, have previously been shown to repress resistin mRNA levels, at least in 3T3-L1 cells (108). Haugen et al. (41) have recently shown that the same effect is seen when 3T3-L1 cells are treated with EPA or arachidonic acid. At 60–250 uM quantities, arachidonic acid was especially effective in reducing resistin mRNA levels by about 80%. Inhibition of the COX-1 and mitogen-activated protein kinase pathways as well as actinomycin D and cycloheximide treatments resulted in attenuation of the PUFA response, which suggests that transcriptional and translational regulation of resistin by PUFAs occurs through a metabolite of arachidonic acid. The authors suggest that this regulation is not mediated by SREBP, but through a decrease in resistin mRNA stability (41).

Upregulation of Gene Expression by Fatty Acids

Fatty acids are key regulators of adipocyte differentiation; thus, they are potent activators of genes and proteins involved in adipogenesis. However, in contrast to their role in gene repression, saturated as well as unsaturated LCFAs seem to be equally effective in upregulating genes involved in adipogenesis (28). Mechanistically, nonmetabolizable fatty acids such as α-bromopalmitate are also capable of effecting changes (37), a finding that indicates a direct role for fatty acids in upregulation of these genes. It is possible that fatty acids directly upregulate gene expression, but in the case of certain genes such as ap2, this is unlikely since upregulation in response to fatty acids is not rapid and is inhibited by cycloheximide, an inhibitor of protein synthesis (3, 4). It is therefore more likely that fatty acids function by affecting the abundance of a transcription factor that mediates the regulation of these genes (28); further studies are required to understand the mechanism by which such regulation occurs.

In adipose tissue, phosphoenolpyruvate carboxykinase (PEPCK) is responsible for supplying glycerophosphate for triglyceride synthesis (Table 1) and is upregulated in response to PUFAs. While glucose inhibits PEPCK in adipose and liver, PUFAs induce PEPCK mRNA in 3T3 adipocytes (31). It seems clear that the induction of PEPCK by fatty acids in adipose tissue does not require protein synthesis or fatty acid metabolism to eicosanoids. This inhibition is specific to adipose tissue; the same response is not seen in the liver, where PEPCK serves in a gluconeogenic capacity (31).

In the liver, several genes have been demonstrated to be upregulated by fatty acids (Table 1); these include apolipoproteins AI and AII (6), acyl-CoA synthetase (28), acyl-CoA oxidase (6), liver fatty acid–binding protein (L-FABP) (71), carnitine palmitoyl transferase (CPT-1) (15), and cytochrome P450 A1 (28). CPT-1 and L-FABP are also upregulated in response to fatty acids in other tissues and

TABLE 1 Effects of polyunsaturated fatty acids on expression of genes involved in lipid metabolism*

Pathway gene	Transcription factor	Effect of PUFAs	References
Lipogenesis			
Stearoyl CoA desaturase 1	SREBP-1c, LXRα	↓	81
Sterol-regulatory element binding protein-1c	LXRα, PPARα	↓	85, 122, 125, 126
Fatty acid synthase	SREBP-1c, LXRα	↓	19, 122
Acetyl CoA carboxylase	SREBP-1c, LXRα	↓	95
Phosphoenol pyruvate carboxykinase (adipose)	PPARα	↓	114, 115
S14	SREBP-1c	↓	19
Fatty acid transport/metabolism			
Acyl CoA synthetase	PPARα	↑	99
Fatty acid transport protein	PPARα	↑	33
Energy utilization/fatty acid oxidation			
Carnitine palmitoyl transferase-1	PPARα	↑	9
Uncoupling protein-1	PPARα	↑	101b
Acyl CoA oxidase	PPARα	↑	6
Cholesterol metabolism			
Cyp7α hydroxylase	HNF-4α, LXR	↓	66
HMG CoA synthase	SREBP	↑	121
ApoCIII	HNF-4α	↓	42
TNFα	NF-κB	↓	128

*Abbreviations: Apo, apolipoprotein; Cyp, cytochrome P; HMG, hydroxymethylglutaryl; HNF-4α, hepatocyte nuclear factor-4 alpha; LXRα, liver X receptor alpha; NF-κB, nuclear factor-kappa B; PPARα, peroxisome proliferator receptor alpha; PUFA, polyunsaturated fatty acid; SREBP-1c, sterol-regulatory element binding protein-1c; TNFα, tumor necrosis factor alpha.

cell culture models, such as the small intestine (FABP) (77) and INS-1 pancreatic β-cell line (CPT-1) (5a).

MECHANISMS FOR PUFA-MEDIATED CHANGES IN GENE EXPRESSION

PUFAs have been shown to affect gene expression through at least three different nuclear receptors, PPAR, liver X receptor (LXR), and hepatocyte nuclear factor-4 alpha (HNF-4α), and through the transcription factors, SREBP, and nuclear factor-κB (NF-κB) (57). Along with various ancillary proteins and partners, several

isoforms of each of these mediators interact with PUFAs and with each other to weave a complex pattern of regulation by fatty acids (Figure 2).

Peroxisome Proliferator-Activated Receptors

STRUCTURE AND FUNCTION PPARs belong to the steroid hormone nuclear receptor superfamily of ligand-activated transcription factors that also includes the retinoic acid receptor (RAR), LXR, and the ubiquitous retinoid X receptor (RXR). Three different isoforms of PPAR, PPARα, PPARβ/δ, and PPARγ, have been identified and are encoded by three different genes; they all function by dimerizing with RXR and binding to a prescribed DNA sequence, termed the PPAR response element (PPRE) (Figure 2) (25). The PPRE comprises direct repeat sequences separated by a single nucleotide spacer (AGGNCA_AGGTCA), and the binding of the PPAR/RXR heterodimer to the PPRE affects transcription of the target gene (50).

PPARα is the major PPAR subtype found in hepatocytes and is involved in regulation of genes of lipid and carbohydrate metabolism. It is required for the clofibrate induction of peroxisomal and microsomal enzymes such as acyl-CoA oxidase (AOX) and CYP4A in the liver (64), CPT-1, the rate-limiting enzyme for β-oxidation of fatty acids (9, 38), as well as fatty acid translocases and transport proteins (75).

PPAR LIGANDS A wide range of structurally diverse natural and synthetic compounds have been shown to bind the various isoforms of PPAR, and because of their role in insulin sensitivity and lipid metabolism, much effort has been directed towards the identification and development of novel PPAR ligands. In general, all the n-3 and n-6 fatty acids activate the three PPAR isoforms. However, their affinities for the receptor vary, which suggests a role for site-specific availability and metabolism of particular fatty acids and differences in their affinity for specific PPAR subtypes. For example, EPA is a much more potent activator of PPARα in primary hepatocytes as compared with arachidonic acid (89). Kliewer et al. (65) showed through transactivation assays that polyunsaturated fatty acids such as linoleic, linolenic, and arachidonic acids were able to activate PPARα at a concentration of 100 uM. Since the concentration of these free fatty acids in human adult serum can be over 1 mM, it was concluded that these fatty acids are potent endogenous ligands of PPARα. Furthermore, these PUFAs were also found to activate PPARγ potently. The authors also demonstrated through transactivation and competitive binding assays that metabolites of PUFAs, such as the naturally occurring eicosanoids 8-HETE and 15 D-J2, were also able to activate PPARα and PPARγ. In fact, these eicosanoids showed more specificity and subtype selectivity than their fatty acid precursors in activating either PPARα or PPARγ more potently (65).

More recently, it has been shown that conjugated linoleic acids (CLA) are also capable of activating PPARγ. Feeding pigs CLA before perturbation of their

gastrointestinal system with enteric bacteria markedly upregulated PPARγ and resulted in decreased mucosal inflammation (45b). These results have been confirmed in the RAW264.7 mouse macrophage cell line, where CLA supplementation results in decreased interferon-γ expression, mediated by upregulation of PPARγ.

Despite this abundance of endogenous ligands for PPARs, of greater therapeutic and commercial value in recent years has been the development of synthetic ligands for the different PPAR isoforms (51). Fibrates, a class of lipid-lowering drugs, are being used to manage high plasma cholesterol levels. Fibrates such as clofibrate and WY 14643 are potent activators of PPARα. Additionally, the insulin-sensitizing thiazolidinedione (TZD) class of drugs is used clinically to manage type II diabetes mellitus; these TZDs, which include the compound troglitazone, preferentially bind to and activate PPARγ. In addition to these ligands, PPARs, like some other nuclear receptors, can also be activated via phosphorylation, for example as induced by insulin through the mitogen-activated protein kinase pathway (25).

REGULATION OF GENES BY PPAR The effect of long-chain PUFAs on expression of a number of genes has been shown to be mediated through PPARs. These include, but are not limited to, acyl-CoA synthase (99), FATP (33), CPT1 (9), PEPCK (114, 115), UCP1 (101b), and SCD1 (73). PPARα is also required for the fish oil–mediated induction of AOX mRNA as well as CYP4A2 mRNA (6). However, the development of the PPARα-null mouse has shown that not all regulation of genes by PUFAs is mediated by PPARα. Bing et al. (7) showed that fish oil, a rich source of n-3 PUFAs, upregulated genes of β-oxidation in wild-type but not in PPARα-null mice. Although this was expected, the authors also observed that fish oil suppressed lipogenic genes such as S14 and FAS (7) in both wild-type and mutant mice, which suggests that the effects of PUFA on lipid oxidation and synthesis were not likely to be mediated by a common transcription factor, i.e., PPARα. The resultant search for a novel transcription factor regulated by PUFAs led to the identification of the SREBPs, discussed below, as key candidates.

In humans, the precise role of PPARα in liver is less clear; it is only weakly expressed and several splice variants of the isoform have been characterized (54, 84a). While the fibrate class of drugs is commonly used clinically as a lipid-lowering agent, there is not much evidence of upregulation of peroxisomal proliferation in human liver. It therefore has been suggested that the actions of fibrates in humans could be mediated through the actions of PPARα in nonhepatic tissues (54).

There is some evidence for PPAR mediated repression of genes through mechanisms involving competition, with the thyroid receptor (TR), for the RXR partner or competitive binding to response elements of other transcription factors such as HNF-4α, discussed further below (43). There is clear evidence, for example, that in the absence of excess RXR, the actions of the thyroid receptor (TR) are mitigated by PPARs. However, a lot of the suppression of lipogenic genes by fatty acids seems to be mediated via the actions of a different transcription factor, SREBP.

Sterol-Regulatory Element Binding Protein

STRUCTURE, FUNCTION AND ISOFORMS SREBPs belong to the helix-loop-helix family of transcription factors and were first identified through their ability to bind to a sterol response element found on genes involved in cholesterolgenesis (82). Three isoforms of SREBP have thus far been identified; SREBP-1a and SREBP-1c are important to regulation of genes of lipid synthesis, while SREBP-2 has been shown to control genes important to cholesterol homeostasis. SREBP-1a and SREBP-1c are transcribed from the same gene locus and differ only at their N-termini; SREBP-2 is encoded by a separate gene. While SREBP-1a is the predominant isoform in sterol-depleted cell lines, SREBP-1c is the major isoform in rodent and human liver and is now recognized as a key regulator of fatty acid and triglyceride synthesis (82).

SREBPs are synthesized as large proteins (125 kDa) and inserted into the ER membrane with two membrane-spanning domains and with their C-terminal end bound to the SREBP cleavage-activating protein (SCAP). When cellular sterol levels drop, the SREBP-SCAP complex moves to the Golgi apparatus, where it undergoes two proteolytic events, giving rise to the smaller (65 kDa), mature form of the protein. The mature protein then transits to the nucleus, where it binds to *cis* elements, termed sterol regulatory elements (SREs), in the promoters of target genes and induces transcription of a variety of genes involved in cholesterol, triglyceride, and fatty acid synthesis (11).

SREBPs themselves are not very potent activators of transcription and require the actions of ancillary proteins to affect transcription of target genes (67). Several such proteins have been identified, including the Sp1 transcription factor in the LDL promoter (97) and the CCAAT-binding factor/nuclear factor-Y (CBF/NF-Y) in the promoters of HMGCoA synthase (27) and farnesyl diphosphate synthase in cholesterol metabolism (29). Teran-Garcia and coworkers (113) have recently shown that mutation of the SREBP1 site in the promoter of the FAS gene results in only a 25% loss of PUFA repression of FAS. Conversely, mutation of the NF-Y site in the FAS promoter decreases PUFA repression of FAS by almost 50%.

EFFECTS OF POLYUNSATURATED FATTY ACIDS There is convincing in vitro (121) as well as in vivo (62, 122) evidence implicating a role for PUFAs in inhibiting maturation of SREBP in a post-translational manner, thereby repressing lipogenic gene expression. Incubation of CHO, HepG2, and CV-1 cells with micromolar quantities of PUFAs resulted in a decrease in transcription of SRE-containing genes in a dose-dependent manner. This regulation by PUFAs was absent in a defective CHO cell line that exhibited loss of SREBP regulation by sterols or in cells transfected with promoters containing mutant SREs. This suggests that PUFAs do not function at the level of SREBP binding to the SRE but rather through modulating intracellular regulatory sterol levels (121). Fasting-refeeding experiments in rodents have established that caloric intake is directly parallel to SREBP nuclear content (46); this trend is consistent with observations that lipogenic gene expression is greatly reduced during fasting and increased upon

refeeding. These findings led to the hypothesis that the inhibitory effect of PUFAs on lipogenic gene expression could occur via repression of SREBP mRNA or inhibition of SREBP maturation. Rats fed fat-free diets supplemented with n-3 or n-6 PUFAs greatly reduce nuclear content of SREBP protein as well as expression of SREBP target genes (62, 122). Also, SREBP overexpression in transgenic mice leads to decreased sensitivity to PUFA-mediated repression of target genes (123). We have shown that while cholesterol feeding does not alter SREBP mRNA levels, it causes an increase in nuclear levels of SREBP protein. Supplementation of the diet with PUFA decreases the nuclear content of SREBP without affecting mRNA levels (61). These findings suggest that PUFAs function by inhibiting maturation of the precursor form of the SREBP protein (Figure 2).

Several mechanisms have been suggested whereby PUFAs can alter the maturation of SREBPs. Since increased intracellular sterol levels seem to result in a decline in SREBP maturation/transit, PUFAs could function by raising cellular cholesterol levels. Indeed, unsaturated fatty acids have been shown to increase sphingomyelinase activity, resulting in increased hydrolysis of sphingomyelin contained in the plasma membrane and increased cholesterol levels in the ER. This results in decreased maturation of SREBP-1 and SREBP-2 but does not affect SREBP mRNA levels (120).

Unsaturated fatty acids have also been shown to affect the hepatic abundance of SREBP-1a and SREBP-1c mRNA, but not that of SREBP-2, which is mainly regulated by sterols (57, 62, 69, 122, 123). This inhibition is dependent on chain length and degree of desaturation, with eicosapentanoate and arachidonate being more potent inhibitors than linoleic or oleic acids. Both reductions in SREBP-1 mRNA stability and decreased transcription of the SREBP-1 gene (Figure 2) have been suggested as possible mechanisms for this inhibition (57).

In HepG2 cells, arachidonic, linoleic, and eicosapentanoic acids all repress transcription of the SCD1 gene, which is known to have an SRE in its promoter region. However, overexpression of a mature SREBP in these cells does not prevent this repression by PUFAs, which suggests an SREBP-independent mechanism in the regulation of SCD1 gene expression (79); one possible candidate mediating this effect is another member of the nuclear receptor superfamily, LXR.

Liver X Receptors

STRUCTURE AND FUNCTION As mentioned above, LXRs are a set of nuclear receptors that have been recently identified as candidates for mediating gene regulation by fatty acids (39, 83). LXRs α and β have been well characterized for their ability to bind oxysterols such as 24, 25-epoxycholesterol as endogenous ligands. They have been shown to regulate diverse processes such as bile acid synthesis and lipogenesis (66, 90). LXRα is expressed mainly in liver, kidneys, intestine, adipose, and adrenal glands, while LXRβ is more ubiquitously expressed (66). LXRs function by heterodimerizing with RXRα and binding to direct repeats with four nucleotide spacers (DR4 elements), termed LXR response elements (LXREs), in the promoter regions of target genes (Figure 2).

REGULATION BY POLYUNSATURATED FATTY ACIDS Oxysterols are the natural endogenous ligands of LXRs α and β. Binding of oxysterols to LXRs has been shown to affect transcription of a variety of genes involved in cholesterol, lipid, and bile acid metabolism (Table 1). These include 7α-hydroxylase (Cyp7α), the rate-limiting enzyme in bile acid synthesis (66), lipoprotein lipase, FAS, ACC, SCD1, and the lipogenic transcription factor, SREBP1c (66, 90).

It has been shown that PUFAs inhibit the prolipogenic actions of LXRs through various mechanisms. One possibility is that competitive binding of PUFAs to LXRs prevents oxysterol binding, thereby antagonizing induction of target genes, notably SREBP1c, and consequently genes containing either LXREs or SREs in their promoter elements (39, 126). Yoshikawa et al. (127) showed that in HEK293 cells, unsaturated fatty acids resulted in decreased SREBP-1c promoter activity, with the order of potency being arachidonic acid > EPA > DHA > linoleic acid. Deletion of the two LXREs in the SREBP-1c promoter abolished the effects of PUFAs on SREBP-1c (127), suggesting an interaction between LXR and SREBP-1c. However, in direct contrast to this study, there is now evidence that repression of SREBP-1c mRNA levels by PUFAs is independent of LXRs. Pawar et al. (85) have shown that treatment of HEK293 cells with EPA results in inhibition of SREBP-1c mRNA in the presence and absence of an LXR agonist. Also, while fish oil enrichment of the diet resulted in repression of SRE-containing genes and induction of PPRE-containing genes in rats, neither EPA (in HEK293 cells) nor fish oil (in the diet) had any effect on classical LXR target genes such as CYP7A1 and ABCG5. The authors suggest that although PUFAs do in fact regulate SREBP-1c levels and consequently SRE-containing genes, this regulation does not extend to LXRE-containing genes and is therefore unlikely to involve an interaction between LXR and SREBP-1c.

Another mechanism that has recently been suggested is the inhibition of LXRs by PUFAs through activation of PPARα and PPARγ. PUFAs, as discussed above, are potent activators of PPARα and PPARγ. It was recently shown that overexpression of PPARα and PPARγ in HEK-293 cells inhibits SREBP-1c promoter activity in a dose-dependent manner (125). Mutation of the two LXREs in the SREBP-1c promoter abolished this effect of PPARs, and gel mobility shift assays showed that the activation of PPARs in fact reduced the binding of the LXR/RXR heterodimer to the LXRE (Figure 2). Supplementation with RXR decreased the repression observed, which suggests that competition between PPAR and LXR for the RXR partner could play a role in the inhibitory effects (125).

Hepatocyte Nuclear Factor-4α

HNF-4α is a member of the hepatocyte nuclear factor family that includes six different isoforms (42). HNF-4α is a highly conserved nuclear receptor that binds to DR1 elements as a homodimer and seems to be indispensable to hepatocyte differentiation and hepatic functions such as cholesterol and lipoprotein secretion. It is expressed mainly in liver, kidney, intestine, and pancreas and is capable of

activating target genes even in the absence of ligand. It has been shown that PUFAs, in their CoA form, can affect HNF-4α activity; fatty acids can also affect HNF-4α activity via PKA-mediated phosphorylation (42). The importance of HNF-4α to glucose and lipid metabolism is underscored by the observation that mutations in the promoter of the HNF-4α gene lead to a form of maturity onset diabetes of the young (MODY1) (124). The development of a liver-specific HNF-4α knockout mouse has provided much insight into the role that this nuclear receptor plays in fatty acid mediated–regulation of hepatic genes (57).

Using mainly cell culture models, it has been demonstrated that HNF-4α is important to the activation of various genes involved in lipid and cholesterol metabolism, including ApoA-I, ApoA-II, ApoB, ApoC-II, ApoE, and ApoC-III (discussed below), as well as CYP7A1 (Table 1). HNF-4α has also been implicated in the regulation of iron metabolism by transferrin, carbohydrate metabolism through PEPCK and L-PK, as well as the cytochrome P450 system (42).

It has been reported that fatty acyl-CoAs are able to bind HNF-4α directly at physiological concentrations; binding of saturated fatty acids like palmitoyl CoA (16:0) and myristoyl-CoA (14:0) results in activation of HNF-4α while the binding of unsaturated fatty acids like α-linolenic acid (18:3n3), EPA (20:5n-3) and docosahexanoic acid (22:6n6) results in repression of HNF-4α (43). Furthermore, Hertz, Bar-Tana, and colleagues (44) have recently shown that the fibrate class of drugs are converted to fatty acyl-CoA thioesters within cells and are then able to repress the HNF-4α nuclear receptor and target genes such as Cyp7A1. In the case of the ApoCIII gene, fibrates also function by activating PPARα, which then competes with HNF-4α for binding to the promoter (44). Ongoing research will no doubt further strengthen the role of HNF-4α in mediating some of the hypolipidemic effects of the increasingly popular fibrate compounds.

Inhibitor-κB/Nuclear Factor-κB

Many other mechanisms of PUFA-mediated gene regulation have also been suggested; one of them includes the regulation of the transcription factor nuclear factor-κB (NF-κB). Inhibitor κB (I-κB) and NF-κB are present in the cytoplasm of cells, in their inactive form, as a heterodimer. Phosphorylation of I-κB degrades it, thereby allowing NF-κB to separate from I-κB, translocate to the nucleus, and affect the transcription of a variety of genes, including Cox2 and interleukin-6 (13, 60). The translocation of NF-κB has been shown to be regulated both positively and negatively by various PUFAs. While arachidonate stimulates NF-κB translocation and thus has a positive effect on the transcription of its target genes, EPA inhibits NF-κB translocation, resulting in lower transcription of NF-κB target genes (14, 128). Fish oil supplementation in humans has been shown to decrease levels of NF-κB target genes such as TNFα (128) by decreasing I-κB phosphorylation and consequent NF-κB translocation. The mechanisms by which EPA inhibits NF-κB translocation are as yet unclear. It has been suggested that because EPA is not

readily metabolized through the COX pathway (63), it could function by inhibiting arachidonic acid metabolism rather than by forming eicosanoid mediators of its own (54). In fact, the addition of an arachidonate metabolism inhibitor, ETYA, to cells mimicked the effects of EPA (109). However, because both EPA and ETYA are also capable of inducing PPARα (65), it has been suggested that the effects observed with fish oil or ETYA treatment could be mediated by PPARα activation (54).

IMPLICATIONS TO HUMAN HEALTH

In the period between 1999 and 2000, an estimated 31% of the U.S. population was classified as obese, as defined by a body mass index of 30 or above. The monetary costs of this epidemic translated to 9.1% of total healthcare spending, a staggering sum of $92.6 billion (30). The effects of this rapidly rising epidemic stem not only from obesity itself, but also from related complications such as diabetes, hyperlipidemia, cardiovascular disease, and stroke. An indication of this is the fact that between the years 1999 and 2000, drugs targeting cardiovascular disease topped the list of pharmaceutical sales, at an estimated $43 billion.

Dietary PUFAs have long been shown to be effective in managing lipid levels and are consequently of benefit in preventing and treating lipid-related disorders. n-3 PUFA feeding has been clearly linked to decreased plasma triglyceride levels as well as decreased VLDL secretion (40, 54, 88). This seems to be controlled at the level of the ApoB and ApoCIII lipoproteins. ApoCIII synthesis has been shown to be modulated by HNF-4α activation; this could occur either through stimulation or inhibition of HNF-4α by saturated or unsaturated fatty acids, respectively, or through competition between PPARα and HNF-4α for binding to the ApoCIII promoter (43). Another mechanism proposed is the modulation of LDL receptor activity by fatty acids (94, 116), which results in decreased levels of ApoB; however, the generation of the LDL-receptor knockout mouse has created some controversy around this hypothesis (72). A recent study (84b) has shown that ApoB degradation is affected not only by n-3 PUFAs, as previously suggested, but also by n-6 PUFAs, and lipid peroxidation of these fatty acids is required for the effect. This study provides new insight into the role that dietary PUFAs could play in regulating ApoB secretion and preventing hyperlipidemia.

The identification and characterization of transcription factors such as SREBP-1c provide further insight into the role that dietary fat may play in the development of obesity and other lipid-related complications. As discussed above, SREBP-1c, which is downregulated in response to PUFAs, binds to response elements in the promoter regions of key lipogenic genes, including FAS, ACC, and SCD-1. Overexpression of SREBP-1a, -1c, -2, or SCAP results in increased lipid synthesis as well as increased liver and plasma lipid levels. Conversely, liver-specific knockouts of SCAP show decreased lipid synthesis and content and decreased expression of SREBP target genes (47). The implication of changes in SREBP target genes is

strengthened further by the observation that global deletion of the SCD1 gene in mice results in the generation of lean mice (81). These mice show increased insulin sensitivity and decreased plasma and liver lipids (87). Furthermore, the SCD1 knockout mice also have increased rates of fatty acid oxidation, decreased fat pad weight, and are protected from diet-induced obesity (81). There are already some initial clues as to the translatability of this evidence from mice to human subjects. It has been shown, for example, that in human subjects with triglycerides ranging from 0.3 to 20 mM, SCD activity in liver, as measured by the plasma desaturation ratio, accounts for one-third of the variance in plasma triglyceride levels (5b).

Also of great importance to human health is the role that PUFAs play in the activation of the PPARs and the consequent increases in lipid clearance and oxidation. In addition to using fibrates and TZDs to clinically lower lipid levels and increase insulin sensitivity, PUFAs could represent a plausible dietary approach to the prevention of lipid-induced chronic disease.

CONCLUSIONS

In recent years, the understanding that fat is more than just a static store of energy has led to the discovery of various pathways whereby fatty acids affect gene expression. This review, although certainly not conclusive, has attempted to sort through the effects that various dietary fats have on some of these genes, especially those involved in lipid metabolism. As discussed, the effects that fatty acids exert differ based on their chain length, degree of desaturation, and extent of cellular metabolism. They can affect gene expression at the nuclear level either directly or through one of their metabolites, or they can alter various signaling cascades within the cell, thereby raising second messenger concentrations and thus affecting gene expression. Some of the well-characterized mediators of fatty acid–induced changes in gene expression include the nuclear receptors PPAR, HNF-4α, and LXR, as well as the transcription factors SREBP and NF-κB. Not only do these factors mediate the effects of fatty acids, there are also examples of crosstalk between them, making fatty acid regulation of gene expression a much more complicated story. The field is not without discrepancies, and it is probable that novel transcription factors and signaling mechanisms will be identified as our understanding of the topic grows.

The research on nuclear effects of fatty acids has certainly progressed well beyond its infancy. However, the increasing prevalence of obesity and lipid-related disorders such as diabetes and cardiovascular disease has no doubt intensified the search for new compounds, both pharmacological and nutritional, to manage these conditions. Now more than ever, there is a need to understand the changes that contribute to the development of metabolic disease. The coming years should bring a greater understanding of the mechanisms and pathways involved in the development of such diseases and an unraveling of the roles of various dietary fats in promoting or abating diet-induced disease.

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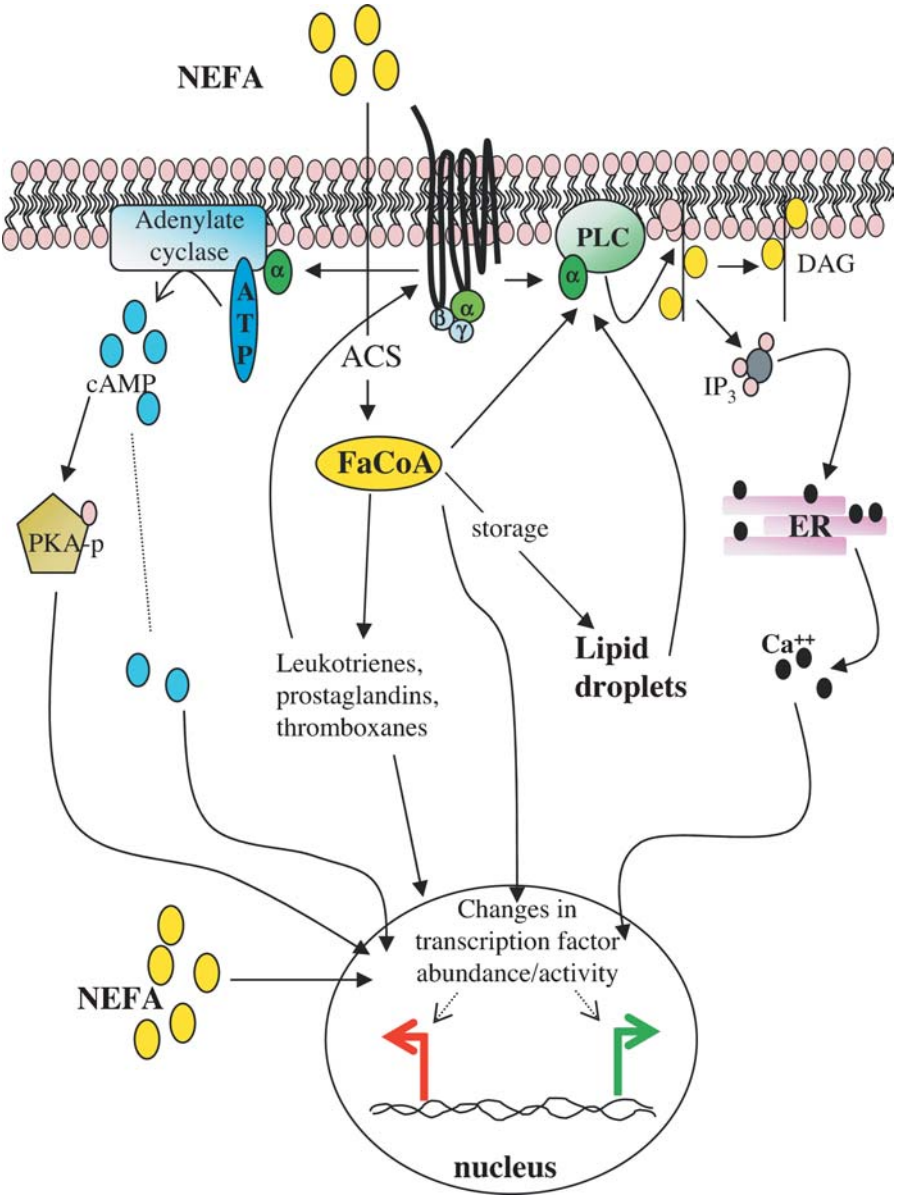

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Figure 1 Fatty acid–mediated changes in cell signaling. Once nonesterified fatty acids (NEFAs) are converted to fatty acyl-CoAs within the cell by acyl-CoA synthetases (ACSs), they can affect cell signaling through various mechanisms. They can be converted to oxidized products such as prostaglandins, thromboxanes, and leukotrienes that can increase cellular cAMP levels through G-protein coupled receptors. cAMP can then phosphorylate protein kinase A (PKA). Alternately, fatty acyl CoAs can be elongated/desaturated and incorporated into plasma membrane phospholipids, where they can be cleaved by phospholipase C (PLC) to form inositol triphosphate (IP_3) and diacylglycerol (DAG). IP_3 mediates calcium release from the endoplasmic reticulum (ER). These various second messengers as well as NEFAs and their metabolites themselves can then affect the nuclear abundance/activity of transcription factors, thereby affecting gene expression.

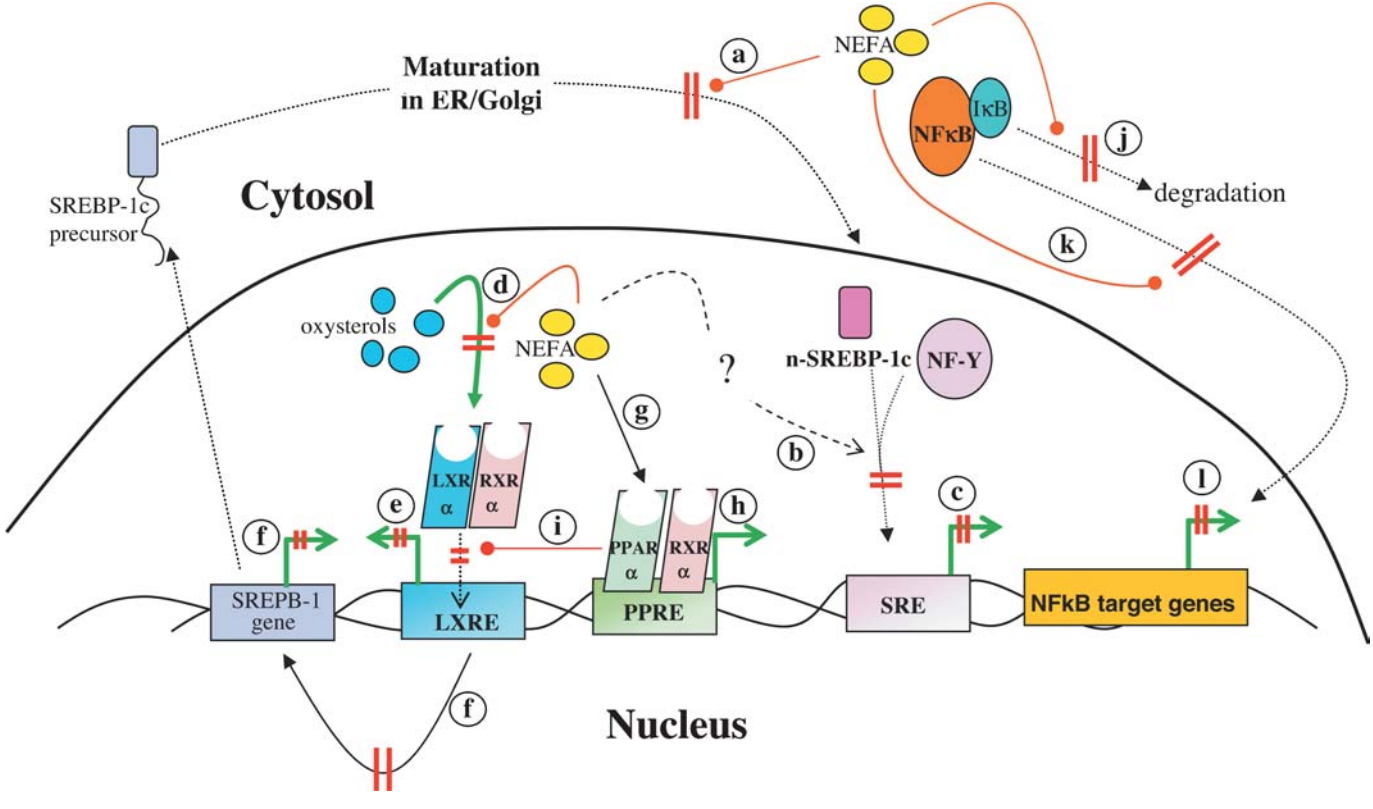

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Figure 2 Nuclear effects of fatty acids. Nonesterified fatty acids (NEFAs) interact with various transcription mediators through several mechanisms to change gene expression patterns. Fatty acids inhibit proteolytic maturation of SREBP-1 from the ER/Golgi (*a*) and subsequent binding of SREBP and coactivators such as NF-Y to SREs (*b*), thereby decreasing transcription of SRE-containing genes (*c*). Fatty acids also compete with oxysterols for binding to LXRα (*d*), thus decreasing transcription of LXR target genes (*e*), including SREBP-1 (*f*). Fatty acids also bind to and activate PPARα (*g*), thereby inducing transcription of PPRE-containing genes (*h*). PPARα activation has also been shown to inhibit LXR binding to the LXRE (*i*), which represents another level of regulation in the transcription of SREBP-1 and LXR target genes. Fatty acids have been shown to inhibit I-κB degradation (*j*), thereby preventing NF-κB translocation to the nucleus (*k*) and subsequent transactivation of NF-κB target genes (*l*). Abbreviations: ER, endoplasmic reticulum; I-κB, inhibitor κB; LXRα, liver X receptor α; LXRE, LXR response element; NF-κB, nuclear factor-κB; NF-Y, nuclear factor-Y; PPARα, peroxisome proliferator-activated receptor-α; PPRE, PPAR response element; RXRα, retinoid X receptor α; SRE, sterol response element; SREBP-1, sterol regulatory element-binding protein-1c.

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