Review article

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Dietary polyunsaturated fatty acid regulation of hepatic gene transcription

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Abstract

Objective: Dietary polyunsaturated fatty acids have a significant impact on the activity and abundance of key transcription factors controlling the expression of proteins involved in major metabolic pathways. The primary objective of this review is to provide an overview of our understanding of how dietary PUFA effects on gene expression lead to changes in hepatic metabolism.

Design: Narrative review. This review will focus on how dietary PUFA control hepatic lipid synthesis and oxidation through the regulation of specific transcription factors. Research articles that address critical issues in fatty acid regulation of gene expression were identified. Information from these papers is summarized.

Results: Four families of hepatic transcription factors have been identified that are regulated by changes in dietary lipid intake or by treating cultured cells with specific fatty acids. These families include: peroxisome proliferator activated receptors (PPARα, β, γ1, γ2), liver X receptors (LXRα and β), hepatic nuclear factor-4 α (HNF-4α) and sterol regulatory element binding protein (SREBP-1a, -1c and –2). Together, these factors control carbohydrate and lipid metabolism, cholesterol and bile acid metabolism and apolipoproteins production.

Conclusions: These studies reveal a complex interaction between dietary lipid, specific transcription factors and hepatic carbohydrate, lipid, cholesterol and bile acid metabolism.

Keywords: *PPAR*, *LXR*, *HNF4*, *SREBP*, *PUFA*, *Transcription*.

Introduction

Dietary fat is an important macronutrient for growth and development of all organisms. Changes in the quantity of dietary fat ingested or its composition impacts cellular processes affecting a many physiological systems and cell types (Table 1). Many of these effects can be linked to changes in membrane lipid composition affecting membrane fluidity or eicosanoid signaling (1). However, nearly a decade ago a family of fatty acid-regulated nuclear receptors was discovered (2, 3), i.e., peroxisome proliferator activated receptors [PPARα, β, γ1, and γ2], a discovery that refocused much of the research in this area around fatty acid effects on gene expression. Since the initial description of PPARs as fatty acid-regulated nuclear receptors, a number of transcription factors have been identified as targets for regulation by fatty acids or their metabolites; including HNF4α, NFκB, RXRα, SREBP-1c and LXRα and β (1–7).

While these factors affect gene expression in many cell types of the body, the focus of this review will be on the liver. The liver plays a central role in whole body lipid metabolism and several of these metabolic pathways are regulated by PUFA, particularly the 20- and 22-carbon n3-PUFA. For example, ingestion of diets enriched in n3-PUFA, e.g., fish oil, or treatment of cells with pure n3-PUFA, i.e., eicosapentaenoic acid (20:5,n3) or docosahexaenoic acid (22:6,n3), induces several genes encoding proteins involved in fatty acid transport and binding, fatty acyl CoA formation, and oxidation (mitochondrial, peroxisomal and microsomal). This treatment also represses many genes encoding enzymes involved in de novo lipogenesis. Enhanced fatty acid oxidation coupled with inhibition of de novo lipogenesis (DNL) likely contributes to the decline triglyceride secretion (as VLDL) from rodent liver as well as the hypolipemic effect of n3-PUFA. This mechanism shifts hepatic lipid metabolism from lipid synthesis and storage to oxidation (1, 7). While hepatic DNL is a minor pathway in humans, feeding humans diets enriched in 20- and 22-carbon n3-PUFA has a significant

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hypolipemic effect (8). This is due to enhanced chylomicron and VLDL clearance as well as diminished hepatic VLDL secretion. Elevated serum triglycerides are risk factors for several chronic diseases including, coronary artery disease, hypertension, and insulin resistance (8, 9). Rodent models have provided considerable insight into the molecular mechanisms for PUFA control of hepatic lipid metabolism. This review will highlight the recent advances in our understanding of PUFA regulation of hepatic gene as it impacts hepatic lipid metabolism.

Fatty acid uptake, metabolism and potential routes for control of transcription factors

Lipid metabolism represents a key facet of fatty acid regulation of gene expression. Fatty acids are delivered to cells either as complex lipoprotein complexes, e.g., chylomicrons or very low density lipoproteins (VLDL), or as non-esterified fatty acids (NEFA), mobilized from storage depots (Fig. 1 and 2). Triglycerides (TAG) in chylomicrons and VLDL are hydrolyzed by the action of lipoprotein lipase (LPL) and NEFA enter cells via fatty acid transporters (FAT). Remnant lipoproteins are taken up by the liver and hydrolyzed by hepatic lipases. Once in cells, NEFA are rapidly converted to fatty acyl-CoA thioesters (FACoA) by acyl CoA synthetases (ACS). The intracellular concentration of NEFA and FACoAs is low ($< 10\ \mu M$) and most NEFA and FACoA is protein-bound to fatty acid- and FACoA-binding proteins. The conversion of NEFA to FACoA by ACS is a rate-determining step for entry of fatty acids into β-oxidation (mitochrondrial or peroxisomal), elongation/desaturation or assimilation into complex lipids, i.e., triglycerides, cholesterol esters or phospholipids. Consequently, factors that affect ACS levels or their activity will impact intracellular NEFA and FACoA levels as well as fatty acid metabolism.

A number of in vitro studies have indicated that certain NEFA or FaCoA serve as ligands regulating the activity of specific transcription factors, e.g., PPAR and LXR families and HNF-4α (3, 4, 10, 11). From these studies it seems reasonable to speculate that fatty acid regulation of transcription factor function involves a change in either the intracellular NEFA or FaCoA levels. Such changes in intracellular lipid levels are likely achieved by increasing the extracellular level of the fatty acid or possibly by differential lipid metabolism. Unlike 16- and 18-carbon fatty acids, CoA thioesters of 20- and 22-carbon PUFA are poor substrates for at least two esterification reactions, i.e., acyl cholesterol acyl transferase [ACAT] and diacylglycerol acyl transferase [DGAT] (12–15). AGAT assimilates fatty acids into cholesterol esters, while DGAT is the terminal step in triglyceride synthesis (Fig. 2). In contrast to PUFA $\leq$ 20-carbons, PUFA $\geq$ 22-carbons, e.g., 22:4,n6, 22:5,n6 and 22:6,n3, require prior peroxisomal β-oxidation to chain shorten the fatty acid before entry into the mitochondrial β-oxidation spiral (16). The delay in assimilation of 20- and 22-carbon PUFA into neutral lipids or their oxidation may lead to a rise in the intracellular NEFA or FACoA levels of specific 20- or 22-carbon PUFA (both n3 and n6) and affect the activity of specific transcription factors.

Although these biochemical studies suggest that 20- or 22-carbon PUFA might accumulate in the intracellular NEFA or CoA pool, little direct evidence supports this concept. There is, however, anecdotal evidence. The fatty acid activation of PPARα illustrates this point. While PPARs can bind

Table 1. Physiological Effects of 20- and 22-carbon N3 PUFA

Target System	Effect	Likely Mechanism
CNS	Improves cognitive functions	Membrane composition; RXRα
Retina	Enhances visual acuity	Membrane composition
Immune	Immunosuppressive	Membrane composition/Rafts
	Anti-inflammatory	Eicosanoid synthesis/Action; NFκB
Cardiovascular	Anti-arrhythmia	Membrane composition/Rafts
	Anti-thrombotic	Eicosanoid synthesis/Action; NFκB
Serum Lipids	Lowers triglycerides	PPARα & γ; SREBP-1c; LXRα & β
Liver	Suppresses lipogenesis	SREBP-1c; LXRα
	Increases fatty acid oxidation	PPARα
	Suppresses VLDL synthesis	PPARα/HNF4α
Skeletal Muscle	Improves insulin sensitivity	Membrane composition
		PPARα & γ; NFκB

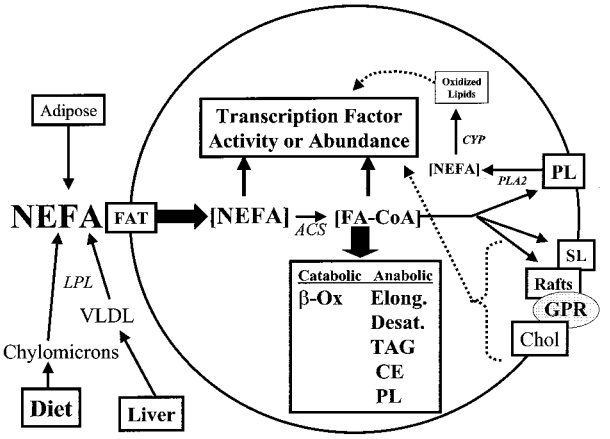

Fig. 1. Multiple mechanisms are involved in the PUFA regulation of transcription factor activity and abundance. Plasma free fatty acids enter cells via transporters and move into a NEFA pool. NEFA are rapidly converted to FACoA and are substrates for assimilation into complex lipids (triglycerides [TAG], phospholipids [PL], cholesterol esters [CE]) as well as for β-oxidation (mitochondria and peroxisome). NEFA and FACoA regulated the activity of specific transcription factors (see Fig. 3). The FACoAs are precursors for phospholipid (PL) and sphingolipid (SL) biosynthesis and are assimilated into membranes. Membranes contain lipid rafts (Rafts), regions of the membrane enriched in sphingomyelin and cholesterol. The inner leaflet of raft membranes bind signaling molecules, like Src-kinases and G-protein-linked receptors (GPR) and are linked to downstream signaling cascades, e.g., Map-kinases (MapK). The activity of SREBPs and PPARs are affected by phosphorylation. Enrichment of PUFA in membrane lipids induces a redistribution of cholesterol, an event that impacts SREBP proteolytic processing in the endoplasmic reticulum and Golgi. Fatty acids released from phospholipids by the actions of PLA2s provide substrates for specific CYP-mediated monooxygenase (CYP). These oxidized lipids might affect the activity of transcription factors like PPARs or exit the cell and bind to specific eicosanoid receptors to alter cellular levels of second messengers, e.g. cAMP or Ca^{+2}.

many fatty acids *in vitro* there appears to be physiological discrimination at the cellular level. As the predominant PPAR-subtype in rat hepatic parenchymal cells, PPARα binds 18:1,n9 and 20:5,n3 with nearly equal affinity, i.e., 0.6 vs 1.1 μM (10). Yet, 20:5,n3, but not 18:1,n9, activates PPARα in rat primary rat hepatocytes (17). The simplest explanation for this difference is that the intracellular NEFA pool available to activated PPARα is subject to metabolic regulation. As indicated above, 20:5,n3-CoA is a poor substrate for AGAT and DGAT. A decrease in the rate of 20:5,n3 assimilation into neutral lipids might elevate intracellular 20:5,n3 to a level sufficient to activate PPARα. The notion that slowly or poorly metabolized fatty acids activate PPAR is the basis for the action of the fibrate class of lipid-lowering drugs (Lopid®, Pizer; Tricor®, Abbott). Fibrates were synthesized originally as metabolically stable analogs of branched chain fatty acids (10). However, when compared to fibrates, 20-carbon PUFA are weak PPARα activators (3, 17).

There are alternate routes for regulation of transcription factor function, either through generation of alternative ligands or activation of kinase signaling cascades (Fig. 1). The incorporation of PUFA into membrane phospholipids affects membrane fluidity and cholesterol content and impacts the generation of signaling molecules (1, 7). Enrichment of PUFA in membrane components associated with lipid rafts (both the phospholipid and acylated protein component) has a significant impact on G-protein-receptor, Src-kinase, Map-kinase as well as Ca^{+2} signaling (1). Map-kinase

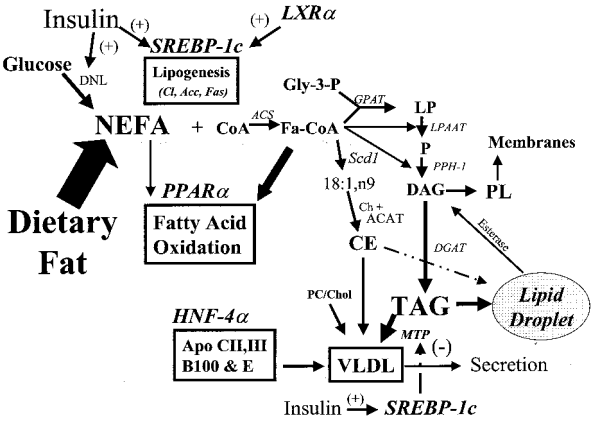

Fig. 2. Possible mechanisms for fatty acid regulation of hepatic triglyceride synthesis, VLDL assembly and secretion. Elevated intracellular NEFA (PUFA) will activate PPARα to enhance fatty acid flow into β-oxidation. NEFA or FACoA suppress the nuclear abundance of SREBP-1c leading to a decline in de novo fatty acid synthesis and may affect steps in triglyceride synthesis (GPAT). SREBP-1c gene transcription is induced by insulin and ligand-activated LXR. PUFA may antagonize LXR and inhibit transcription of the SREBP-1c gene. PUFA enhanced degradation of $mRNA_{SREBP\text{-}1c}$ leads to a decline in nSREBP-1c and a suppression of lipogenic gene expression. SREBP-1c also inhibits hepatic microsomal transfer protein levels, a mechanism that may account for the decline in VLDL secretion with excess glucose/insulin. In this situation, fatty acids assimilated into triglycerides and cholesterol esters are stored as lipid droplets rather than being assembled into VLDL for secretion. Triglycerides in lipid droplets are degraded to 1,3-DAG and re-acylated with fatty acids. This route leads to VLDL assembly and secretion. DNL, de novo lipogenesis; PPAR, peroxisome proliferator activated receptor; HNF-4α, hepatic nuclear factor-4α; LXR, liver X receptor; SREBP, sterol regulatory element binding protein; CL, ATP-citrate lyase; ACC, acetyl CoA carboxylase; FAS, fatty acid synthase; GPAT, glycerol phosphate acyl transferase; Apo CII, CIII, B100 and E, apolipoproteins CII, CIII, B100, E; SCD1, stearoyl CoA desaturase; MTP, microsomal transfer protein. LPAAT, lysophosphatidate acyltransferase; PPH-1, phosphatidate phosphohydrolase; Ch, cholesterol; CE, cholesterol ester; PC, phosphatidyl choline; LP, lysophosphatidate; P, phosphatidate; DAG, diacylglycerol; PL, phospholipids. (+) Activation; (−) Inhibition.

phosphorylation of PPAR, SREBPs and HNF-4α affect their activity (18–21). Elevation of PUFA into membrane phospholipids also affects membrane cholesterol levels (22). Cholesterol is typically found associated with membrane sphingolipid. Increased PUFA in phospholipids displaces cholesterol to the cytoplasm and also activates a sphingolipid signaling mechanism. Together, these factors inhibit microsomal processing of sterol response element binding proteins (SREBP) to the form found in the nucleus.

PUFA also impact the synthesis of bioactive lipids generated by the cyclooxygenase [COX1 and COX2] and the lipoxygenase [5-LOX, 12-LOX, 15-LOX] (Fig. 1). Both COX and LOX products of PUFA bind and affect the activity of PPARs, particularly PPARγ (1). In contrast to arachidonic acid (20:4,n6), 20:5,n3 is also a poor substrate for both COX (COX1 and COX 2) and LOX (5-, 12-, 15-LOX). In addition, eicosanoids originating from 20:5,n3 display weak bioactivity. Finally, n3-PUFA enhanced catabolism of bioactive eicosanoid metabolism through increased peroxisomal degradation. Together, these mechanisms account for the anti-inflammatory effects of n3-PUFA on biological systems (1).

In contrast to hepatic Kupffer and endothelial cells, hepatic parenchymal cells express little or no COX 1 and COX2 (23). Consequently, PUFA regulation of hepatic gene expression is independent of cyclooxygenase and lipoxygenase. Hepatic parenchymal cells possess prostaglandin receptors and can respond to prostaglandins generated by Kupffer or endothelial. In fact, prostaglandins, like PGE2 or PGF2α, mimic the actions of n3 and n6 PUFA on hepatic lipogenesis. Hepatic parenchymal cells generate eicosanoids through an alternative pathway; the NADPH-dependent cytochrome P450 monooxygenase pathway [CYP] (24). This pathway generates hydroxy-, dihydroxy- and epoxy-fatty acids from both n6 and n3 PUFA. Investigators have reported little or no differential effect of n3 versus n6 PUFA on the expression of hepatic genes (1). Our unpublished studies have shown that both n6 and n3 PUFA are good substrates for CYP-mediated oxidation. Whether this pathway plays a role in liver gene expression remains to be determined.

Direct effects of NEFA and FACoA on transcription factor activity

The emphasis of this review is on the regulation of transcriptions factors involved in the control of hepatic lipid metabolism. Figure 2 is a schematic illustrating where specific fatty acid-regulated transcription factors likely impact hepatic lipid metabolism. In this section, I will review the evidence for fatty acid regulation of these specific transcription factors.

Peroxisome proliferator activated receptors

PPARs are the most extensively characterized fatty acid-regulated transcription factors (3). PPARs are class II nuclear receptors that bind cis-regulatory elements [peroxisome proliferator regulatory element (PPRE), a direct repeat of a hexamer separated by 1 nucleotide (DR1) with a 5′ extension; murine acyl CoA oxidase: AACTAGGNCAAAGGTCA] in promoters of responsive genes. PPARs bind PPREs as a heterodimer with RXRα. Studies with the PPARα-null mouse have indicated that PPARα is required for the peroxisome proliferator or fatty acid regulation of many hepatic genes involved in glucose and lipid homeostasis including fatty acid transport, fatty acid binding proteins, fatty acyl CoA synthesis, microsomal, peroxisomal and mitochondrial oxidation, ketogenesis and Δ5, Δ6, and Δ9-desaturation, at least 1 glycolytic enzyme, i.e., L-pyruvate kinase and several apolipoproteins, e.g., ApoCII and CIII (3, 17, 25–27).

All PPAR subtypes bind 20:5,n3 (IC_{50} [or K_d] of $\sim$1–4 μM). Structural analysis of PPARβ shows that 20:5,n3 occupies 300 Å^3 of the 1300 Å^3 hydrophobic binding pocket (10). Fatty acid binding induces a change in the structure of the ligand binding domain (LBD) to facilitate co-activator binding to the PPAR-LBD. Helix 12 of the LBD contains an activator function (AF-2) that interacts with other helices within the LBD when agonist is bound. This structural change in the LBD is required for ligand-mediated activation of gene transcription. Fatty acids $\leq$ 14-carbons or longer than 20-carbons do not bind well to PPARs and do not stabilize the AF-2 helix, lessening the likelihood for co-activator recruitment and gene activation. Based on these structural studies, 20:5,n3, but not 22:6,n3, is a PPAR ligand. Consequently, for 22:6, n3 to activate PPARs, it will require prior retro-conversion to 20:5, n3, a process involving peroxisomal β-oxidation.

Liver X Receptors

Like PPARα, liver X receptors (LXRα and LXRβ) are class II nuclear receptors that bind direct repeats [LXRE, a DR-4, murine Cyp7A1: TGGTCActcaAGTTCA] as a heterodimer with RXRα (28). Oxysterols, like 22 (R)-hydroxycholesterol and 24,25-epoxycholesterol, bind and activate LXRs. LXR/RXR heterodimers bind LXREs in promoters

of enzymes involved in hepatic bile acid synthesis, e.g., 7α-hydroxylase [Cyp7A], the main route for cholesterol elimination from the body. Cholesterol loading of cells generates oxidized cholesterol (oxysterols) and oxysterols are feed-forward inducers of bile acid synthesis and cholesterol elimination. LXRs also regulate lipogenic gene expression through two mechanisms (29–32). One involves the regulation of transcription of the gene encoding SREBP-1c, a key transcription factor involved in the insulin-mediated induction of lipogenesis. The second mechanism involves direct binding of LXR/RXR heterodimers to promoters of certain lipogenic genes, e.g., fatty acid synthase.

It has been known for many years that excessive cellular cholesterol is esterified by an unsaturated fatty acid, like 18:1,n9, to form cholesterol esters by acyl-CoA: cholesterol acyltransferase (ACAT). Cholesterol esters are either stored in cells or exported by VLDL (Fig. 2). Such studies establish a linked between cholesterol and fatty acid metabolism. Interestingly, oxysterols suppress cellular levels of SREBP-2, but not SREBP-1 (33). Consequently, SREBP-2 dependent genes like the LDL-receptor and HMG-CoA reductase are repressed by oxysterols, while SREBP-1 regulated genes, e.g., ATP-citrate lyase, acetyl CoA carboxylase, fatty acid synthase, stearoyl CoA desaturase and glycerophosphate acyl transferase are activated. This represents a second feed forward mechanism to quench the toxic effects of excessive accumulation of cellular cholesterol.

Recently, LXRs were identified as targets for fatty acid regulation (11, 34). Unsaturated fatty acids antagonize oxysterol binding by LXRα in Hek 293 and hepatoma and inhibit LXRα action. The half-maximal effect of 20:4.n6 on 24,25-epoxycholesterol binding to the LXR-ligand binding domain is 1.5 μM. This level of inhibitory fatty acid is similar to Kd for 20-carbon PUFA binding to PPARα (10). Thus, both PPARα and LXRs can be viewed as intracellular sensors of NEFA and respond accordingly by regulating specific pathway metabolic pathways to prevent fatty acid and cholesterol overload. The hierarchy for the fatty acid effect on LXR is 20:4,n6 > 18:2,n6 > 18:1,n9; saturated fatty acids have no effect. Interestingly, transcription of the LXRα gene, but not the LXRβ gene, is induced by PPARα activators (35). PPARα/RXRα bind PPREs in the LXRα gene promoter. Thus, PUFA can potentially induce LXRα levels in cells, while inhibiting its action. Whether this regulatory scheme is operative in vivo needs to be established.

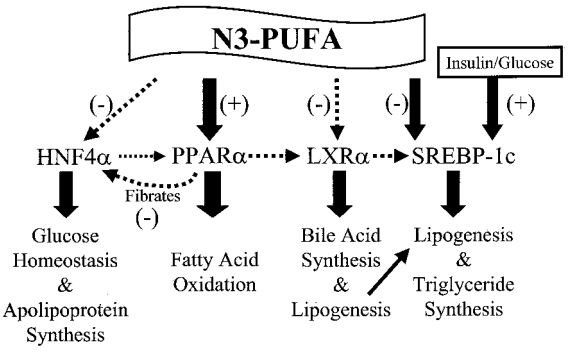

Fig. 3. Overview of N3-PUFA effects on hepatic metabolism through control of specific transcription factors. N3-PUFA, either as NEFA or FACoA, are potential regulators of nuclear receptors (PPARα, LXRα and β or HNF-4α). It is not clear if NEFA or FACoA controls the turnover of $mRNA_{SREBP\text{-}1c}$, a mechanism that affects the nuclear content of SREBP-1c (nSREBP-1c). Nevertheless, n3-PUFA control of the activity and abundance of several transcription factors can potentially impact several hepatic metabolic pathways. (+) Activation; (−) Inhibition. Thick arrows represent well-established observations. Thin or dashed arrows represent observation needing further study.

Hepatic Nuclear Factor-4α (HNF-4α)

HNF-4α is a class III nuclear (orphan) receptor that binds DR-1 motifs (rat L-pyruvate kinase: TGGACTcTGGCCC) as a homodimer. The cre-lox conditional HNF-4α null mouse has revealed a wide array of hepatic genes is controlled either directly or indirectly by HNF-4α (36). These include the genes encoding transcription factors (PPARα, HNF-1), apolipoproteins (ApoCII, CII, A1, AII), enzymes involved in iron (transferrin) and carbohydrate metabolism (L-pyruvate kinase, phosphoenolpyruvate carboxykinase), cytochrome P450 monooxygenases and bile acid synthesis (Fig. 2 and 3) (34, 36).

Hertz and Bar-Tana were the first to report the direct binding of FACoA regulated HNF-4α activity on the ApoCIII promoter. Palmitoyl-CoA (Kd ~2.6 μM) bound the putative HNF4α LBD at levels near the free in vivo FA-CoA concentration (1, 4). While binding of saturated fatty acids (14:0-CoA or 16:0-CoA) activated HNF-4α, binding of 18:3,n3-CoA, 20:5,n3-CoA or 22:6,n3-CoA inhibited HNF4α effects on gene transcription.

Fibrates interfere with HNF-4α function and lead to a fall in cellular levels of HNF-4α (37). PPARα/RXRα heterodimers compete with HNF-4α for binding to certain DR-1 motifs, e.g., ApoC-III and transferring promoters, but not in the 7α-hydroxylase (Cyp7A1) or L-pyruvate kinase promoters (26, 37). More recently, Bar-Tana and colleagues have reported that cells convert fibrates

to CoA thioesters. These fibrate-CoA thioesters bind HNF-4α and inhibited HNF-4α-mediated activation of gene transcription (38). This observation suggests that fibrate action may extend beyond the PPARα regulatory network and to include effects on HNF-4α regulatory networks.

Sterol Regulatory Element Binding Proteins (SREBP).

SREBPs are helix-loop helix transcription factors involved in the transcription of genes involved in cholesterol and lipid synthesis (39). Three SREBPs have been described. SREBP-1a and 1c are transcribed from the same gene locus, but because of differential promoter and exon usage, SREBP-1 differs in the N-terminal amino acids. A separate gene encodes SREBP-2. Transgenic mice over expressing SREBP-1a develop fatty liver because of the induction of lipogenesis and triglyceride synthesis and repression of VLDL secretion (1, 39). SREBP-1 inhibits microsomal transfer protein expression, a protein required for VLDL assembly and secretion. Similar, but less dramatic, effects are seen in animals over expressing SREBP-1c. Animals over expressing SREBP-2 display enhanced expression of genes involved in cholesterol synthesis. Although there is some cross over regulation, it appears that SREBP-1 regulates fatty acid and triglyceride synthesis, while SREBP-2 regulates cholesterol synthesis. SREBP-1c is the predominant subtype of SREBP-1 expressed in rodent and human liver.

SREBPs are translated as large precursors ($\sim$ 125 kd) tethered to the endoplasmic reticulum where SREBP is bound at the C-terminal end to SREBP cleavage activating protein [SCAP] (39). With sterol depletion both SREBP and SCAP move to the Golgi where proteases (site 1 protease and site 2 protease) cleave the protein to release a mature transcriptionally active form ($\sim$65 kd; nSREBP) that travels to the nucleus to bind to sterol regulatory elements (SREs) in promoters of specific genes. nSREBPs increase the transcription of genes involved in cholesterol and lipid synthesis. Elevated intracellular cholesterol down regulates the site 1 protease effectively reducing the formation of nSREBPs. Thus, cholesterol is a feedback regulator controlling SREBP nuclear levels and impacting cholesterol and lipid homeostasis.

Once in the nucleus, SREBPs bind sterol regulatory elements (SRE, consensus YCAYNYCAY) in promoters of genes involved in both cholesterol and lipid homeostasis. While SREBP-1a and SREBP-2 bind co-activators like CBP, SREBP-1c has a reputation for interacting poorly with co-activators (39). SREBP-1c functionally interacts with ancillary transcription factors, like NF-Y and/or Sp1, which bind near the SRE in many regulated genes. This interaction alone is not sufficient to recruit co-activators to promoters. Instead, if co-activators (CBP, GCN5 or p/CAF) are recruited to the promoter by nuclear receptors binding the same promoter, e.g., thyroid hormone receptors (40), SREBP-1c functionally interacts with these co-activators to augment gene transcription. Many lipogenic enzymes are induced by thyroid hormone [T_3, 3,5,3′-triiodothyronine]. SREBP-1c may play an important role in the regulation of lipogenic gene expression by nuclear receptors binding cis-regulatory elements in promoters of lipogenic genes.

Unsaturated fatty acids regulate nuclear levels of SREBPs, by controlling the transcription, $mRNA_{SREBP}$ turnover and processing of SREBP precursor protein (6, 34, 41–43). The hierarchy for fatty acid regulation of nSREBP-1c levels is 20:5,n3 = 20:4,n6 > 18:2,n6 > 18:1,n9. The transcriptional mode of action involves a fatty acid interference with oxysterol-activated LXRα induction of SREBP-1c gene transcription (34). This mechanism has been described in Hek 293 cells, but not in primary hepatocytes or in vivo (41–43). Unsaturated fatty acids also have been reported to suppress cellular levels of the mRNAs encoding SREBP-1 (both 1a and 1c), but not SREBP-2, by enhancing RNA turnover. This phenomenon is seen in livers of animals fed diets containing various fats as well as in primary hepatocytes and established cell lines. Our unpublished observation indicate that while the 3′ untranslated region of SREBP-1c contains elements that enhance RNA turnover, these elements alone do not confer PUFA-regulated control to the RNA. The molecular basis for PUFA regulation of SREBP-1c RNA turnover remains unresolved. Finally, a recent study reported that PUFA treatment of cultured HepG2 cells inhibits proteolytic cleavage of SREBPs with no effect on SREBP mRNAs (27). The mechanism involves PUFA enrichment of membrane phospholipids that both activates a ceramide signaling pathway and displaces cholesterol from membranes to the endoplasmic reticulum where it affects SREBP processing in the endoplasmic reticulum and Golgi.

In addition to these nutrient-mediated mechanisms, SREBP-1c gene transcription is regulated by

insulin and glucagon (1, 39, 44). Insulin induces and glucagon suppresses transcription of this gene. Over expression of SREBP-1c overrides the requirement for insulin to induce lipogenic gene expression. The molecular basis for this regulation, as well as the interaction between insulin-regulated signals and LXR interaction on the SREBP-1c promoter has not been described.

Concluding remarks

Considerable progress has been made in our understanding of fatty acid regulated gene expression over the last decade. At least 4 families of hepatic transcription factors have been identified that play a major role in glucose homeostasis and apolipoproteins synthesis, fatty acid oxidation, bile acid, fatty acid and triglyceride synthesis (Fig. 3). Such exquisite control of lipid and cholesterol homeostasis at the transcriptional level represents a compensatory mechanism to changes in lipid uptake from the plasma. Both ingested dietary lipid as well as fatty acids mobilized from depots influence plasma lipid composition. Such changes are necessary in order to maintain a proper balance of cholesterol and fatty acids for membrane composition and function, metabolism, cell growth and differentiation and to prevent the toxic effects associated with lipid or cholesterol overload. These mechanisms likely impact VLDL synthesis and secretion as well as whole body management of lipid and cholesterol metabolism. Since these transcription factors are regulated by intracellular NEFA or FaCoA levels, factors that influence lipid metabolism are likely to have a significant impact on the regulation of these transcription factors and the metabolic networks they control.

Whether these same mechanisms are operative in humans is unclear. Treatment of human hyperlipemic patients with fibrates dramatically lowers plasma triglycerides (8). However, cultured human liver cells (HepG2) expresses low levels of PPARα and are refractory to fibrate induction of peroxisomal enzymes (45). Nonetheless, mitochondrial HMG CoA synthase, carnitine palmitoyl transferase I, and acyl-CoA synthetases are inducible by fibrates in human liver cell cultures if PPARα levels are elevated. Recent studies with the PPARα null mouse have indicated that n3-PUFA regulation of serum triglycerides may extend beyond its effects on PPARα (27). PUFA regulation of SREBP-1c is PPARα independent (42). PUFA-mediated suppression of SREBP-1c likely impacts DNL and TG synthesis as well as VLDL secretion. PUFA-regulated PPARα-independent mechanisms may extend beyond the liver and involve enhanced clearance of serum lipids; an area that is poorly understood. LXR agonists have dramatic effects on bile acid synthesis and lipogenesis in rodents (29), but the promoter for human7α-hydroxylase (CYP7A) does not possess an LXRE (33, 37). Instead, the human Cyp7A promoter binds HNF-4α at a DR1 and fibrates interfere with HNF-4α regulation of Cyp7A in human hepatoma cells. Whether fatty acids regulated LXRs in vivo in rodents or humans has yet to be reported. Finally, hepatic de novo lipogenesis (DNL) has traditionally been viewed as a minor pathway in humans (46–48). Yet, recent studies in humans indicate that in when fed low fat-high carbohydrate diets, hepatic DNL and plasma VLDL-TG increase. In fact, this phenomenon has been labeled carbohydrate-induced hypertriglyceridemia. The increase in DNL also affects VLDL-TG composition; palmitate increases, while essential fatty acids like linoleate decrease in VLDL-TG. This change in TG-rich lipoproteins may cause an increase in small dense LDL and a decrease in HDL cholesterol (HDL-C), a lipid profile that may accelerate atherosclerosis (49, 50). The rodent model has provided insight into the molecular basis for this control where PUFA-enriched diets suppress levels of SREBP-1c leading to a decline in DNL and its end products, i.e., 16:0, to VLDL-TG composition.

Clearly, considerable progress has been made in our understanding of how fatty acids regulate gene expression. But more studies are required to gain a full understanding of how fatty acids, particularly the n3-PUFA, control major metabolic pathways. A better understanding of these mechanisms will allow us to take full advantage of the unique properties of 20- and 22-carbon PUFA in improve human health.

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