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DIETARY POLYUNSATURATED FATTY ACID REGULATION OF GENE TRANSCRIPTION

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INTRODUCTION

Several different disease states, including atherosclerosis, insulin-resistant diabetes, and certain types of cancer, have been linked to the type and amount of fat we ingest (6, 61, 72, 78). Dietary fats appear to influence the onset and

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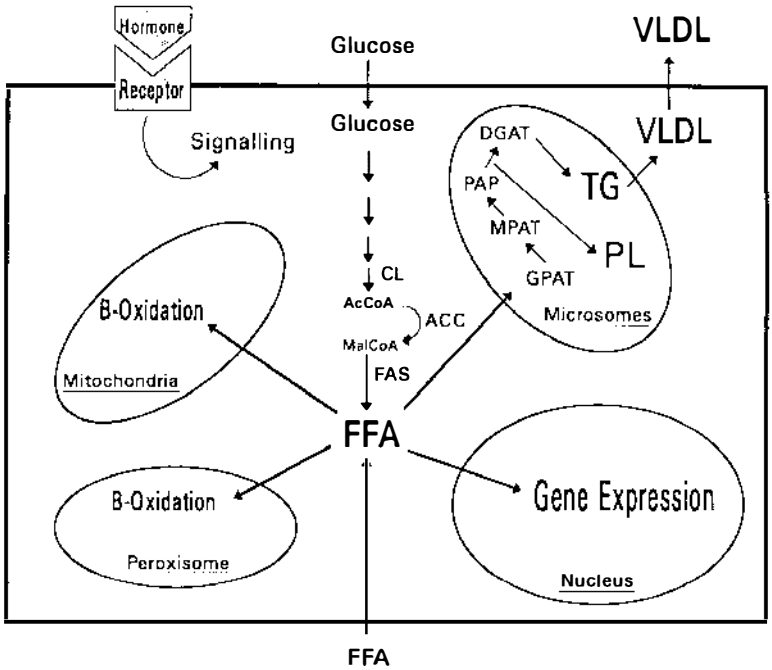

Figure 1 Schematic view of hepatic fatty acid metabolism illustrating the flow of free fatty acids into various hepatocyte compartments, i.e. cytosol, nucleus, mitochondria, microsome, and peroxisome. The two principal sites for polyunsaturated fatty acid (PUFA) control of hepatocyte function are (*a*) a rapid mechanism by which fatty acids (or metabolites) affect gene expression; and (*b*) an adaptive mechanism by which enrichment of membrane lipids with PUFA causes changes in signal transduction. FFA, free fatty acids; TG, triglyceride; PL, phospholipid; VLDL, very low-density lipoproteins; GPAT, glycerol phosphate acyltransferase; MPAT, monoacylglycerol phosphate acyltransferase; PAP, phosphatidic acid phosphatase; DGAT, diacylglycerol acyltransferase; AcCoA, acetyl CoA; MalCoA, malonyl CoA; CL, citrate lyase; ACC, acetyl CoA carboxylase; FAS, fatty acid synthase.

progression of these various diseases by exerting an effect at two levels: (*a*) changes in membrane phospholipid composition (6, 8, 71); and (*b*) direct control of the nuclear events that govern gene transcription (4, 5, 10–12, 40). Thus the beneficial as well as the detrimental effects that dietary fats exert on various diseases may involve a combination of interactive regulatory mechanisms: (*a*) rapid changes in gene expression and (*b*) a long-term adaptive modulation of membrane composition, which leads to changes in hormone signaling. Figure 1 illustrates the interaction of these two events.

In this scenario, dietary fats high in polyunsaturated fatty acids (PUFAs) enrich hepatic plasma and microsomal membranes with long-chain PUFAs. This enrichment subsequently alters hormone binding to cell-surface receptors and

affects signaling mechanisms, which in turn modify carbohydrate and lipid metabolism (8, 19, 23, 71). These PUFAs also affect nuclear mechanisms that change the expression of various genes encoding enzymes involved in lipid metabolism. These enzymes include FAS (5, 11, 12), malic enzyme (43, 68), Δ^9 desaturase (57, 73), acetyl-CoA carboxylase (42), and the S14 protein (5, 11, 12, 40).

PUFAs also reduce the activities of microsomal enzymes involved in fatty acid desaturation and triglyceride synthesis (7, 20, 22, 32, 53, 58, 71). These integrated mechanisms result in a reduction of substrate availability for triglyceride synthesis and a diversion of fatty acids into the mitochondria and peroxisomal β-oxidative pathways (24). The long-term benefit of this process is the lowering of VLDL-triglyceride, a known risk factor for coronary heart disease. The concept depicted in Figure 1 can be applied to a number of different disease states that may be responsive to the composition of dietary fat.

Although the role of membrane phospholipid fatty acid composition in hormone signaling and receptor activity has been extensively reviewed by others (8, 71), the notion that fatty acids rapidly regulate the transcription of specific genes is a rather recent discovery. For this reason, this review focuses on the effects of dietary fats on gene expression.

SUPPRESSION OF HEPATIC LIPOGENESIS BY (*n*–3) AND (*n*–6) POLYENOIC FATTY ACIDS

Allmann & Gibson (3) first discovered that within two days of adding only 2% 18:2(*n*–6) to a high-carbohydrate, fat-free diet of mice, the rate of hepatic fatty acid synthesis and the activities of FAS, malic enzyme, and glucose-6-phosphate dehydrogenase were depressed by 70%. In contrast, a diet of palmitate (16:0) or oleate [18:1(*n*–9)] did not affect hepatic fatty acid synthesis. Over the past 25 years, several investigators have demonstrated that dietary polyenic fatty acids of the (*n*–3) and (*n*–6) families suppress hepatic lipogenesis, whereas saturated and monounsaturated fatty acids have no inhibitory capability (4, 5, 9, 11–19, 25, 31, 34, 36, 42, 43, 56–58, 63–66, 68, 69, 75, 76, 79, 80). This action of (*n*–6) and (*n*–3) fatty acids is independent of carbohydrate intake (16, 56, 75) and occurs at a level of (*n*–6) fatty acid intake four- to fivefold greater than that needed to fulfill the essential fatty acid requirement for optimal growth (14). Moreover, the lipogenic response to (*n*–6)/(*n*–3) fatty acids appears to be liver specific, since (*n*–6)/(*n*–3) fatty acids did not selectively suppress adipose and lung lipogenic rates (14–17).

INHIBITION OF ENZYME SYNTHESIS BY (*n*–3) AND (*n*–6) POLYENOIC FATTY ACIDS

Inhibition of enzymatic activities by PUFA does not represent a fatty acid–mediated impairment in enzyme catalytic efficiency, e.g. allosteric inhibition

of acetyl-CoA carboxylase (80), but rather reflects a decrease in enzyme content as a result of inhibited enzyme protein synthesis (14, 25, 69, 75). For example, dietary safflower oil or corn oil suppressed the synthesis of hepatic FAS by 80%, whereas dietary hydrogenated coconut oil or hydrogenated cottonseed oil had no inhibitory effect (25, 69). Two mechanisms may explain this inhibition of enzyme synthesis: (*a*) interference with the translation rate of mRNA and/or (*b*) a reduction in the amount of mRNA encoding the enzyme. This issue was addressed by focusing on the PUFA effects on the two genes encoding for FAS and the S14 protein, a putative lipogenic protein (11, 12, 14).

Dietary PUFAs regulate the synthesis of hepatic FAS and S14 primarily via a reduction in the amount of mRNA encoding these proteins (5, 11, 12, 40). When young growing rats were fed a diet containing 10% safflower oil for six days, the mRNAs encoding FAS and S14 were decreased 30 and 50%, respectively, below the values observed with a fat-free, high-glucose diet. Comparable amounts of dietary monounsaturated (e.g. triolein) or saturated fat had no suppressive effect. PUFA not only impaired *FAS* and *S14* gene expression in growing and adult rodents, but also blocked the 20- to 30-fold rise in mRNA levels associated with weaning rats onto a high-carbohydrate diet. The weanling animal appeared more sensitive to the inhibitory actions of PUFA than the growing or adult rat: 3% 18:2(*n*–6) suppressed $mRNA_{FAS}$ and $mRNA_{S14}$ abundance 70–90% in the 30-day-old rat, whereas 10% 18:2(*n*–6) suppressed $mRNA_{FAS}$ and $mRNA_{S14}$ 60–65% in the 3-month-old rat. The regulation of gene expression by dietary PUFA is not unique to *FAS* and *S14* but extends to the suppression of the genes for acetyl-CoA carboxylase (42), glucose-6 phosphate dehydrogenase (75), stearoyl-CoA desaturase-1 (57), stearoyl-CoA desaturase-2 (73), and pyruvate dehydrogenase (18). Thus dietary PUFAs appear to coordinately suppress the level of transcripts encoding lipogenic enzymes. The consequence of this action is a decrease in lipogenic enzymes, which leads to reduced hepatic lipogenic capacity and diminished synthesis of Δ^9 desaturase products.

POTENCY AND STRUCTURAL REQUIREMENTS FOR FATTY ACID INHIBITORS

The inhibitory potency of a dietary fat depends on the concentration and structure of the fatty acid constituents of the dietary lipid. To inhibit the expression of genes encoding lipogenic proteins, a dietary fatty acid must contain a minimum of 18 carbons and possess at least 2 conjugated double bonds located at the 9 and 12 positions (9, 14, 21). Thus, neither polyenic fatty acids of the (*n*–9) family nor the unusual fatty acids such as 5c,13c-22:2 suppress fatty acid biosynthesis (14). However, a fatty acid may contain

additional double bonds and still retain inhibitory potency. For example, dietary columbinic acid (5t,9c,12c-18:3) and α-linolenic acid (9c,12c,15c-18:3) both reduce the abundance of FAS mRNA and the rate of lipogenesis (15, 69). With respect to double-bond configuration, one of the two double bonds may be in the *trans* configuration, but 9t,12t-18:2 does not lower the level of hepatic FAS mRNA (21).

Although dietary 18:2(*n*–6) and 18:3(*n*–3) markedly reduce the abundance of hepatic FAS and S14 mRNAs (11, 12), the actual intracellular modulator of gene expression is probably not 18:2(*n*–6) or 18:3(*n*–3) per se (2, 9, 14). This view is based on the finding that in order for these fatty acids to suppress the expression of genes encoding lipogenic enzymes, they must first undergo desaturation by Δ^6 desaturase. When Δ^6 desaturation was blocked by the fatty acid analog, eicosatetraynoic acid (ETYA), the suppression of $mRNA_{FAS}$ level by dietary safflower oil, corn oil, or 18:2(*n*–6) was prevented. This treatment dramatically increased hepatic 18:2(*n*–6) levels. Adding the product of the Δ^6 desaturase (e.g. 6c,9c,12c-18:3) completely reinstated inhibition of gene expression (S Abraham & SD Clarke, unpublished data). Although 20:4(*n*–6) and 20:5(*n*–3) inhibit *FAS* and *S14* gene expression, the formation of these fatty acids is not an a priori requirement for polyenic fatty acid regulation of gene expression (14).

The potent suppression of FAS and S14 mRNA levels by fish oil rich in (*n*–3) fatty acids, together with the observation that the formation of 20:4(*n*–6) is not required for the regulation of gene expression, suggests that prostanoids may not be involved in the PUFA mechanism. This view is supported by the report that several prostanoid inhibitors fail to prevent PUFA-mediated suppression of *FAS* gene expression (2, 14, 25). Similarly, columbinic acid, which is not a substrate for prostanoid synthesis, continued to suppress FAS enzyme activity (69). However, the inability to detect an effect does not necessarily imply that such an effect does not exist. Selective regulation of gene transcription by fatty acids may involve high-affinity binding of a PUFA mediator to nuclear *trans*-acting proteins. Such selectivity may require modification of the fatty acid by mechanisms such as hydroxylation or oxidation. Because the structure and activity of this hypothetical fatty acid metabolite could embrace numerous possibilities, elucidation of the nuclear proteins that regulate *FAS* and *S14* gene expression will greatly help identify the fatty acid metabolite responsible for controlling gene expression.

REGULATION OF GENE TRANSCRIPTION BY (*n*–3) AND (*n*–6) FATTY ACIDS

The suppression of FAS and S14 mRNA abundance in rat liver as a consequence of ingestion of PUFA could result from: (*a*) inhibition of gene tran-

scription or (*b*) interference with a posttranscriptional mechanism involving transcript maturation or rate of mRNA degradation. Nuclear run-on analysis with nuclei prepared from livers of rats fed a diet containing either 10% tripalmitin or 10% menhaden oil demonstrated that PUFA inhibition of transcription of these genes parallels the decline in FAS and S14 mRNAs (5). The fact that transcription rates for β-actin, PEP-carboxykinase, and tyrosine aminotransferase were unchanged by this treatment indicated that the PUFA action was gene specific (5, 40). Although these studies do not exclude an effect of PUFA on mRNA maturation and turnover, the principal target for PUFA action on these genes is at the level of gene transcription.

Our early understanding of the mechanisms by which PUFAs suppress lipogenesis and the activities of lipogenic enzymes suggested that this process was a long-term adaptation that required either peripheral metabolism of the ingested PUFA to an active derivative or the release of a hormone(s) that suppressed hepatic lipogenesis (14–16, 76). However, these early kinetic studies relied heavily on changes in the activities of lipogenic enzymes. Because the average half-life of a lipogenic enzyme is 36–48 h, we could not evaluate the early changes that might occur in gene transcription or in mRNA abundance following PUFA ingestion. Thus the inhibitory effects of PUFA would by definition appear to involve long-term events. This view is consistent with the enrichment of hepatic membrane phospholipids following polyenic fatty acid ingestion. This enrichment could possibly enhance hepatocyte responsiveness to hormones that activate adenylate cyclase (8, 19). Such a shift in hormonal balance could lead to increased cAMP-dependent protein kinase activity and subsequent suppression of *FAS* and *S14* gene transcription (19, 60).

However, two lines of evidence suggest that PUFAs act directly on the liver to rapidly regulate the transcription of genes encoding lipogenic proteins. The first line comes from an in vivo kinetic study in which rats were meal-fed a high-glucose diet containing 10% triolein or 10% menhaden oil. The effects of these diets on hepatic *FAS* and *S14* gene transcription were examined (40). Rats meal-trained to the 10% triolein oil diet significantly induced both *FAS* and *S14* gene transcription within 90 min of the initiation of meal ingestion. When rats were switched to a meal containing menhaden oil, the induction of *S14* and *FAS* transcription rates that occurs with meal ingestion was inhibited by ≥ 50%. The inhibitory effect of menhaden oil reached its nadir after consumption of the second meal. The decline in transcription rates paralleled the decline in mRNA, indicating that the early target of PUFA action was at the level of gene transcription. The effects of menhaden oil were rapidly (within 3 h) reversed when menhaden oil was removed from the diet (12).

The rapidity of PUFA suppression and reinduction following PUFA removal from the diet refutes the proposal that significant changes in membrane phospholipid content must precede PUFA effects on gene expression. Feeding rats

PUFA not only leads to increased glucagon-stimulated adenylate cyclase activity (19), but also enhances insulin sensitivity (8, 23). In addition, the dose of corn oil required to elicit a stimulation of adenylate cyclase was only 2% of the diet (19), a level far below that needed to maximize the effects of corn oil or safflower oil (i.e. 5–10%) on *S14* or *FAS* gene expression (12, 40). Thus the effects of PUFA feeding on adenylate cyclase and insulin-stimulated pathways are inconsistent with the pattern of PUFA control of lipogenic gene transcription. Moreover, dietary PUFA had no effect on the transcription of the gene for PEP-carboxykinase, which is highly sensitive to increases in cellular cAMP (5, 40).

Studies with cultured primary hepatocytes have demonstrated that PUFAs act directly on these cells to effect changes in gene expression (4, 40). In this in vitro system, induction of both FAS and S14 mRNA requires several hormones, e.g. insulin, T_3, and glucocorticoids. When albumin-bound fatty acids are included in the hormonally defined, serum-free media, we observed that 20:4(*n*–6) at levels ranging from 50–500 μM resulted in progressive suppression of $mRNA_{FAS}$ accumulation (4). However, levels of $mRNA_{FAS}$ in control cultures were unaffected by the addition of C20:1(*n*–9). Interestingly, $mRNA_{\beta actin}$ increases slightly in response to C20:4(*n*–6) treatment. As occurs with FAS expression, treating cultured hepatocytes with C20:4(*n*–6) or C20:5(*n*–3) inhibits the T_3-stimulated increase in $mRNA_{S14}$. This inhibition is a dose-dependent mechanism with an $ED_{50} \leq 100$ μM (40).

Although it has been suggested that lipid hydroperoxides derived from albumin-bound PUFA lead to cytotoxicity (29, 54), our data do not support this argument (4, 40, 65, 66). For example, we have found that after a 48-h treatment of cells with PUFA, the hepatocytes are >90% viable, as evidenced by lactate dehydrogenase release from the cells. Therefore, our data collectively indicate that PUFA inhibition of gene expression is rapid and does not require extrahepatic metabolism of the PUFA. Moreover, the mechanism of this inhibition does not involve extrahepatic factors, e.g. gut hormones.

Pretranscriptional Control by PUFA

Fatty acid control of *FAS* and *S14* gene transcription suggests that PUFA-regulated transcription factors target key *cis*-regulatory elements associated with these genes. In order to further address the mechanism by which (*n*–6) and (*n*–3) fatty acids govern gene transcription, we have cloned from a rat genomic cosmid library a 39-kb insert that contains the entire *FAS* gene plus 2.5 kb of 5′-flanking sequence. The region −2495 to +1500 of the *FAS* gene has been fully sequenced and contains several nucleotide motifs characteristic of a promoter region.

In addition to the basal promoter elements, transfection analysis studies with the region −2195 to +65 bp of the *FAS* gene have revealed that this region

contains potential response elements to glucocorticoids and insulin (55). Furthermore, a series of 5′-deletions has localized a potential insulin-responsive region within the *FAS* gene, i.e. −332 to +65 bp (55). Finally, preliminary results suggest that the region −2195 to +65 of the *FAS* gene may also contain a nucleotide sequence responsive to PUFA. For example, chloramphenicol acetyl transferase (CAT) expression was inhibited by 20:4(*n*−6) but not by 20:1(*n*−9) (A Milner & SD Clarke, unpublished results).

Unlike the *FAS* gene, the 5′-flanking region of the *S14* gene has been extensively characterized, and a number of *cis*-acting elements have been identified (37, 40, 50, 51, 84). Recently, we localized a PUFA-response region, which is resident in the proximal promoter of the *S14* gene (40). When rat liver cells in primary culture were transfected with the gene for S14-CAT fusion (−4315 to +19 bp) and cotransfected with a thyroid hormone receptor β_1 ($TR\beta_1$) expression vector to install T_3 responsiveness, T_3 treatment of the hepatocytes induced S14-CAT activity 100-fold (40). More importantly, 20:5(*n*−3) (at 300 μM) attenuated this induction by 80%, which indicates that PUFA-RE were located within 4315 bp of the 5′ end of the *S14* gene (40). The ED_{50} for this response was ≤ 100 μM, and both 20:4(*n*−6) and 20:5(*n*−3) were equipotent. In contrast, 18:1(*n*−9) had little effect on S14-CAT activity. Moreover, neither 18:1(*n*−9), 20:4(*n*−6), nor 20:5(*n*−3), affected the expression of CAT driven by the RSV enhancer/promoter. Therefore, the effects of fatty acids on S14-CAT expression were gene specific and did not result from toxic or nonspecific effects of PUFA on hepatocytes. These transfection results correlated with the effects of PUFA on $mRNA_{S14}$ expression.

Deletion analysis localized the PUFA-RE to within 290 bp of the 5′ end of the *S14* gene. However, the −290 to +19 bp region of the *S14* gene has weak promoter activity. Therefore, in order to localize the PUFA-RE within the promoter, the S14 thyroid hormone response element (TRE) (−2.9 to −2.5 kb) was ligated just upstream from the promoter to elevate CAT expression. The level of T_3-stimulated CAT and PUFA attenuation of this activity in this truncated construct was comparable to that found in plasmids containing the full-length *S14* regulatory region (−4315 to +19 bp). A series of deletions within the promoter indicated that the PUFA-sensitive region was located between −220 and −80 bp (Figure 2).

The *S14* proximal promoter contains four functional elements involved in initiating and potentiating gene transcription: (*a*) a modified TATA-box at −27 bp, (*b*) an NF-1/CTF-like binding site (−63 to −48 bp), and (*c*) regions B (−115 to −88 bp) and C (−244 to −227 bp) that bind tissue-specific factors (51, 52). The B region also functions to potentiate T_3 activation of *S14* gene transcription (40). The location of the PUFA-sensitive region either within or adjacent to the B region suggests that PUFA-regulated factors interfere

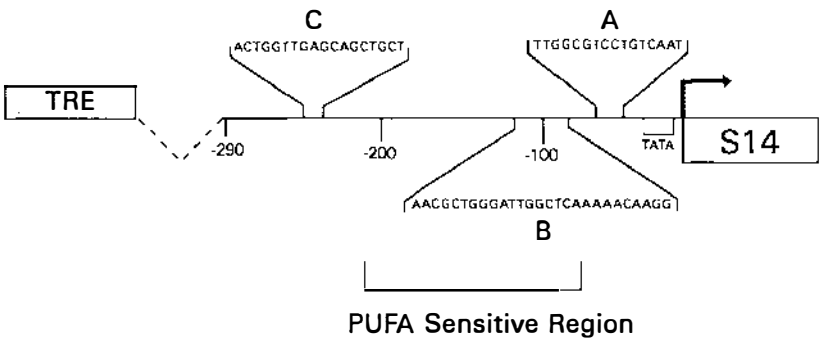

Figure 2 Map of functional *cis*-acting elements within the *S14* proximal promoter. The *S14* proximal promoter contains a modified TATA-box at −27 bp. Regions A (−48 to −63 bp), B (−88 to −113 bp), and C (−227 to −244 bp) represent DNase I–footprinted regions (51). Hepatic NF-1 (or a related factor) binds region A. *Trans*-acting factors binding to regions B and C are tissue specific. The S14 TRE (−2502 to −2900 bp) is ligated at −290 bp. The PUFA-responsive region is located between −80 and −220 bp.

with the T_3 control of this gene. However, ligation of the S14 TRE or a consensus TRE (AGGTCAggacAGGTCA) upstream from the thymidine kinase (TK) promoter did not confer PUFA control to the TK promoter. Thus the PUFA-mediated attenuation of T_3 action was promoter specific. Although the molecular details for this interference remain unclear, these studies suggest that PUFAs do not interfere with the action of the T_3 regulatory network on other genes, i.e. T_3-receptor expression or T_3-receptor transactivation.

Insulin, dietary carbohydrate, glucocorticoids, and retinoic acid also induce *S14* gene transcription (37–41). These factors target the region between −1.6 and −1.4 kb from the 5′ end of the *S14* gene. Because this region does not harbor PUFA-RE, we can infer that PUFA may not interfere with the action of these endocrine-regulatory pathways controlling transactivation of the *S14* gene.

Prospective Mechanisms for PUFA Control of Gene Transcription

Analysis of the *S14* model suggests that PUFA control of gene transcription is conferred by the gene and not by the signaling mechanism. This concept is illustrated by the finding that PUFAs had no effect on T_3 activation of a TRE in the context of the TK promoter but significantly attenuated T_3 action when the TRE was in the context of the *S14* promoter (40).

With respect to these observations, how might these PUFAs specifically

regulate gene transcription? Three possibilities merit consideration: (*a*) by generation of the PUFA-regulated signal, (*b*) by PUFA-regulation of the *trans*-acting factor(s), and (*c*) by interaction of the *trans*-acting factor(s) with the target gene. In keeping with these potential scenarios, we proposed a simplistic model to explain the regulation of gene transcription by PUFA. Currently, our data indicate that Δ^6 desaturation of 18 carbon (*n*–6/*n*–3) fatty acids is a prerequisite step in the generation of the bioactive regulator for lipogenic gene transcription. This mandatory desaturation can be circumvented by feeding (*n*–6) or (*n*–3) fatty acid products of Δ^6 desaturase (14). After the (*n*–6/*n*–3) fatty acid has undergone Δ^6 desaturation, we propose that the PUFAs (or a PUFA metabolite) are transferred to the nucleus, where they function as ligands or modifiers of a nuclear fatty acid–receptor protein (PUFA-BP). Following PUFA-dependent modification of the PUFA-BP, PUFA-BP interacts with a *cis*-acting element in the target gene that governs gene transcription.

We can only speculate as to the mechanisms by which PUFAs modulate a nuclear protein and regulate DNA/protein interactions. One possibility is that the PUFAs (or a metabolite) act as ligands for a specific *trans*-acting protein, e.g. peroxisomal proliferator–activated receptor (PPAR) (26–28, 35, 46, 49). Alternatively, an oxidized product of the PUFA could modify the redox state of a nuclear *trans*-acting amplifier protein, e.g. c-*fos* or c-*jun* (1, 30, 81). A third possibility is that the PUFA may influence the phosphorylation state of a specific nuclear protein that governs the transcription of genes coding for lipogenic proteins (33, 45, 48). Finally, a PUFA-BP may not be a DNA-binding protein per se but may nevertheless function as an auxiliary protein that interacts with a DNA-binding protein. In this way, a PUFA-BP could modulate other *trans*-acting proteins that govern *FAS* and *S14* gene transcription.

Although the existence of a PUFA-BP is hypothetical at this time, recent evidence indicates that the nuclei of several tissues contain a collection of proteins that are members of the superfamily of steroid-like receptors (26–28, 62, 67) and that are activated by fatty acids (27, 44, 67). This family of putative fatty acid–activated proteins includes the PPAR of mouse (35, 77) and rat (27, 83), a steroid hormone–like receptor from human osteosarcoma (67), *apo*-AI regulatory protein-1 cloned from rat liver, and possibly the *apo*-B transcription factor HNF-4 (70).

Studies with PPAR and its isoforms provide the strongest evidence indicating that PUFAs govern gene transcription by regulating the activity of these steroid receptor–like proteins (27, 44, 67). PPAR is activated by a diverse group of compounds, including amphipathic carboxylates, herbicides, plasticizers, and possibly PUFAs (28, 49, 63, 74). Interestingly, peroxisomal proliferators also suppress the expression of *FAS* and inhibit hepatic fatty acid biosynthesis (24). Moreover, the time course for induction of acyl-CoA oxidase by peroxisomal proliferating agents is comparable to that for the suppression of *FAS* gene

expression (12, 40, 59). Recently, transcription *trans*-activation assays revealed that PPAR and PPAR-like proteins are activated by a variety of fatty acids (27, 44, 67). However, studies with nafenopin (28) suggest that peroxisomal proliferators may not bind directly to PPAR but may instead affect a metabolic pathway that generates a regulator of PPAR. In this regard, peroxisomal proliferators reportedly stimulate the conversion of long-chain fatty acids to dicarboxylic fatty acids via the ω-hydroxylase pathway (46). However, whether these modified fatty acids bind to PPAR or to other nuclear proteins or influence the transcription of lipogenic genes remains to be determined. Nevertheless, these data collectively suggest that fatty acids may function as modifiers of nuclear proteins that operate in modulating gene expression (26, 47).

Although PPAR-like proteins may well be the prospective mediators for PUFA regulation of hepatic gene transcription, several lines of evidence argue against PPAR per se as the nuclear fatty acid receptor. First, saturated and monounsaturated fatty acids do not affect the expression of lipogenic proteins (4, 12, 14, 40). In contrast, saturated, monounsaturated, and polyunsaturated fatty acids all function as activators of PPAR and induce PPAR-mediated gene transcription (27, 44, 67). Thus the PPAR-like proteins do not appear to exhibit the selectivity required to explain PUFA control of gene expression. Second, the 20:4 (*n*–6) analog, eicosatetraynoic acid (ETYA), reportedly activates PPAR and affects transcription (27, 44). However, ETYA has no effect on *FAS* gene expression (2, 9). Finally, PPAR-like *trans*-acting proteins seem to be present in several nonhepatic tissues, including bone, heart, skin, lung, and kidney (28, 35, 67). However, PUFA suppression of genes coding for lipogenic enzymes has only been observed in hepatic tissue (14, 17). Therefore, the role of PPAR-like proteins in mediating PUFA control of gene transcription remains unclear.

An alternative (or possibly supplemental) view to the ligand-activated mechanism is the suggestion that PUFA may regulate gene transcription via its effect on covalent modification (30, 45, 82). For example, PUFA may regulate the phosphorylation state of specific transcription factors. Support for PUFA modulation of phosphorylation comes from recent reports that cytosolic protein kinase C (PKC) can be directly activated by unsaturated—but not saturated—fatty acids (45, 82). Fatty acid regulation of PKC did not require the fatty acid to be incorporated into phospholipids, nor did it require PKC translocation to the membrane. However, both mono- and polyunsaturated fatty acids activate PKC equally well (45, 82). Clearly, the specificity of PUFA control of gene transcription is not reflected in the fatty acid specificity for PKC activation. Therefore, although fatty acid–dependent activation of protein kinases may have far-reaching effects on gene transcription (33), any proposed mechanism involving protein phosphorylation must maintain the specificity of action for dietary PUFA.

SUMMARY

We have known for nearly 30 years that dietary polyenoic (*n*–6) and (*n*–3) fatty acids potently inhibit hepatic fatty acid biosynthesis. The teleological explanation for this unique action of PUFAs resides in their ability to suppress the synthesis of (*n*–9) fatty acids. By inhibiting fatty acid biosynthesis, dietary PUFAs reduce the availability of substrate for Δ^9 desaturase (7, 22, 34, 36) and in turn reduce the availability of (*n*–9) fatty acids for incorporation into plasma membranes. In this way, essential biological processes dependent on essential fatty acids (e.g. reproduction and *trans*-dermal water loss) continue to operate normally. Therefore, if essential fatty acid intake did not regulate (*n*–9) fatty acid synthesis, the survival of the organism would be threatened.

During the past 20 years, we have gradually elucidated the cellular and molecular mechanisms by which dietary PUFAs modulate fatty acid biosynthesis and (*n*–9) fatty acid availability. Central to this mechanism has been our ability to determine that dietary PUFAs regulate the transcription of genes coding for lipogenic enzymes (12, 40). The potential mechanisms by which PUFAs govern gene transcription are numerous, and it is unlikely that any one mechanism can fully elucidate the nuclear actions of PUFA. The difficulty in providing a unifying hypothesis at this time stems from: (*a*) the many metabolic routes taken by PUFAs upon entering the hepatocyte (Figure 1); and (*b*) the lack of identity of a specific PUFA-regulated *trans*-acting factor. However, the studies described above indicate that macronutrients, like PUFA, are not only utilized as fuel and structural components of cells, but also serve as important mediators of gene expression (12, 14, 40). As regulators of gene expression, PUFAs (or metabolites) are thought to affect the activity of transcription factors, which in turn target key *cis*-linked elements associated with specific genes. Whether this targeting involves DNA-protein interaction or the interaction of PUFA-regulated factors is unclear. A better understanding of the nuclear actions of PUFA will clarify the role of these compounds in lipid metabolism and lead to a better understanding of the role of PUFAs in disease processes such as insulin-resistant diabetes and certain forms of cancer.

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