Nonalcoholic Steatosis and Steatohepatitis. I. Molecular mechanism for polyunsaturated fatty acid regulation of gene transcription

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Clarke, Steven D. Nonalcoholic Steatosis and Steatohepatitis. I. Molecular mechanism for polyunsaturated fatty acid regulation of gene transcription. Am J Physiol Gastrointest Liver Physiol 281: G865–G869, 2001.—This review addresses the hypothesis that polyunsaturated fatty acids (PUFA), particularly those of the n-3 family, play pivotal roles as “fuel partitioners” in that they direct fatty acids away from triglyceride storage and toward oxidation and they enhance glucose flux to glycogen. In doing this, PUFA may reduce the risk of enhanced cellular apoptosis associated with excessive cellular lipid accumulation. PUFA exert their beneficial effects by upregulating the expression of genes encoding proteins involved in fatty acid oxidation while simultaneously downregulating genes encoding proteins of lipid synthesis. PUFA govern oxidative gene expression by activating the transcription factor peroxisome proliferator-activated receptor-α. PUFA suppress lipogenic gene expression by reducing the nuclear abundance and DNA binding affinity of transcription factors responsible for imparting insulin and carbohydrate control to lipogenic and glycolytic genes. In particular, PUFA suppress the nuclear abundance and expression of sterol regulatory element binding protein-1 and reduce the DNA binding activities of nuclear factor Y, stimulatory protein 1, and possibly hepatic nuclear factor-4. Collectively, the studies discussed suggest that the fuel “repartitioning” activity of PUFA has been observed in humans as well as various animal models (2, 6, 18), but the amount of n-6 and n-3 fatty acids and the best n-6 to n-3 ratio required for optimum metabolic benefit are unknown. However, as little as 2–5 g of 18:3(n-3) or 20:5 and 22:6(n-3) lower blood triglycerides and reduce the risk of fatal ischemic heart disease (reviewed in Ref. 3).

The fuel repartitioning effect of PUFA requires that 18:2(n-6) and 18:3(n-3) undergo δ-6 desaturation (16). Interestingly, loss of δ-6 desaturase activity is associated with liver failure in humans (Blake WL and Clarke SD, unpublished data). The recent cloning of the human δ-6 and -5 desaturase genes will allow us to ascertain whether low δ-6/δ-5 desaturation plays a causative role in the development of fatty liver syndrome in humans (4, 5).

Some of the beneficial effects of PUFA are caused by changes in membrane fatty acid composition and subsequent alterations in hormonal signaling. However, fatty acids themselves exert a direct, membrane-independent influence on molecular events that govern gene expression (Fig. 1). We believe that the regulation of gene expression by dietary fats has the greatest impact on the development of disorders in lipid and glucose metabolism. More importantly, determination of the cellular and molecular mechanisms regulated by PUFA may identify novel sites for pharmacological intervention.
PUFA INDUCTION OF LIPID OXIDATION: ROLE OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-α

One of the first steps in the PUFA-dependent partitioning of metabolic fuels involves an immediate reduction in the production of hepatic malonyl-CoA (3). Malonyl-CoA is a negative metabolite effector of carnitine palmitoyltransferase (24). Consequently, a PUFA-mediated decrease in hepatic malonyl-CoA favors fatty acid entry into the mitochondria and peroxisomes and leads to enhanced fatty acid oxidation (24). Whether PUFA suppress malonyl-CoA levels in skeletal muscle and heart remains to be determined, but such a mechanism would be consistent with the higher rates of fatty acid oxidation observed in humans and animals fed diets rich in PUFA (6, 18).

The reduction in hepatic malonyl-CoA is paralleled by a PUFA-dependent induction of genes encoding proteins involved in fatty acid oxidation and ketogenesis (3, 7, 11). These changes in gene transcription occur too quickly to be explained simply by altered hormone signaling resulting from modifications of the membrane lipid environment. Rather, the changes are more consistent with the idea that PUFA directly (e.g., ligand binding) regulate the activity or abundance of a nuclear transcription factor. In 1990, peroxisome proliferator-activated receptor (PPAR)-α, a novel lipid-activated transcription factor, was cloned (10). PPAR-α is a member of the steroid receptor superfamily, and like other steroid receptors it possesses a DNA binding domain and a ligand binding domain (10, 13–15). Interaction of PPAR-α with its DNA recognition site is markedly enhanced by ligands such as the hypotriglyceridemic fibrate drugs, conjugated linoleic acid, and PUFA (10, 13–15, 20). In general, PPAR-α activation leads to the induction of several genes encoding proteins involved in lipid transport, oxidation, and thermogenesis including hepatic carnitine palmitoyltransferase, hepatic and skeletal muscle peroxisomal acyl-CoA oxidase, and muscle uncoupling protein-3 (1, 3, 13, 20). The n-3 PUFA are more potent than the n-6 PUFA as in vivo activators of PPAR-α (13–15), but neither family of PUFA is a particularly strong PPAR-α activator. However, PUFA metabolites such as eicosanoids or oxidized fatty acids have one to two orders of magnitude greater affinity for PPAR-α and are consequently far more potent transcriptional activators of PPAR-α-dependent genes (15).

The importance of PPAR-α to overall glucose and fatty acid homeostasis has been clearly demonstrated in PPAR-α knockout mice (4, 22). Because PPAR-α−/− mice lack the ability to increase rates of fatty acid oxidation during periods of food deprivation, they develop characteristics of adult-onset diabetes including fatty liver, elevated blood triglycerides, and hyperglycemia (13). The essentiality of PPAR-α to lipid oxidation was further underscored when hyperglycemia was found to suppress PPAR-α expression, induce PPAR-γ expression, increase β-cell and cardiomyocyte lipids, and accelerate cell death (25). Such “lipotoxicity” may be a contributing factor to the complications of cellular lipid overload (12, 25). Clearly, PPAR-α is emerging as a pivotal player in both fatty acid and glucose metabolism. More importantly, its regulation by PUFA, particularly n-3 PUFA and possibly conjugated linoleic acid, may offer an explanation for the reported benefits of these fatty acids in protecting individuals from developing the detrimental characteristics of non-insulin-dependent diabetes (reviewed in Ref. 3).

PUFA SUPPRESSION OF LIPOGENESIS: ROLE OF STEROL REGULATORY ELEMENT BINDING PROTEIN-1, NUCLEAR FACTOR Y, AND HEPATIC NUCLEAR FACTOR-4

Dietary PUFA inhibit hepatic lipogenesis by suppressing the expression of a number of hepatic en-
zymes involved in glucose metabolism and fatty acid biosynthesis including glucokinase, pyruvate kinase, glucose-6-phosphate dehydrogenase, citrate lyase, acetyl-CoA carboxylase, fatty acid synthase, stearoyl-CoA desaturase, and the δ-6 and δ-5 desaturases (2–5, 8, 11, 17, 22, 23). The discovery of PPAR-α led quickly to the idea that PPAR-α was a "master switch" transcription factor that is targeted by PUFA to coordinately suppress genes encoding proteins of lipid synthesis and induce genes encoding proteins of lipid oxidation. This attractive hypothesis was strengthened by reports that potent pharmacological activators of PPAR-α modestly reduced lipogenic gene transcription (11, 20). However, PPAR-α does not appear to interact with PUFA response sequences of lipogenic genes (3, 11, 22). Moreover, PUFA continue to suppress the transcription of hepatic lipogenic genes in PPAR-α−/− mice (11). Thus the inhibition of lipogenic gene transcription associated with PPAR-α activation is indirect and may simply reflect the PPAR-α-dependent induction of the δ-6 desaturase pathway (Ref. 16; Tang Z, Cho HP, Nakamura MT, and Clarke SD, unpublished data).

PUFA response sequences have been well characterized in only three genes: fatty acid synthase, S14, and L-type pyruvate kinase (3, 11, 22). The rat fatty acid synthase gene contains two independent PUFA regulatory sequences that are located between −118 and −43 and between −7250 and −7035 (Teran-Garcia M and Clarke SD, unpublished data). Approximately 65% and 35% of the PUFA control can be attributed to the proximal and distal elements, respectively. Interestingly, the proximal PUFA response region of the fatty acid synthase gene has characteristics that are very similar to the PUFA response region of the S14 gene (−220 to −80), whereas the distal PUFA response region of the fatty acid synthase has similarities to the L-type pyruvate kinase PUFA response region (−160 to −97) (11).

The proximal PUFA response region of the fatty acid synthase gene imparts insulin responsiveness to the gene and contains DNA binding sites for sterol regulatory element binding protein (SREBP)-1, upstream stimulatory factor (USF), stimulatory protein 1 (Sp1), and nuclear factor Y (NF-Y) (21, 22). The nuclear abundance of USF is unaffected by dietary PUFA (22). In contrast, PUFA rapidly reduce the nuclear content of hepatic SREBP-1, and this is associated with a decrease in the rate of fatty acid synthase and S14 gene transcription (7, 8, 17, 22, 23). SREBP-1 and SREBP-2 are a family of transcription factors (i.e., SREBP-1a, -1c, and -2) that were first isolated as a result of their properties for binding to the sterol regulatory element (2, 17). SREBP-2 is a regulator of genes encoding proteins involved in cholesterol metabolism (2). SREBP-1 exists in two forms, 1a and 1c. SREBP-1a is the dominant form in cell lines and is a regulator of genes encoding proteins involved in both lipogenesis and cholesterologenesis. SREBP-1c constitutes 90% of the SREBP-1 found in vivo and is a determinant of lipogenic gene transcription (2, 17, 23).

SREBP-1 is synthesized as a 125-kDa precursor protein that is anchored in the endoplasmic reticulum membrane (2, 17). Proteolytic release of the 68-kDa mature SREBP-1 occurs in the golgi system, and movement of SREBP-1 from the endoplasmic reticulum to the golgi requires the trafficking protein SREBP cleavage-activating protein (SCAP) (2, 8). Once released, mature SREBP-1 translocates to the nucleus and binds to the classic sterol response element and/or to a palindrome CATG sequence. In the case of fatty acid synthase, SREBP-1 interacts with a CATG palindrome that also functions as an insulin response element (2). Overexpression of mature SREBP-1a in liver is associated with high rates of fatty acid biosynthesis and the development of fatty liver (2, 17). In contrast, the ablation of the SREBP-1 gene results in low expression of lipogenic genes (2, 7, 17). These observations led us to hypothesize that PUFA inhibit lipogenic gene transcription by impairing the proteolytic release of SREBP-1c and/or by suppressing SREBP-1c gene expression (22, 23). Diets rich in 18:2(n-6) or 20:5 and 22:6(n-3) were found to reduce the hepatic nuclear and precursor content of mature SREBP-1 65% and 90% and 60% and 75%, respectively (22). The decrease in SREBP-1c was accompanied by a comparable decrease in the transcription rate of hepatic fatty acid synthase (22). Unlike PUFA, saturated and monounsaturated fats had no effect on the nuclear content or precursor content of SREBP-1 or on lipogenic gene expression (8, 22, 23). The PUFA-dependent reduction in hepatic content of SREBP-1 may explain how PUFA inhibit the transcription of several genes encoding proteins involved in hepatic glucose metabolism and fatty acid biosynthesis including glucokinase, acetyl-CoA carboxylase, stearoyl-CoA desaturase, and the δ-6 and δ-5 desaturases (4, 5, 7, 11, 22). Interestingly, the inhibition of lipogenic gene expression that reportedly occurs in adipose tissue with the ingestion of fish oil does not involve an SREBP-1-dependent mechanism (30).

PUFA reduce the nuclear content of SREBP-1 by a two-phase mechanism. The first phase is a rapid (<60 min) inhibition of the proteolytic release process (8, 23). The second phase involves an adaptive (∼48 h) reduction in the hepatic content of SREBP-1 mRNA that is followed by a reduction in the amount of precursor SREBP-1 protein (22, 23). The mechanism by which PUFA acutely inhibit the proteolytic processes is unknown. However, nuclear run-on assays suggested that PUFA reduce the hepatic content of SREBP-1 mRNA by postranscriptional mechanisms (22, 23). Using rat liver cells in primary culture, we determined that PUFA reduces the half-life of SREBP-1c mRNA from 11 h to <5 h (23). The mechanism by which PUFA control the half-life of SREBP-1 is unknown, but it may require the synthesis of a rapidly turning over PUFA-dependent protein (23).

SREBP-1c by itself possesses weak trans-activating power, but the binding of SREBP-1c to its recognition sequence enhances the upstream DNA binding of NF-Y and Sp1, which in turn amplifies the trans-activating activities of the three transcription factors (17). NF-Y
is a heterotrimeric nuclear protein that reportedly plays a role in regulating chromatin structure by way of its interaction with histone acetyl transferases. The binding sites for NF-Y are essential for fatty acid synthase (21) and S14 promoter activity (11). Mutations within the Y box region of −104 to −99 of the S14 gene eliminated promoter activity by preventing NF-Y from interacting with upstream T3 (−2800 to −2500) and carbohydrate response (−1600 to −1400) regions (11). Similarly mutating the Y box motif between −90 and −80 of the rat fatty acid synthase gene eliminated 80% of the promoter activity, and mutating the adjacent Sp1 site (−80) reduced promoter activity by >90% (Teran-Garcia M and Clarke SD, unpublished data). In contrast, eliminating the SREBP-1 site (−67 to −53) reduced fatty acid synthase promoter activity by only 40%. More importantly, only 35% of the PUFA inhibition of fatty acid synthase promoter activity was lost with the SREBP-1 site mutation. On the other hand, mutating the NF-Y site eliminated nearly 70% of the PUFA suppression of fatty acid synthase promoter activity. Moreover, the near 90% inhibition in hepatic fatty acid synthase gene transcription associated with the ingestion of a diet rich in fish oil was accompanied by a 50–60% reduction in DNA binding affinity for NF-Y and Sp1 (Teran-Garcia M and Clarke SD, unpublished data).

The insulin response region and its associated transcription factors (i.e., SREBP-1, NF-Y, and Sp1) are not the only nuclear factors regulated by PUFA. Transfection-reporter analyses indicate that PUFA exert a negative influence on the carbohydrate response element of the L-type pyruvate kinase (4) and fatty acid synthase genes (Teran-Garcia M and Clarke SD, unpublished data). The nature of the transcription factors and the mechanism by which PUFA regulate them are not well defined. One hepatic protein that may be a PUFA target is hepatic nuclear factor (HNF)-4. HNF-4 is a member of the steroid receptor superfamily. HNF-4 enhances the glucose/insulin induction of L-type pyruvate kinase transcription by binding as a homodimer to a direct repeat-1 motif (9). Like PPAR-α, HNF-4 has a ligand binding domain that interacts with acyl-CoA esters, but unlike PPAR-α, fatty acyl-CoA binding to HNF-4 decreases its DNA binding activity (9). This suggests that PUFA may exert part of its negative influence on gene transcription by reducing HNF-4 DNA binding activity. Linker scanner mutations through the carbohydrate response region of the L-type pyruvate kinase promoter (i.e., −183 to −97) did in fact reveal that the HNF-4 recognition elements were essential for PUFA suppression of the promoter (11). Recently, we found that sequences between −7242 and −7150 of the fatty acid synthase gene were required for glucose to induce fatty acid synthase gene transcription (19). Subsequent studies have demonstrated that the −7242 to −7150 region contains DNA recognition sites for HNF-4 and a novel carbohydrate response factor (19). Moreover, deleting this sequence eliminated 30–40% of the total PUFA suppression of the fatty acid synthase promoter (Teran-Garcia M and Clarke SD, unpublished data). Thus PUFA may exert part of their suppressive effects on gene transcription by interfering with the glucose-mediated trans-activation processes that in part involve reduction of HNF-4 DNA binding activity.

**SUMMARY**

PUFA have been known for nearly 40 years to uniquely suppress lipid synthesis. PUFA, particularly n-3, accomplish this by coordinating an upregulation of lipid oxidation and a downregulation of lipid synthesis. In other words, PUFA function as metabolic fuel “repartioners.” Such fuel repartitioning may protect cells against the accelerated rates of apoptosis reportedly observed with excessive triglyceride accumulation (12, 25). PUFA exert their effects on metabolic pathways by governing the DNA binding activity and nuclear abundance of select transcription factors responsible for regulating the expression of genes encoding key regulatory proteins of lipid and glucose metabolism. With respect to their role in fatty acid oxidation, PUFA increase the fatty acid oxidative capacity of tissues through their ability to function as ligand activators of PPAR-α and thereby induce the transcription of several genes encoding proteins affiliated with fatty acid oxidation. On the other hand, PUFA suppress lipid synthesis by inhibiting transcription factors that mediate the insulin and carbohydrate control of lipogenic and glycolytic genes. In this regard, PUFA rapidly generate an intracellular signal that immediately suppresses the proteolytic release of mature SREBP-1 from its membrane-anchored precursor and simultaneously reduces the DNA binding activities of NF-Y and Sp1. Within a matter of minutes after PUFA treatment, the nuclear content of SREBP-1c is greatly reduced. The drop in nuclear content of SREBP-1c further contributes to the reduction in DNA binding of NF-Y and Sp1. Continued ingestion of PUFA subsequently lowers SREBP-1 mRNA levels by accelerating transcript decay, which in turn results in a lower hepatic content of precursor endoplasmic reticulum-anchored SREBP-1. With regard to the carbohydrate response element, PUFA may also mediate reductions in the DNA binding activity of pivotal transcription factors (e.g., HNF-4), but the nature of the affected transcription factors remains to be unequivocally established. Without question, the missing final chapter in the entire PUFA-regulatory story is the nature of the intracellular signal responsible for regulating the various affected transcription factors.

**REFERENCES**


