Polyunsaturated Fatty Acid
Regulation of Gene Transcription: A
Molecular Mechanism to Improve
the Metabolic Syndrome1,2

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ABSTRACT 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 that they enhance glucose flux to
glycogen. In doing this, PUFA may protect against the ad-
verse symptoms of the metabolic syndrome and reduce the
risk of heart disease. PUFA exert their beneficial effects by
up-regulating the expression of genes encoding proteins
involved in fatty acid oxidation while simultaneously down-
regulating genes encoding proteins of lipid synthesis. PUFA
govern oxidative gene expression by activating the tran-
scription factor peroxisome proliferator-activated receptor
α. PUFA suppress lipogenic gene expression by reducing
the nuclear abundance and DNA-binding affinity of tran-
scription factors responsible for imparting insulin and carbo-
hydrate control to lipogenic and glycolytic genes. In par-
ticular, PUFA suppress the nuclear abundance and
expression of sterol regulatory element binding protein-1
and the DNA-binding activities of nuclear factor Y,
Sp1 and possibly hepatic nuclear factor-4. Collectively, the
studies discussed suggest that the fuel “repartitioning” and
gene expression actions of PUFA should be considered
among criteria used in defining the dietary needs of (n-6)
and (n-3) and in establishing the dietary ratio of (n-6) to (n-3)
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Dietary (n-6) and (n-3) polyunsaturated fatty acids
(PUFA)4 reduce triglyceride accumulation in skeletal muscle
and potentially in cardiomyocytes and β cells (1,2). Lower
tissue lipids are associated with improvements in the metabolic
syndrome, such as increased insulin sensitivity (1,3). PUFA
elicit their effects by coordinately suppressing lipid synthesis
in the liver, up-regulating fatty acid oxidation in liver and ske-
etal muscle and increasing total body glycogen storage (3–8).
Δ-6 Desaturation of 18:2(n-6) and 18:3(n-3) is required for
this “repartitioning” of metabolic fuel (9), and on a carbon-
for-carbon basis, (n-3) fatty acids are more potent than (n-6)
fatty acids. The repartitioning activity of PUFA, particularly
(3) PUFA, has been observed in humans as well as various
animal models (3,10–13). Unfortunately, the amount of (n-6)
and (n-3) and the best (n-6)-to-(n-3) ratio required for opti-
mum metabolic benefit are unknown. However, as little as 2–5
of 18:3(n-3) or 50.5 and 22:6(n-3) lower blood triglyceride
concentrations and reduce the risk of fatal ischemic heart
disease (12,13). Some of the beneficial effects of PUFA are due
to changes in membrane fatty acid composition and subse-
quent alterations in hormonal signaling (1). However, fatty
acids themselves exert a direct, membrane-independent influ-
ence on molecular events that governs gene expression. It is
the regulation of gene expression by dietary fats that we
believe has the greatest impact on the development of insulin
resistance and its related pathophysiologies (i.e., the metabolic
syndrome). More importantly, determination of the cellular
and molecular mechanisms regulated by PUFA may identify
novel sites for pharmacological intervention.

PUFA Induction of Lipid Oxidation: The Role of
Peroxisome Proliferator-activated Receptor α. One of
the first steps in the PUFA-dependent repartitioning of met-
abolic fuels involves an immediate reduction in the production
of hepatic malonyl coenzyme A (CoA) (14). Malonyl-CoA
is a negative metabolite effector of carnitine palmitoyltransfer-
ase (15). 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.
Whether PUFA suppress malonyl-CoA levels in skeletal
muscle and heart remains to be determined, but such a mech-
anism would be consistent with the higher rates of fatty acid
oxidation observed in humans and animals fed diets rich in
PUFA (10,11).

The reduction in hepatic malonyl-CoA is paralleled by a
PUFA-dependent induction of genes encoding proteins involved
in fatty acid oxidation and ketogenesis (3,4,7). 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, PPARα, a novel lipid-activated transcription
factor, was cloned (16). PPARα is a member of the steroid
receptor superfamily, and like other steroid receptors, it possesses
a DNA-binding domain and a ligand-binding domain (7,8,16).
The interaction of PPARα with its DNA recognition site is
markedly enhanced by ligands such as the hypotriglyceremic

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4 Abbreviations used: CoA, coenzyme A; PUFA, polyunsaturated fatty acids;
PPAR, peroxisome proliferator-activated receptor; SREBP-1, sterol regulatory
element binding protein-1; NF-Y, nuclear factor Y; HNF-4, hepatic nuclear fac-
tor-4.
PUFA Suppression of Lipogenesis: The Roles 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 enzymes 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 (4–6,24,25). The discovery of PPARα led quickly to the idea that PPARα was a “master switch” transcription factor that was targeted by PUFA to coordinately suppress genes encoding proteins of lipid synthesis and to induce genes encoding proteins of lipid oxidation. This attractive hypothesis was strengthened by reports that potent pharmacological activators of PPARα modestly reduced lipogenic gene transcription (4,20). However, PPARα does not interact with PUFA response regions identified in four different genes (3,4,6,9). Moreover, PUFA continue to suppress the transcription of hepatic lipogenic genes in PPARα mice (26). Thus, the inhibition of lipogenic gene transcription associated with PPARα activation is indirect and may simply reflect the PPARα-dependent induction of the Δ-6 desaturase pathway (9,27).

PUFA response sequences have been well characterized in only three genes: fatty acid synthase, S14 and L-type pyruvate kinase (3,4,20,28,29). The rat fatty acid synthase gene contains two independent PUFA regulatory sequences that are located between −118 and −43 and between −7250 and −7035 (M. Teran-Garcia and S. D. Clarke, unpublished data). Approximately 65 and 35% of the PUFA control can be attributed to the proximal and distal elements, respectively. Interestingly, the proximalPUFA 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) (4).

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-1 (SREBP-1), upstream stimulatory factor (USF), Sp1 and nuclear factor Y (NF-Y) (20,29). The nuclear abundance of USF and its DNA-binding activity is unaffected by dietary PUFA (20). 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 (20,29–31). SREBP-1 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 (32,33). SREBP-2 is a regulator of genes encoding proteins involved in cholesterol metabolism (32,33). 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 cholesterogenesis. SREBP-1c constitutes 90% of the SREBP-1 found in vivo and is a determinant of lipogenic gene transcription (32,33).

SREBP-1 is synthesized as a 125-kDa precursor protein that is anchored in the endoplasmic reticulum membrane (32,33). 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 (33). Once released, mature SREBP-1 translocates to the nucleus and binds to the classical sterol response element and/or to a palindrome CATG sequence. In the case of fatty acid synthase, SREBP-1 interacts with a CATG palindrom that also functions as an insulin response element (32). Overexpression of mature SREBP-1a in liver is associated with high rates of fatty acid biosynthesis, and ablation of the SREBP-1 gene results in low expression of lipogenic genes (32,33). These observations led us to the hypothesis that PUFA inhibit lipogenic gene transcription by impairing the proteolytic release of SREBP-1c and/or by suppressing SREBP-1c gene expression. 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 by 65 and 90% and by 60 and 75%, respectively (20). The decrease in SREBP-1 was accompanied by a comparable decrease in the transcription rate of hepatic fatty acid synthase (20). Unlike PUFA, saturated and monounsaturated fatty acids had no effect on the nuclear content or precursor content of SREBP-1 or on lipogenic gene expression (20,29–31,34). 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 and stearoyl-CoA desaturase (4). 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 via a two-phase mechanism. The first phase is a rapid (<60-min) inhibition of the proteolytic release process (34). The second phase involves an adaptive (~48-h) reduction in the hepatic content of SREBP-1 mRNA that is subsequently followed by a reduction in the amount of precursor SREBP-1 protein (20,35). 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 through post-transcriptional mechanisms (20,35). Using rat liver cells in primary culture, we determined that PUFA reduced the half-life of SREBP-1c mRNA from 11 h to <5 h (35). The mechanism by which PUFA control the half-life of SREBP-1 is unknown but may require the synthesis of a rapidly turning over PUFA-dependent protein (35).

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 (32,36). 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 (4). The binding sites for NF-Y are essential for fatty acid synthase (M. Teran-Garcia and S. D. Clarke, unpublished data) and S14 promoter activity (4). 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 (4). 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% (M. Teran-Garcia and S. D. Clarke, unpublished data). In contrast, eliminating the SREBP-1 site (~67 to ~53) reduced fatty acid synthase promoter activity by only 40%. More important, only 35% of the PUFA inhibition of fatty acid synthase promoter activity was lost with the SREBP-1 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 (M. Teran-Garcia and S. D. Clarke, 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 indicated that PUFA exert a negative influence on the carbohydrate response element of the 8.4-kb rat pyruvate kinase (4) and fatty acid synthase genes (M. Teran-Garcia and S. D. Clarke, 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-4 (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 (4). Like PPARα, HNF-4 has a ligand binding domain that interacts with acyl-CoA esters, but unlike PPARα, fatty acyl-CoA binding to HNF-4 decreases its DNA-binding activity (37). This suggests that PUFA may exert part of their negative influence on gene transcription by reducing HNF-4 DNA-binding activity. Linker scanner mutations through the response element 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 (4). 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 (38). Subsequent studies have demonstrated that the –7242 to –7150 sequence contains DNA recognition sites for HNF-4 and a novel carbohydrate response factor (38). Moreover, deleting this sequence eliminated 30–40% of the total PUFA suppression of the fatty acid synthase promoter (M. Teran-Garcia and S. D. Clarke, unpublished data). Thus, PUFA may exert part of their suppressive effects on gene transcription by interference with the glucose-mediated trans-activation processes that in part involve reducing HNF-4 DNA-binding activity.

Summary. For nearly 40 y, PUFA have been known to uniquely suppress lipid synthesis. PUFA, particularly (n-3), accomplish this by coordinating an up-regulation of lipid oxidation and a down-regulation of lipid synthesis. In other words, PUFA function as metabolic fuel repartitioners. The outcome is an improvement in the symptoms of the metabolic syndrome and a reduced risk of heart disease. PUFA control these 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. PUFA increase the fatty acid oxidative capacity of tissues through their ability to function as ligand activators of PPARα and thereby induce the transcription of several genes encoding proteins affiliated with fatty acid oxidation.

PUFA suppress lipid synthesis by inhibiting transcriptional factors that mediate the insulin and carbohydrate control of lipogenic and glycolytic genes. With respect to the insulin response element, 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.
LITERATURE CITED


