THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 274, No. 33, Issue of August 13, pp. 23577–23583, 1999

Printed in U.S.A.

Sterol Regulatory Element Binding Protein-1 Expression Is Suppressed by Dietary Polyunsaturated Fatty Acids

A MECHANISM FOR THE COORDINATE SUPPRESSION OF LIPOGENIC GENES BY POLYUNSATURATED FATS*

(Received for publication, March 5, 1999, and in revised form, May 19, 1999)

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Polyunsaturated fatty acids (PUFA) coordinately suppress the transcription of a wide array of hepatic lipogenic genes including fatty acid synthase (FAS) and acetyl-CoA carboxylase. Interestingly, the over-expression of sterol regulatory element binding protein-1 (SREBP-1) induces the expression of all of the enzymes suppressed by PUFA. This observation led us to hypothesize that PUFA coordinately inhibit lipogenic gene transcription by suppressing the expression of SREBP-1. Our initial studies revealed that the SREBP-1 and FAS mRNA contents of HepG2 cells were reduced by 20:4(*n*-6) in a dose-dependent manner (*i.e.* EC_{50} ~10 μM), whereas 18:1(*n*-9) had no effect. Similarly, supplementing a fat-free, high glucose diet with oils rich in (*n*-6) or (*n*-3) PUFA reduced the hepatic content of precursor and nuclear SREBP-1 60 and 85%, respectively; however, PUFA had no effect on the nuclear content of upstream stimulatory factor (USF)-1. The PUFA-dependent decrease in nuclear content of mature SREBP-1 was paralleled by a 70–90% suppression in FAS gene transcription. In contrast, dietary 18:1(*n*-9), *i.e.* triolein, had no inhibitory influence on the expression of SREBP-1 or FAS. The decrease in hepatic expression of SREBP-1 and FAS associated with PUFA ingestion was mimicked by supplementing the fat-free diet with the PPARα-activator, WY 14,643. Interestingly, nuclear run-on assays revealed that changes in SREBP-1 mRNA abundance were not accompanied by changes in SREBP-1 gene transcription. These results support the concept that PUFA coordinately inhibit lipogenic gene transcription by suppressing the expression of SREBP-1 and that the PUFA regulation of SREBP-1 appears to occur at the post-transcriptional level.

Dietary polyunsaturated fatty acids (PUFA)[1] are effective hypolipidemic agents (1), and they exert this effect by coordinately suppressing hepatic lipid synthesis and secretion while inducing hepatic and skeletal muscle fatty acid oxidation (2–10). Dietary PUFA coordinately decrease the transcription of hepatic genes encoding glycolytic and lipogenic enzymes (fatty acid synthase, acetyl-CoA carboxylase, stearoyl-CoA desaturase, malic enzyme, L-pyruvate kinase, and glucokinase) (3, 11–15), whereas they concomitantly increase the transcription of genes encoding enzymes involved in fatty acid oxidation (carnitine palmitoyltransferase (16) and acyl-CoA oxidase (9)). The outcome is a decrease in hepatic lipogenesis and an increase in hepatic fatty acid oxidation and ketogenesis. Genes encoding the oxidative enzymes appear to be regulated by a common transcription factor, peroxisomal proliferator-activated receptor (PPAR) (9, 16–21). Because PPARs are lipid-activated transcription factors, they have often been proposed as the "master switches" that regulate the expression of enzymes involved in lipid synthesis and degradation (19–21). However, several lines of evidence, including studies with PPARα knock-out mice, indicate that the PUFA suppression of lipogenic gene transcription does not directly involve PPARα (22, 23).

Despite several years of investigation, the molecular mechanisms responsible for the PUFA regulation of genes encoding enzymes of lipid synthesis remain poorly defined. Functional mapping studies have identified candidate response regions in the S14 (24), pyruvate kinase (14), and stearoyl-CoA desaturase genes (25), but the identity of the transcription factors affected by PUFA remain unclear. Recently, sterol regulatory element binding protein-1 (SREBP-1) was identified as a transcription factor that appears to play a pivotal role in the expression of lipogenic genes (26–30). SREBPs are transcription factors that were first isolated as a result of their properties for binding to the sterol regulatory element and conferring sterol regulation to several genes involved with cholesterol synthesis (31). SREBPs are synthesized as 125-kDa precursor proteins that contain two transmembrane domains for insertion into the endoplasmic reticulum membrane (31). The N-terminal domain, which is a 68-kDa, helix-loop-helix leucine zipper transcription factor (*i.e.* mature SREBP), is released for nuclear translocation by a sterol-dependent proteolytic cascade (31). Over-expression of mature SREBP-1 in transgenic mice greatly increases the hepatic abundance for numerous lipogenic enzymes including fatty acid synthase and acetyl-CoA carboxylase (28, 30). Moreover, the nuclear abundance of SREBP-1 has been found to be reduced by fasting and greatly increased by carbohydrate refeeding (29). In addition, changes in the nuclear content of SREBP-1 resulting from starving-refeeding displayed a temporal pattern that was similar to the pattern of change oberved for fatty acid synthase gene transcription (11, 29). Further support for the role of SREBP-1 in lipogenic gene

* This work was supported by National Institutes of Health Grants DK 52573 and DK 53872 (to S. D. C.) and DK 09723 (to M. T. N.) and by the sponsors of the M. M. Love Chair in Nutritional, Cellular and Molecular Sciences at the University of Texas at Austin (to S. D. C.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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[1] The abbreviations used are: PUFA, polyunsaturated fatty acids; FAS, fatty acid synthase; SREBP, sterol regulatory element binding protein; PPAR, peroxisomal proliferator activated receptor; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; USF-1, upstream stimulatory factor-1.

expression was demonstrated by the discovery that the region of −71 to −54 of the rat fatty acid synthase appears to contain SREBP-1 response sequences and that binding of SREBP-1 to this region enhances fatty acid synthase gene promoter activity (26). In light of these collective data, we hypothesized that PUFA coordinately suppress the transcription of hepatic lipogenic and glycolytic genes by suppressing the expression of SREBP-1. In this report, we demonstrate that PUFA reduced the hepatic concentration of precursor and mature SREBP-1 protein and concomitantly lowered the hepatic abundance of SREBP-1 mRNA. Moreover, the reduction in SREBP-1 protein was paralleled by a comparable decrease in the transcription of hepatic fatty acid synthase.

EXPERIMENTAL PROCEDURES

In Vivo and in Vitro Regulation of SREBP-1 and Fatty Acid Synthase Gene Expression by Fatty Acids—The impact of fatty acids on the hepatic expression of SREBP-1 and fatty acid synthase was examined in HepG2 cells treated with varying concentrations of albumin-bound 18:1(*n*-9) or 20:4(*n*-6) and in rats fed a high carbohydrate diet containing triolein, safflower oil, or fish oil. HepG2 cells (ATCC no. HB-8065) were plated onto dry collagen-coated tissue culture plates and maintained in minimum essential medium (Life Technologies) supplemented with 2 mM L-glutamine, 1 mM pyruvate, and 10% fetal bovine serum (32). After the cells reached confluence, serum was removed from the medium. After 48 h of serum stavation, 1 μM insulin and dexamethasone were added to the medium, and the cells (n = 4 plates per treatment) were subsequently treated with 0, 10, 50, 100, or 200 μM albumin-bound (fatty acid/albumin molar ratio 4/1) 18:1(*n*-9) or 20:4(*n*-6) (Nu-Chek Prep) for 24 h. Total RNA was extracted for Northern analyses using phenol-guanidinium isothiocyanate (33). To examine the influence that dietary PUFA exert on SREBP-1 and fatty acid synthase gene expression, male Harlan Sprague-Dawley rats (Harlan Sprague-Dawley) were adapted to a 3 h/day meal-feeding regimen (2) using a high glucose, fat-free diet (Dyets). After a 7-day adaptation period, the rats were randomly assigned to dietary treatments (n = 4–5 rats per diet) that consisted of the fat-free diet; the fat-free diet supplemented (10 g/100 g of diet) with triolein (99% 18:1(*n*-9)), safflower oil (65% 18:2(*n*-6)), or sterol-free, menhaden fish oil (35% 20:5 and 22:6(*n*-3)); or the fat-free diet supplemented with 0.1% WY 14,643 (Chemsyn Science Labs). Following an additional 5-day feeding period, rats were killed immediately after the last 3-h meal. This dietary design was employed in three separate dietary studies.

The abundance of a variety of hepatic transcripts described in the figures was determined by Northern analysis using total RNA extracted by the phenol-guanidinium isothiocyanate procedure (2, 33). The cDNA probes for hybridization were labeled with [α-^{32}P]dCTP (NEN Life Science Products) using either polymerase chain reaction radiolabeling or random prime labeling (Life Technologies). Corrections for variations in RNA loading of each lane were made using glyceraldehyde-3-phosphate dehydrogenase mRNA as the reference transcript.

Nulcear Run-on Assays—The impact of various dietary fats on the *in vivo* transcription of the hepatic fatty acid synthase, SREBP-1, SREBP-2, and acyl-CoA oxidase was determined by nuclear run-on assays using nuclei isolated from the livers of rats from the various dietary treatments (11, 34, 35). Briefly, liver was homogenized in 20 ml of Buffer A (15 mM Tris, pH 7.5, 60 mM KCl, 15 mM NaCl, 0.15 mM spermidine, 0.5 mM dithiothreitol, 2 mM EDTA, and 0.5 mM EGTA) containing 0.34 M sucrose. The homogenate was centrifuged at 600 × g for 10 min, and the resulting pellet was resuspended in 10 ml of Buffer B (Buffer A containing 1 M sucrose and 0.5% Triton X-100). The resuspended pellet was centrifuged for 10 min at 2500 × g. The resulting pellet was resuspended in 20 ml of Buffer C (Buffer A containing 0.25 M sucrose, 0.1 mM EDTA, 0.1 mM EGTA, and 0.5% Triton X-100) and centrifuged for 10 min at 2500 × g. The nuclear pellet was then resuspended in nuclear storage buffer (40% glycerol, 75 mM Hepes, pH 7.5, 60 mM KCl, 15 mM NaCl, 0.15 mM spermidine, 0.5 mM spermine, 0.5 mM dithiothreitol, 0.1 mM EDTA, and 0.1 mM EGTA) and the nuclei were stored at −80 °C. The trancription assay was conducted by incubating the nuclei with transcription reaction mixture for 20 min at 26 °C in a 300-μl reaction volume. The final reaction mixture contained 20% glycerol, 75 mM Hepes (pH 7.5), 60 mM KCl, 15 mM NaCl, 3 mM $MgCl_2$, 1 mM spermidine, 0.5 mM spermine, 0.5 mM dithiothreitol, 0.5 mM EDTA, 0.1 mM EGTA, 260 units/ml RNasin (Promega), 10 μM creatine phosphate, 16 units/ml creatine kinase, 0.5 mM CTP, 0.5 mM GTP, 1 mM ATP, 0.1 μM UTP, and 100 μCi [α-^{32}P]UTP (NEN Life Science Products). Labeled nascent RNA transcripts were extracted with organic phenol/chloroform and precipitated by ethanol. Nascent transcripts for fatty acid synthase, acyl-CoA oxidase, SREBP-1, and SREBP-2 were quantified by their hybridization to their respective cDNAs. RNA hybrids were quantified by cutting out each slot and counting the membrane slots by liquid scintillation counting.

Cellular Abundance of Precursor / Mature SREBP-1 and USF-1—The effect of dietary fat on the membrane (precursor) and nuclear (mature) content of SREBP-1 was determined by isolating microsomal and nuclear proteins from liver freshly removed from rats fed the various types of fat described previously (36, 37). To prevent proteolysis of precursor and mature SREBP-1, all buffers contained 50 μg/ml *N*-acetyl-leucyl-leucyl-norleucinal, 24 μg/ml pefabloc, 5 μg/ml pepstatin A, 10 μg/ml leupeptin, and 2 μg/ml aprotinin. Briefly, liver was homogenized in 30 ml of Buffer A (10 mM Hepes (pH 7.6), 25 mM KCl, 1 mM sodium EDTA, 2 M sucrose, 10% (v/v) glycerol, 0.15 mM spermine, 2 mM spermidine, and protease inhibitors). The homogenate was layered over a 10-ml cushion of Buffer A and was centrifuged in a SW-27 rotor (Beckman) at 75,000 × g for 1 h at 4 °C. The resulting pellet was resuspended in 1 ml of Buffer B (10 mM Hepes, pH 7.6, 100 mM KCl, 2 mM $MgCl_2$, 1 mM EDTA, 1 mM dithiothreitol, 10% (v/v) glycerol, and protease inhibitors). After the addition of ammonium sulfate (4 M, pH 7.9) the suspension was centrifuged at 257,000 × g for 45 min at 4 °C. The resulting supernatant was collected as nuclear protein extract. For membrane protein extraction, the liver was homogenized in 20 mM Tris/HCl, pH 8, 150 mM NaCl, and 1 mM $CaCl_2$ plus protease inhibitors. The homogenate was centrifuged at 800 × g for 10 min at 4 °C. Microsomal membranes were collected by centrifuging the 800 × g supernatant for 1 h, 100,000 × g at 4 °C (SW-55 rotor, Beckman). The pellet was rinsed briefly with homogenization buffer and subsequently resuspended in 1.5 ml of 250 mM Tris/HCl (pH 6) and 2 mM $CaCl_2$ plus protease inhibitors. The membrane proteins were extracted by adding an equal volume of 2 mM $CaCl_2$, 320 mM NaCl, 2% Triton X-100,and protease inhibitors to the membrane pellet, mixing, and subsequently centrifuging for 45 min at 100,000 × g at 4 °C. The abundance of SREBP-1 and USF-1 was determined by Western blotting (36–38) following the procedure provided for the enhanced chemiluminescence Western blotting detection system kit (Amersham Pharmacia Biotech). Immunoreactive SREBP-1 was identified using monoclonal anti-SREBP-1 (IgG-2A4) prepared from hybridoma cells (ATCC, CRL 2121), and immunoreactive USF-1 was identified by incubating with buffer containing 0.1 μg/ml anti-USF-1 (Santa Cruz Biotechnology). Bands were quantified for relative intensity using the Ambis imaging system.

RESULTS

SREBP-1 and Fatty Acid Synthase Expression in HepG2 Cells Are Suppressed by 20:4(n-6)—The possibility that PUFA may inhibit the expression of SREBP-1 was initially examined by treating HepG2 cells with varying concentrations of 20:4(*n*-6). The pattern for the dose-dependent reduction in SREBP-1 and fatty acid synthase mRNA abundance elicited by 20:4(*n*-6) was almost identical for both transcripts (Fig. 1). Maximum reduction in SREBP-1 and fatty acid synthase mRNA occurred at approximately 50 μM. The amount of 20:4(*n*-6) required to achieve a 50% reduction in both SREBP-1 and fatty acid synthase mRNA was approximately 10–15 μM (Fig. 1), which is within the physiological range for the plasma unesterfied 20:4(*n*-6). The expression of SREBP-1 and fatty acid synthase in HepG2 cells was not inhibited by 18:1(*n*-9), which was consistent with numerous dietary studies showing that monounsaturated fatty acids do not suppress lipogenic gene transcription (2, 3, 5). The suppression of SREBP-1 and fatty acid synthase expression by 20:4(*n*-6) was paralleled by a marked decrease in fatty acid synthase promoter activity (data not shown).

Dietary PUFA Reduce the Amount of Hepatic SREBP-1 Protein and mRNA—To ascertain if the PUFA suppression of SREBP-1 expression extended to the intact animal, rats were fed a fat-free, high glucose diet that was supplemented with a variety of fats that varied in number and location of the double bonds. Relative to the fat-free diet, both safflower oil, which contains 65% of its fatty acid as 18:2(*n*-6), and sterol-free fish oil, which is rich in 20:5 and 22:6(*n*-3), significantly reduced the microsomal content of precursor SREBP-1 (Fig. 2, *A* and *C*) and

the nuclear content of mature SREBP-1 (Fig. 2, *B* and *C*). On the other hand, supplementing the fat-free diet with triolein, which provided only 18:1(*n*-9), had no effect on the amount of either the precursor or the mature form of SREBP-1 (Fig. 2). In general, the nuclear content of SREBP-1 reflected the amount of precursor SREBP-1 (Fig. 2). However, the ingestion of cholesterol-free fish oil was associated with an 85% decrease in the nuclear content of mature SREBP-1, whereas the content of membrane-bound precursor SREBP-1 was reduced only 60% (Fig. 2). These data suggest that fish oils may impair the proteolytic release of mature SREBP-1. Neither triolein nor PUFA altered the nuclear content of USF-1 (Fig. 3).

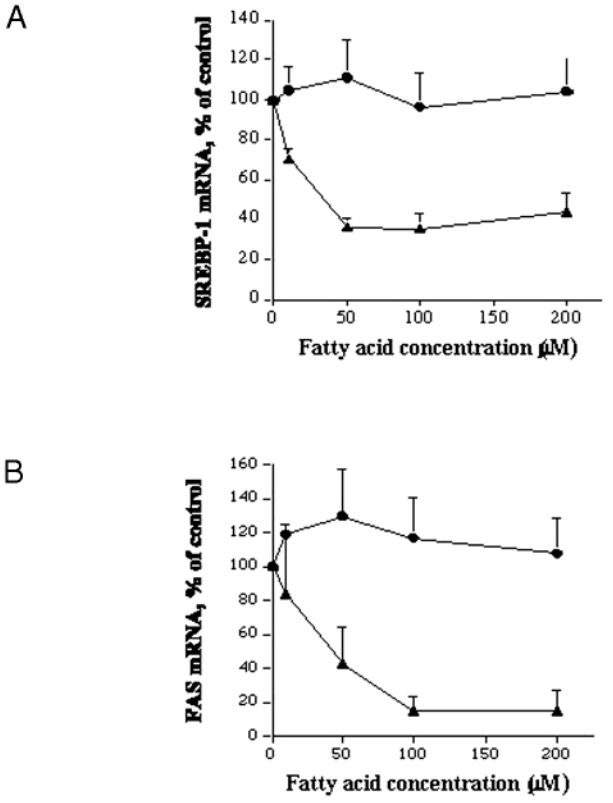

FIG. 1. **SREBP-1 and fatty acid synthase (FAS) mRNA abundance was suppressed by 20:4(*n*-6) in a dose-dependent manner.** *A* and *B* depict the changes in the abundance of SREBP-1 and FAS mRNA in HepG2 cells treated for 24 h with 0, 10, 50, 100, and 200 μM albumin-bound 18:1(*n*-9) ( ) or 20:4(*n*-6) (▲). Data are expressed relative to the level of SREBP-1 and FAS mRNA in cells treated with no fatty acid. Data are means ± S.E.; $n = 4$ plates/point.

The PUFA-dependent reduction in hepatic content of precursor and mature SREBP-1 was accompanied by a comparable decrease in the amount of hepatic SREBP-1 mRNA (Fig. 4). Moreover, the hepatic abundance of SREBP-1 mRNA was positively correlated with the hepatic abundance of fatty acid synthase mRNA (Fig. 4).

Is the Suppression of SREBP-1 Expression by PUFA Mediated by PPARα?—Although the PUFA suppression of lipogenic genes does not appear to directly involve a PPARα-mediated mechanism (22, 23), PUFA activation of hepatic PPARα could be responsible for the suppression of SREBP-1 expression. Such a mechanism would provide a unifying explanation for how PUFA induce genes of hepatic lipid oxidation and concomitantly suppress genes of lipogenesis. To examine this hypothesis, the expression of SREBP-1 was examined in rats fed the fat-free diet supplemented with WY 14,643, a potent activator of PPARα. As expected, the ingestion of WY 14,643 greatly increased the level of mRNA for the PPARα-regulated gene, peroxisomal acyl-CoA oxidase (Fig. 5*B*). Consistent with the possibility that PUFA suppressed SREBP-1 expression by functioning as ligand activators for PPARα, we found that the hepatic level of SREBP-1 was reduced 50% by the ingestion of the PPARα-specific activator WY 14,643 (Fig. 5*A*). Moreover, the decrease in hepatic abundance of SREBP-1 mRNA was paralleled by a significant reduction in the hepatic abundance of fatty acid synthase mRNA (Fig. 5).

Do Dietary PUFA and WY 14,643 Suppress SREBP-1 Gene Transcription?—Nuclear run-on assays were employed to determine whether the reduction in the hepatic abundance of fatty acid synthase and SREBP-1 mRNA was accompanied by a decrease in fatty acid synthase and SREBP-1 gene transcription (Table I and Fig. 6). Consistent with our earlier observations (11), hepatic fatty acid synthase gene transcription was markedly reduced by the ingestion of PUFA, whereas the con-

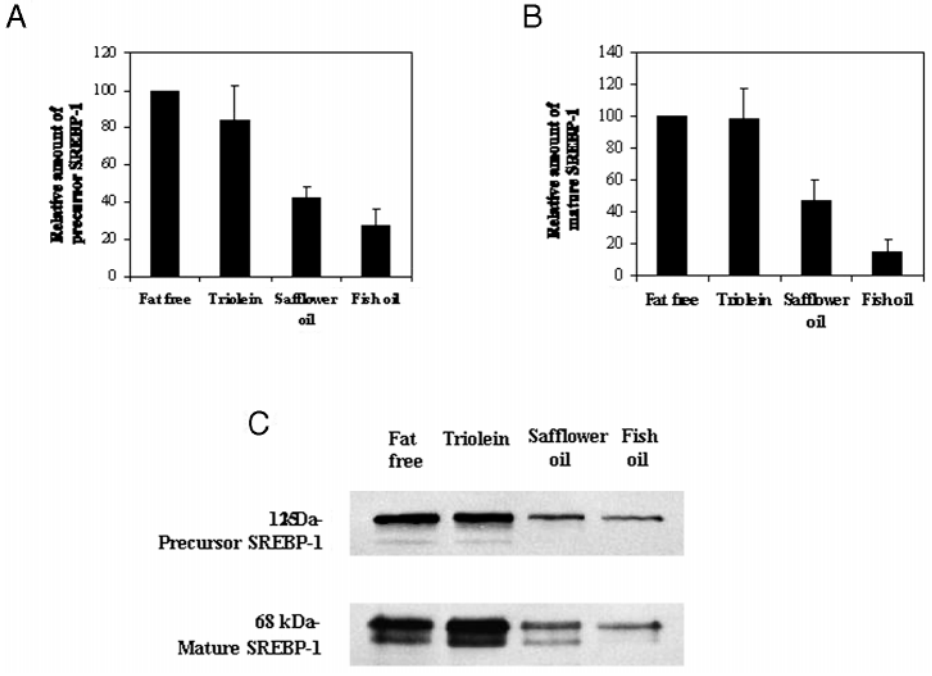

FIG. 2. **Dietary (*n*-6) and (*n*-3) PUFA suppressed the hepatic content of precursor and mature SREBP-1.** *A* and *B*, the influence of dietary oils rich in (*n*-9), (*n*-6), and (*n*-3) fatty acids (*i.e. Triolein*, *Safflower oil*, and *Fish oil*, respectively) on the hepatic concentration of precursor (*A*) and mature SREBP-1 (*B*) was determined using Western blot analysis. Values are expressed relative to the abundance of precursor and mature SREBP-1 found in the livers of rats fed the fat-free diet. Data are expressed as the mean ± S.E.; $n = 4$ rats/treatment. Similar results were observed in three different dietary studies. *C*, a representative Western (25 μg protein/lane) for precursor (membrane-associated) and mature (nuclear) SREBP-1.

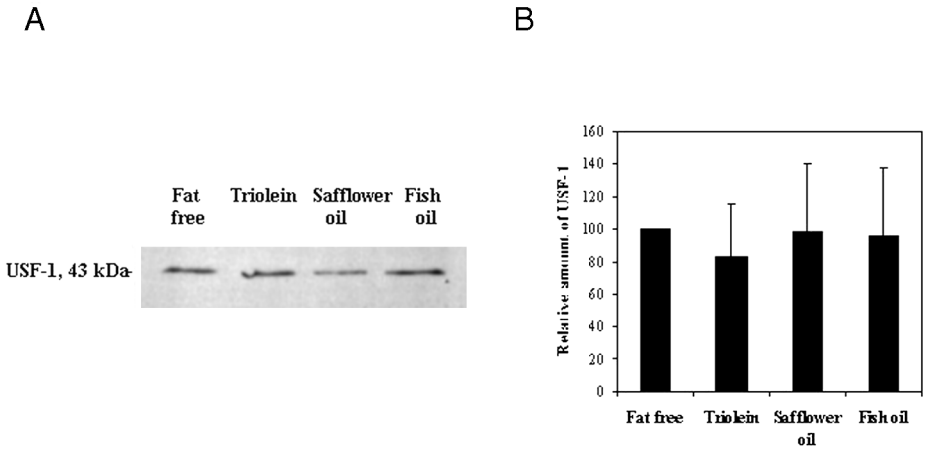

FIG. 3. **The hepatic abundance of nuclear USF-1 was unaffected by dietary (*n*-6) and (*n*-3) PUFA.** The influence of dietary oils rich in (*n*-9), (*n*-6), and (*n*-3) fatty acids (*i.e. Triolein*, *Safflower oil*, and *Fish oil*, respectively) on the hepatic concentration of USF-1 was determined by Western analysis using the same nuclear protein extracts depicted in Fig. 2. *A*, a representative USF-1 Western (25 μg protein/lane). *B*, hepatic content of USF-1 relative to that observed in rats fed the fat-free diet. Data are means ± S.E.; n = 4 rats/ treatment. Similar results were observed in two separate dietary studies.

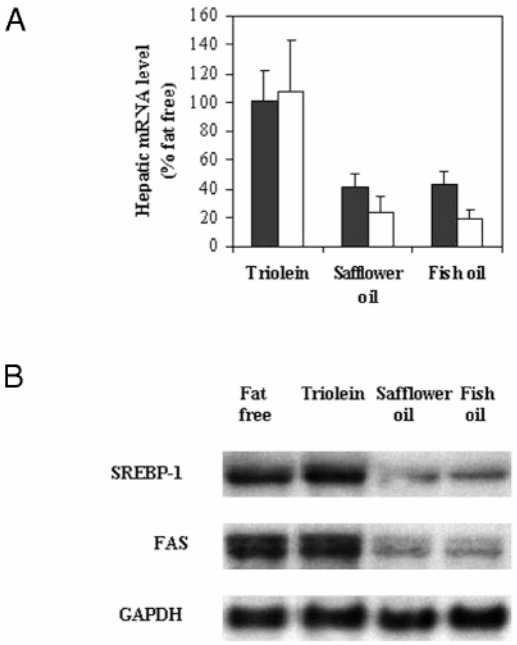

FIG. 4. **Dietary (*n*-6) and (*n*-3) PUFA suppress the hepatic abundance of SREBP-1 and fatty acid synthase mRNA.** *A*, the hepatic abundance of mRNA for SREBP-1 (*black bars*) and fatty acid synthase (FAS) (*white bars*) in rats fed dietary oils rich in (*n*-9), (*n*-6), and (*n*-3) fatty acids (*i.e. Triolein*, *Safflower oil*, and *Fish oil*, respectively). Data are expressed relative to the values found in rats fed the fat-free diet, and are means ± S.E.; n = 4 rats/treatment. The hepatic content of precursor and mature SREBP-1 protein within these same animals is depicted in Fig. 2. *B*, a representative Northern blot (30 μg of RNA/lane) using equal amounts of RNA pooled from each of the 4 rats within a cited dietary group.

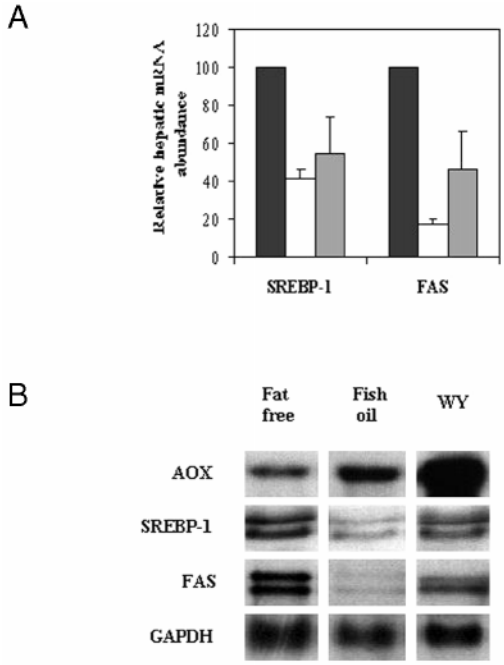

FIG. 5. **Ingestion of the PPARα activator WY 14,643 reduced the hepatic abundance of SREBP-1 and fatty acid synthase mRNA.** *A*, relative abundance of SREBP-1 and FAS mRNA in rats fed the fat-free diet (*black bars*) or the fat-free diet supplemented with 10% menhaden fish oil (*white bars*) or 0.1% WY 14,643 (*gray bars*). Data are expressed as means ± S.E.; n = 4 rats/treatment. *B*, a representative Northern blot (30 μg/lane). *WY*, WY 14,643.

sumption of 18:1(*n*-9) had no effect (Table I and Fig. 6). Interestingly, WY 14,643 suppressed fatty acid synthase gene transcription approximately 70%, which was similar to the degree of suppression associated with the consumption of safflower oil (Table I and Fig. 6). On the other hand, WY 14,643 induced the transcription of acyl-CoA oxidase severalfold. which is consistent with prior findings that ligand activation of PPARα induces peroxisomal acyl-CoA oxidase promoter activity (18). Despite the fact that PUFA and WY 14,643 both significantly reduced the hepatic abundance of SREBP-1 mRNA, SREBP-1 gene transcription was not suppressed by the ingestion of either PUFA or WY 14,643 (Table I and Fig. 6). Similarly, SREBP-2 gene transcription was not inhibited by either PUFA or WY 14,643 (Table I and Fig. 6). These data suggest that PUFA and WY 14,643 may govern the level of SREBP-1 mRNA by regulating the stability of SREBP-1 and -2 transcripts.

DISCUSSION

Supplementing a high carbohydrate diet with oils rich in (*n*-6) and (*n*-3) PUFA results in an inhibition of hepatic gene transcription for a wide array of lipogenic enzymes including fatty acid synthase, acetyl-CoA carboxylase, citrate lyase, malic enzyme, and stearoyl-CoA desaturase (3, 11–15). Maximum inhibition of gene expression occurs when the diet contains approximately 20% of its calories as PUFA, but as little as 5% of the dietary energy as PUFA is sufficient to inhibit lipogenic gene expression 50% (39). The dose-response curve and the time course for the PUFA inhibition of gene expression indicate that PUFA coordinately regulate all lipogenic genes, which further suggests that these genes may share a common transcriptional control point (2, 3, 6, 39). In our search for a "master switch" mechanism to explain the PUFA regulation of lipogenic gene expression, we were intrigued by the reports that overexpression of the transcription factor SREBP-1 in transgenic mice was accompanied by a large increase in the expression of

several hepatic lipogenic enzymes including fatty acid synthase and acetyl-CoA carboxylase (28, 30). Moreover, the suppression and induction of hepatic lipogenic gene transcription observed with starving and starving-refeeding appeared to follow a temporal pattern that paralleled the decrease and increase in nuclear abundance of mature SREBP-1 (29). Both of these scenarios suggested that the nuclear content of mature SREBP-1 might be the key determinant that coordinates the up- and down-regulation of genes encoding a wide array of lipogenic enzymes, *e.g.* fatty acid synthase. Consistent with this idea we found that feeding a diet rich in (*n*-6) or (*n*-3) fatty acids reduced the hepatic nuclear content of SREBP-1 protein 50 and 85%, respectively. More importantly, the decrease in the nuclear content of mature SREBP-1 was paralleled by comparable decreases in fatty acid synthase gene transcription and mRNA abundance (Figs. 4 and 5). On the other hand, supplementing the fat-free diet with 18:1(*n*-9), *i.e.* triolein, did not decrease the nuclear content of mature SREBP-1 nor did it reduce the expression of fatty acid synthase, which was very consistent with numerous reports demonstrating that saturated and monounsaturated fatty acids do not possess the ability to suppress hepatic lipogenic gene transcription (2–6, 11–15).

The nuclear content of mature SREBP-1 is dependent upon the synthesis of SREBP-1 precursor and/or the proteolytic release of the mature SREBP-1 from its precursor (31). SREBP-1 is synthesized as a 125-kDa precursor that contains two transmembrane domains that allow the protein to be anchored in the membrane of the endoplasmic reticulum (31). The 480-amino acid N-terminal domain corresponds to the mature SREBP-1 transcription factor and is released from the endoplasmic reticulum membrane by a two-step proteolytic cascade (31). Recent reports indicate that fatty acids, including 18:1(*n*-9) and (*n*-6)/(*n*-3) PUFA, may possibly enhance the sterol suppression of the proteolytic cascade and/or directly inhibit the SREBP-1 proteolytic cascade (40, 41). The consequence of this fatty acid regulation was found to be a decrease in the nuclear content of mature SREBP-1 but no detectable change in membrane content of precursor SREBP-1 (40, 41). However, our results do not appear to fully support these conclusions. First, the decrease in the nuclear content of mature SREBP-1 associated with PUFA ingestion was paralleled by a comparable reduction in the membrane content of precursor SREBP-1 (Fig. 2). This finding indicates that PUFA primarily function as suppressors of SREBP-1 precursor synthesis rather than as regulators of the proteolytic release of mature SREBP-1. Consistent with this conclusion was our observation that the reduction in the amount of precursor SREBP-1 protein was nearly identical to the reduction in hepatic SREBP-1 mRNA abundance (Figs. 2–4). Second, unlike the observations of Worgall *et al.* (40), we found that treating HepG2 cells with 18:1(*n*-9) did not suppress the expression of either SREBP-1 or fatty acid synthase. These results were very consistent with our observations that 18:

TABLE I

Effects of dietary PUFA and WY 14,643 on hepatic gene transcription activity

Nuclear run-on assays were performed to determine the influence of triolein, safflower oil, fish oil, or WY 14,643 on the transcription of SREBP-1, SREBP-2, FAS, and peroxisomal acyl-CoA oxidase (AOX). Transcription (dpm/transcript/10^6 dpm total RNA) for SREBP-1, SREBP-2, FAS, and AOX in rats fed the fat-free diet was 12 ± 3, 4 ± 1, 127 ± 15, and 8 ± 5, respectively. The responses to dietary fats and WY 14,643 are expressed as a percentage of the fat-free values after correcting for nonspecific hybridization to the pBS vector. Data are means ± S.E.; n = 5 rats/treatment.

Hepatic gene transcript	Transcription activity			
	Triolein	Safflower oil	Fish oil	WY 14,643
SREBP-1	125 ± 19	118 ± 36	104 ± 22	96 ± 14
SREBP-2	80 ± 20	168 ± 52	92 ± 15	84 ± 39
FAS	106 ± 16	34 ± 8	12 ± 2	33 ± 6
AOX	84 ± 14	135 ± 41	100 ± 21	1576 ± 223

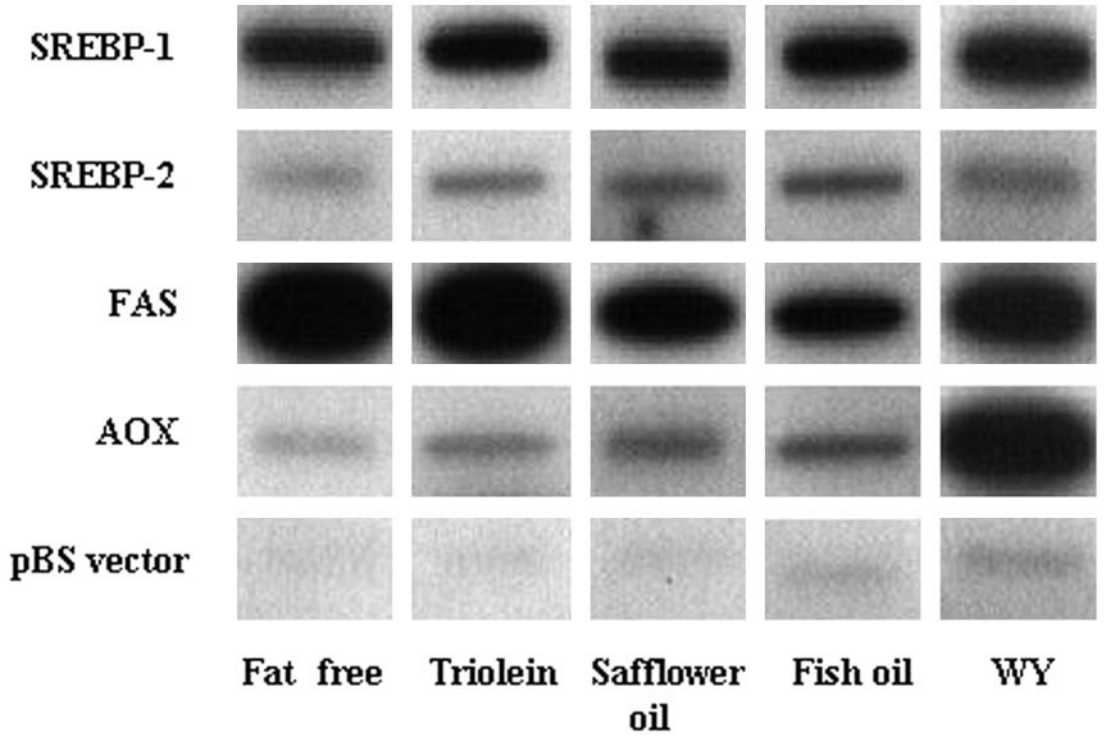

FIG. 6. **The reduction in hepatic abundance of SREBP-1 mRNA associated with the ingestion of PUFA and WY 14,643 is not accompanied by a decrease in SREBP-1 gene transcription.** Nuclear run-on assays were conducted using nuclei isolated from rats fed a fat-free diet supplemented with oils rich in (*n*-9), (*n*-6), or (*n*-3) fatty acids (*i.e. Triolein*, *Safflower oil*, or *Fish oil*, respectively) or with WY 14,643 (*WY*). *AOX*, acyl-CoA oxidase. A summary of the nuclear run-on assays is presented in Table I.

1(*n*-9) did not reduce the hepatic content of mature SREBP-1,nor did 18:1(*n*-9) suppress the hepatic expression of SREBP-1 and fatty acid synthase. Our results were consistent with the vast amount of *in vivo* and *ex vivo* data demonstrating that 18:1(*n*-9) has no effect on the expression of a wide array of glycolytic and lipogenic enzymes (3, 5, 13–15, 39). Differences in methodological approaches may explain the differences in outcomes between our studies and those of Worgall *et al.* (40). First, our HepG2 cells were serum-starved for 48 h prior to transfection, and they were treated with both insulin and glucocorticoid. This may have enhanced triglyceride synthesis and secretion, which in turn may have decreased the inhibitory influence of 18:1(*n*-9). Second, our HepG2 cells were grown on collagen, which allows them to form monolayers. Finally, and most important, we did not transfect the cells until they had reached confluence, because we have found that if nonconfluent HepG2 cells are treated with albumin-bound free fatty acids a large release of intracellular lactate dehydrogenase occurs, and such cell damage occurs even though β-galactosidase expression remains unaffected (unpublished data).

PUFA are potent ligand activators of a family of nuclear transcription factors called PPARs (16–21). The dominant PPAR in the liver is PPARα, and fatty acid activation of PPARα appears to coordinately induce genes encoding enzymes involved in fatty acid oxidation and ketogenesis (8, 16–18). PUFA activation of PPARs has also been proposed to suppress the expression of lipogenic genes, but direct involvement of PPARs in the PUFA suppression of lipogenic gene expression has not been demonstrated (22, 23). However, it is possible that PUFA activation of PPARα could lead to the suppression of a pivotal transcription factor (*e.g.* SREBP-1) that is common to all lipogenic enzymes and, in this way, indirectly lead to the inhibition of lipogenic gene expression. Consistent with this hypothesis, we found that feeding rats the potent PPARα-specific activator WY 14,643 reduced the hepatic abundance of hepatic SREBP-1 mRNA to a level comparable with that found in rats fed diets containing PUFA (Fig. 5). Moreover, the decrease in SREBP-1 mRNA associated with the ingestion of WY 14,643 was accompanied by a marked decrease in fatty acid synthase gene transcription (Fig. 5). These data suggest that ligand activation of PPARα may play a role in the suppression of lipogenic genes via PPARα regulation of SREBP-1 expression. Such a conclusion is not consistent with the observation that dietary fish oils continued to suppress hepatic lipogenic gene transcription in PPARα null mice (23). However, one must keep in mind that the liver contains other PPAR isoforms (19), *e.g.* PPARδ, and it is very possible that PUFA continue to regulate lipogenic genes by activating PPARs other than PPARα. In addition, it is very possible that C-20 and C-22(*n*-3) PUFA of fish oil suppresses the proteolytic release of mature SREBP-1. This conclusion is based on the observation that dietary fish oil decreased the level of precursor SREBP-1 65%, whereas the amount of mature, nuclear SREBP-1 was reduced nearly 90% (Fig. 2). If the release and/or nuclear transclocation of mature SREBP-1 is in fact suppressed by the (*n*-3) PUFA of fish oil, then it would still be possible for dietary fish oil to inhibit lipogenic gene transcription even in PPARα null mice. However, it remains to be determined whether dietary fish oil will in fact lower the nuclear content of SREBP-1 in PPARα null mice.

Because PPARα is a well characterized transcription factor that regulates the transcription of several genes encoding proteins involved in lipid metabolism (16–19), we anticipated that the decrease in hepatic abundance of SREBP-1 mRNA resulting from PUFA and WY 14,643 ingestion would be accompanied by a reduction in SREBP-1 gene transcription. However, to our surprise, nuclear run-on assays revealed that neither dietary PUFA nor WY 14,643 inhibited the transcription of SREBP-1 (Table I and Fig. 6). These results suggested that PUFA and WY 14,643 may have reduced the hepatic content of SREBP-1 mRNA possibly by accelerating the rate of mRNA degradation. Although this is a speculative conclusion, there is evidence that PUFA enhance the degradation rate of mRNA for stearoyl-CoA desaturase (42), acetyl-CoA carboxylase (12), and malic enzyme (15). How PUFA and WY 14,643 may alter the stability of the SREBP-1 transcript remains to be determined. However, it is interesting to note that significant quantities of PPAR are located in the cytosol of some cells (43, 44). Thus, it is tempting to speculate that PPARs may regulate gene expression by influencing both transcriptional and post-transcriptional events.

Finally, it appears that PUFA suppress the expression of both SREBP-1a and -1c. This conclusion is based on the knowledge that nearly 50–70% of the SREBP-1 found in HepG2 cells is of the SREBP-1a type (28, 30, 31, 45), whereas approximately 70–90% of the SREBP-1 found in rodent liver is SREBP-1c (45). Consequently, even though the monoclonal antibody and cDNA used to quantify changes in SREBP-1 protein and mRNA could not distinguish between SREBP-1a and -1c, PUFA was found to reduce the expression of SREBP-1 both in HepG2 cells (*i.e.* SREBP-1a) and in the intact liver (SREBP-1c).

In conclusion, we have presented evidence demonstrating that SREBP-1 plays a key role in the PUFA regulation of lipogenic gene transcription. Specifically, we report that PUFA reduce the hepatic abundance of SREBP-1 mRNA and the membrane (precursor) and nuclear (mature) content of SREBP-1 protein by 60–85% and that this inhibition of SREBP-1 expression is paralleled by a marked decrease in the transcription of hepatic fatty acid synthase. In light of these data, and in light of the reports indicating that over-expression of mature SREBP-1 induces the expression of all of the same lipogenic enzymes that are suppressed by PUFA (10–15, 28–30), we propose that SREBP-1 is the pivotal transcription factor responsible for coordinating the PUFA suppression of lipogenic gene transcription.

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