# Suppression of fatty acid synthase promoter by polyunsaturated fatty acids

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**Abstract** **Dietary polyunsaturated fat is known to suppress expression of fatty acid synthase (FAS), a central enzyme in de novo lipogenesis. The sterol regulatory element-binding protein (SREBP) has recently been shown to be involved in this suppression. We previously reported that the first 2.1 kb of the FAS promoter are sufficient for transcriptional induction by a high carbohydrate diet as well as suppression by polyunsaturated fat in transgenic mice. Here, we first examined the DNA sequences responsible for SREBP-mediated suppression of FAS promoter activity by polyunsaturated fatty acids (PUFA) in vivo. Feeding polyunsaturated fat prevented both the low-level activation of the −278 FAS promoter which contains the −150 sterol response element (SRE), as well as the maximal activation of the longer −444 FAS promoter. We observed that ectopic expression of the activated form of SREBP in liver prevented PUFA-mediated suppression of both the endogenous FAS and FAS promoter-reporter transgene expression. We also found that the promoter region required for PUFA suppression in vivo is located between −278 to −131, where SREBP functions. Using HepG2 cells, we further examined the specific FAS promoter elements required for PUFA suppression. We found that the −150 SRE, as well as the –65 E-Box, contribute to PUFA suppression of the FAS promoter, at least in vitro.**—Moon, Y. S., M-J. Latasa, M. J. Griffin, and H. S. Sul. **Suppression of fatty acid synthase promoter by polyunsaturated fatty acids.** ***J. Lipid Res.*** **2002.** 43: **691–698.**

**Supplementary key words** SREBP • transgenic mice • −150 SRE • −65 E-box • HepG2

Fatty acid synthase (FAS) plays a central role in de novo lipogenesis in mammals (1). By the action of its seven active sites, FAS catalyzes all the reactions in the conversion of acetyl-CoA and malonyl-CoA to palmitate. FAS activity is not known to be regulated by allosteric effectors or covalent modification. However, FAS concentration is exquisitely sensitive to nutritional and hormonal status in lipogenic tissues such as liver and adipose tissue (1–3), being undetectable in the livers of fasted mice and dramatically induced upon refeeding a high carbohydrate, fat-free diet. We have shown that nutritional regulation of FAS occurs mainly via changes in FAS gene transcription (4, 5). Increased circulating insulin and decreased glucagon levels may participate in the regulation of FAS expression. Transcription of the FAS gene was not detectable in fasted or refed streptozotocin-diabetic mice but was increased by feeding a high-carbohydrate diet or by insulin administration (5). Using 3T3-L1 adipocytes, we mapped an insulin response sequence (IRS) to −71 to −50 that contains a core E-box where upstream stimulatory factor (USF)1 and USF2 can bind to mediate insulin regulation of the FAS gene (6–11). We also demonstrated that the 2.1 kb 5′-flanking sequence is sufficient for tissue-specific and hormonal/nutritional regulation of the FAS gene in the in vivo context (12). However, using transgenic mice bearing various 5′-deletion FAS promoter-chloramphenicol acetyltransferase (CAT) reporter genes, we found that the first 131 bp promoter region (containing the −65 E-Box) could not confer nutritional and insulin regulation to the CAT reporter gene in vivo (13).

More recently, we showed that two upstream 5′ promoter regions were required for transcriptional regulation of FAS in vivo. The first region, located between −278 and −131, confers FAS promoter activation upon refeeding/insulin treatment, but a second region, from −444 to −278, is required for maximal activation similar to the expression level observed in mice carrying the longer 2.1 kb FAS promoter region (14). We demonstrated that binding of sterol regulatory element binding protein (SREBP) to the −150 sterol response element (SRE) is responsible for low-level induction, whereas occupancy of the −332 E-box by USF is necessary for high-level activation of the FAS promoter. The potential role of SREBP has also been demonstrated by others when mRNA levels for FAS, along with those for other lipogenic enzymes, were detected to be higher in the livers of transgenic mice expressing a truncated active form of SREBP as compared with control mice (15–17). Furthermore, FAS induction by high carbohydrate/insulin was found to be severely impaired in USF and SREBP knockout mice, demonstrating a requirement for both of these transcription factors (18, 19).

Abbreviations: CAT, chloraphenicol acetyltransferase; FAS, fatty acid synthase; PEPCK, phosphoenolpyruvate carboxykinase; SREBP, sterol regulatory element binding protein; USF, upstream stimulatory factor.

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In contrast to a high-carbohydrate diet, polyunsaturated fat in the diet causes suppression of several genes involved in lipid metabolism, including lipogenic enzymes such as FAS (20–22). A sustained expression of mature SREBP-1 prevented suppression of lipogenic gene expression by polyunsaturated fat (23). Expression of SREBP has been shown to be suppressed by polyunsaturated fat as well. Feeding fish oil decreased the mature form of SREBP-1 through a decrease in the mRNA levels for the SREBP-1c isoform (24). However, a more recent report indicated that polyunsaturated fatty acids (PUFA) downregulate both SREBP-1a and 1c (25, 26). PUFA were reported to accelerate SREBP-1 mRNA decay and to reduce the cleavage of SREBP-1 precursor protein (23, 27, 28). These in vitro and in vivo studies strongly suggest that suppression of lipogenic gene transcription by PUFA is secondary to a decrease in SREBP levels or activity. In this regard, using primary hepatocytes, Jump and coworkers reported that FAS promoter activity was suppressed by PUFA and that cotransfection of SREBP-1 caused an increase in the activity of the FAS promoter spanning from −265 to +16 (29).

There are two potential sites present in the FAS promoter for SREBP function. As described above, for induction of the FAS promoter activity by carbohydrate/insulin, we found that SREBP probably functions via the −150 SRE. On the other hand, other laboratories have shown that SREBP can also function via the −65 E-box due to the dual binding specificity of SREBP-1 for both SREs and E-Boxes (29–32). In contrast, Fukuda and coworkers, using primary hepatocytes, reported the DNA sequence responsible for PUFA-mediated suppression of the FAS gene to be located at −57 to −35, which does not contain any putative SRE or E-box sequences (33). The DNA elements responsible for PUFA suppression of the FAS promoter, therefore, need to be further characterized.

Here, we report in vivo and in vitro examination of the FAS promoter region responsible for its regulation by PUFA to define the element(s) implicated in PUFA suppression. The weak but detectable promoter activity in transgenic mice expressing CAT driven by the −278 FAS promoter containing the −150 SRE, as well as the maximal CAT expression driven by the longer −444, −644, and −2.1 kb FAS promoter constructs are strongly suppressed by dietary polyunsaturated fat. An activated form of SREBP, when expressed in liver, prevents this suppression of FAS promoter activity in vivo. Transient transfection into HepG2 cells in vitro also demonstrates the involvement of SREBP and the importance of the −150 SRE. However, the −65 E-box could contribute to PUFA suppression of FAS promoter activity at least in vitro.

## MATERIALS AND METHODS

### Production of transgenic mice and plasmids construction

Transgenic mice containing −2,100 to +67 of the rat FAS gene linked to the CAT reporter gene have been described previously (12). Transgenic mice harboring −644-FAS-CAT were generated by injection of an agarose gel purified *Nar*I-*Kpn*I fragment from the −2,100-FAS-CAT plasmid containing −644 to +67 of the FAS promoter, CAT, and SV40 polyadenylation sequences into pronuclei of fertilized mouse embryos (Harvard Medical School, Brigham and Women's Hospital Transgenic Mice Facility). Similarly, transgenic mice harboring −444-FAS-CAT, −278-FAS-CAT, and −131-FAS-CAT were produced using *Dra*III-*Kpn*I, *BsaH*I-*Kpn*I, and *Asc*I-*Kpn*I fragments, respectively, from the −2,100-FAS-CAT plasmid. These various 5′-deletions of the FAS promoter region of the CAT fusion constructs are shown in **Fig. 1**. Heterozygous transgenic progenies (F1) were obtained by breeding the founders to C57BL/6 wild-type mice. The F1 male and female littermates were bred back to C57BL/6 wild-type mice. As previously described, multiple independent founder lines were used to eliminate the effects of integration site and copy number of transgenes on transgene expression (13). Subsequent transgene-positive progenies were used for experiments. Transgenic mice carrying the FAS promoter/CAT gene were identified by PCR amplification of tail DNA using the primers 5′-GCCAAGCTGTCAGCCCATGT-3′ and 5′-AATGTGAATAAAGGCCGGATAAAA-3′, which amplify a 390 bp fragment spanning the junction between the FAS promoter and the CAT reporter gene. The SREBP-1a transgenic mice were obtained from Jackson Laboratory and they were bred to the different FAS transgenic mice in order to generate various double FAS-CAT/SREBP-1a transgenic mice. To identify the double-transgenic mice, the primers 5′-CATCCCTGTGACCCCTCC-3′ and 5′-CTCCAAACCACCCCCCTC-3′ were used to amplify a 151 bp fragment of the human growth hormone polyadenylation signal sequence, which is part of the SREBP-1a transgene. We used the F1 double-transgene-positive mice for our studies.

The reporter construct −444-FAS-luciferase (LUC), which contains the −444/+67 of the rat FAS promoter fused to the LUC reporter plasmid pGL2 basic (Promega), was previously described (14). The reporter gene constructs for −278-FAS-LUC and −278(−65)-FAS-LUC containing the −278/+67 of the rat FAS promoter fused to the LUC reporter plasmid pGL2-basic (Promega) in the absence or presence of the mutation of −65/−60, were generated by ligating the *BsaH*I–*Xho*I fragment from −2,100-CAT (8) or −2,100(−65)-CAT plasmids, respectively, to the pGL2basic vector after digestion with *Sma*I and *Xho*I. −2,100(−65)-CAT was generated by site-directed mutagenesis of the −65/−60 in the −2,100-CAT plasmid (9). The reporter gene constructs for −125-FAS-LUC and −125(−65)-FAS-LUC containing the −125/+67 of the rat FAS promoter fused to the LUC reporter plasmid pGL2-basic (Promega) in the absence or presence of the mutation of −65/−60, were generated by ligating the *Xma*I–*Xho*I fragment from −2,100-CAT or −2,100(−65)-CAT plasmids, respectively, to the pGL2basic vector after digestion with the same restriction enzymes.

### Animal treatments

The animals had access ad libitum to food pellets containing 58% carbohydrate. The mice were fed a synthetic

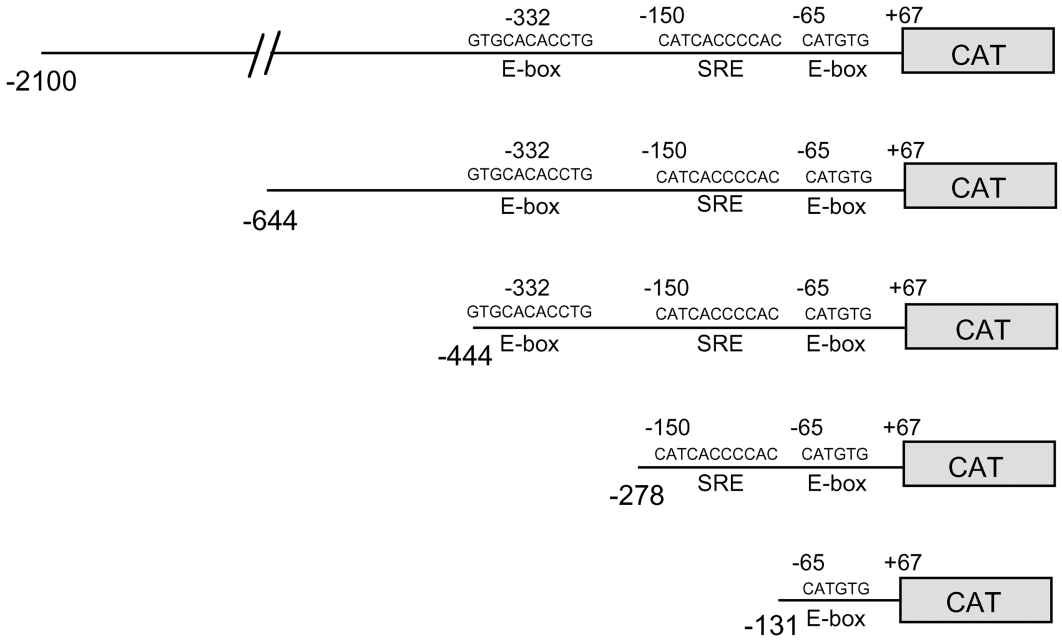


**Fig. 1.** Nested deletion of the 5′-flanking sequences of the fatty acid synthase (FAS) gene used to drive the chloraphenicol acetyltransferase (CAT) expression in transgenic mice. Putative E-boxes and sterol response element (SRE) for upstream stimulatory factor (USF) and sterol regulatory element binding protein (SREBP) binding are shown.

low carbohydrate protein diet (No. 5789C; Purina Mills) containing 71% (w/w) casein and 4.25% (w/w) sucrose for two weeks (14). Mice were first trained to eat the high carbohydrate, fat-free or high protein, fat-free diet (No. 57QL: Purina Mills) supplemented with 10% triolein (Sigma) from 9 AM until 12 PM for 10 days as described previously (12, 22). Butylated hydroxytoluene (Sigma) was added at 0.1% to prevent oxidation of the polyunsaturated fatty acid diet. One group was maintained on the triolein diet for an additional 7 days, while the other group was switched to menhaden oil (Zapata-Protein, Reedville, VA) diet for 7 days. Two hours after the last meal, the mice were sacrificed for the tissue dissection.

### RNA isolation, Northern blot analysis, and RNase protection assays

Tissues excised by rapid dissection and frozen in liquid nitrogen were pulverized using a ceramic mortar and pestle. Total RNA was isolated from the frozen tissues using TRIzol (Gibco-BRL) following the manufacturer's procedure. For Northern blot analysis, 20 μg of total RNA were resolved by electrophoresis in 1% formaldehyde-agarose gels in 2.2 M formaldehyde, 20 mM MOPS, 1 mM EDTA, stained with ethidium bromide, and transferred to Hybond N (Amersham Corporation). After UV cross-linking, membranes were incubated at 42°C for at least 4 h in 50% formamide, 5× salt-sodium citate (SSC), 5× Denhardt's solution, 0.5% SDS, and 50 μg/ml herring sperm DNA, and then hybridized under identical conditions to the $^{32}$P-random primed cDNA probe for at least 16 h. The plasmid pSREBP-1a (kindly provided by Drs. Michael S. Brown and Joseph L. Goldstein, University of Texas, Southwestern Medical Center) containing a 4,154 bp fragment of the human SREBP-1a cloned into the *Eco*RI-*Sal*I restriction site in pBluescript II was used as cDNA probe. Post-hybridization washes were carried out for 30 min at room temperature in 2× SSC, 0.1% SDS, and then in 0.1× SSC, 0.1% SDS at 50°C for 1 h, after which filters were exposed to X-ray film with intensifying screen at −80°C. In order to perform RNase protection assays, a 150 bp fragment of the mouse FAS cDNA sequence was amplified with the primers FAS-*Hind*III (5′-TTTTTTAAGCTTAGGGGTCGA-CCTGGTCCTCA-3′) and FAS-*Xba*I (5′-GCCATGTCTAGAG-GGTGGTTGTTAGAAAGAT-3′) using Pfu DNA polymerase. The PCR product was digested with *Hind*III and *Xba*I, and cloned into pcDNA3.0 to generate the plasmid pFAS-RPA. The CAT-RPA plasmid was generated by inserting into pcDNA3 a 180 bp PCR product amplified using CAT-*Hind*-III (5′-AAGCACAAGCTTTATCCGGCCTTTATTCAC-3′) and CAT-*Xba*I (5′-ATTCACTCTAGAGCGATGAAAACGTT-TCAGTT-3′) as primers subsequent to *Hind*III and *Xba*I digestion, and gel purification. The control plasmid (pTRI-actin-mouse) contained a 250 bp *Kpn*I-*Xba*I fragment from the mouse β-actin gene (Ambion). Antisense RNA probes were generated by in vitro transcription of the *Hind*III-digested plasmids using SP6 polymerase and [α-$^{32}$P] UTP. Ten micrograms of total RNA were subjected to the RNase protection assay using an RPA II kit (Ambion) following the manufacturer's procedure. The protected probes (120 bp for FAS and 150 bp for CAT probe) were separated on a 5% denaturing polyacrylamide-urea gel. Gels were dried and exposed to X-ray film.

### Transient transfections

HepG2 cells were plated ($2.5 \times 10^5$ cells/well) in collagen coated 6-well plates (Becton Dickinson) and maintained

in DMEM containing 10% FBS for 24 h. For transient transfections, cells were co-transfected with the FAS-promoter-luciferase constructs (2.5 μg/well) and CMV-β-galactosidase (5 ng/well) as a control for transfection efficiency using Lipofectamine (4 μl/well) (Life Technologies). Varying amounts of either SREBP-1a or -1c expression vectors or equivalent amounts of pcDNA3.1 (Invitrogen) for control were also used. Cells were washed twice with 1× PBS 3 h after transfection and incubated for 16 h with DMEM containing 10% delipidated calf serum. Fatty acids were dissolved in 100% ethanol and complexed to fatty acid-free BSA by stirring for 1 h at 37°C. Cells were treated with DMEM containing 150 μM albumin-bound (fatty acid-albumin molar ratio 5:1) oleic acids or arachidonic acids (Sigma), 1 μM insulin, and dexamethasone for 24 h. After harvesting, cell extracts were used for measurement of luciferase and β-galactosidase activities by the Dual-Light Reporter Gene Assay (Tropix). Transfections were carried out in triplicate and at least two different preparations for each plasmid were used.

## RESULTS AND DISCUSSION

### Regulation of the CAT reporter gene driven by nested 5′-deletions of the FAS promoter region by dietary polyunsaturated fat in transgenic mice

FAS expression in liver has been known to be regulated by different types of dietary fat (20–22). PUFA decrease transcription of the FAS gene, while monounsaturated fatty acids do not have a significant effect. Previously, we have shown that the first 2.1 kb of the 5′-flanking region of the FAS gene are sufficient in transgenic mice for nutritional regulation, including suppression by polyunsaturated fat (12). Recently, we found that two upstream 5′-regions of the FAS promoter are required for carbohydrate/insulin regulation in vivo: one at −278 to −131 that accounts for a low level induction, and the other at −444 to −278 for maximal induction (13).

To identify the sequences responsible for regulation of the FAS gene by dietary polyunsaturated fat in an in vivo context, transgenic mice carrying −2,100-FAS-CAT, −644-FAS-CAT, −444-FAS-CAT, −278-FAS-CAT, and −131-FAS-CAT fusion genes were fed either menhaden oil (which is rich in the PUFA eicosapentaenoic and docosahexaenoic acids) or triolein. Hepatic FAS and CAT mRNA levels were measured simultaneously by RNase protection assay to compare the expression levels among the different transgenic mice (**Fig. 2**). As a control, actin mRNA levels were also determined in the same reaction, and they remained essentially the same whether the animals were fed a triolein or menhanden oil diet. As shown in Fig. 2, the endogenous FAS mRNA levels in the livers of animals fed menhaden oil were approximately 70% lower than those in the livers of animals fed triolein. Expression of the CAT driven by the −2,100, −644, and −444 of the FAS 5′-flanking sequence was high when the transgenic mice were fed triolein. Expression of the CAT driven by the −278 FAS-promoter, on the other hand, reached only about 10% of the level observed in mice carrying the three longer constructs. Likewise, the transgenic mice carrying the −131-FAS-CAT showed extremely low transgene expression levels, which were detectable only after a prolonged exposure of the gel.

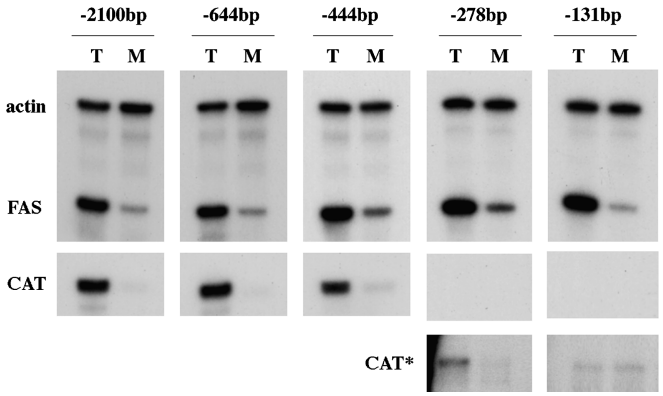


**Fig. 2.** Suppression by polyunsaturated fatty acids of CAT activity driven by the various FAS promoter regions in transgenic mice. mRNA levels for the endogenous FAS and CAT transgene were analyzed by RNase protection assays using total liver RNA as described under Materials and Methods in transgenic mice carrying the −2,100-FAS-CAT, −644-FAS-CAT, −444-FAS-CAT, −278-FAS-CAT, and −131-FAS-CAT. Suppression of menhaden diet for endogenous FAS in −2,100, −644, −444, −278, and −131-FAS-CAT transgenic mice was 80%, 81%, 66%, 67%, and 84%, respectively; suppression for CAT transgene was 96%, 99%, 87%, 69%, and 0%, respectively. Actin mRNA was used as a control. The same results were obtained from three independent experiments. T and M indicate triolein or menhaden oil fed, respectively. * Represents an autoradiogram on BioMax film (Kodak) exposed longer: 3 days for −278-FAS-CAT and 1 week for −131-FAS-CAT.

When mice were fed menhaden oil, CAT expression decreased dramatically to almost undetectable levels in transgenic mice carrying the reporter gene driven by the three longer FAS promoter fragments, −2,100-FAS-CAT, −644-FAS-CAT, and −444-FAS-CAT. The degree of repression in the latter two lines was similar to the high level suppression observed in the −2,100-FAS-CAT transgenic mice. The suppression of CAT expression in these mice by polyunsaturated fat feeding was more robust than the 70% decrease observed in the endogenous FAS mRNA levels. We do not have an explanation for this discrepancy, although it is possible that the FAS mRNA could have a longer half-life than the CAT mRNA. The low level expression of the CAT in −278-FAS-CAT transgenic mice on the triolein diet was further decreased by approximately 90% when the transgenic mice were fed the menhaden oil diet. These data indicate that, while the −444 to −278 region was required for maximal FAS promoter activity that could be suppressed upon polyunsaturated fat feeding, the −278-FAS promoter region was still capable of mediating PUFA suppression. On the other hand, the transgenic mice containing the shortest −131-FAS-CAT reporter construct showed the same extremely low expression levels for CAT mRNA whether the animals were fed triolein or menhaden oil. This indicates that the region responsible for PUFA-suppression of the FAS gene is no longer present in the −131-FAS-CAT construct.

Taken together, these results show that deletion of the promoter sequence from −278 to −131 causes complete loss of regulation of CAT expression by polyunsaturated fat feeding and this region, therefore, is required for PUFA suppression of FAS transcription. The sequences required for suppression by dietary polyunsaturated fat overlap with those for induction by carbohydrate/insulin in vivo. Sequences present between −131 to −278 are required for suppression of the FAS promoter by PUFA. Moreover, promoter activity that reaches the maximal level via −278 to −444 is still suppressed, probably via the putative PUFA response element located between −131 to −278.

### Involvement of SREBP in the regulation of the FAS promoter by polyunsaturated fat

We previously characterized the transcription factors that likely function in the two regions that were identified for maximal carbohydrate/insulin regulation of the FAS gene. SREBP, by binding to the −150 SRE present in the region between −278 to −131, mediates a low level induction by carbohydrate/insulin, while USF, by binding to the −332 E-box present in the region between −444 to −278, brings about maximal induction (13, 14). It has recently been shown in rodents that feeding polyunsaturated fat decreased expression of SREBP-1c as well as the level of mature SREBP in the nucleus (23, 24). Furthermore, it has been shown that SREBP was required for the decrease observed in mRNA levels for lipogenic enzymes during polyunsaturated fat feeding, and that a sustained expression of mature SREBP-1 in transgenic mice prevented this decrease (23). In order to examine in vivo the involvement of SREBP in the regulation of FAS promoter activity by polyunsaturated fat, we generated double transgenic mice carrying both the CAT reporter gene driven by the various 5′-deletion fragments of the FAS promoter that we previously described (−644, −444, −278, and −131 bp) and a truncated active form of SREBP-1a driven by the phosphoenolpyruvate carboxykinase (PEPCK) promoter.

We first examined SREBP expression in the livers of these double transgenic mice (**Fig. 3**). The levels for endogenous SREBP-1 mRNA in the livers of animals fed menhaden oil were approximately 50% lower than those of the animals fed triolein. [The SREBP-1a sequence used as a probe in the northern blot detects both the SREBP-1a and -1c isoforms (14).] SREBP transgene expression, on the other hand, did not vary with dietary unsaturated fat consumption. This was expected since, contrary to lipogenic enzymes, the PEPCK promoter used to drive SREBP transgene expression is not known to be regulated by dietary polyunsaturated fat.

As presented in the RNase protection analysis in **Fig. 4**, unlike in the FAS-CAT single transgenic mice, all FAS-CAT/SREBP double transgenic mice showed high levels for endogenous FAS mRNA whether the animals were fed triolein or menhaden oil. The constitutively active form of SREBP produced by the transgene probably activated the endogenous FAS promoter regardless of the types of dietary fat consumed by the mice. Similarly, in −644-FAS-CAT/SREBP and −444-FAS-CAT/SREBP double transgenic mice, CAT mRNA levels remained high on both the triolein and menhaden oil diets. In −278-FAS-CAT/SREBP double transgenic mice, CAT mRNA levels were also the same whether the animals were fed triolein or menhaden oil, but the expression levels were similar to those observed in the −278-FAS-CAT transgenic mice on the triolein diet.

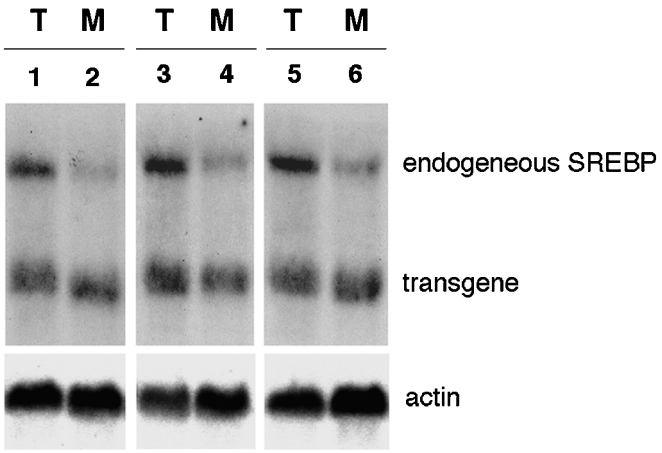


**Fig. 3.** Northern blot analysis for endogenous SREBP and the SREBP transgene in −644-FAS-CAT/SREBP-1a transgenic animals. Transgenic mice were either fed triolein or menhaden oil for 7 days. Twenty micrograms of total RNA isolated from liver were subjected to Northern blot analysis as described under Materials and Methods and exposed to X-ray film (Fuji) for 1 day. T and M indicate triolein and menhaden oil fed, respectively. Suppression of the endogenous SREBP gene was 58% for samples one and two, 60% for samples three and four, and 57% for samples five and six. Actin mRNA levels were used as a control.

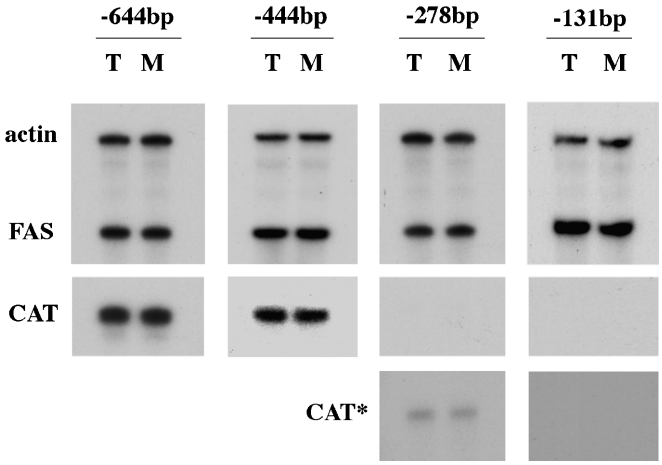


**Fig. 4.** Ectopic SREBP-1a expression prevents suppression of the FAS promoter by polyunsaturated fat in vivo. mRNA levels for the endogenous FAS and CAT transgene were analyzed by RNase protection assays using total liver RNA as described under Materials and Methods in double transgenic mice carrying the −644-FAS-CAT, −444-FAS-CAT, −278-FAS-CAT, and −131-FAS-CAT along with the SREBP transgene driven by the phosphoenolpyruvate carboxykinase (PEPCK) promoter. Actin mRNA was used as a control. Essentially the same results were obtained from three independent experiments. T and M indicate triolein and menhaden oil fed, respectively. * Represents an autoradiogram on BioMax film (Kodak) exposed for 3 days.

As expected, the −131-FAS-CAT/SREBP double transgenic mice did not express readily detectable levels of CAT mRNA on either diet, indicating that deletion of the promoter sequence from −278 to −131 caused loss of CAT expression as well as induction by the SREBP transgene. This indicates that the region up to −131 lacks the sequences sufficient for activation of the FAS promoter by SREBP and that the region between −278 and −131 is required for the SREBP mediated activation observed at a low level with the −278 FAS promoter. This region contains the −150 SRE and coincides with the region we previously characterized as the minimal sequence required for nutritional/insulin regulation (14). As we demonstrated previously, however, an upstream regulatory region containing the −332 E-box where USF could bind was required for the maximal expression of CAT (13). Moreover, the SREBP transgene could restore CAT expression to maximal levels in −444-FAS-CAT/SREBP and −644-FAS-CAT/SREBP transgenic mice during menhaden oil feeding.

### Suppression of the FAS promoter by PUFA in HepG2 cells

SREBP-1c, the major isoform present in liver and adipose tissue, is known to be responsible for regulation of lipogenic genes by PUFA (24, 29). Recent reports, however, showed that unsaturated fatty acids downregulate both SREBP-1a and 1c (25). In addition, PUFA was shown to be effective in suppressing FAS expression in HepG2 cells where SREBP-1a is the major isoform expressed (34). We therefore employed HepG2 cells to further examine the DNA sequences responsible for PUFA suppression of the FAS gene. When cells were treated with arachidonic acid, luciferase activity driven by the −444 FAS promoter was 60% of that observed in cells treated with oleic acid. Upon cotransfection of increasing amounts of the truncated active form of SREBP-1a or -1c, suppression of the FAS promoter by PUFA steadily diminished up to the level where the luciferase activity was the same whether the cells were treated with arachidonic acid or oleic acid (**Fig. 5**). The presence of increasing amounts of constitutively active SREBP also induced the basal promoter activity (Fig. 5, inset). Furthermore, the amounts required of SREBP-1a and SREBP-1c for preventing PUFA suppression of FAS promoter differed, reflecting their transactivation potencies. A greater amount of SREBP-1c, being less potent than SREBP-1a, was required in order to fully prevent PUFA suppression of FAS promoter activity. This further indicates the role of SREBP in PUFA suppression of FAS promoter activity.

We, as well as others, have previously shown that SREBP can bind, at least in vitro, to both the −150 SRE and −65 E-box of the FAS promoter (14, 29–32, 35, 36). HepG2 cells enabled us to further examine the role of these elements in PUFA suppression of FAS promoter activity. Luciferase activity driven by the −278 FAS promoter was approximately 50% lower when cells were treated with arachidonic acid as compared with that observed during oleic acid treatment (**Table 1**). When we further deleted the FAS promoter to −125, we observed a 23% inhibition of reporter activity by PUFA treatment. Because the PUFA

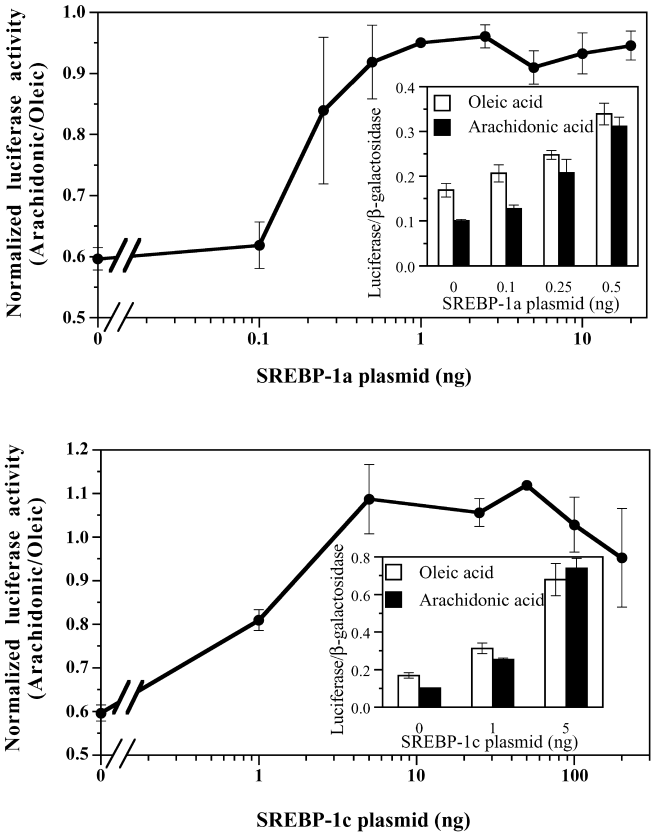


**Fig. 5.** Cotransfection of SREBP-1a and SREBP-1c prevents suppression of the FAS promoter by polyunsaturated fatty acids in HepG2 cells. The FAS −444 Luc construct (2.5 μg) was cotransfected with the indicated amounts of an expression vector for either SREBP-1a or SREBP-1c into HepG2 cells and then the cells were treated with either oleic or arachidonic acids (150 μM) for 24 h. The values represent the normalized luciferase activity ratio of arachidonic acid to oleic acid treatments. The actual normalized values of the luciferase activity for the most relevant points are shown in the insets and they represent the mean ± SD of three samples. Essentially the same results were obtained in two independent experiments.

suppression was not completely abolished by deletion of the region from −278 to −125, we conclude that the −150 SRE present in this region probably is the target site for SREBP. However, an additional PUFA response sequence is present in the first −125 of the FAS promoter. We hypothesized that the −65 E-box, where we found USF to function (but where SREBP can also bind in vitro), could contribute to PUFA suppression. Unlike in transgenic mice, transient transfection of −125 Luc into HepG2 cells showed easily detectable levels of luciferase activity, allowing us to address the role of the −65 E-box. When we mutated the −65 E-box in the context of the −125 FAS promoter (thereby lacking the SRE at −150), there was a complete loss of suppression by PUFA. This clearly demonstrates that, at least in vitro, both the −150 SRE and −65 E-box contribute to PUFA suppression of FAS promoter activity. Using primary hepatocytes, Fukuda and coworkers previously reported that suppression of the FAS promoter occurs via the element present between

TABLE 1. Sequence requirement for PUFA suppression in vitro

| Construct | Oleic | Arachidonic | % Suppression |
|---|---|---|---|
| | Luciferase/β-galactosidase | | |
| –278 Luc | 1.65 | 0.86 | 47.7 |
| –125 Luc | 3.19 | 2.43 | 23.6 |
| –278(–65) Luc | 2.05 | 1.45 | 29.4 |
| –125(–65) Luc | 0.84 | 0.80 | 4.7 |

The indicated constructs (2.5 μg) were transiently transfected into HepG2 cells and then treated with the different fatty acids. (−65) denotes mutation of the −65 E-box. The values represent the mean of three samples. % Suppression was obtained by subtracting the arachidonic to oleic ratio from 1, and then multiplying the result by 100. Essentially the same results were obtained from three independent experiments.

−57 to −35 (33). We do not know the reason for the discrepancy between their and our present results. Nevertheless, the prevention of PUFA suppression of FAS promoter activity by SREBP in vivo and the presence of the SREBP sites support our conclusion. In vivo assessment of the putative contribution of the −65 E-box to suppression of the FAS promoter by polyunsaturated fat, however, was not possible because of the extremely low level of FAS promoter activity observed in the −131-FAS-CAT transgenic mice.

Overall, our present studies demonstrate that SREBP is involved in PUFA-mediated suppression of both endogenous FAS and CAT transgene expression, and we have defined the regions in the FAS promoter necessary for this repression. We conclude that SREBP probably functions via the −150 SRE and that the −65 E-box may also contribute to suppression, as demonstrated in HepG2 cells. The reported decrease in SREBP-1 gene expression and mature SREBP by polyunsaturated fat supports the notion that SREBP is limiting during feeding of polyunsaturated fat, which in turn prevents activation of the FAS promoter (24–26). When polyunsaturated fat is not present in the diet, the SREBP level increases and, by binding to the −150 SRE, supports the regulated activation of the FAS promoter, albeit at a low level. Binding of USF to the upstream −332 E-box, which probably does not change with various nutritional/hormonal conditions, brings about maximal activation of the FAS promoter. In this regard, dietary oil rich in polyunsaturated fatty acids has been shown not to affect the USF-1 levels but to decrease SREBP-1 levels (24–26). To understand the in vivo role of the −65 E-box in PUFA suppression, mutation of this element in the context of a longer FAS promoter fragment in transgenic mice will be required.

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