Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 8454–8458, September 1993
Biochemistry

Polyunsaturated fatty acids inhibit S14 gene transcription in rat liver and cultured hepatocytes

(menhaden oil/eicosapentaenoic acid/thyroid hormone/transfection)

DONALD B. JUMP*†, STEVEN D. CLARKE‡, ORMOND MACDOUGALD*§, AND ANNETTE THELEN*

*Genetics, Cell and Molecular Biology Programs, Physiology Department, Michigan State University, East Lansing, MI 48824; and ‡Department of Food Science and Human Nutrition, Colorado State University, Fort Collins, CO 80523

Communicated by M. Daniel Lane, June 1, 1993

ABSTRACT **Polyunsaturated fatty acids (PUFAs) have been shown to have significant effects on hepatic lipogenic gene expression. The S14 gene has been used as a model to examine the effects of PUFAs on hepatic lipogenic gene expression. *In vivo* studies showed that feeding rats a high carbohydrate diet containing menhaden oil rapidly (within hours) and significantly (≥50%) attenuates hepatic S14 gene transcription and S14 mRNA abundance. The suppressive effect of menhaden oil was both gene and tissue specific. The effect of PUFAs on expression of the S14 mRNA and a transfected S14 fusion gene (i.e., S14CAT4.3) was examined in cultured hepatocytes in the presence of triiodothyronine (T_3), insulin, dexamethasone, and albumin under serum-free conditions. Whereas T_3 stimulated both S14 mRNA (>40-fold) and S14CAT4.3 (>100-fold), eicosapentaenoic acid (C20:5ω3) significantly attenuated (≥80%) both S14 mRNA and S14CAT activity in a dose-dependent fashion. The effects of C20:5 on hepatocyte gene expression were both gene and fatty acid specific. Deletion analysis of transfected S14CAT fusion genes indicated that the S14 thyroid hormone response element (at −2.5 to −2.9 kb) was not sensitive to C20:5 control. The cis-linked PUFA response elements were localized to a region within the S14 proximal promoter (at −80 to −220 bp). This region also contains cis-acting elements that potentiate T_3 activation of S14 gene transcription. These studies suggest that C20:5 (or its metabolites) regulates factors within the S14 proximal promoter region that are important for T_3 activation of S14 gene transcription.**

Dietary polyunsaturated fatty acids (PUFAs), particularly those rich in 20- and 22-carbon (ω3 and ω6) fatty acids, have several unique metabolic effects including suppression of very low density lipoprotein production, reduction of cholesterol synthesis, diminution of blood pressure, and modulation of the growth of certain carcinomas (1–3). A particularly intriguing feature of PUFAs is their ability to regulate the expression of several genes involved in lipid metabolism, such as the genes encoding apolipoprotein AI (4), low density lipoprotein receptors (5), glucose-6-phosphate dehydrogenase (6), and fatty acid synthase and the S14 protein (7–9).

PUFAs suppress the hepatic mRNAs coding for the S14 protein (pI 4.9; 17 kDa) in both adult and weaning rats by inhibiting S14 gene transcription (7–9). S14 gene transcription is induced by 3,5,3′-triiodothyronine (T_3) (10, 11), glucocorticoids (12, 13), retinoic acid (14, 15), insulin, carbohydrate (16–19), and tissue-specific factors (20) and suppressed by factors that elevate hepatic cAMP levels (16). While the molecular basis for PUFA-mediated inhibition of gene transcription remains unclear, our understanding of the regulation of S14 gene expression makes this an attractive model to examine specific aspects of PUFA action at the molecular level.

In this report, the effects of triolein and menhaden oil on the regulation of hepatic S14 gene expression were examined. Triolein contains essentially oleic acid (C18:1ω9), a monounsaturated fatty acid. Menhaden oil is enriched in long chain $n-3$ fatty acids [e.g., eicosapentaenoic acid (EPA; C20:5ω3) and docasahexaenoic acid (DHA; C22:6ω3)], which have potent suppressive effects on hepatic lipogenesis (21–23). *In vivo* studies examined the kinetics of menhaden oil suppression of S14 gene expression as well as the gene and tissue specificity of menhaden oil action. Studies with cultured hepatocytes examined the effects of C18:1 and C20:5 on S14 gene expression. Our results show that PUFAs rapidly inhibit hepatic S14 gene transcription *in vivo* and that the PUFA-mediated action is directed against a 140-bp region flanking the 5′ end of the S14 gene.

METHODS AND MATERIALS

Animals. Male Sprague–Dawley rats (125–150 g) were obtained from Charles River Breeding Laboratories. Rats (one rat per cage) were meal-fed a high carbohydrate (HiCHO; 58% glucose; ICN, Cleveland, OH)/fat-free diet from 9 a.m. until 12 noon daily (9). Diets were supplemented (at 10% wt/wt) with either triolein [1,2,3-tri(*cis*-9-octadecenoyl)-glycerol; >95%; Sigma] or menhaden oil (MaxEPA, Scherer, FL). All diets contained butylated hydroxytoluene at 0.1% wt/wt (9, 21). Gas chromatographic analysis showed that menhaden oil contained 433.5 mg of polyenes per g as ω3 fatty acids (347.1 mg/g) and ω6 fatty acids (26.6 mg/g). EPA and DHA were present at 160.9 mg/g and 112.4 mg/g, respectively.

RNA Extraction/Transcription. Liver, epididymal fat, and hepatocyte total RNA were isolated (24) and used for measurement of mRNAs coding for S14, β-actin, and phospho*enol*pyruvate carboxykinase (PepCk) by Northern and dot-blot analyses (10, 12). Hepatic nuclei were isolated for run-on gene transcription analysis (17).

Hepatocytes. Sprague–Dawley rats (150–350 g) maintained on Teklad chow were fasted for 48 hr prior to hepatocyte preparation. Primary hepatocytes were prepared using the collagenase perfusion method (25) as modified by Jacoby *et al.* (18). Cells were maintained at 3×10^6 cells per 60-mm plastic tissue culture dish (Primaria) in modified Williams' E medium supplemented with 25 mM glucose, 10 nM dexa-

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Abbreviations: PUFA, polyunsaturated fatty acid; PUFA-RE, PUFA response element; T_3, 3,5,3′-triiodothyronine; TRE, thyroid hormone response element; CAT, chloramphenicol acetyltransferase; EPA, eicosapentaenoic acid; DHA, docasahexaenoic acid; PepCk, phospho*enol*pyruvate carboxykinase; HiCHO, high carbohydrate; TK, thymidine kinase.

†To whom reprint requests should be addressed.
§Present address: Department of Biological Chemistry, Johns Hopkins University, 725 North Wolf Street, Baltimore, MD 21205-2185.

methasone, 1 μM insulin, 26 mM sodium bicarbonate, 2 mM L-glutamine, 100 units of penicillin per ml, and 100 μg of streptomycin per ml. Hepatocytes were transfected using Lipofectin (Life Technologies, Grand Island, NY). The formation of liposome–DNA complexes was carried out by mixing 1–5 μg of plasmid DNA with lipofectin to give a lipofectin-to-DNA ratio of 6.6:1 (wt/wt) in 4 ml of medium (18, 26). This mixture was added directly to each dish. After treatment with the DNA–liposome mixture for 12 hr, medium was replaced with serum-free experimental Williams' E medium.

Hepatocytes were transfected with S14–chloramphenicol acetyltransferase (CAT) fusion genes. S14CAT4.3, S14CAT1.6, and S14CAT0.29 have 3′ end points at +19 bp and 5′ end points at −4315, −1601, and −290 bp, respectively, relative to the 5′ end of the rat S14 gene (15, 35). Transcription of the S14 gene in hepatocytes is driven by thyroid hormone response elements (TREs) located between −2502 and −2900 bp upstream from the 5′ end of the gene (10, 11, 17). This region was amplified by PCR using oligonucleotides (DJ29: 5′-TATTATTGGATCCTGACGTAGCGGAGGATAGAGAT and DJ30: 5′-TATTATTGGATCCTTTCCATGACTACAACCCTGTGGAG) prepared at the Michigan State University Biochemistry Department Macromolecular Structure Facility. The resultant DNA fragment was inserted into the *Bam*HI sites of the thymidine kinase (TK)–CAT fusion gene and the S14CAT0.29.

Proximal Promoter Deletions. Deletions of the proximal promoter region were made by preparing four sense oligonucleotides—DJ40 (5′-AATTGCCTGCAGTCAAGTGTACTGGGT), DJ41 (5′-ATATATCTGCAGTTGGCCGCCCACTGA), DJ42 (5′-ATATATCTGCAGGCGACCAAACGCTGGGAT), and DJ45 (5′-ATATATCTGCAGATCCAGTGACTGGGTTTGGCGT)—and one antisense oligonucleotide—DJ44 (5′-ATATATCTCGAGGTGCTTCCTTTCTCAGAG)—for use in PCR. The four sense oligonucleotides represent S14 genomic DNA ending at −290, −220, −121, and −80 bp, respectively. The location of these 5′ end points was based on the DNase I footprint pattern of the S14 promoter (20). The antisense sequence ends at +19 relative to the 5′ end of the S14 gene. The synthesized DNAs were inserted (directionally) into a CAT plasmid containing no promoter but with the S14 TRE located upstream.

Hepatocytes were cotransfected with S14CAT chimeric genes and the MLVTRβ_1 expression vector. MLVTRβ_1 contains the murine leukemia virus promoter and the rat liver β_1 thyroid hormone receptor gene (obtained from V. Mahdavi; ref. 27). RSVCAT (obtained from S. Conrad, Michigan State University) was used as a control for nonspecific effects of fatty acids on hepatocytes.

Cells were treated with T_3 (1 μM; vehicle: 0.001% dimethyl sulfoxide/1 μM NaOH) in the presence of albumin or albumin-bound fatty acids. Oleic acid (C18:1ω9) and EPA (C20:5ω3) (free acids) were obtained from Sigma and Nu Chek Prep (Elysian, MN). The fatty acids were resuspended in Williams' E medium supplemented with 2 mM albumin (Boehringer Mannheim; fatty acid free) to give a 100 mM solution. The fatty acid/albumin solution was neutralized with NaOH, and butylated hydroxytoluene and α-tocopherol were added to 0.1% and 20 μM, respectively, and the solution was stored under nitrogen at −80°C to prevent oxidation (21). The fatty acid stock solutions were diluted into Williams' E medium to give fatty acid concentrations of 50, 100, or 300 μM with corresponding albumin concentrations of 8.3, 16, or 50 μM. The fatty acid-to-albumin molar ratio was 6:1. Cells were treated with medium containing the fatty acid/albumin mixture in the presence of T_3 (1 μM) for 48 hr with one change of medium after 24 hr. Fatty acid-containing medium was prepared fresh daily. Cells were harvested for protein assay (28) and CAT activity (29).

RESULTS

Effects of Triolein and Menhaden Oil on S14 Gene Expression. In rats fed the triolein diet, food ingestion induced S14 mRNA nearly 3-fold (Fig. 1). This is due to the HiCHO-mediated elevation of S14 gene transcription (17). In contrast, rats fed the menhaden oil diet showed both a suppressed (>90%) premeal level of hepatic S14 mRNA and virtually no induction of S14 mRNA after feeding. Menhaden oil abrogates the stimulatory effects of the HiCHO diet on hepatic S14 gene expression.

Kinetics of Menhaden Oil Action on Hepatic S14 Gene Expression. The rapidity of the menhaden oil effect on hepatic S14 gene expression was examined in rats meal-trained on a triolein diet and then switched to a menhaden oil diet. In contrast to the rats maintained on the triolein diet, rats switched to the menhaden oil diet showed a progressive decline in both S14 gene transcription and S14 mRNA (Fig. 2). A single meal of menhaden oil suppressed S14 gene expression by 50%. After 2 and 5 days on the menhaden oil diet, S14 gene expression was suppressed by >75% and >90%, respectively. S14 transcription rates paralleled the decline in S14 mRNA, indicating that the principal site of menhaden oil action was at the level of S14 gene transcription.

Gene and Tissue Specificity of Menhaden Oil Action. The specificity of menhaden oil action was examined by measuring changes in hepatic PepCk and β-actin mRNA. The level of hepatic PepCk and β-actin mRNA in triolein fed rats was 0.19 ± 0.04 units (mean ± SE) and 2.5 ± 0.07 units, respectively. The corresponding levels of run-off transcription for PepCk and β-actin were 62 ± 18 ppm and 4.1 ± 1.3 ppm, respectively. Menhaden oil feeding did not consistently affect hepatic PepCk or β-actin expression on either the short-term (1–2 days) or long-term (5 day) feeding studies.

S14 mRNA levels were examined in white adipose tissue, a tissue where S14 is expressed at high levels and is hormonally regulated (30). S14 mRNA levels fell (by 50%) only after 5 days of feeding the menhaden oil diet. Taken together, these studies show that the rapid effects of menhaden oil on hepatic S14 gene expression were gene and tissue specific.

Effects of Oleic Acid and EPA on S14 Gene Expression in Cultured Hepatocytes. The inhibitory action of menhaden oil on hepatic lipid metabolism has been attributed to the pres-

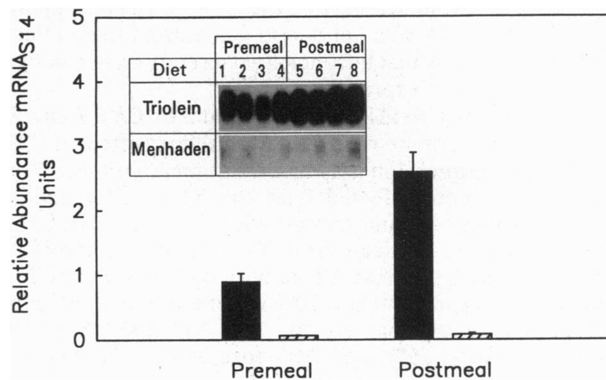

FIG. 1. Effect of meal-feeding dietary fats on hepatic S14 gene expression. Two groups of rats were meal-fed a HiCHO plus 10% triolein diet for 10 days. One group was maintained on the triolein diet for an additional 5 days (solid bars), while the other group was switched to a HiCHO plus 10% menhaden oil diet (hatched bars) for 5 days. (*Inset*) Separation of S14 mRNA by denaturing electrophoresis. The "premeal" group is in lanes 1–4 and the "postmeal" group is in lanes 5–8. Rats received food from 9 a.m. until 12 noon. The premeal group was killed at 9 a.m. prior to receiving food, and the postmeal group was killed at 5 p.m. Livers were isolated for total RNA extraction and analysis of S14 mRNA. Results are expressed as mean ± SE units (n = 4 per group).

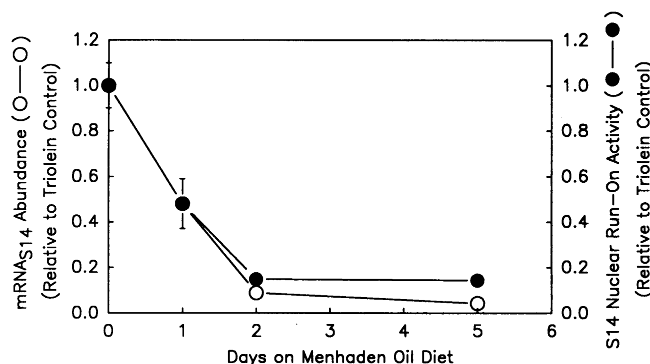

FIG. 2. Kinetics of menhaden oil inhibition of S14 gene expression. Rats were meal-fed a triolein diet as described in Fig. 1. Rats were either maintained on the triolein diet or switched to a menhaden oil diet for one, two, or five meals (days). Rats were killed 90 min after food removal. Hepatic RNA and nuclei were isolated for S14 mRNA (○) and transcription (●) analysis. Results are expressed as means ± SE relative to the triolein control. Hepatic S14 mRNA (3.96 ± 0.27 units) and transcription rates (52.8 ± 6.1 ppm) in the triolein-fed rats did not vary significantly over the 5-day experiment. Three animals were in each group. Similar results were obtained when rats were killed 5 hr after removal of food.

ence of highly unsaturated fatty acids, like EPA (C20:5ω3) and DHA (C22:6ω3), in fish oils (21–23). Primary hepatocyte cultures were treated with a monounsaturated fatty acid, oleic acid (C18:1ω9), or a polyunsaturated fatty acid, C20:5, to determine whether these fatty acids acted directly on hepatocytes to affect gene expression.

The effects of C18:1 and C20:5 on S14 gene expression were assessed in the presence of T_3. T_3 induced S14 mRNA ≥40-fold in the absence of fatty acids. Accordingly, hepatocyte monolayers were treated with serum-free medium containing insulin (1 μM), dexamethasone (10 nM), and T_3 (1 μM) in the absence and presence of albumin-bound fatty acids. When compared to control cultures receiving only albumin, C18:1 treatment of hepatocytes only marginally (<20%) suppressed S14 mRNA levels over the concentration range tested (Fig. 3). In contrast, C20:5 at 100 and 300 μM suppressed S14 mRNA by 80% and 95%, respectively, when compared to the C18:1 controls. Neither C18:1 nor C20:5 influenced hepatocyte β-actin mRNA levels, indicating that the C20:5-mediated suppression of S14 mRNA was not due to generalized toxic effects. These studies show that fatty acids act directly on hepatocytes to inhibit S14 gene expression.

Effects of Fatty Acids on Transfected S14 CAT Gene Expression. To determine whether the inhibitory effect of C20:5 on S14 gene expression was transcriptional, cultured hepatocytes were cotransfected with the S14CAT4.3 and the MLVTRβ_1 plasmids and treated with T_3 (Fig. 4). In C18:1-treated cells, T_3 induced S14CAT activity ≥100-fold. In contrast, T_3 induced S14CAT activity only 30-fold in C20:5-treated cells, representing a 70% decline in the T_3 effect on S14 gene transcription. At 50, 100, and 300 μM, C20:5 promoted a 16%, 56%, and 70% dose-dependent decline in S14CAT activity, respectively, with an apparent ED_{50} of ≤100 μM. Similar effects were seen with arachidonic acid (C20:4ω6) on both S14 mRNA and S14CAT activity. These studies show that the inhibitory effect of C20:5 S14CAT activity paralleled the suppression of S14 mRNA, suggesting that C20:5 inhibited endogenous S14 gene expression at the transcriptional level.

The specificity of the C20:5 effect was examined by transfecting hepatocytes with RSVCAT (Fig. 4). RSVCAT was expressed at high levels after C18:1 treatment and decreased by 9% in C20:5-treated hepatocytes. The lack of any significant effect of C20:5 on RSVCAT-transfected cells

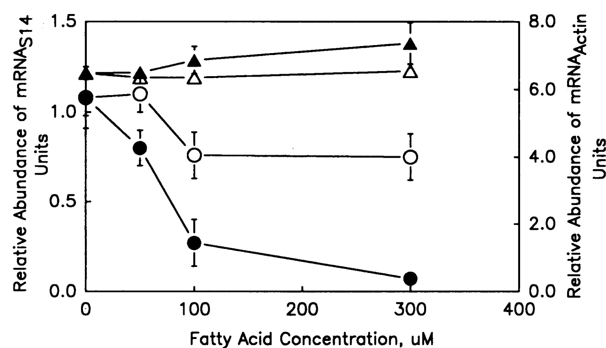

FIG. 3. Effect of oleic acid and EPA on S14 and β-actin mRNA levels in primary hepatocytes. Control cultures of hepatocyte monolayers were treated with 50 μM albumin. Experimental hepatocyte cultures were treated with oleic acid (C18:1ω9) (○ and △) or EPA (C20:5ω3) (● and ▲) at 50, 100, and 300 μM. The corresponding albumin concentrations were 8.4, 16.7, and 50 μM, respectively. All cultures received T_3 (1 μM). Cells were harvested after 48 hr of treatment for extraction of total RNA for the analysis of S14 mRNA (○ and ●) and β-actin mRNA (△ and ▲) levels. Results are expressed as mean ± SE units ($n \geq 3$).

indicates that PUFAs (i) are not toxic to hepatocytes at ≤300 μM, (ii) do not impede the transcription of the CAT gene driven by the Rous sarcoma virus promoter, and (iii) do not interfere with the analysis of CAT activity. Thus, the inhibitory action of C20:5 on S14 gene transcription was specific.

Localization of PUFA Response Elements (PUFA-REs) by Deletion Analysis. The 5′ flanking region of the S14 gene harbors a series of response elements controlled by hormones, nutrients, and tissue-specific factors. These elements are localized within three major DNase I hypersensitive sites (10, 13, 15–17). One is located near the 5′ end of the S14 gene (−290 to +1) and contains several cis-acting elements that function in the organization of the transcriptional preinitiation complex (15, 20). A second site located between −1400 and −1600 bp

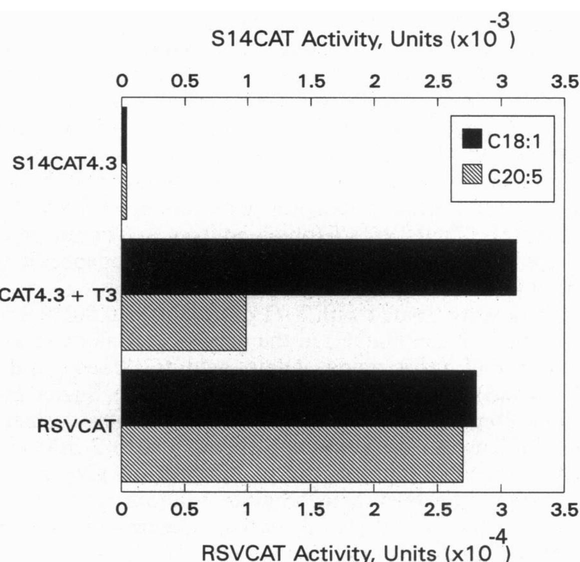

FIG. 4. Effect of oleic acid and EPA on S14CAT and RSVCAT activity. Hepatocytes were transfected with either S14CAT4.3 plus MLVTRβ_1 or RSVCAT. S14CAT4.3-transfected cells were treated without and with T_3 (1 μM). Both S14CAT4.3- and RSVCAT-transfected cells were treated with albumin-bound fatty acids for 48 hr [C18:1 (solid bars) or C20:5 (hatched bars) at 300 μM]. CAT activity was measured and expressed as units (cpm of ^{14}C-acetylated chloramphenicol per hr per 100 μg of protein). This study is representative of three separate studies with three samples per group. The standard deviation within each group was <20%.

contains a series of response elements that are regulated by insulin, carbohydrate, glucocorticoids, retinoic acid, and tissue-specific factors (13–18). A third site located between −2500 and −2900 bp contains putative TREs (10, 11).

Hepatocytes were cotransfected with the MLVTRβ_1 thyroid hormone receptor expression vector and either S14CAT4.3, S14CAT1.6, or S14CAT0.29 fusion genes (Fig. 5). Cells were treated with T_3 to maximally induce S14CAT activity. Each group was treated with either C18:1 or C20:5 at 300 μM. In C18:1-treated cells, T_3 induced CAT activity from the S14CAT4.3, S14CAT1.6, and S14CAT0.29 genes 100-, 18-, and 3.2-fold, respectively. C20:5 inhibited the T_3 stimulation of S14CAT4.3, S14CAT1.6, and S14CAT0.29 by 68%, 81%, and 62%, respectively. This pattern of inhibition suggests that the principal target for PUFA control may not be within the two enhancers, at −1.4 to −1.6 kb and −2.5 to −2.9 kb, but within the S14 proximal promoter (+19/−290).

Because the S14 proximal promoter has weak activity in the absence of a strong enhancer, the S14 TRE (−2500/−2900) was inserted upstream from the proximal promoter (+19 to −290 bp) to assess further the effects of C20:5. T_3 induced high levels (>80-fold) of CAT activity in C18:1-treated cells, and C20:5 suppressed the T_3-stimulated activity by 65%. These results are similar to the effects of T_3 and C20:5 on S14CAT4.3 activity.

To determine whether the S14 TRE was a target for C20:5 control, the S14 TRE was ligated to the TK promoter (Fig. 5). The TKCAT gene is weakly (<20%) inhibited by C20:5. Addition of the S14 TRE conferred high levels of T_3 control to the TKCAT gene (75-fold), and C20:5 suppressed TKCAT activity by <20%, a level of inhibition similar to that seen with the TK promoter alone. Based on these studies, the S14 TRE is not sensitive to C20:5 inhibition. Thus, our studies suggest that elements within the S14 proximal promoter harbor PUFA-RE.

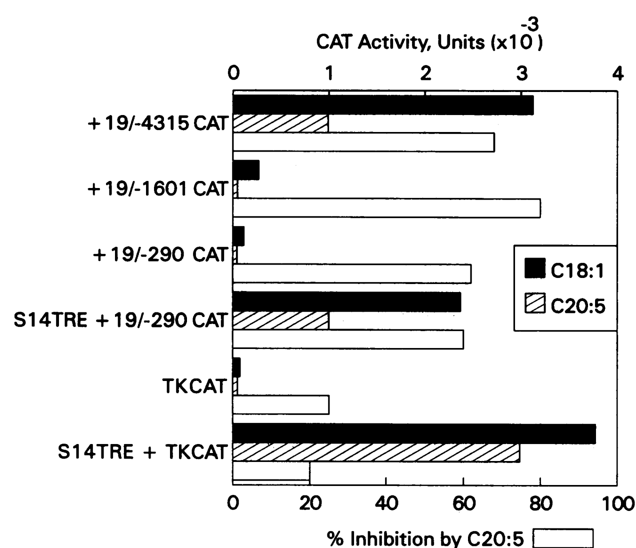

FIG. 5. Deletion analysis of the S14 5′ flanking DNA. Hepatocytes were transfected with S14CAT genes with 5′ end points at −4315, −1601, or −290 or with the S14 TRE (−2500/−2900) ligated upstream from the S14 promoter (−290) or upstream from the TK promoter. CAT activity was measured in cells transfected with a TKCAT construct without and with the S14 TRE ligated upstream. Hepatocytes were cotransfected with MLVTRβ_1 and treated with T_3. Cells were also treated with either 300 μM C18:1 (solid bar) or C20:5 (hatched bar) for 48 hr. CAT activity was measured and expressed as units. The percent inhibition by C20:5 is shown by the open bars. These results represent the mean of three separate studies with three samples per group. The standard deviation in each group was <15%.

Deletion Analysis of the S14 Proximal Promoter. To localize the PUFA-RE within the S14 proximal promoter, a series of promoter deletions were constructed. The promoter fragments (+19/−290, +19/−220, +19/−121, and +19/−80) were inserted in normal orientation into a plasmid containing the S14 TRE located upstream (5′) of the CAT gene (Fig. 6). T_3 stimulated CAT activity in C18:1-treated cells by 149-fold, while in C20:5-treated cells, T_3 stimulated S14CAT activity 56-fold, representing a 64% decline in the T_3-mediated induction of S14CAT activity. T_3 and C20:5 induced similar effects on S14CAT activity with 5′ promoter end points at −220. However, when the promoter was deleted to −121 bp, the degree of C20:5 inhibition declined to 24%. This plasmid was still highly sensitive to T_3 induction (150-fold). Truncating the promoter to −80 bp had two effects: (*i*) a decline in T_3 response to only 37-fold and (*ii*) an abrogation of C20:5 sensitivity. This pattern of control suggests that the 140-bp region between −80 and −220 bp contains two functional elements. One potentiates T_3 activation of S14 gene transcription, and the other is a target for PUFA attenuation of gene transcription.

DISCUSSION

We have used the S14 gene as a model to examine the regulation of lipogenic gene expression by dietary PUFAs. This study confirms and extends previous reports (7–9) by showing that (*i*) feeding rats menhaden oil rapidly attenuates (within hours) S14 gene transcription leading to suppression of hepatic S14 mRNA levels, (*ii*) specific ω3 and ω6 PUFAs act directly on hepatocytes to suppress S14 mRNA and inhibit S14CAT activity, (*iii*) C20:5 attenuates T_3-stimulated S14 gene transcription, and (*iv*) ω3 fatty acids target cis-response elements located within the S14 proximal promoter (−80 and −220 bp).

The potent suppressive action of menhaden oil is attributed to the presence of PUFAs, like C20:5ω3 and C22:6ω3 (21–23, 31, 32). While treating hepatocytes with C18:1 had little effect on S14 gene expression, both C20:4 and C20:5 suppressed expression of the endogenous gene (S14 mRNA) and the transfected S14CAT genes in a dose-dependent fashion, $ED_{50} \leq 100$ μM (Fig. 3). PUFA action on hepatic S14 gene

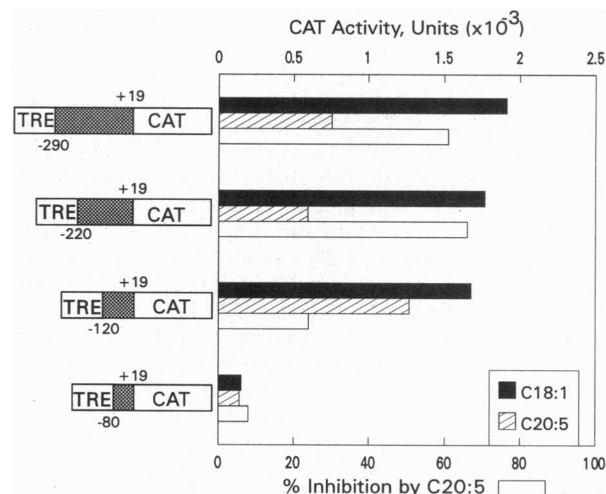

FIG. 6. Deletion analysis of the S14 proximal promoter. Proximal promoter deletions were constructed as described in *Materials and Methods*. The S14 TRE was ligated upstream from each promoter construct to amplify CAT activity. Cells were cotransfected with MLVTRβ_1 and treated with T_3 to evaluate hormone responsiveness. Cells were also treated with either oleic acid (solid bars) or EPA (hatched bars) for 48 hr. The percent inhibition by EPA is shown by the open bars. This study represents the mean of two separate studies each with three samples per group. The standard deviation in each group was <15%.

expression is direct and does not require extrahepatic mediators (e.g., gut hormones, glucagon, or extrahepatic metabolism of ω3 fatty acids).

The *in vivo* feeding studies illustrate the rapidity of PUFA action on hepatic gene expression. In contrast to white adipose tissue, the liver is an early target for PUFA action. Fatty acid synthase (7–9, 32), malic enzyme (33), steroyl-CoA desaturase (34), and glucose-6-phosphate dehydrogenase (6, 33) are also inhibited by dietary fat. Finding that transcription of these genes is also rapidly suppressed might suggest that PUFA feeding activates a coordinate regulatory mechanism directed at several genes involved in lipid metabolism. This effect is specific because mRNAs coding for PepCk and β-actin were not consistently affected by these dietary fats. Understanding this coordinate regulatory mechanism will be important for defining the overall mechanism of PUFA regulation of hepatic lipid synthesis.

The PUFA suppression of S14 gene expression at the transcriptional level suggests a role for PUFA-regulated trans-acting factors. While this study has not identified such factors, we have localized the cis-linked targets of PUFA action to a 140-bp region within the proximal promoter of the S14 gene (i.e., −80 to −220 bp). This region does not contain any known hormone response elements controlling S14 gene transcription. However, this region contains elements that function to initiate S14 gene transcription (15, 20). These PUFA-REs are regulated by C20:4 and C20:5 and interfere with the T_3 control of S14 gene transcription. T_3 is a major activator of hepatic S14 gene transcription and regulates S14 gene transcription through TREs located far upstream from the promoter, at −2.5 to −2.9 kb (10, 11, 17). The S14 TRE was insensitive to PUFA control when inserted upstream from either the TK promoter or the truncated S14 promoter (+19 to −80 bp). Finding that the S14 TRE was not a target for PUFA action suggested that PUFAs may not have generalized effects on other T_3-regulated genes.

The S14 proximal promoter (+19 to −220 bp) contains three cis-acting elements that function in the initiation of gene transcription: (*i*) a modified TATA box (at −27 bp), (*ii*) a NF-1/CTF-like binding site (at −48 to −63 bp), and (*iii*) a cis-acting element (region B, at −88 to −113 bp) that binds tissue-specific factors (20). The B-region also functions to augment S14 gene transcription (15, 20). Deletion of the B region results in an >80% decline in T_3 response and a loss of PUFA sensitivity (Fig. 6). Deletion of the S14 promoter to −120 bp yields a promoter that remains highly sensitive to T_3, but is only moderately (≤24%) sensitive to PUFA control. This pattern of regulation suggests that factors binding to the B-region function to augment T_3 control of transcription. Factors binding upstream from the B-region or within the B-region are targets for C20:5 action. DNase I footprinting and gel shift analysis have not detected specific DNA–protein interaction within the −113 to −220 bp region (data not shown). This is not unexpected if the PUFA-regulated trans-acting factor is a low abundance protein (e.g., a ligand-activated receptor). While the PUFA-regulated trans-acting factors still remain to be identified, a possible candidate might be the peroxisome proliferator-activated receptor [i.e., PPAR (36)]. Peroxisomes are induced in liver by fatty acids (37, 38), and recent studies have reported that some fatty acids activate PPAR (37, 38). PPAR is a member of the steroid/thyroid/retinoid superfamily of nuclear receptors (36).

In conclusion, we have shown that ω3 fatty acids in menhaden oil can effectively inhibit S14 gene transcription *in vivo* and in cultured primary hepatocytes. A cis-acting target for PUFA control (i.e., a PUFA-RE) has been localized to the proximal promoter region (−80 to −220 bp) of the S14 gene. The next step will be to identify the trans-acting factors that bind these elements and to determine how PUFA (or their metabolites) regulate these factors.

The authors wish to thank H. Towle, R. Hanson, L. Kedes, S. Conrad, and V. Mahdavi for the plasmids used in these studies and H. Towle for the helpful advice on hepatocyte cultures and transfections. The authors also wish to thank Pat Fair and her colleagues at the Southeast Fisheries Science Center–Charleston Laboratory (Charleston, SC) for the fatty acid analyses of the MaxEPA used in this study. This work was supported by the National Institutes of Health (DK43320).

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