THE JOURNAL OF BIOLOGICAL CHEMISTRY Vol. 272, No. 43, Issue of October 24, pp. 26827–26832, 1997
Printed in U.S.A.

Polyunsaturated Fatty Acid Suppression of Hepatic Fatty Acid Synthase and S14 Gene Expression Does Not Require Peroxisome Proliferator-activated Receptor α*

(Received for publication, March 21, 1996, and in revised form, June 20, 1997)

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Dietary polyunsaturated fatty acids (PUFA) induce hepatic peroxisomal and microsomal fatty acid oxidation and suppress lipogenic gene expression. The peroxisome proliferator-activated receptor α (PPARα) has been implicated as a mediator of fatty acid effects on gene transcription. This report uses the PPARα-deficient mouse to examine the role of PPARα in the PUFA regulation of mRNAs encoding hepatic lipogenic (fatty acid synthase (FAS) and the S14 protein (S14)), microsomal (cytochrome P450 4A2 (CYP4A2)), and peroxisomal (acyl-CoA oxidase (AOX)) enzymes. PUFA ingestion induced $mRNA_{AOX}$ (2.3-fold) and $mRNA_{CYP4A2}$ (8-fold) and suppressed $mRNA_{FAS}$ and $mRNA_{S14}$ by ≥80% in wild type mice. In PPARα-deficient mice, PUFA did not induce $mRNA_{AOX}$ or $mRNA_{CYP4A2}$, indicating a requirement for PPARα in the PUFA-mediated induction of these enzymes. However, PUFA still suppressed $mRNA_{FAS}$ and $mRNA_{S14}$ in the PPARα-deficient mice. Studies in rats provided additional support for the differential regulation of lipogenic and peroxisomal enzymes by PUFA. These studies provide evidence for two distinct pathways for PUFA control of hepatic lipid metabolism. One requires PPARα and is involved in regulating peroxisomal and microsomal enzymes. The other pathway does not require PPARα and is involved in the PUFA-mediated suppression of lipogenic gene expression.

Dietary polyunsaturated fatty acids (PUFAs),[1] in particular highly unsaturated *n*-3 fatty acids, suppress hepatic lipogenesis and triglyceride synthesis/secretion while inducing peroxisomal and microsomal fatty acid oxidation (1–12). These effects on lipid metabolism are due to changes in gene expression leading to induction or suppression of mRNAs encoding key metabolic enzymes (4, 13–19). PUFA administration *in vivo* or to cultured rat hepatocytes rapidly inhibits the transcription of genes encoding fatty acid synthase, stearoyl-CoA desaturase, the S14 protein, and L-type pyruvate kinase (15–19). While the molecular mediators for PUFA regulation of these genes have not been defined, studies on the regulation of peroxisomal enzymes suggest that fatty acids activate a nuclear receptor to control gene transcription (20–24).

Peroxisome proliferators encompass a wide variety of compounds including hypolipidemic drugs (WY14,643, gemfibrozil, and clofibrate), plasticizers (di-(2-ethyhexyl)phthalate), steroids (dehyrdoepiandrosterone and dehyrdoepiandrosterone-sulfate), and dietary fatty acids (25–27). Collectively, peroxisome proliferator-induced changes in gene expression are mediated by activating a nuclear receptor, the peroxisome proliferator-activated receptor (PPAR). PPARs are members of the steroid/thyroid superfamily of nuclear receptors (20–28). Activation of PPAR-mediated transcription is achieved through PPAR-RXR heterodimers which bind DNA motifs called peroxisome proliferator response elements (PPRE) located in promoters of target genes (20–33). PPARs also have inhibitory effects on gene transcription. For example, apolipoprotein CIII and transferrin gene expression is inhibited by PPAR-RXR competition for an HNF-4 binding site within the promoters of these genes (34, 35). PPARα has also been shown to interfere with thyroid hormone action by sequestering RXRα, a factor required for thyroid hormone receptor binding to DNA (36–38).

The fact that PPARs are activated by fatty acids in conjunction with the known effects of PUFA on peroxisomal and microsomal fatty acid oxidation suggest that PUFA regulation of these pathways might utilize a common transcriptional mediator, *i.e.* PPAR. While several PPAR subtypes (α, β (also known as δ, Nuc1, FAAR), γ1, and γ2) have been identified in rodents, PPARα is the predominant form in rodent liver (22–24, 28, 31, 39). Recent gene targeting studies clearly demonstrate that PPARα is required for the pleiotropic response to peroxisome proliferators including an increase in hepatic mRNAs encoding peroxisomal and microsomal enzymes (40).

This report examines the role of PPARα in PUFA-regulation of mRNAs encoding hepatic lipogenic, microsomal and peroxisomal enzymes. In this work, we assessed PUFA regulation of the *S14* gene and fatty acid synthase, models for lipogenic gene expression, and acyl-CoA oxidase (AOX) and cytochrome P450A2 (CYP4A2), enzymes involved in peroxisomal and microsomal fatty acid oxidation, respectively. The recently developed PPARα-null mouse (40) was used to determine whether PPARα mediates PUFA regulation of hepatic *AOX*, *CYP4A2*, *S14*, and *FAS* gene expression. This work shows that while PPARα is required for the PUFA-mediated induction of both *AOX* and *CYP4A2* gene expression, it is not required for the PUFA-mediated inhibition of either *S14* or *FAS* gene expression. These and other studies indicate that PUFA regulation of hepatic gene transcription involves at least two distinct pathways, a PPARα-dependent and a PPARα-independent pathway.

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[1] The abbreviations used are: PUFA, polyunsaturated fatty acids; PPAR, peroxisome proliferator-activated receptor; AOX, acyl-CoA oxidase; RXR, retinoid X receptor; FAS, fatty acid synthase; CYP4A2, cytochrome P450 4A2; PPRE, peroxisome proliferator response element; TK, thymidine kinase; CAT, chloramphenicol acetyltransferase; PCR, polymerase chain reaction.

MATERIALS AND METHODS

Animals and Diets—Male Sprague-Dawley rats (125–150 g) were obtained from Charles River Breeding Laboratories (Kalamazoo, MI). Male C57BL/6N × Sv/129 mice (25–35 g), F_6 homozygote wild-type (+/+) or knockout (−/−) were used for one of the feeding studies (40). Rats and mice were maintained on Teklad chow diet. In all feeding studies, rats and mice were meal-trained to a high carbohydrate diet as described previously (16, 17). The test diets consisted of a high carbohydrate (58% glucose) diet (ICN, Cleveland, OH) supplemented with either 10% (w/w) of complex fats (triolein, olive oil, fish (menhaden)), fatty acid ethyl esters (eicosapentaenoic acid or docosahexaenoic acid (Southeast Fisheries Science Center, Charleston, SC)), or 0.2% gemfibrozil (Sigma). All diets were supplemented with 0.1% butylated hydroxytoluene (17).

RNA Analysis—Total RNA from rat or mouse livers or from cultured rat primary hepatocytes was isolated using the guanidinium isothiocyanate procedure. The following cDNA probes were used to measure specific mRNAs: S14, pExoPEII6 (16); acyl-CoA oxidase, pTZ18R obtained from T. Osumi, Himeji Institute, Japan (41); fatty acid synthase, pFAS1 obtained from H. S. Sul, University of California-Berkeley (42), and β-actin, pRBA-1, obtained from L. Kedes, Stanford University, Palo Alto, CA.

The cDNA for *CYP4A2* was cloned by differential display screening (43) of rat liver RNA. First strand cDNA was prepared (Delta RNA Fingerprinting Kit, K1801–1, CLONTECH) from rat liver RNA of rats fed olive oil and fish oil for 5 days. PCR was performed using a Perkin-Elmer DNA thermal cycler 9600 as follows: one cycle of 94 °C, 10 s; 40 °C, 5 min; 68 °C, 5 min; two cycles of 94 °C, 5 s; 40 °C, 5 min, 68 °C, 5 min; 25 cycles of 94 °C 5 s; 60 °C, 1 min; and 68 °C, 2 min for 30 cycles of 95 °C/min; 60 °C/min; 72 °C/min. ^{35}S-Labeled PCR products were separated by denaturing polyacrylamide gel electrophoresis and visualized by autoradiography. A band present in the fish oil derived cDNA and diminished in the olive oil derived cDNA was excised from the gel, reamplified by PCR, and cloned into pGEM-T (Promega). The insert was sequenced by dideoxynucleotide sequencing (44). Computer assisted analysis (GCG, University of Wisconsin) of the cloned sequence with the GenBank™ data base indicated that the cloned sequence was identical to a region of the rat cytochrome P450 4A2 sequence (accession no. 57719) at nucleotides 12624–12728 for the rat gene. This sequence was in the noncoding 3′-untranslated region. To obtain the sequence unique to *CYP4A2* and containing the translated sequence, two PCR primers (5′-primer, 5′-atatatgaattc^{7370}CACATGGGACCACCTGG; 3′-primer, 5′-atatatatcgat^{12650}GCTGGGAAGGTG TCTGGAGT (underlined sequence represents *Eco*RI and *Cla*I cloning sites, respectively; the superscripted numbers represent the position of the nucleotide in the rat *CYP4A2* gene)) were used to generate a cDNA from liver RNA of fish oil-fed rats by reverse transcriptase-PCR. The identity of the clone (pCYP4A2-2) was verified by DNA sequencing and used to measure *CYP4A2* mRNA.

mRNA levels were measured by dot and Northern blot analyses (17) and the level of hybridization was quantified using a Molecular Dynamics PhosphorImager (Sunnyvale, CA) or by videodensitometry using an Agfa-2 scanner linked to a Macintosh computer with NIH Image software.

Plasmid Construction and Primary Hepatocytes—The construction of the reporter gene with the rat AOX PPRE fused upstream from the thymidine kinase promoter (TKCAT223) was described previously (38).

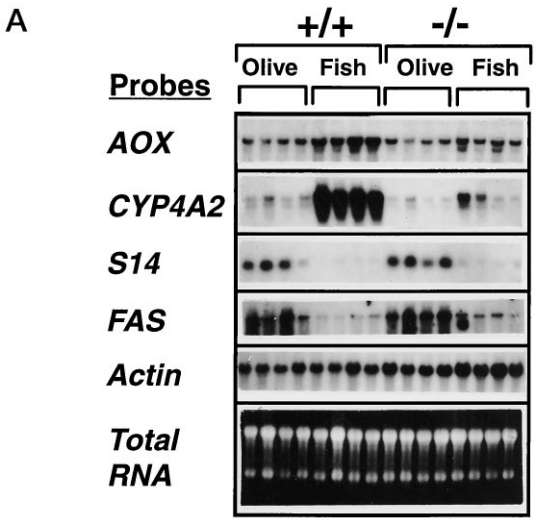
Primary hepatocytes were obtained from rat liver by the collagenase perfusion method and transfected with specific DNAs in the presence of Lipofectin (16). Hepatocytes were treated with triiodothyronine along with specific fatty acids or peroxisome proliferators (WY14,643 or gemfibrozil dissolved in Me_2SO) (16, 38). After 48 h of treatment, hepatocytes were analyzed for protein and CAT activity (38). CAT activity is defined as CAT units = counts/min of ^{14}C-butylated chloramphenicol/h/100 μg of protein.

Statistical Analysis—All data are presented as the mean ± S.E. Statistical comparisons were made by a single-factor factorial analysis of variance using Microsoft Excel version 7.

RESULTS

The Effects of Olive and Fish Oil on Hepatic Gene Expression in Wild-type (+/+) and PPARα Null (−/−) Mice—PPARα is the predominant PPAR subtype in rodent liver and has a central role in regulating the transcription of genes encoding hepatic peroxisomal and microsomal enzymes (24, 28, 40). To determine whether PPARα mediates PUFA regulation of hepatic gene expression wild type (+/+) and PPARα null (−/−), mice were fed an olive oil or fish oil diet for 5 days. Northern analyses show that feeding (+/+) mice fish oil for 5 days resulted in a ~2-fold ($p < 0.003$) and ~9-fold ($p < 0.001$) increase in hepatic $mRNA_{AOX}$ and $mRNA_{CYP4A2}$, respectively (Fig. 1, *A* and *B*). In contrast, fish oil did not significantly induce $mRNA_{AOX}$ and $mRNA_{CYP4A2}$ in the PPARα null (−/−) mice. These results indicate that PPARα is required for the PUFA-mediated induction of *AOX* and *CYP4A2* mRNAs. While hepatic β-actin mRNA was elevated in the (−/−) mice when compared with the (+/+) mice, it was not affected by dietary manipulation.

Analysis of mRNAs encoding S14 and FAS shows both mRNAs were suppressed (≥70%) in both the wild type (+/+) and PPARα null (−/−) mice following fish oil feeding. Since



B

Relative mRNA Abundance

	+/+			-/-		
	Olive	Fish	ANOVA	Olive	Fish	ANOVA
AOX	1 ± 0.1	2.3 ± 0.5	p<0.003	1.1 ± 0.2	1.4 ± 0.3	N.S.
CYP4A2	1 ± 0.3	8.9 ± 1.2	p<0.001	0.5 ± 0.3	1.3 ± 1.1	N. S.
S14	1 ± 0.2	0.05 ± 0.03	p<0.001	1.1 ± 0.2	0.23 ± 0.01	p<0.001
FAS	1 ± 0.4	0.2 ± 0.1	p<0.012	1.6 ± 0.4	0.4 ± 0.3	p<0.013
β-Actin	1 ± 0.2	1.3 ± 0.3	N. S.	1.7 ± 0.2	2.1 ± 0.5	N. S.

FIG. 1. **The effects of olive and fish oil feeding on levels of $mRNA_{AOX}$, $mRNA_{CYP4A2}$, $mRNA_{S14}$, and $mRNA_{FAS}$ from both wild-type (+/+) and PPARα null (−/−) mice.** Eight mice of each genotype were meal-fed with diets supplemented with 10% olive oil for 10 days. Four of each genotype were switched to a diet supplemented with 10% fish oil for 5 days. Total RNA was prepared from mouse livers and measured for mRNAs encoding *AOX*, *CYP4A2*, *S14*, *FAS*, and β-actin by Northern analysis. Ethidium bromide staining of the RNA on a typical agarose gel indicates that the RNA was evenly loaded. The results of the hybridization are displayed (*A*) and quantified (*B*). *A*, The *AOX*, *CYP4A2*, *S14*, and *FAS* and β-actin blots were exposed to x-ray film for 36, 18, 24, 24, and 18 h, respectively. *B*, the level of hybridization was quantified by a PhosphorImager and normalized against the level of expression of the corresponding mRNAs in olive oil-fed mouse liver; $n = 4$. The statistical comparison (analysis of variance, *ANOVA*) was for olive oil *versus* fish oil-fed mice. *N.S.*, not significant.

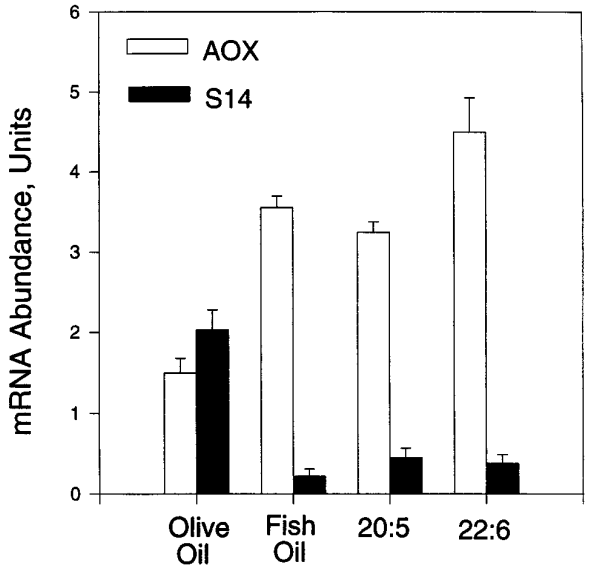


FIG. 2. **A comparison of the effect of olive oil and fish oil on hepatic *S14* and *AOX* gene expression *in vivo*.** Rats were meal-fed with diets supplemented with 10% (w/w) olive oil, menhaden (fish) oil, eicosapentaenoic acid (20:5), or docosahexaenoic acid (22:6) for 5 days. Total hepatic RNA was prepared and examined by dot-blot analysis for the effect of feeding on $mRNA_{AOX}$ and $mRNA_{S14}$ levels. The results were quantified and normalized against the level of hepatic mRNA expressed in chow-fed rats, *i.e.* 1 unit. Analysis of variance for both $mRNA_{AOX}$ and $mRNA_{S14}$ levels for menhaden oil-, 20:5-, and 22:6-fed *versus* olive oil-fed, $p < 0.002$. These results are representative of two separate studies; $n = 4$.

PUFA rapidly inhibits the transcription of both the S14 and the *FAS* gene (16, 17), these observations indicate that PPARα is not required for the PUFA-mediated suppression of transcription of these genes.

The Effects of Olive Oil and Fish Oil on Hepatic AOX and S14 Gene Expression—Previous studies have shown that *S14* and *FAS* are regulated by PUFA and peroxisomal proliferators in rat liver or primary hepatocytes (17, 45). The studies described below compare the PUFA and peroxisome proliferator regulation of *S14* and *AOX* in rat liver and primary hepatocytes. These *in vivo* and *in vitro* (rat primary hepatocytes) studies were performed to gain additional support for the idea that PUFA regulation of lipogenic gene expression is a result of a different pathway than PUFA regulation of AOX gene expression.

Rats were meal-fed diets supplemented with 10% olive oil, fish oil, eicosapentaenoic acid (20:5), or docosahexaenoic acid (22:6) for 5 days. When compared with chow-fed rats, hepatic $mRNA_{AOX}$ is elevated ~40% in olive oil-fed rats and ~3-fold in fish oil-, 20:5-, and 22:6-fed rats (Fig. 2). $mRNA_{CYP4A2}$ was induced >10-fold under similar conditions.[2] While $mRNA_{S14}$ is induced ~2-fold by the olive oil feeding, fish oil, 20:5, and 22:6 suppressed $mRNA_{S14}$ by ≥78%. Hepatic $mRNA_{FAS}$ is also suppressed in fish oil-fed rats (17). Feeding mice (Fig. 1) or rats (Fig. 2) fish oil or their highly unsaturated fatty acid constituents (20:5 (*n*-3) or 22:6 (*n*-3)) leads to a pronounced induction of $mRNA_{AOX}$ and $mRNA_{CYP4A2}$ while inhibiting expression of $mRNA_{S14}$ and $mRNA_{FAS}$.

Time Course of Fish Oil and Gemfibrozil Effects on Hepatic S14 and AOX Gene Expression in Vivo—The rapidity of fish oil action on hepatic $mRNA_{AOX}$ and $mRNA_{S14}$ was examined in rats fed fish oil for 1 to 5 days (Fig. 3*A*). Rats were meal-fed a high carbohydrate diet supplemented with10% triolein oil for 10 days. Subsequently, half of the rats were maintained on this

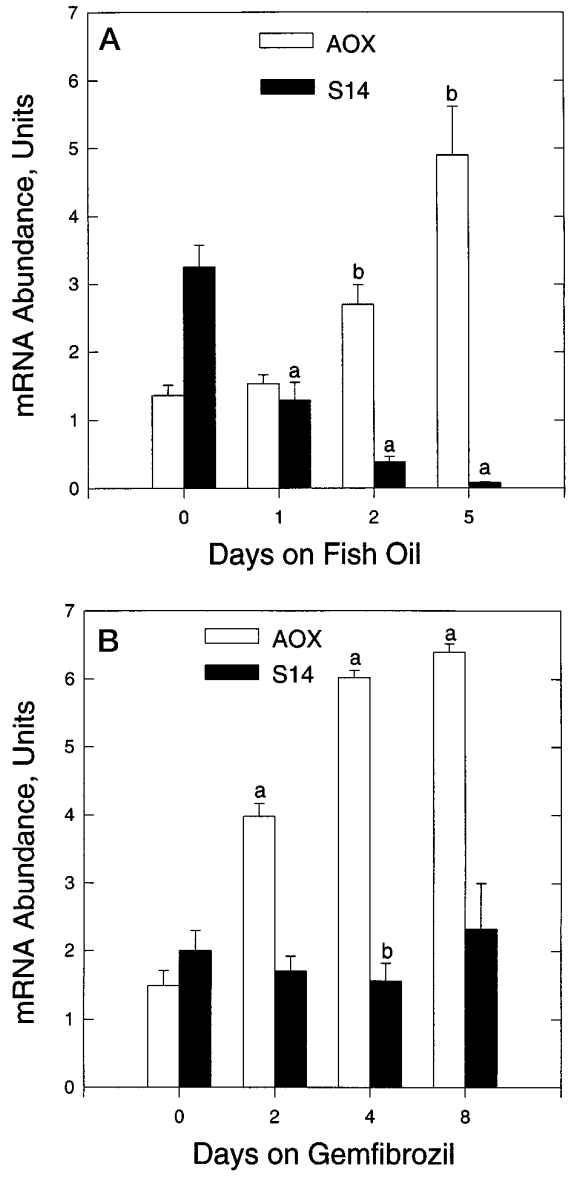


FIG. 3. **Time course of fish oil and gemfibrozil effects on rat hepatic *S14* and *AOX* gene expression.** *A*, rats were meal-fed diets supplemented with 10% (w/w) triolein oil for 10 days. Half of the rats were maintained on triolein oil, while the other half were switched to the fish oil diet. Both triolein- and fish oil-fed rats were killed after 1, 2, and 5 days. Total liver RNA was prepared and examined by dot-blot analysis for $mRNA_{AOX}$ and $mRNA_{S14}$ levels; $n = 4$–10 per time point. These results are representative of two separate studies. The results were quantified and normalized against the level of hepatic mRNA expressed in chow-fed rats, *i.e.* 1 unit. Analysis of variance: *a*, 1-, 2-, and 5-day fish oil feeding on $mRNA_{S14}$ *versus* triolein oil fed $mRNA_{S14}$, $p < 0.001$; *b*, 2- and 5-day fish oil feeding on $mRNA_{AOX}$ *versus* triolein oil fed $mRNA_{AOX}$, $p < 0.001$. *B*, after meal-training rats to a high carbohydrate fat-free diet, half of the rats were maintained on the high carbohydrate, fat-free diet, while the other half was switched to a diet supplemented with 0.2% (w/w) gemfibrozil for 2, 4, and 8 days. Dot-blot analysis was used to measure the $mRNA_{AOX}$ and $mRNA_{S14}$ levels. The results were quantified and normalized against the level of hepatic mRNA expressed in chow-fed rats, *i.e.* 1 unit; $n = 3$–4 per time point. These results are representative of two separate studies. Analysis of variance: *a*, 2, 4, and 8 days, gemfibrozil *versus* control diet fed, $mRNA_{AOX}$, $p < 0.004$; *b*, 4-day gemfibrozil fed *versus* control diet fed, $mRNA_{S14}$, $p = 0.002$.

diet and half were switched to a high carbohydrate diet supplemented with 10% fish oil. Fish oil feeding induced a rapid suppression of $mRNA_{S14}$; a 60% suppression was observed within 1 day of switching the diet from triolein to fish oil. Similar effects on mRNAs encoding FAS and L-pyruvate kinase

[2] B. Ren, A. Thelen, and D. B. Jump, unpublished results.

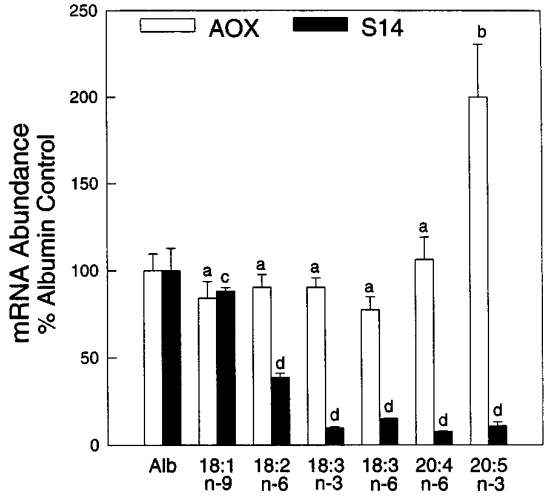


FIG. 4. **A comparison of various fatty acids on *S14* and *AOX* gene expression in primary rat hepatocytes.** Primary rat hepatocytes prepared by the collagenase perfusion were plated into 100-mm Primaria tissue culture plates in the presence of 1 μM triiodothyronine, 1 μM insulin, and 50 μM albumin. The cells were maintained in medium containing different fatty acids (250 μM) for 48 h (17). Total RNA was prepared from the hepatocytes and analyzed by dot blot analysis for mRNA coding for *AOX* and *S14*. The results were quantified and normalized against the level of *AOX* or *S14* mRNA expressed in hepatocytes receiving no fatty acid treatment (*albumin control*). Analysis of variance: *a*, 18:1 (*n*-9)-, 18:2 (*n*-6)-, 18:3 (*n*-3)-, 18:3 (*n*-6)-, or 20:4 (*n*-6)-treated $mRNA_{AOX}$ *versus* albumin-treated $mRNA_{AOX}$, $p \geq 0.07$; *b*, 20:5 (*n*-3)-treated $mRNA_{AOX}$ *versus* albumin-treated $mRNA_{AOX}$, $p = 0.006$; *c*, 18:1-treated $mRNA_{S14}$ *versus* albumin-treated $mRNA_{S14}$, $p = 0.01$; *d*, 18:2 (*n*-6)-, 18:3 (*n*-3)-, 18:3 (*n*-6)-, 20:4 (*n*-6)-, or 20:5 (*n*-3)-treated $mRNA_{S14}$ *versus* albumin-treated $mRNA_{S14}$, $p \leq 0.0004$. These results are representative of two separate studies; $n = 4$.

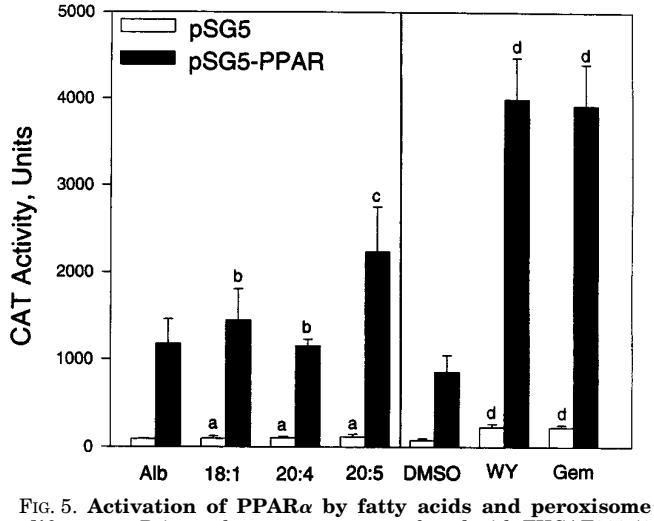


FIG. 5. **Activation of PPARα by fatty acids and peroxisome proliferators.** Primary hepatocytes co-transfected with TKCAT223 (1 μg) in the presence of 0.5 μg of pSG5 (*open bars*) or pSG5-PPARα (*closed bars*). The cells were treated with different fatty acids at the concentration of 250 μM in the presence of 50 μM albumin. A second group of cells were treated with either 100 μM WY14,643 or 100 μM gemfibrozil. Medium was replaced after 24 h, and cells were harvested after 48 h of treatment and assayed for protein levels and CAT activity. Analysis of variance: *a*, without PPARα, 18:1, 20:4, or 20:5 *versus* albumin, $p \geq 0.3$; *b*, with PPARα, 18:1, or 20:4 *versus* albumin, $p \geq 0.1$; *c*, with PPARα, 20:5 *versus* albumin, $p = 0.04$; *d*, with or without PPARα, $p \leq 0.007$. These results are representative of at least two separate studies; $n = 3$/study.

have were reported previously (17). In contrast, $mRNA_{AOX}$ remained unaffected after 1 day on the fish oil diet, yet was induced 2- and 3.5-fold after 2 and 5 days, respectively. Such studies indicate that changes in S14 mRNA precede changes in AOX mRNA following initiation of fish oil feeding, but they do not argue against PPARα as a common mediator for the PUFA-regulation of *AOX* and *S14*.

In an effort to separate the induction of *AOX* from the suppression of *S14*, the peroxisome proliferator, gemfibrozil was fed to rats at 0.2% (w/w) for up to 8 days (Fig. 3*B*). $mRNA_{AOX}$ was induced ~4-fold after 4 days on gemfibrozil, a level comparable to the level of $mRNA_{AOX}$ after 5 days on fish oil. In contrast, gemfibrozil did not significantly suppress $mRNA_{S14}$ (Fig. 3*B*) or $mRNA_{FAS}$ (not shown). Only a modest 22% inhibition of $mRNA_{S14}$ was seen after 4 days of gemfibrozil feeding. These results show that mRNAs encoding both *S14* and *AOX* are affected by PUFA within 2 days of initiating fish oil feeding. However, the absence of a significant inhibition of $mRNA_{S14}$ following 8 days of gemfibrozil feeding argues against PPARα as a common mediator for PUFA regulation of both *AOX* and *S14* gene expression.

Effect of Fatty Acids on AOX and S14 Gene Expression in Primary Hepatocytes—Primary hepatocytes provide a method to assess the direct effects of PUFA on hepatic gene expression (16, 17). To examine the effects of fatty acids on *S14* and *AOX* mRNAs, primary rat hepatocytes were treated with albumin alone or albumin plus various fatty acids (Fig. 4). Treatment of primary hepatocytes with 18:1, 18:2, 18:3 (both *n*-3 and *n*-6), and 20:4 did not induce $mRNA_{AOX}$. Only 20:5 treatment induced $mRNA_{AOX}$ (~2-fold). This finding is consistent with the effects of highly unsaturated fatty acids on *AOX* gene expression *in vivo* (Figs. 1 and 2). Oleic acid (18:1) did not affect *S14* gene expression when compared with controls. However, 18:2 (*n*-6) resulted in ~50% decline and 18:3 (both *n*-3 and *n*-6), 20:4 (*n*-6) and 20:5 (*n*-3) treatment resulted in >80% suppression of hepatocyte $mRNA_{S14}$ levels. These findings demonstrate that a broader spectrum of fatty acids affect *S14* gene expression than *AOX* gene expression in primary rat hepatocytes.

Fatty Acid Effects on Reporter Gene Activity in Primary Hepatocytes—To determine if fatty acids activate PPARα in liver, primary hepatocytes were transfected with a reporter gene containing the *AOX* PPRE fused to the thymidine kinase promoter, *i.e.* TKCAT223 (38). Primary hepatocytes were co-transfected with pSG5 (empty vector) or pSG5-mPPARα, a PPARα expression vector (Fig. 5). In the absence of co-transfected PPARα, TKCAT223 CAT activity was expressed at low levels (<150 CAT units) and this activity was marginally affected by fatty acid or peroxisome proliferator (WY14,643, gemfibrozil) treatment. Co-transfection with pSG5-PPARα led to at least a 10-fold stimulation of the TKCAT223 activity. Treatment of PPARα-transfected hepatocytes with 18:1 and 20:4 had no effect on CAT activity, while 20:5 treatment induced CAT activity by ~2-fold. This pattern of fatty acid regulation of PPARα is consistent with the effects of these fatty acids on $mRNA_{AOX}$ (Fig. 4). By comparison, both WY14,643 and gemfibrozil induced CAT activity by 4-fold. These studies show that long chain unsaturated fatty acids such as 18:1, 18:2, 18:3 (*n*-3 and *n*-6), and 20:4 do not activate PPARα in primary hepatocytes. Only the highly unsaturated fatty acid, 20:5, *n*-3 activates PPARα, albeit to a level less than WY14,643 or gemfibrozil. This pattern of control contrasts with the known effects of fatty acids on $mRNA_{S14}$ (16, 17) (Fig. 4) and S14CAT activity[3] in primary hepatocytes where 18:2 (*n*-6), 18:3 (*n*-3 and *n*-6), 20:4 (*n*-6), and 20:5 (*n*-3) inhibit *S14* gene expression 50–80%.

DISCUSSION

PPARα is the predominant PPAR subtype expressed in rat liver and it plays a central role in the induction of hepatic

[3] D. B. Jump, A. Thelen and M. Badin, manuscript in preparation.

peroxisomal and microsomal fatty acid oxidation (24, 40). Since several peroxisomal, microsomal and lipogenic enzymes are regulated by PUFA at the pretranslational level, we tested the hypothesis that dietary PUFA regulate hepatic fatty acid oxidation and *de novo* lipogenesis through a common mediator, *i.e.* PPARα. Interestingly, all studies reporting on fatty acid regulation of PPARα have been carried out by over expressing receptors in established cell lines. No studies have directly examined the role PPARα may have in fatty acid-regulated hepatic gene transcription. The PPARα-null mouse allows such an analysis. Coupling this genetic approach with other studies has allowed us to show for the first time that 1) PPARα is required for PUFA-mediated induction of hepatic $mRNA_{AOX}$ and $mRNA_{CYP4A2}$ (Fig. 1); 2) PPARα is not required for PUFA-mediated suppression of $mRNA_{S14}$ or $mRNA_{FAS}$ (Fig. 1); 3) while 18:2 (*n*-6), 18:3 (*n*-6 and *n*-3), 20:4 (*n*-6) and 20:5 (*n*-3) suppress $mRNA_{S14}$ and $mRNA_{FAS}$, only 20:5 (*n*-3) induces $mRNA_{AOX}$ in primary hepatocytes (Fig. 4); 4) while gemfibrozil induces hepatic $mRNA_{AOX}$, it has little or no effect on $mRNA_{S14}$ or $mRNA_{FAS}$ (Fig. 3). Taken together, these studies indicate that PUFA control of peroxisome/microsomal fatty acid oxidation and *de novo* lipogenesis in rat liver does not involved PPARα as a common mediator. The differential effect of specific fatty acids, *i.e.* 18:2, 18:3 (*n*-3 and *n*-6), 20:4 (*n*-6), *versus* gemfibrozil underscores the lack of coordinate regulation of these pathways in rat liver. Such studies indicate that PUFA regulates at least two pathways in liver, one involves PPARα and controls expression of genes encoding proteins involved in peroxisomal and microsomal fatty acid oxidation. The other mechanism is PPARα-independent and is involved in the PUFA-mediated suppression of lipogenic gene expression.

PUFA suppress hepatic $mRNA_{S14}$ and $mRNA_{FAS}$ levels by inhibiting gene transcription (15–17). From the data reported above, this inhibitory mechanism does not require PPARα. Although the mechanism of PUFA induction of hepatic $mRNA_{AOX}$ and $mRNA_{CYP4A2}$ has not been established, the following studies implicate transcription as the principal mode of PUFA regulation of *AOX* and *CYP4A*: 1) peroxisomal proliferators rapidly induce transcription of genes encoding AOX, the bifunctional enzyme, thiolase and CYP4A subtypes 1–3 (26, 40); 2) PPARα is required for the induction of these genes (40); 3) PPARα binds PPREs as PPAR/RXR heterodimers in the promoters of these genes and stimulates transcription of cis-linked reporter genes (20–33, 38); 4) fatty acids activate PPARα and stimulate transcription of cis-linked reporter genes (20–33) (Fig. 5); and 5) PPARα is required for the PUFA induction of hepatic $mRNA_{AOX}$ and $mRNA_{CYP4A2}$ (Fig. 1).

Previous efforts to examine the involvement of PPARα in PUFA regulation of lipogenic gene expression showed that the cis-regulatory targets for PUFA and PPAR in the *S14* promoter (38, 45) did not converge. Analysis of stearoyl-CoA desaturase 1 gene expression indicated that peroxisome proliferators/PPAR induced by PUFA-suppressed transcription (46). Such studies argued against PPAR as a mediator of PUFA effects on lipogenic gene transcription. However, the over expression of receptors does not necessarily reflect physiologically relevant processes. The use of the PPARα-null mouse allows us to directly evaluate the role PPARα plays in PUFA regulation of hepatic gene expression. In contrast to (+/+) mice, hepatic $mRNA_{AOX}$ and $mRNA_{CYP4A2}$ was not significantly induced in PPARα (−/−) mice by the PUFA diet indicating a requirement for PPARα in the PUFA-mediated induction of these enzymes. The fact that hepatic $mRNA_{S14}$ and $mRNA_{FAS}$ was suppressed in both (+/+) and (−/−) mice provides strong evidence against a requirement for PPARα for PUFA-mediated suppression of *S14* and *FAS* gene transcription. While these studies confirm our earlier suggestion that PPAR did not mediate PUFA suppression of *S14* gene transcription, they provide new information on the requirement for PPARα in the PUFA-induction of *AOX* and *CYP4A2* and the lack of involvement of PPARα in PUFA-mediated suppression of *FAS* gene transcription or L-pyruvate kinase gene expression.[2] While other PPAR subtypes (PPARγ and PPARδ) (24, 39) are expressed in liver, Northern analyses suggests PPARγ and δ are minor subtypes in rodent liver. However, their role in PUFA control of hepatic gene expression cannot be excluded.

An important outcome of these studies is the finding that of all the PUFA tested, only 20:5 (*n*-3) activates PPARα in liver. Several groups have reported on fatty acid activation of PPARs in established cells lines like CV-1 and HeLa (20–24). Recently three groups reported that specific fatty acids, *i.e.* 18:2 (*n*-6), 18:3 (*n*-3 and *n*-6), and 20:4 (*n*-6) are ligands for PPARα (47–49). These same ligands do not activate PPARα or induce $mRNA_{AOX}$ in primary hepatocytes (Figs. 4 and 5). Feeding animals soybean or corn oil, oils containing 18:2 and 18:3 fatty acids, does not induce peroxisomal enzymes (4). This apparent conflict can be reconciled by the fact that primary hepatocytes have a high capacity for fatty acid oxidation, triglyceride synthesis and very low density lipoprotein secretion (7). We speculate that these pathways prevent intracellular fatty acids from accumulating to levels that activate PPARα. Interestingly, 20:5 (*n*-3) was the only PUFA tested here that activated PPARα. 20:5 (*n*-3) is reported to be poorly oxidized in mitochondria and poorly incorporated into complex lipids, such as triglycerides (7). Thus, 20:5 (*n*-3) might accumulate in the cell and mimic a state of fatty acid overload in the liver. Fatty acid overload resulting from high fat feeding (>50% calories as fat), uncompensated diabetes and liver disease have all been reported to increase peroxisomal β-oxidation (26, 50, 51). Alternatively, 20:5 (*n*-3) might be metabolized to an active ligand. Recent studies have suggested that the leukotriene, LTB4, is a ligand for PPARα (52). Indeed, LTB4 is derived from 20:4 (*n*-6) by the action of 5-lipoxygenase and LTA4 hydrolase. If this pathway were operative, we would expect 20:4 (*n*-6) treatment of hepatocytes to activate PPARα and induce $mRNA_{AOX}$. The lack of a 20:4 (*n*-6) effect on $mRNA_{AOX}$ and PPARα along with the low LTA4 hydrolase activity associated with liver cells (53) suggest that LTB4 is not the operative ligand for 20:5 (*n*-3) activation of PPARα. However, the *in vitro* model used in this work may lack factors present in the *in vivo* system.

In summary, PUFA induce peroxisomal and microsomal fatty acid oxidation and suppress *de novo* lipogenesis (2–15). This apparent coordinate regulation of lipid metabolism does not involve PPARα as a common mediator. While highly unsaturated *n*-3 fatty acids, such as 20:5, can activate PPARα resulting in increased $mRNA_{AOX}$ and $mRNA_{CYP4A2}$, PPARα does not mediate the suppressive effects of 18:2, 18:3 (*n*-3 and *n*-6), 20:4 (*n*-6), or 20:5 (*n*-3) on lipogenic gene expression. Thus, PUFA suppression of *S14* and *FAS* gene transcription is mediated by a pathway that is independent of PPARα. The underlying mechanism of this alternative pathway is currently under investigation.

Acknowledgments—We thank Michelle Mater, Karl Olson, and David Pan for critical review of the manuscript. We also thank Drs. T. Osumi, H. S. Sul, and L. Kedes for the cDNA probes used in these studies.

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Polyunsaturated Fatty Acid Suppression of Hepatic Fatty Acid Synthase and S14 Gene Expression Does Not Require Peroxisome Proliferator-activated Receptor α

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J. Biol. Chem. 1997, 272:26827-26832.
doi: 10.1074/jbc.272.43.26827