Peroxisome proliferator-activated receptor α is required for feedback regulation of highly unsaturated fatty acid synthesis[1]

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Abstract **Δ6 desaturase (D6D), the rate-limiting enzyme for highly unsaturated fatty acid (HUFA) synthesis, is induced by essential fatty acid-deficient diets. Sterol regulatory element-binding protein-1c (SREBP-1c) in part mediates this induction. Paradoxically, D6D is also induced by ligands of peroxisome proliferator-activated receptor α (PPARα). Here, we report a novel physiological role of PPARα in the induction of genes specific for HUFA synthesis by essential fatty acid-deficient diets. D6D mRNA induction by essential fatty acid-deficient diets in wild-type mice was diminished in PPARα-null mice. This impaired D6D induction in PPARα-null mice was not attributable to feedback suppression by tissue HUFAs because PPARα-null mice had lower HUFAs in liver phospholipids than did wild-type mice. Furthermore, PPARα-responsive genes were induced in wild-type mice under essential fatty acid deficiency, suggesting the generation of endogenous PPARα ligand(s). Contrary to genes for HUFA synthesis, the induction of other lipogenic genes under essential fatty acid deficiency was higher in PPARα-null mice than in wild-type mice even though mature SREBP-1c protein did not differ between the genotypes. The expression of PPARγ was markedly increased in PPARα-null mice and might have contributed to the induction of genes for de novo lipogenesis. Our study suggests that PPARα, together with SREBP-1c, senses HUFA status and confers pathway-specific induction of HUFA synthesis by essential fatty acid-deficient diets.**—Li, Y., T. Y. Nara, and M. T. Nakamura. **Peroxisome proliferator-activated receptor α is required for feedback regulation of highly unsaturated fatty acid synthesis. *J. Lipid Res.* 2005.** 46: **2432–2440.**

Supplementary key words Δ6 desaturase • arachidonic acid • docosahexaenoic acid • essential fat deficiency • liver • peroxisome proliferator-activated receptor α-null mouse • polyunsaturated fatty acid • sterol regulatory element-binding protein-1c

In mammals, highly unsaturated fatty acids (HUFAs) such as arachidonic acid (20:4 n-6) and docosahexaenoic acid (22:6 n-3) are present in membrane phospholipids and are required for many physiological functions, including eicosanoid signaling, skin and hair integrity, vision and brain functions, cardioprotection, and the regulation of gene expression (1). Tissue HUFAs are maintained in a narrow concentration range to perform these functions. Although mammals are unable to synthesize HUFAs from acetyl-CoA, they are capable of synthesizing HUFAs from precursor PUFA, linoleic (18:2 n-6) and α-linolenic (18:3 n-3) acids (1). Δ6 desaturase (D6D) catalyzes the first and rate-limiting reaction of HUFA synthesis (2) and thus is subject to regulation by dietary fatty acids. D6D is induced when animals are fed a diet devoid of all n-6 and n-3 fatty acids (an essential fat-deficient diet), whereas D6D is suppressed by diets containing either a substrate or a product, the latter exerting stronger suppression (3, 4).

The underlying mechanism of this D6D regulation began emerging only recently. The enzyme activity of D6D largely parallels the expression of mRNA (4), which is regulated mainly at the transcriptional level (5). Two transcription factors, sterol regulatory element-binding protein-1c (SREBP-1c) and peroxisome proliferator-activated receptor α (PPARα), are involved in the regulation of the D6D gene. SREBP-1c, a transcription factor of the basic-helix-loop-helix-leucine-zipper family, induces a set of genes for fatty acid and glycerolipid synthesis (6), including all three mammalian desaturases: stearoyl-CoA desaturase (SCD) for monounsaturated fatty acid synthesis (7, 8) and D6D and Δ5 desaturase (D5D) for HUFA synthesis (9, 10). SREBP-1c

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Abbreviations: ACBP, acyl-coenzyme A binding protein; AOX, acyl-coenzyme A oxidase; CYP, cytochrome P450; D5D, Δ5 desaturase; D6D, Δ6 desaturase; HUFA, highly unsaturated fatty acid; PK, pyruvate kinase; PPAR, peroxisome proliferator-activated receptor; PPRE, peroxisome proliferator response element; SCD, stearoyl-coenzyme A desaturase; SREBP, sterol regulatory element-binding protein; TRB3, mammalian tribbles homolog.

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is synthesized as a precursor protein that is inserted into the endoplasmic reticulum membrane. Proteolytic cleavage is required to produce mature SREBP-1c protein, which then enters the nuclei and activates target genes (11). Dietary PUFAs suppress the induction of lipogenic genes in liver, whereas essential fat-deficient diets such as fat-free diet and triolein diet induce these genes (12, 13). SREBP-1c was identified as the factor that at least in part mediates this PUFA effect by binding the PUFA response sequence in promoters of FAS (14), S-14 (15), SCD (7, 16), and D6D (10). Dietary PUFAs exert their effects by reducing both mRNA and proteolytic activation of SREBP-1c (14, 17, 18). However, the mechanism of differential regulation among these SREBP-1c target genes has not been well characterized.

PPARα, a member of the nuclear receptor family, induces genes involved with mitochondrial and peroxisomal fatty acid β-oxidation as well as ketogenesis in liver (19, 20). Studies using targeted disruption of the PPARα gene have demonstrated that PPARα is essential for the upregulation of genes for fatty acid oxidation during fasting (21–23). Various long-chain fatty acids and hypolipidemic drugs called peroxisome proliferators bind and activate PPARα as agonistic ligands (24, 25). Although SREBP-1c and PPARα induce almost mutually exclusive sets of genes, three mammalian desaturases, SCD-1, D6D, and D5D, are induced not only by essential fat deficiency but also paradoxically by peroxisome proliferators in rats (26–28) and to a lesser extent in pigs (29). In rodents, peroxisome proliferators cause peroxisome proliferation and hepatomegaly in addition to the induction of fatty acid oxidation enzymes (20, 30). Thus, increased degradation of HUFAs and demand for membrane phospholipids may at least in part account for the marked induction of D6D by peroxisome proliferators. Consistent with this hypothesis, the induction of desaturases by peroxisome proliferator was slower than the induction of fatty acid oxidation enzymes (27, 28). In addition to the possible indirect induction, peroxisome proliferators can also directly, albeit modestly, induce SCD-1 (27) and D6D (5) in cell culture through peroxisome proliferator response element (PPRE) present in the promoters of these genes. The physiological role of PPRE in desaturases is unclear at present. As Matsuzaka et al. (9) implied, a possible function of PPARα could be to maintain HUFA synthesis in fasting conditions, when SREBP-1c is drastically decreased (31). It has yet to be determined whether PPARα has a previously unidentified essential function in the feedback regulation of HUFA synthesis.

Thus, the objectives of this study were to elucidate an in vivo regulatory role of PPARα in *1*) HUFA synthesis under essential fat deficiency and *2*) differential regulation between genes for HUFA synthesis and genes involved with other lipid metabolism.

METHODS

Animals and diets

Breeding pairs of PPARα-null mice (129S4/SvJae-Pparatm^{1Gonz}) (32) and wild-type mice (129S4/SvJae) were purchased from Jackson Laboratory (Bar Harbor, ME). Eight week old male mice were housed in individual cages and maintained at 24°C with a 12 h light/dark cycle and provided free access to food during the dark cycle. Before treatment, mice were fed a standard diet, AIN93G (33), for 1 week. Then, animals were divided into five groups (n = 5–6) and fed the following diets for 1 week: control (AIN93G, 7% soybean oil; fed), fasting (AIN93G; fasted), fat-free (soybean oil was replaced with isocaloric anhydrous D-glucose; fed), triolein [soybean oil was replaced with an equal amount of triolein, 99% purity (Sigma Chemical Co., St. Louis, MO); fed], or Wy [AIN93G containing 0.1% Wy14643 (Chemsyn Science Laboratories, Lenexa, KS); fed]. All animals except the fasting group were euthanized at the end of the 12 h meal period. Mice in the fasting group were euthanized after 24 h of fasting. For SREBP-1c protein analysis, liver was processed immediately. For the analyses of RNA and fatty acids in phospholipids, liver was frozen in liquid nitrogen and stored at −80°C. For the analysis of nonesterified fatty acids in liver, blood was expelled from liver by perfusing liver with phosphate-buffered saline through the portal vein, and then liver was immediately frozen in liquid nitrogen. The animal protocol was reviewed and approved by the Institutional Animal Care and Use Committee.

RNA analysis

A detailed procedure has been described elsewhere (29). Briefly, total RNA was prepared using TRIzol reagent (Invitrogen, Carlsbad, CA) and was reverse-transcribed using random hexamers. The mRNA was analyzed by real-time quantitative PCR using SYBR Green fluorescence dye (Applied Biosystems, Foster City, CA). The abundance of mRNA was expressed relative to 18S rRNA.

SREBP-1c protein analysis

Microsomal and nuclear extracts of mouse liver were prepared with a slight modification of the method of Sheng et al. (34). Fresh liver of individual mice was homogenated, and nuclear protein was extracted. Aliquots of liver homogenate for nuclear protein extraction were also used for microsomal protein preparation. Nuclear and microsomal protein was quantitated using the BCA protein assay kit (Pierce Biotechnology, Inc., Rockford, IL). Thirty micrograms of each protein was loaded for Western blotting. Precursor and mature protein were detected by monoclonal anti-SREBP-1 antibody (IgG-2A4) (ATCC, Manassas, VA).

Fatty acid analysis

This procedure was described previously (35). Briefly, total liver lipids were extracted using chloroform-methanol (2:1, v/v). To prevent hydrolysis of fatty acid esters, a piece of frozen liver was homogenized without thawing in the extraction solvent using Polytron PT1200C (Kinematica). C17:0 phosphatidylcholine and free fatty acids were added as internal standards at the extraction step. The extract was separated by thin-layer chromatography into phospholipids and free fatty acids, which then were methylated using methanolic HCl (Supelco, Bellefonte, PA). Each fatty acid species was quantified by HP5890 gas chromatography (Agilent Technologies, Wilmington, DE) with a 30 m × 0.25 mm PAG capillary column (Supelco).

Statistical analysis

Statistical analysis was performed using a commercial software package (Statview 5.0.1; SAS Institute, Cary, NC). Student's *t*-test was used for comparisons between two groups. One-way ANOVA followed by Fisher's protected least-squares difference posthoc test were used for comparisons among multiple treatment groups. Two-way ANOVA was used to determine genotype and diet effects. $P < 0.05$ was considered statistically significant.

TABLE 1. Liver weight of wild-type and PPARα-null mice fed various diets for 1 week

Mouse	Control	Fasting	Fat-Free	Triolein	Wy14643
Wild type	1.13 ± 0.10 b	0.80 ± 0.10 c	1.31 ± 0.20 b	1.21 ± 0.03 b	2.19 ± 0.26 a
PPARα-null	1.15 ± 0.17 b	0.63 ± 0.05 c	1.16 ± 0.16 b	1.14 ± 0.19 b	1.26 ± 0.19 b

PPARα, peroxisome proliferator-activated receptor α. Wild-type and PPARα-null mice were fed control (AIN93G), essential fat-deficient (fat-free and triolein), or Wy (control + 0.1% Wy14643) diets for 1 week. The fasting group was fed the control diet for 1 week and then fasted for 24 h before termination. Values shown are means ± SD (n = 4–5 animals). Groups bearing different letters differ significantly ($P < 0.05$) by Fisher's protected least-squares difference (PLSD) test.

RESULTS

D6D mRNA induction by essential fat-deficient diets was diminished in PPARα-null mice

Both fat-free and triolein diets were essential fat-deficient and were devoid of both n-6 and n-3 fatty acids. The amount of dietary fat in the triolein diet was kept the same as in the control diet, although all fat was provided as nonessential fat (18:1 n-9). There was no significant difference in body weight between wild-type and PPARα-null mice. Animals fasted for 24 h weighed 10% less than mice fed the control diet in both genotypes (data not shown). The liver weight of fasted animals was significantly lower than that of the control group (**Table 1**). The liver weight of other diet groups was unchanged with the exception that the wild-type mice fed Wy14643, a PPARα ligand, showed a marked increase in liver weight as a consequence of peroxisome proliferation in these animals (Table 1). Morphological changes in liver, including fatty liver, were not observed in any of the groups other than liver enlargement in the wild-type group fed Wy14643.

We measured the expression of D6D in the liver of wild-type and PPARα-null mice fed essential fat-deficient diets to determine whether PPARα is required for D6D induction under essential fat deficiency. Compared with the control diet, D6D expression in wild-type mice was significantly increased by fat-free and triolein diets (**Fig. 1A**). In contrast, the induction of D6D by fat-free and triolein diets was diminished in PPARα-null mice, indicating that PPARα is required for full induction of D6D in essential fat deficiency. Wy14643 induced D6D in wild-type mice, whereas D6D induction as well as hepatomegaly were abolished in PPARα-null mice (Table 1, Fig. 1A), an observation consistent with a study by Guillou et al. (36), who reported failed induction of D6D by fenofibrate in PPARα-null mice. A similar pattern was observed in the expression of D5D, another desaturase in the HUFA synthesis pathway. The D5D mRNA was increased in the fat-free- and triolein-fed wild-type mice (Fig. 1B), whereas no induction of D5D by essential fat deficiency occurred in PPARα-null mice. In contrast to rats (26, 28), D5D in mice was not significantly induced by peroxisome proliferators

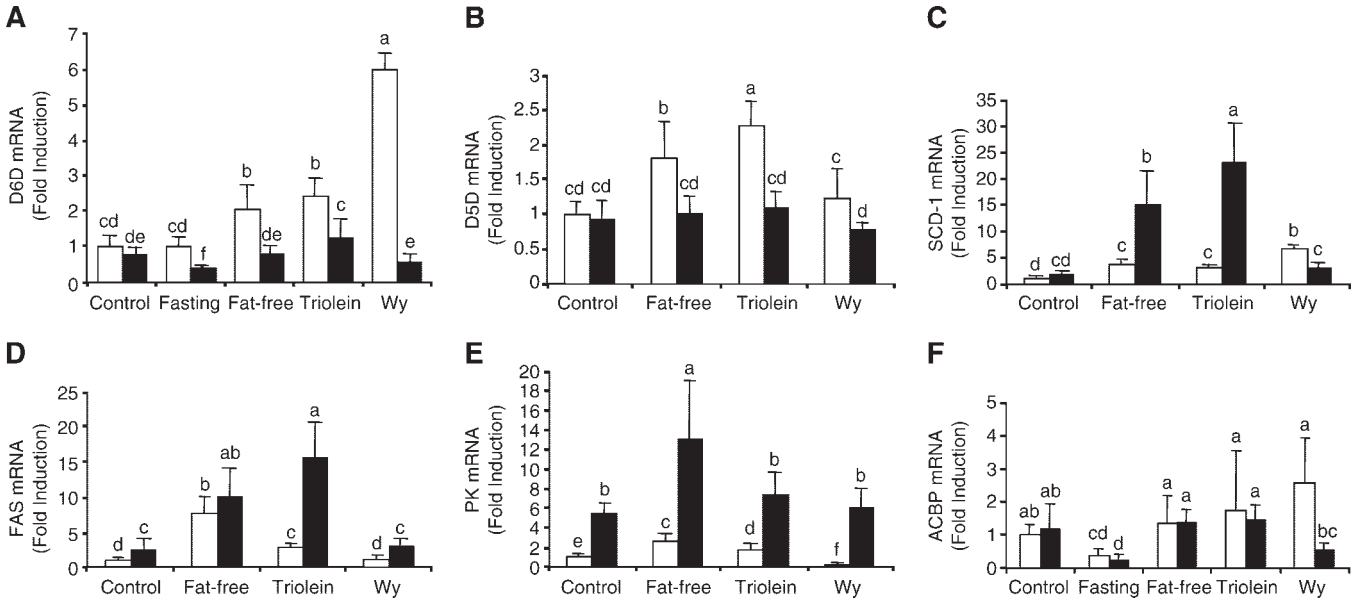

Fig. 1. Peroxisome proliferator-activated receptor α (PPARα)-null mice were unable to fully induce desaturases for highly unsaturated fatty acid (HUFA) synthesis in liver under essential fat-deficient conditions. Wild-type and PPARα-null mice were fed control, essential fat-deficient (fat-free and triolein), or Wy (control + 0.1% Wy14643) diets for 1 week. The fasting group was fed the control diet for 1 week and then fasted for 24 h before euthanasia. The abundance of mRNA was measured by quantitative PCR and expressed as fold increase over the mean value of the control group. Error bars show SD from four to five animals. Groups bearing different letters differ significantly ($P < 0.05$) by Fisher's protected least-squares difference (PLSD) test. Open bars, wild-type mice; closed bars, PPARα-null mice. A: Δ6 desaturase (D6D). B: Δ5 desaturase (D5D). C: Stearoyl-CoA desaturase-1 (SCD-1). D: FAS. E: Liver-type pyruvate kinase (PK). F: Acyl-CoA binding protein (ACBP).

in the present study (Fig. 1B), consistent with other results (36), suggesting possible species differences. This low response could be attributable to PPARα-independent high basal expression of D5D mRNA in mice.

We also quantified the expression of other mRNAs encoding lipogenic enzymes that are involved in nonessential unsaturated fatty acid synthesis but not in HUFA synthesis. SCD-1, which catalyzes monounsaturated fatty acid synthesis, was induced by essential fat-deficient diets as well as by Wy14643 in wild-type mice (Fig. 1C). In PPARα-null mice, SCD-1 induction by Wy14643 was abolished, whereas in contrast to D6D and D5D (Fig. 1A, B), SCD-1 was induced even more in PPARα-null mice than in wild-type mice by essential fat-deficient diets (Fig. 1C). Induction of FAS showed a similar pattern to SCD-1 except for the lack of induction by Wy14643 in wild-type mice (Fig. 1D). Liver-type pyruvate kinase (PK) shared the same induction trend with FAS, but PK was highly induced in PPARα-null mice on all diets, suggesting increased glucose utilization as a fuel source in PPARα-null mice (Fig. 1E). The mRNA analysis has demonstrated that PPARα is required specifically for the induction of genes for HUFA synthesis, and not for other lipogenic genes under essential fat deficiency.

Recently, the acyl-CoA binding protein (ACBP) gene was reported to be induced by both PPARα and SREBP-1c (37). Consistent with that study, ACBP expression by Wy14643 was lower in PPARα-null mice than in wild-type mice (Fig. 1F). However, in contrast to the D6D and D5D genes, essential fat-deficient diets had no effect on ACBP in either wild-type or PPARα-null mice (Fig. 1F).

PPARα-null mice had less HUFA in liver phospholipids than did wild-type mice under essential fat deficiency

Because HUFAs are primarily degraded in peroxisomes (38), targeted disruption of the PPARα gene may impair HUFA oxidation, resulting in HUFA accumulation. If this were the case, the lack of D6D induction by essential fat-deficient diets could be attributable to the presence of HUFAs above the normal level in PPARα-null mice. Thus, we next examined the amount of HUFAs in liver phospholipids, in which the majority of HUFAs are incorporated. As shown in **Table 2**, the major products of the D6D pathway, 20:4 n-6 and 22:6 n-3, were significantly lower in PPARα-null mice than in wild-type mice, indicating that HUFA composition in liver does not account for the impaired induction of D6D in PPARα-null mice. Moreover, low HUFA levels in PPARα-null mice further support an essential role of PPARα in the induction of synthesis to maintain tissue HUFAs. One week of feeding essential fat-deficient diets led to a significant reduction of 22:6 n-3, whereas 20:4 n-6 was not yet significantly reduced.

SREBP-1c nuclear protein was not different between genotypes under essential fat deficiency

Both mRNA and mature protein of SREBP-1c are increased by essential fat-deficient diets and are suppressed by dietary HUFAs (14); thus, SREBP-1c contributes to the feedback regulation of the D6D gene by HUFAs through the sterol regulatory element in the D6D promoter (10). We determined by Western blot analysis whether disruption of the PPARα gene impairs the activation of SREBP-1c under essential fat deficiency. The results revealed that there was no difference of SREBP-1c mature protein between the genotypes (**Fig. 2A**, **B**), demonstrating that the lack of D6D induction in PPARα-null mice is not mediated by SREBP-1c. Furthermore, although SREBP-1c mature protein was increased equally by fat-free diets in both wild-type and PPARα-null mice (Fig. 2), D6D was not induced in PPARα-null mice (Fig. 1A), indicating that SREBP-1c protein alone is not sufficient, and PPARα must be present, to induce D6D.

PPARα-responsive genes were induced by essential fat deficiency

Because our results revealed the requirement of PPARα in D6D induction under essential fat deficiency, we reasoned that essential fat-deficient diets would activate PPARα

TABLE 2. Fatty acids in liver phospholipids

	Control		Fat-Free		Triolein		*P*	
Fatty Acid	Wild Type	PPARα-Null	Wild Type	PPARα-Null	Wild Type	PPARα-Null	Diet	Genotype
16:0	27.6 ± 0.85	24.7 ± 1.14	30.7 ± 2.17	26.0 ± 5.57	28.8 ± 1.94	26.6 ± 0.93	NS	<0.01
18:0	15.1 ± 0.87	17.3 ± 2.30	11.7 ± 1.38	14.0 ± 5.08	10.6 ± 0.62	13.1 ± 1.62	<0.01	<0.05
18:1 n-9	7.38 ± 0.62	7.49 ± 1.89	13.1 ± 3.96	12.2 ± 5.82	20.6 ± 0.61	20.3 ± 4.18	<0.001	NS
18:2 n-6	21.0 ± 0.42	23.6 ± 0.78	15.5 ± 4.42	23.2 ± 9.44	11.4 ± 0.56	13.3 ± 2.03	0.001	<0.05
20:3 n-6	1.67 ± 0.13	1.17 ± 0.09	1.61 ± 0.64	0.73 ± 0.61	2.06 ± 0.24	1.51 ± 0.18	<0.05	<0.01
20:4 n-6	13.7 ± 1.36	13.2 ± 0.87	13.1 ± 0.79	11.2 ± 1.28	13.3 ± 0.52	12.6 ± 1.11	NS	<0.05
20:5 n-3	0.48 ± 0.05	0.53 ± 0.12	0.31 ± 0.11	0.29 ± 0.03	0.35 ± 0.04	0.44 ± 0.09	<0.01	NS
22:6 n-3	11.0 ± 0.62	9.32 ± 0.39	10.6 ± 1.00	8.22 ± 0.47	9.27 ± 0.21	8.94 ± 0.95	<0.05	<0.001
Other Sat.	0.61 ± 0.01	0.54 ± 0.09	0.34 ± 0.12	0.38 ± 0.01	0.35 ± 0.06	0.40 ± 0.07	0.001	NS
Other Unsat.	1.70 ± 0.28	2.13 ± 0.47	3.15 ± 0.86	3.86 ± 1.13	3.28 ± 0.23	2.86 ± 0.68	<0.01	NS
Total	100	100	100	100	100	100	—	—
Total Sat.	43.2 ± 0.45	42.5 ± 1.17	42.2 ± 1.08	40.3 ± 3.75	40.4 ± 1.30	40.3 ± 0.88	NS	NS
Total Unsat.	56.8 ± 0.37	57.5 ± 1.17	57.8 ± 1.08	59.7 ± 3.79	59.6 ± 0.52	59.7 ± 0.87	NS	NS
Total HUFA	26.8 ± 1.35	24.3 ± 0.96	25.5 ± 1.09	20.4 ± 1.49	25.0 ± 0.91	23.5 ± 1.93	<0.05	<0.001

Values shown are mol% (means ± SD; n = 3–4 animals per group). The animals were treated as described for Fig. 1. Two-way ANOVA was used to test the effects of diet, genotype, and the interaction between diet and genotype. HUFA, highly unsaturated fatty acid (20:3 n-6, 20:4 n-6, 20:5 n-3, 22:6 n-3); Sat., saturated fatty acid; Unsat., unsaturated fatty acid.

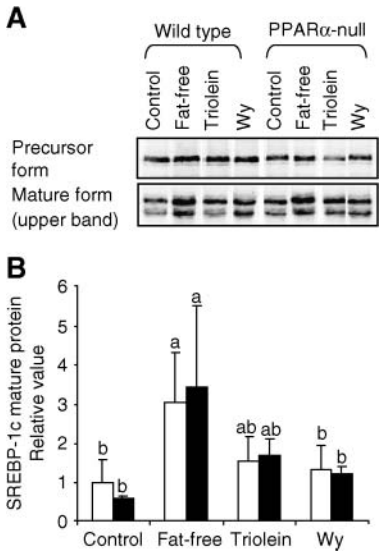

Fig. 2. Sterol regulatory element-binding protein-1c (SREBP-1c) nuclear protein amount was unaffected by disruption of the PPARα gene in mouse liver. The animals were treated as described for Fig. 1. A: A representative Western blot (pooled samples of five mice) of SREBP-1c precursor protein (upper panel) and mature protein in the nucleus (lower panel). B: Quantitation of SREBP-1c mature protein. The abundance of protein was quantified using Western blots of individual mice and expressed as fold increase over the mean value of the control group. Open bars, wild-type mice; closed bars, PPARα-null mice. Error bars show SD from five animals per group. Groups bearing different letters differ significantly ($P < 0.05$) by Fisher's PLSD test.

and induce other PPARα-responsive genes. As shown in **Table 3**, mRNAs of acyl-CoA oxidase (AOX), cytochrome P450 4A10, and L-bifunctional protein were indeed small but significantly induced when wild-type mice were fed essential fat-deficient diets. The induction of these PPARα target genes suggests that endogenous PPARα ligands are generated under essential fat deficiency.

Nonesterified fatty acids were decreased by the fat-free diet in wild-type mice

Nonesterified fatty acids are considered natural ligands of PPARα because they bind and activate PPARα in vitro (24, 25). Under prolonged essential fat deficiency, nonessential HUFAs, mead acid (20:3 n-9) in particular, increase in tissues (39). We quantitated nonesterified fatty acids in liver of wild-type mice to determine whether there was an increase in any fatty acid species that could act as a PPARα ligand under essential fat deficiency. None of the nonesterified fatty acid species above the detection limit (>1 nmol/g liver) increased in animals fed the fat-free diet (**Table 4**), and 20:3 n-9 remained below the detection limit in both phospholipid and nonesterified fatty acid fractions after 1 week of feeding the fat-free diet (Tables 2, 4). Instead, total as well as most individual nonesterified fatty acids were decreased by the fat-free diet (Table 4), raising the possibility that the endogenous ligand of PPARα under essential fat deficiency may not be a fatty acid.

Expression of both PPARγ and mammalian tribbles homolog was increased in PPARα-null mice

We investigated two candidate genes that may be responsible for the increased expression of lipogenic genes in PPARα-null mice (Fig. 1C–E). Studies showed that the expression of PPARγ, another member of the PPAR family, was increased in PPARα-null mice (40), and that overexpression of PPARγ in the liver of PPARα-null mice induced lipogenic genes (41). Consistent with these reports, a large increase in PPARγ mRNA was observed in PPARα-null mice in all dietary treatments (**Fig. 3A**).

Insulin plays critical roles in the induction of lipogenic genes (42). Another candidate gene, mammalian tribbles homolog (TRB3), counters insulin signaling by inhibiting Akt/protein kinase B (43). Moreover, the expression of TRB3 is reportedly dependent on PPARα (44). However, contrary to that report (44), TRB3 mRNA was higher in PPARα-null mice than in wild-type mice (Fig. 3B). This result was reproducible with the same primer set used by Koo et al. (44) (data not shown). Our study suggests that the modulation of insulin signaling by TRB3 is not the mechanism that causes the high induction of SCD-1, FAS, and PK in PPARα-null mice.

DISCUSSION

PPARα has been shown to play a key role in the metabolic adaptation to fasting by inducing fatty acid oxidation

TABLE 3. Induction of PPARα-responsive genes by essential fat-deficient diets in liver of wild-type mice

Gene	Control	Fat-Free	Triolein	Wy14643
Acyl-CoA oxidase				
Wild type	1.00 ± 0.14 d, e	1.93 ± 0.73 c	2.95 ± 0.47 b	14.0 ± 5.80 a
PPARα-null	0.65 ± 0.07 e	1.20 ± 0.34 d	0.99 ± 0.39 d, e	0.66 ± 0.12 e
Cytochrome P450 4A10				
Wild type	1.00 ± 0.40 c, d	1.58 ± 0.65 c	1.91 ± 1.10 b	134 ± 12.2 a
PPARα-null	0.39 ± 0.26 e	0.41 ± 0.10 e	0.37 ± 0.12 e	0.53 ± 0.18 d, e
L-Bifunctional protein				
Wild type	1.00 ± 0.27 d	1.20 ± 0.33 c, d	1.96 ± 0.52 b, c	173 ± 38.4 a
PPARα-null	2.80 ± 2.40 b	3.23 ± 1.02 b	2.16 ± 1.17 b, c	2.54 ± 0.61 b

The animals were treated as described for Fig. 1. Total RNA was isolated, and mRNA was quantified by real-time quantitative PCR. The abundance of mRNA was expressed as fold increase over the mean value of the wild type animals fed control diet. Values shown are means ± SD (n = 4–5 animals). Groups bearing different letters differ significantly ($P < 0.05$) by Fisher's PLSD test.

TABLE 4. Nonesterified fatty acids in liver from wild-type mice fed a control or fat-free diet

Fatty Acid	Control	Fat-Free
16:0	238 ± 100	126 ± 26[a]
16:1	29.5 ± 10.5	21.2 ± 3.5
18:0	23.2 ± 8.9	12.3 ± 2.3[a]
18:1 n-9	124 ± 44	71.6 ± 12.4[a]
18:1 n-7	20.9 ± 7.3	15.5 ± 4.1
18:2 n-6	58.6 ± 13.8	7.9 ± 2.2[a]
18:3 n-3	5.5 ± 1.3	N/D
Unknown	20.3 ± 3.7	22.4 ± 4.0
20:4 n-6	8.1 ± 4.5	N/D
Total	528 ± 181	277 ± 44[a]

Values shown are nmol/g liver (means ± SD; n = 4–6 animals). N/D, not detectable (<1.0 nmol/g liver).

[a] Significantly different between treatments by Student's *t*-test ($P < 0.05$).

pathways (21–23). By using PPARα-null mice, we demonstrated that PPARα is also required for the induction of D6D and D5D genes under essential fat deficiency (Fig. 1A, B). The requirement of PPARα for D6D gene induction is consistent with the presence of functional PPRE in the human D6D promoter (5). The critical role of PPARα in HUFA synthesis is further supported by lower total HUFA levels in PPARα-null mice than in wild-type mice (Table 2).

Sophisticated transcriptional regulation of genes in multicell organisms can be achieved by the involvement of multiple transcription factors that respond to different signals (45). Simultaneous activation of two transcription factors, SREBP-1c and PPARα, is the likely mechanism by which liver can both sense the demand of HUFAs and induce synthesis. This proposed mechanism would instill a specific induction of the genes for HUFA synthesis among large groups of genes targeted by SREBP-1c and PPARα. Among the SREBP-1c-targeted genes tested, the presence of PPARα was required only for the induction of D6D and D5D under essential fat deficiency (Fig. 1), suggesting that this dependence of D6D and D5D genes on PPARα confers pathway-specific regulation. Likewise, the differential regulation of desaturases from β-oxidation enzymes could be achieved by the presence of sterol regulatory elements in desaturases. Although functional PPRE is present in the promoters of D6D (5) and SCD-1 (27), fasting does not induce desaturases in the fed state (Fig. 1A) (28), whereas fatty acid oxidation enzymes are induced in a PPARα-dependent manner during fasting (21–23). Both protein and mRNA of SREBP-1c in liver decrease drastically during fasting and increase upon refeeding (18, 31). Thus, low SREBP-1c would prevent the desaturases from full induction during fasting.

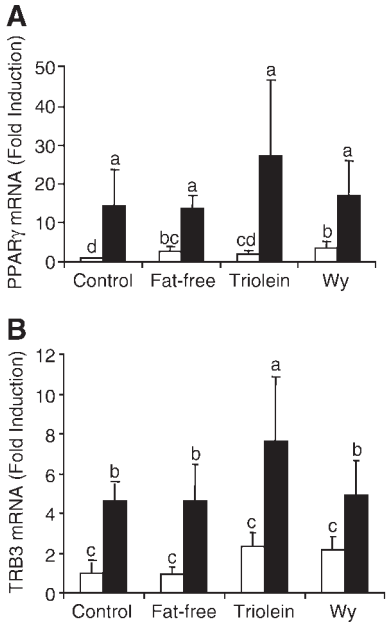

Fig. 3. Increased expression of both PPARγ and mammalian tribbles homolog (TRB3) genes in liver of PPARα-null mice. The animals were treated as described for Fig. 1. A: PPARγ mRNA. B: TRB3 mRNA. Open bars, wild-type mice; closed bars, PPARα-null mice. Gene expression was measured by quantitative PCR. The abundance of mRNA was expressed as fold increase over the mean value of the control group. Error bars show SD from four to five animals. Groups bearing different letters differ significantly ($P < 0.05$) by Fisher's PLSD test.

The proposed mechanism of D6D regulation by simultaneous activation of SREBP-1c and PPARα could further explain the robust induction of D6D by Wy14643 (Fig. 1A). Because the peroxisome proliferator was administered in the fed state, PPARα was activated under the presence of SREBP-1c, resulting in a strong induction of D6D compared with the state in which only either SREBP-1c (Fig. 2, control) or PPARα (fasted state) is active. An increased demand of HUFAs attributable to peroxisome proliferation and hepatomegaly (Table 1) by Wy14643 administration could also contribute to the marked induction of D6D (Fig. 1A) (28). However, smaller but significant induction of desaturases by a peroxisome proliferator was observed in pigs without hepatomegaly (29), further supporting a direct role of PPARα in desaturase induction. Moreover, differential effects of peroxisome proliferators and HUFAs in fish oil on desaturase expression can also be explained by the proposed requirement of the simultaneous activation of PPARα and SREBP-1c for the full induction of D6D. Both peroxisome proliferators and dietary fish oil induce peroxisomal β-oxidation enzymes (46–49), and the effect of fish oil is also dependent on PPARα (49). Thus, excess dietary HUFAs in fish oil are likely to act as PPARα ligands in vivo, inducing their own degradation pathway. However, in contrast to peroxisome proliferators, dietary fish oil strongly suppresses D6D expression (4). It is conceivable that the differential effects of fish oil and peroxisome proliferators on D6D are the result of strong inhibition of SREBP-1c activity by fish oil (14, 15), whereas peroxisome proliferators had no effect on SREBP-1c (Fig. 2).

Peroxisomal enzymes were induced under essential fat deficiency (Table 3). This induction may contribute to the upregulation of docosahexaenoic acid synthesis, because the last step of its synthesis, one cycle of β-oxidation, is performed in peroxisomes (50–52). PPARα-dependent induction of PPARα target genes (Table 3) suggests the production of endogenous ligands under essential fat defi-

ciency. On the other hand, AOX mRNA in the fat-free group was higher than in the control group even in the absence of PPARα (Table 3), suggesting that an unidentified, PPARα-independent mechanism may also be present and upregulate AOX in response to low docosahexaenoic acid in PPARα-null mice fed an essential fat-deficient diet (Table 2).

Postulated PPARα ligands generated under essential fat deficiency have yet to be identified. Decreased nonesterified fatty acids in the liver from the animals fed a fat-free diet (Table 4) suggest that, unlike under fasting, a molecule other than a fatty acid may act as a ligand of PPARα under essential fat deficiency. Lysophospholipids represent a candidate for the endogenous ligands of PPARα in an essential fat-deficient condition. A recent report has shown that lysophosphatidic acids act as a ligand of PPARγ (53). Membrane phospholipids undergo a deacylation/reacylation cycle through hydrolysis to PUFAs and 1-acyl 2-lyso phospholipids followed by their reesterification (54). When an essential fat-deficient diet was fed, the most notable change in nonesterified fatty acid fractions was a drastic reduction of precursors (18:2 n-6 and 18:3 n-3) and a product, 20:4 n-6 (Table 4). The marked decrease in nonesterified PUFAs may shift the deacylation/reacylation equilibrium toward increased lysophospholipids, which then may act as a PPARα ligand.

Desaturases supply unsaturated fatty acids that are required to maintain the physical properties of biological membranes (1). The present study revealed that total unsaturated fatty acids in liver phospholipids were remarkably well maintained regardless of genotype and diet (Table 2). However, this stability of unsaturation in liver phospholipids does not explain the much higher induction of genes for 18:1 synthesis (SCD-1, FAS, and PK) in PPARα-null mice than in wild-type mice when essential fat-deficient diets were fed (Fig. 1C–E). First, despite the large difference in gene expression between genotypes (Fig. 1C–E), there was no difference in 18:1 in phospholipids between genotypes (Table 2). Furthermore, although high 18:1 in phospholipids in the triolein-fed groups (Table 2) suggests a sufficient supply of 18:1 from the diet for phospholipid synthesis, SCD-1 mRNA was much higher in triolein-fed PPARα-null mice than in wild-type mice (Fig. 1C). Together, our results suggest that the induction of lipogenic genes in PPARα-null mice fed essential fat-deficient diets may be attributable to dysregulation of lipid synthesis rather than to compensatory upregulation.

PPARγ and TRB3 are candidates that could be responsible for the dysregulation of lipogenesis in PPARα-null mice. Although the expression of PPARγ is low in normal liver, PPARγ is induced in PPARα-null mice (Fig. 3A) (40), and ectopic expression of PPARγ in liver induces lipogenic genes (41). Thus, the compensatory increase in PPARγ mRNA in the PPARα-null mouse (Fig. 3A) may be responsible for the hyperinduction of lipogenic genes (Fig. 1C–E). On the other hand, TRB3, a proposed insulin signaling inhibitor (43), was increased in PPARα-null mice in our study (Fig. 3B), whereas another study reported decreased TRB3 in PPARα-null mice (44). The reason for the opposite response of TRB3 in PPARα-null mice in the two studies is currently unclear. However, a marked difference in feeding protocols should be noted. Koo et al. (44) fed diets ad libitum, whereas we fed animals for 12 h/day during the dark cycle. Although PPARα-null mice fed ad libitum grow normally, they are unable to adapt to fasting (21–23) and lose body weight even under 3 h/day meal feeding (unpublished data). Thus, when PPARα-null mice are fed ad libitum, their eating pattern is likely to shift from a dark-cycle eater to an all-day nibbler, resulting in a loss of the diurnal rhythm of lipogenic gene induction seen in wild-type animals (40). Our feeding protocol was designed to minimize the difference in eating patterns between genotypes. Thus, it is possible that TRB3 expression could be regulated in the same manner as lipogenic genes, resulting in the opposite outcome in two different feeding protocols.

In conclusion, this study has demonstrated that *1*) PPARα is specifically required for the full induction of D6D and D5D genes under essential fat deficiency, but not for other SREBP-1c-targeted genes; *2*) PPARα is also required to maintain HUFA levels in liver; and *3*) essential fat deficiency induces PPARα-responsive genes, suggesting the generation of endogenous ligand. In contrast, genes for monounsaturated fatty acid synthesis were increased in PPARα-null mice under essential fat deficiency without affecting the abundance of the product in membrane phospholipids. Increased PPARγ may be involved with the induction of lipogenic genes in PPARα-null mice. Simultaneous activation of two transcription factors, SREBP-1c and PPARα, is the likely mechanism by which liver can sense the deprivation of dietary essential fatty acids and specifically induce genes for HUFA synthesis.

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