Long-Term n-3 FA Deficiency Modifies Peroxisome Proliferator-Activated Receptor β mRNA Abundance in Rat Ocular Tissues

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ABSTRACT: Peroxisomal proliferator-activated receptors (PPAR) are a FA-response system involved in diverse cellular responses. FA regulate PPAR activity and modulate PPAR mRNA abundance. Increasing evidence indicates that PUFA are required for optimal neuronal development and function. To gain insight into the mechanism for nutrition-induced impairment of neuronal development and function we investigated the effect of chronic n-3 FA deficiency on PPAR mRNA levels in rat brain and ocular tissues. Rats were fed for three generations a diet designed to reduce DHA levels in tissues, and the abundance of PPARα and PPARβ transcripts was measured by hybridization with specific probes. Chronic consumption of the α-linolenic acid (LNA)-insufficient diet caused a remarkable modification in DHA content in membrane phospholipids. The results reported here indicate that PPARα mRNA levels did not exhibit significant variation in ocular, hepatic, or nervous tissues from rats fed the experimental diet. In contrast, PPARβ mRNA normalized to β-actin mRNA was 21% higher in ocular tissue from F3 generation rats consuming the LNA-deficient diet but was independent of diet in hepatic and nervous tissues. The absolute abundance of PPARβ transcripts showed a 17% increase in ocular tissue from rats consuming the LNA-deficient diet (F3 generation). The biological significance of the reported changes in PPARβ mRNA in ocular tissue remains to be determined.

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FA are one of the main energy substrates for most mammalian species. In addition, FA serve as components of membrane phospholipids, precursors for the synthesis of molecules involved in cellular signaling, and modulators of gene expression (1–3). An inadequate FA balance, particularly a deficit of long-chain PUFA, is associated with reduced fetal growth and impaired neurodevelopment and nerve transmission in animal models (4–6). Furthermore, dietary studies in humans indicate that PUFA status affects sensory and cognitive development in premature and term infants (7–10).

Investigations over the past decades support the involvement of peroxisome proliferator activated-receptors (PPAR) in many of the physiological responses to FA. PPAR are ligand-dependent transcription factors, initially recognized as mediators of FA effects on lipid metabolism, controlling gene expression for β-oxidation and lipid biosynthesis. Lately, it became apparent that PPAR not only participate in the regulation of FA oxidation and lipogenesis but also play a role in a wide array of cellular responses, including inflammation, thermogenesis, and cell differentiation (3,11,12).

Three PPAR isoforms encoded by individual genes, namely, α, β and γ, have been described. These isoforms display a tissue-selective pattern of expression (13). PPARα is expressed in liver, brown adipose tissue, skeletal muscle, kidney, and adrenal glands. PPARγ is mainly expressed in white adipose tissue and, to a lesser extent, in spleen, gut, and the immune system. PPARβ displays a broad pattern of expression with relatively higher levels in skeletal muscle, testis, placenta, and neuronal tissues (i.e., cerebellum, oligodendrocytes) (14–17). The presence of both PPARα and PPARβ transcripts has been reported both in outer and inner layers of rat retina (13). Regarding PPAR function, the α isoform appears primarily to regulate the transcription of several FA-metabolizing enzymes in hepatic mitochondria and peroxisomes. PPARγ is implicated in the control of lipogenesis, regulating the maturation of preadipocytes and the accumulation of lipid droplets in the cytoplasm of fat cells (18,19). The physiological role of PPARβ remains unclear, although it reportedly regulates acylCoA synthetase 2 in rat brain cultures (20), but it also seems to participate in early differentiation of adipocyte precursor cells (21). Furthermore, PPARβ-null mice display alterations in development, epidermal cell proliferation, myelination of the corpus callosum, and lipid metabolism (22). Recently, PPARβ has also been implicated in colorectal cancer (23), in bone resorption (24), and in the stimulation of reverse cholesterol transport (25).

PPAR activity is mainly controlled by ligand binding (26). Different FA and their derivatives (such as conjugated FA, eicosanoids, and prostaglandins) are able to activate PPAR isoforms (2,26). Among FA, DHA has been reported as a potent PPAR activator (27). FA activation of PPARα seems to account for many of the short-term effects of dietary fat on gene expression in liver, at least in rodents. However, there is increasing evidence that under different nutritional states, not

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Abbreviations: DPAn-6, docosapentaenoate; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; LA, linoleic acid; LNA, α-linolenic acid; PPAR, peroxisome proliferator-activated receptor; RXR, retinoic acid receptor.

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only PPAR activity but also its mRNA abundance are modified. After feeding, PPARγ increases in adipose tissue in response to sterol regulatory element binding protein 1 (28,29), thus stimulating uptake of FA and their conversion to TG, whereas, PPARγ diminishes in murine liver during fasting periods (28). Starvation causes a decrease in PPARα mRNA levels in liver (30). In contrast, fasting results in high expression of PPARα (30,31), increasing the ketonemia and thus providing fuel for different tissues. The recently identified fasting-induced adipose factor gene (FIAF) seems to be a target for the PPARα-mediated response to fasting (32).

This work addresses the effect of long-term n-3 FA deficiency on the abundance of PPAR mRNA in rat tissues. Rats were chronically fed diets sufficient in linoleic acid (LA: 18:2n-6) but low or adequate in α-linolenic acid (LNA: 18:3n-3). Consumption of diets containing oils low in LNA significantly reduced DHA (22:6n-3) content in F2 generation rat brain and liver FA. PPARα and PPARβ mRNA levels were analyzed in nervous, ocular, and hepatic tissues from F2 and F3 generation rats. Our results show a moderate increase in PPARβ mRNA in ocular tissues from rats with an inadequate dietary supply of n-3 FA. In contrast, levels of neither PPARα mRNA in all three tissues nor PPARβ mRNA in hepatic or nervous tissues were significantly modified.

MATERIALS AND METHODS

Diets. Diets based on the AIN93 diet (33) were designed to contain 10% total lipids and either deficient or adequate amounts of LNA (34). The experimental diets were composed of (in g/100 g diet): 20 vitamin-free casein, 60 carbohydrates (15 cornstarch, 10 sucrose, 20 dextrose, and 15 maltose-dextrin), 10 cellulose vitamins/minerals, and 10 lipids. Safflower oil was used to provide an adequate amount of LA (18:2n-6), and flaxseed oil was the source of LNA (18:3n-3) in the LNA-sufficient diet. The LNA-deficient diet contained 8.1 g/100 g of hydrogenated coconut oil (abundant in lauric and myristic acids) and 1.9 g/100 g of safflower oil. The LNA-adequate diet contained 7.75 g/100 g of hydrogenated coconut oil, 1.77 g/100 g of safflower oil, and 0.48 g/100 g of flaxseed. Fatty acyl composition analysis of the diets showed that 18:3n-3 was 3.1 and 0.04% of total FA in the LNA-sufficient and the LNA-deficient diet, respectively (34).

Experimental design. Female Long-Evans rats (Charles River, Portage, MI) were randomly divided into two groups of 12 rats each and reared on n-3 adequate or deficient diets beginning at 21 d of life. These females (F1 generation) were mated with chow-fed males and similarly, F2 generation females were mated with chow-fed males. Their male offspring (F2 and F3 generations) were weaned to the diet of the dam and maintained on this diet until sacrifice at 12 wk of age. No obvious differences in reproduction efficiency or pup size were observed in this study between dietary groups. Animals were killed by decapitation and the tissues rapidly excised and stored at −80°C until analysis. Cerebellum, liver, and whole eyeballs were collected from the F2 and F3 generation rats. All animal procedures were approved by the NIAAA Animal Care and Use Committee, NIH.

FA analysis. FA composition was determined in rat brain and liver. Lipids were extracted in the presence of tricosanoic acid (23:0) as internal standard (35). FA were transmethylated and analyzed by GC (36). The identification of individual FA was based on the retention time, and their content was expressed as a weight percentage of total FA (37).

Slot blot analysis. Frozen tissue samples (approximately 100 mg) were ground in a prechilled mortar. Total RNA was extracted with guanidine isothiocyanate/phenol (TRIZOL reagent; GIBCO BRL, Bethesda, MD) according to the manufacturer's instructions, precipitated in ethanol, and stored at −80°C. Before use, samples were resuspended in diethyl pyrocarbonate-treated doubly distilled water and incubated for 45 min at 37°C with 0.03 units of RNase-free RQ1 DNase (Promega Corp., Madison, WI) per microgram of RNA.

Total RNA (0.5 to 1 μg) was denatured at 68°C in 0.21 M sodium citrate, 0.021 M sodium chloride, pH 7.0, 69% deionized formamide, 9% formaldehyde, and transferred to nitrocellulose membranes (Schleicher & Schuell GmbH, Dassel, Germany) by filtration under negative pressure. RNA was fixed to membranes using a UV cross linker (Stratagene, La Jolla, CA) and hybridized with ^{32}P-labeled cDNA probes for PPARα, PPARβ, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), or β-actin. Probes for PPARα, PPARβ, GAPDH, and β-actin transcripts were obtained by reverse transcription-PCR using primers specific for the corresponding cDNA sequences available on databases (PPARα, Genebank accession # M88592; PPARβ, Genebank accession # U40064; β-actin, Genebank accession # V01217; GAPDH Genebank accession # X02231 X00972). PCR-amplified fragments, cloned into pCR II (Invitrogen, Carlsbad, CA) or pBluescript (Stratagene) vectors, constituted nucleotides 1030 to 1775 of PPARα cDNA, nucleotides 86 to 392 of PPARβ cDNA, nucleotides 1253 to 2381 of β-actin gene, and nucleotides 87 to 626 of GAPDH cDNA. The identity of cloned fragments was confirmed by direct cDNA sequencing. The specificity of all probes used in slot blot hybridizations was verified by Northern analysis. Unique bands of the predicted size were observed in autoradiograms. Hybridization with nonlimiting amounts of radioactive probes was carried out for 24 h at 42°C in 0.02 M sodium phosphate buffer, pH 6.5, containing 6× SSC (0.9 M sodium chloride, 0.09 M sodium citrate pH 7.0), 5× Denhardt's solution (0.1% wt/vol polyvinylpyrrolidone, 0.1% wt/vol Ficoll type 400, 0.1% wt/vol BSA), 5% wt/vol dextran sulfate in formamide, 0.1 mg/mL denatured salmon sperm DNA, and ^{32}P-labeled probes. Labeling of the probes was carried out by the PCR Radioactive Labeling System (GIBCO BRL) and α-^{32}P-dCTP (111 TBq/mmol, 370 MBq/mL; NEN Life Science Products, Inc., Boston, MA). Membranes were washed with a solution containing 0.2 × SSC (0.015 M sodium chloride, 0.0015 M sodium citrate, pH 7.0), and 0.1% SDS at 60, 63, or 68°C for PPARβ, PPARα, or β-actin and GAPDH, respectively. Membranes were exposed to X-ray films using intensifying screens, and densitometric analysis of autoradio-

grams was carried out with a Bio-Rad GS-670 image densitometer. PPAR signal intensities for each tissue in individual animals were standardized with those of the reference mRNA measured in parallel. The mean ± SD of two experiments was determined for each dietary group. Hybridizations to mRNA from different tissues were carried out independently. To compare PPAR mRNA abundance among samples and experiments, β-actin and GAPDH mRNA were used as an internal reference. Thus, results for PPARα and PPARβ mRNA were expressed relative to β-actin and GAPDH mRNA.

Statistical analysis. Statistical differences between the mean values of dietary groups were assessed by the two-tailed unpaired *t*-test and were considered significantly different at $P < 0.05$.

RESULTS

Effect of diet on FA composition. Analysis of liver and brain lipids from rats fed LNA-deficient or LNA-adequate diets shows significant differences in FA composition (Table 1), particularly in DHA, which accounts for over 99 and 85% of total n-3 FA in brain and liver, respectively. A fivefold lower level of DHA was found in brain and an 11-fold DHA decrease in liver from rats fed the LNA-deficient diet. A concomitant compensatory increase in 22-carbon n-6 FA, particularly in docosapentaenoate (DPAn-6, 22:5n-6) and, to a lesser extent, in 22:4n-6 was detected in the lipids of hepatic and nervous tissue. Thus, the total n-6 to n-3 ratio increased almost 10-fold in brain lipids and 14-fold in liver lipids.

TABLE 1
FA Composition[a] of Total Lipid Extracts from Rat Liver and Brain

	Liver		Brain	
FA	LNA-deficient	LNA-adequate	LA-deficient	LNA-adequate
	(% total FA)			
Saturated				
14:0	1.60 ± 0.17*	1.20 ± 0.21	0.30 ± 0.20	0.60 ± 0.40
16:0	17.1 ± 1.00*	19.8 ± 1.90	16.30 ± 0.20	17.0 ± 0.90
18:0	18.9 ± 0.80	17.9 ± 1.90	18.50 ± 0.20	18.6 ± 0.50
20:0	0.09 ± 0.01*	0.08 ± 0.00	0.70 ± 0.09	0.60 ± 0.07
22:0	0.30 ± 0.20*	0.20 ± 0.10	0.90 ± 0.06*	0.80 ± 0.05
24:0	0.65 ± 0.06*	0.44 ± 0.03	1.70 ± 0.20	1.60 ± 0.20
Total	38.60 ± 1.10	39.60 ± 1.00	38.50 ± 0.60	39.20 ± 1.20
Monounsaturated				
14:1	0.02 ± 0.01	0.02 ± 0.02	ND[b]	ND
16:1	1.80 ± 0.40	2.40 ± 0.70	0.30 ± 0.03	0.40 ± 0.06
18:1n-9	7.80 ± 1.30	9.90 ± 1.40	15.3 ± 0.50*	16.2 ± 0.40
18:1n-7	4.70 ± 0.60	5.10 ± 0.80	3.70 ± 0.10*	3.60 ± 0.10
20:1	0.19 ± 0.02	0.16 ± 0.04	2.00 ± 0.20	1.90 ± 0.30
22:1	0.004 ± 0.01	ND	0.20 ± 0.02	0.20 ± 0.03
24:1	0.30 ± 0.07*	0.21 ± 0.20	3.30 ± 0.30	3.30 ± 0.30
Total	14.80 ± 1.90	17.90 ± 2.60	24.90 ± 0.70	25.50 ± 0.90
n-6 series				
18:2n-6	10.5 ± 1.20	11.0 ± 1.20	4.60 ± 0.30*	5.10 ± 0.40
18:3n-6	0.04 ± 0.01*	0.07 ± 0.01	ND	ND
20:3n-6	0.60 ± 0.10*	0.90 ± 0.10	0.30 ± 0.03*	0.30 ± 0.02
20:4n-6	22.3 ± 0.80*	19.2 ± 1.60	8.80 ± 0.30	8.40 ± 0.40
22:4n-6	0.80 ± 0.10*	0.27 ± 0.20	3.60 ± 0.08*	2.70 ± 0.09
22:5n-6	5.30 ± 0.70*	0.20 ± 0.05	9.00 ± 0.50*	0.40 ± 0.05
Total	39.90 ± 2.10*	31.90 ± 2.40	22.30 ± 0.70*	12.40 ± 0.50
n-3 series				
18:3n-3	0.006 ± 0.005*	0.28 ± 0.05	ND	ND
20:5n-3	ND	0.47 ± 0.10	ND	ND
22:5n-3	0.07 ± 0.03*	0.50 ± 0.09	0.008 ± 0.02*	0.13 ± 0.01
22:6n-3	0.60 ± 0.02*	6.60 ± 0.50	2.30 ± 0.10*	11.8 ± 0.70
Total	0.70 ± 0.05*	7.80 ± 0.60	2.30 ± 0.10*	11.90 ± 0.70
20:3n-9	0.17 ± 0.02	0.14 ± 0.03	0.08 ± 0.003	0.06 ± 0.01
18:2n-6/18:3n-3	1062 ± 83*	39.9 ± 5.3	NA[b]	NA
22:5n-6/22:6n-3	8.8 ± 1.1*	0.03 ± 0.006	3.9 ± 0.2*	0.03 ± 0.004
22:5 + 22:6	5.9 ± 0.7*	6.8 ± 0.48	11.3 ± 0.6	12.2 ± 0.7
Total n-6/total n-3	58.7 ± 5.0*	4.1 ± 0.3	9.6 ± 0.5*	1.0 ± 0.04
Total n-6 + total n-3	40.6 ± 2.1	39.8 ± 2.8	24.6 ± 0.7	24.4 ± 1.1

[a]Data are mean ± SD for F2 generation rats. *Statistically significant differences $P < 0.05$.
[b]NA, not available; ND, not detectable; LNA, α-linolenic acid; LA, linoleic acid. Values may not equal 100% due to unidentified peaks.

Changes in lipid composition were not evaluated in ocular tissue because the whole sample was used for mRNA quantification, and other studies show that retinal responses are similar to those of brain with respect to DHA loss and replacement with DPAn-6 (4,38,39).

Effect of diets on PPAR mRNA abundance. GAPDH and β-actin transcripts are widely accepted as reference genes for expression analysis. Because changes in either GAPDH or β-actin mRNA levels have been reported under some experimental conditions (40–42), the abundance of both transcripts was evaluated in the tissues analyzed here (Table 2). Data are expressed as the mean value of β-actin and GAPDH mRNAs in tissues from rats fed the LNA-deficient diet and as a percentage of the value obtained from the LNA-adequate group. GAPDH mRNA levels in different tissues were independent of the dietary supply of LNA, except for a 10% decrease in nervous tissue in F3 generation rats fed the LNA-deficient diet. In addition, significantly lower levels of β-actin mRNA were detected in ocular (10%), nervous (20%), and hepatic (42%) tissues from F2 generation rats fed the LNA-deficient diet as compared to those that consumed the LNA-adequate diet. Therefore, data normalized to β-actin mRNA in all tissues from F2 generation rats and to GAPDH mRNA in nervous tissue (F3 generation) were not included for analysis. In addition, a lower level (37%) of β-actin transcripts was also found in liver from F3 generation rats. However, this difference was not statistically significant, probably owing to variability within the group.

The abundance of PPAR mRNA in nervous and ocular tissues was evaluated and expressed relative to both β-actin and GAPDH mRNA levels in the same tissue, as pointed out in the Materials and Methods section (Tables 3 and 4). Analysis of mRNA in hepatic tissue was included as a control, because of reported high levels of PPARα mRNA expression. As shown in Table 3, most of the differences detected in the relative abundance of PPARα mRNA in ocular, nervous, and hepatic tissue from F2 and F3 generation rats fed LNA-deficient or LNA-adequate diets were not statistically significant. PPARα mRNA abundance normalized to β-actin mRNA was 61% higher in hepatic tissue from F3 generation rats consuming the LNA-deficient diet than that measured in rats fed the LNA-adequate diet. However, the latter could be explained, at least in part, by the lower β-actin mRNA measured in hepatic tissue from these rats (Table 2). This is confirmed when results are examined relative to GAPDH mRNA. In this case, PPARα levels do not significantly differ in the liver samples. As illustrated in Table 4, PPARβ mRNA abundance also displayed significantly higher levels when expressed relative to β-actin mRNA but not to GAPDH mRNA.

The results of the PPARβ mRNA abundance determination in nervous and hepatic tissues shown in Table 4 indicate no significant changes, except a 51% increment in PPARβ mRNA normalized to β-actin mRNA in the F3 generation (possibly influenced by the decrease observed in β-actin transcripts in liver). In ocular tissue, significantly higher PPARβ mRNA abundance relative to β-actin transcripts (21%) was found in F3 generation rats from the LNA-deficient group (Table 4). It is likely that the increase in the normalized PPARβ level in ocular tissue arises mainly from a moderately higher absolute abundance of PPARβ mRNA (17%) in the rats consuming the n-3-deficient diet (Table 4).

DISCUSSION

Effects of dietary FA at the level of gene expression are part of an adaptive metabolic response to the amount and type of fat. These are mediated, at least in part, by the PPAR system. Although the activity of PPAR isoforms is mainly regulated by ligand binding and phosphorylation status, control at the transcriptional level is also apparent. Changes in the abundance of PPARα, which influences the expression of enzymes for FA oxidation, have been detected in liver during fasting periods, with a concomitant increase in the level of PPARα mRNA (31). Moreover, food intake increases PPARγ mRNA levels, whereas PPARγ abundance decreases during fasting periods (28). Changes in PPARα mRNA levels in response to total dietary fat intake and to FA composition have also been documented. Feeding rats a diet rich in LNA for 12 wk after weaning caused a decrease in PPARα mRNA abundance in the epididymal fat pads (43). In contrast, an increased expression of PPARα mRNA in rat liver was observed in 5-wk-old rats fed a high-fat diet (250 g/kg of either coconut oil or olive oil or safflower oil) over a 4-wk period (44).

TABLE 2
β-Actin and GAPDH mRNA Abundance in LNA-Deficient Rats

Tissue	Rat generation	β-Actin[a]	%[b]	GAPDH[a]	%[b]
Ocular	F2	23.3 ± 1.1 (6)	90.1 ± 4.8*[,c]	11.5 ± 0.2 (6)	99.1 ± 1.7
	F3	28.0 ± 1.8 (6)	97.6 ± 6.4	13.3 ± 1.1 (6)	92.7 ± 8.3
Nervous	F2	16.1 ± 2.5 (5)	80.1 ± 15.4*[,d]	10.5 ± 0.6 (6)	98.5 ± 5.8
	F3	18.5 ± 2.3 (5)	110.4 ± 12.2	10.5 ± 0.4 (6)	90.4 ± 3.6*[,e]
Hepatic	F2	1.4 ± 0.4 (5)	57.5 ± 30.4*[,f]	4.4 ± 0.6 (6)	84.3 ± 14.0
	F3	1.3 ± 0.6 (6)	62.9 ± 46.5	5.3 ± 0.3 (6)	97.8 ± 5.9

[a]Data are mean ± SD. In parentheses are the number of samples. *Statistically significant differences: [c]$P = 0.03$, [d]$P = 0.04$, [e]$P = 0.0008$, [f]$P = 0.02$.
[b]The mean value of β-actin mRNA and GAPDH mRNA for the LNA-deficient diet was expressed as a percentage of the value for the group fed the LNA-adequate diet. GAPDH, glyceraldehyde-3-phosphate dehydrogenase; for other abbreviation see Table 1.

TABLE 3
PPARα mRNA Relative Abundance[a] in Tissues from LNA-Deficient Rats

Tissue	Generation	PPARα[b]	%[c]	PPARα/β-actin[b]	%[c]	PPARα/GAPDH[b]	%[c]
Ocular	F2	0.78 ± 0.19	109 ± 24	0.02 ± 0.01 (6)	118 ± 25	0.04 ± 0.01 (6)	111 ± 22
	F3	0.98 ± 0.09	113 ± 9	0.02 ± 0.002 (6)	123 ± 10	0.04 ± 0.003 (6)	122 ± 7
Nervous	F2	1.69 ± 0.10	106 ± 6	0.06 ± 0.01 (5)	133 ± 18*,d	0.16 ± 0.02 (5)	108 ± 9
	F3	1.69 ± 0.10	105 ± 6	0.05 ± 0.01 (4)	98 ± 15	0.16 ± 0.01 (5)	121 ± 7*,e
Hepatic	F2	0.38 ± 0.15	98 ± 4	0.28 ± 0.07 (5)	171 ± 26*,f	0.06 ± 0.02 (6)	116 ± 33
	F3	0.34 ± 0.05	95 ± 15	0.31 ± 0.14 (5)	161 ± 45*,g	0.04 ± 0.01 (6)	105 ± 15

[a]Because hybridization assays to mRNA from different tissues were carried out independently (see Materials and Methods section), the comparison of absolute PPARα mRNA values should be restricted to the same tissue.
[b]Data are mean ± SD. In parentheses are the number of samples. *Statistically significant differences: [d]$P = 0.007$, [e]$P = 0.0027$, [f]$P = 0.0009$, and [g]$P = 0.02$.
[c]The mean value of PPARα mRNA relative to β-actin or to GAPDH mRNA for the LNA-deficient diet was expressed as percentage of the value for the group fed the LNA-adequate diet. PPARα, peroxisome proliferator-activated receptor α; for other abbreviations see Tables 1 and 2.

The effect of high- and low-fat diets on PPARα mRNA abundance in kidney of 3-wk-old rats also has been investigated previously (45). In this study, pups were kept on a low-fat diet (less than 1% fat) from day 16 to 21; then a group was placed on a high-fat diet (supplemented with 25% coconut oil) for 24 h. Despite marked changes in the mRNA levels for enzymes involved in β-oxidation in the kidney cortex, the authors reported no significant modification of PPARα mRNA abundance, suggesting that modulation of the expression of the PPARα gene is not involved in this physiological response to a high-fat diet. A diet high in fat causes a small increase in PPARγ in rat adipose tissue (28). However, the infusion of lipids (Intralipid) in humans results in a marked increase in subcutaneous adipose tissue PPARγ mRNA (46). Considering the PPARβ ligand-binding profile (26), it is likely that FA are physiological regulators of its activity as a transcription factor. However, information regarding the regulation of PPARβ gene expression is not yet available.

Many investigations support the role of n-3 FA, particularly DHA, on the development and function of the nervous system in humans (4,38,47,48), particularly in the visual system (7,10,47). High DHA levels are found in the inner membranes of photoreceptor outer segments, in certain brain areas, and in specific neuronal cell types. Dietary n-3 FA deficiency alters rat retinal function (49). Moreover, DHA promotes differentiation of developing photoreceptors in culture (50). Interestingly, PPARβ mRNA is more prominent than other PPAR isoforms in many areas of the rat adult brain (15) and its level is particularly high during embryonic development (51). Expression of PPARβ is also abundant in differentiating oligodendrocytes, cells involved in myelin sheath formation (16). Consistently, PPARβ null mice show alterations in myelination of the corpus callosum and in lipid metabolism (22). As in other cell types, it likely that PPAR transcriptional activity is modulated by FA in neuronal cells although their PPAR-responsive genes have not been identified.

The purpose of this study was to explore whether long-term modification of FA intake, specifically n-3 FA deficiency, affects the abundance of PPARα and β mRNA in neural tissues. It is well known that the content and composition of dietary lipids can alter FA composition in biological membranes (38,39,52–54). These studies demonstrate that when two or more generations of animals are maintained on an n-3-deficient diet, a marked modification of the FA composition in the nervous system occurs. Therefore, in the present study, rats were fed the experimental diet for three gener-

TABLE 4
PPARβ mRNA Relative Abundance[a] in Tissues from LNA-Deficient Rats

Tissue	Generation	PPARβ[b]	%[c]	PPARβ/β-actin[b]	%[c]	PPARβ/GAPDH[b]	%[c]
Ocular	F2	1.63 ± 0.33	122 ± 20	0.04 ± 0.01 (6)	136 ± 14*,d	0.09 ± 0.02 (6)	123 ± 20
	F3	1.59 ± 0.10	117 ± 6*,e	0.03 ± 0.004 (6)	121 ± 12*,f	0.07 ± 0.01 (6)	126 ± 14
Nervous	F2	2.14 ± 0.26	96 ± 12	0.07 ± 0.01 (5)	122 ± 14	0.20 ± 0.02 (5)	97 ± 9
	F3	1.65 ± 0.10	100 ± 6	0.06 ± 0.01 (5)	104 ± 9	0.16 ± 0.01 (5)	112 ± 4*,g
Hepatic	F2	0.18 ± 0.06	75 ± 33	0.12 ± 0.02 (5)	130 ± 14*,h	0.04 ± 0.01 (6)	87 ± 31
	F3	0.35 ± 0.09	117 ± 12	0.27 ± 0.10 (5)	151 ± 36*,i	0.04 ± 0.01 (6)	112 ± 2

[a]Because hybridization assays to mRNA from different tissues were carried out independently (see Materials and Methods section), the comparison of absolute PPARβ mRNA values should be restricted to the same tissue.
[b]Data are mean ± SD. In parentheses are the number of samples. *Statistically significant differences: [d]$P = 0.001$, [e]$P = 0.005$, [f]$P = 0.004$, [g]$P = 0.03$, [h]$P = 0.02$, and [i]$P = 0.02$.
[c]The mean value of PPARα mRNA relative to β-actin or to GAPDH mRNA for the LNA-deficient diet was expressed as a percentage of the value for the group fed the LNA-adequate diet. For abbreviations see Tables 1–3.

ations. The effects of the diet deficient in LNA were compared to those of a diet providing adequate levels of this FA. One would expect that n-3 FA deficiency would resemble genetic diseases associated with impaired DHA synthesis, which preferentially affect the function of excitable tissues such as brain, retina, and muscle (55,56). Despite no apparent differences in body or brain weight, siblings of these rats that consumed the n-3-deficient diet showed poorer performance in spatial and olfactory-cued learning tests than those that received the control diet (33,57,58). As shown here, long-term inadequate dietary provision of n-3 fat sources results in a remarkable decrease in DHA in rat brain phospholipids (fivefold) and in liver (11-fold), and a concomitant increase in DPAn-6 FA (>20-fold).

In the present study, quantitation of PPAR mRNA in hepatic, ocular, and nervous tissues from rats fed the LNA-deficient or -adequate diet was referred to both β-actin and GAPDH mRNA abundance. Results shown here indicate that reference transcripts did not differ significantly in most tissues from rats consuming the LNA-deficient diet. However, β-actin mRNA levels in the F2 but not in the F3 generation were consistently lower in the LNA-deficient rats for all three tissue types analyzed here. In addition, GAPDH mRNA was also significantly lower in nervous tissue of F3 generation rats fed the LNA-deficient diet. We do not have a clear explanation for the apparent selective decrease in β-actin mRNA. As a matter of speculation, we propose that the expression of β-actin and GAPDH genes is sensitive, to a different extent, to an undefined factor that is dissimilarly present in F2 vs. F3 generation rats fed the LNA-deficient diet. Therefore, taking into account the results discussed above, PPAR data normalized to β-actin mRNA (F2 generation) and GAPDH mRNA (F3 generation, nervous tissue) were excluded from the analysis.

Our findings indicate that the marked decrease in DHA levels in tissues did not correlate with substantial changes in the level of PPARα or PPARβ transcripts in rat liver or brain. We found a modest increase in the abundance of PPARβ mRNA in ocular tissues from rats consuming the LNA-deficient diet compared to those fed the LNA-adequate diet. In F3 generation rats, the PPARβ mRNA level was 17% higher, and PPARβ mRNA normalized to β-actin showed a 21% increase in ocular tissues from the n-3 FA deficient group.

Given that the biological activity of PPAR transcription factors is primarily controlled by ligand binding, the functional relevance of an increment in the abundance of the corresponding transcripts remains a matter of speculation. Considering that the precise function of PPARβ has not been determined, the theoretical outcome of an eventual increase in PPARβ protein (as a consequence of higher mRNA levels) could be inferred from emerging information that supports the role of PPARβ as a dietary lipid sensor and modulator of lipid homeostasis. Reportedly, PPARβ-null mice have reduced adipose tissue depots. Moreover, the advent of a selective PPARβ ligand has provided evidence for its role as a regulator of the expression of the gene encoding the ABCA1 reverse cholesterol transporter (25). Therefore, changes in PPARβ mRNA absolute abundance in ocular tissue could hypothetically stimulate the efflux of cholesterol from cells. However, modulation of other cellular pathways cannot be excluded.

Our results indicate that PPAR gene expression is moderately sensitive to long-term n-3 FA deficiency in the whole-body experimental model tested here. The dietary intervention in this study is highly specific to the n-3 FA series, preserving the total amount of fat as well as the amount of n-6 FA. The response to this dietary intervention conceivably includes mechanisms other than the transcriptional control of PPAR genes. It is possible that the consequences of dietary intake of n-3 FA primarily involve modulation of the activity rather than amount of PPAR. In addition, other transcription factors may be involved in the response to n-3 deficiency. Recent experiments using cell lines show that DHA is a potent activator of the transcriptional activity of 9-*cis* retinoic acid receptor (RXR) isoforms. Moreover, the reported effect was highly specific to DHA in comparison to other C_{22}, C_{20}, and C_{18} unsaturated FA (59). Given the fact that RXR responsiveness to DHA is affected by heterodimerization and that PPAR and RXR are common partners, it is possible that DHA deficiency alters cellular pathways under the transcriptional control of both RXR and PPAR or other heterodimerization partners.

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REFERENCES

1. Clarke, S.D., and Jump, D.B. (1994) Dietary Polyunsaturated Fatty Acid Regulation of Gene Transcription, *Annu. Rev. Nutr. 14*, 83–98.
2. Kliewer, S.A., Sundseth, S.S., Jones, S.A., Brown, P.S., Wisely, G.B., Koble, C.S., Devchand, P., Wahli, W., Willson, T.M., Lenhard, J.M., and Lehmann, J.M. (1997) Fatty Acids and Eicosanoids Regulate Gene Expression Through Direct Interactions with Peroxisome Proliferator-Activated Receptor α and γ, *Proc. Nat. Acad. Sci. USA 94*, 4318–4323.
3. Jump, D.B., and Clarke, S.D. (1999) Regulation of Gene Expression by Dietary Fat, *Annu. Rev. Nutr. 19*, 63–90.
4. Neuringer, M., Anderson, G.J., and Connor, W.E. (1988) The Essentiality of n-3 Fatty Acids for the Development and Function of the Retina and Brain, *Annu. Rev. Nutr. 8*, 517–541.
5. Bourre, J.M., Francois, M., Youyou, A. Dumont, O., Piciotti, M., Pascal, G., and Durand, G. (1989) The Effects of Dietary α-Linoleic Acid on the Composition of Nerve Membranes, Enzymatic Activity, Amplitude of Electrophysiological Parameters, Resistance to Poison and Performance of Learning Tasks in Rats, *J. Nutr. 119*, 1880–1892.
6. Carlson, S.E. (2000) Behavioral Methods Used in the Study of Long-Chain Polyunsaturated Fatty Acid Nutrition in Primate Infants, *Am. J. Clin. Nutr. 71*, 268S–274S.
7. Uauy, R., Birch, E.E, Birch, D.G., and Peirano, P. (1992) Visual and Brain Function Measurements in Studies of n-3 Fatty Acid Requirements of Infants, *J. Pediatr. 120*, S168–S180.
8. Carlson, S.E., Werkman, S.H., Rhodes, P.G., and Tolley, E.A. (1993) Visual-Acuity Development in Healthy Preterm Infants: Effect of Marine-Oil Supplementation, *Am. J. Clin. Nutr. 58*, 35–42.
9. Neuringer, M., Connor, W.E., Van Petten, C., and Barstad, L. (1984) Dietary Omega-3 Fatty Acid Deficiency and Visual Loss in Infant Rhesus Monkeys, *J. Clin. Invest. 73*, 272–276.

10. Birch, E.E., Hoffman, D.R., Uauy, R., Birch, D.G., and Prestidge, C. (1998) Visual Acuity and the Essentiality of Docosahexaenoic Acid and Arachidonic Acid in the Diet of Term Infants, *Pediatr. Res. 44*, 201–209.
11. Devchand, P.R., Keller, H., Peters, J.M., Vázquez, M., González, F.J., and Wahli, W. (1996) The PPARα–Leukotriene B4 Pathway to Inflammation Control, *Nature 384*, 39–43.
12. Kersten, S., Desvergne, B., and Wahli, W. (2000) Roles of PPARs in Health and Disease, *Nature 405,* 421–424.
13. Braissant, O., Foufelle, F., Scotto, C., Dauça, M., and Wahli, W. (1996) Differential Expression of Peroxisome Proliferator Activated Receptor (PPARs): Tissue Distribution of PPAR-α, -β and -γ in the Adult Rat, *Endocrinology 137*, 354–366.
14. Mukherjee, R., Jow, L., Croston, G.E., and Paterniti, J.R., Jr. (1997) Identification, Characterization, and Tissue Distribution of Human Peroxisome Proliferator-Activated Receptor (PPAR) Isoforms PPARγ2 versus PPARγ1 and Activation with Retinoid Receptor Agonists and Antagonists, *J. Biol. Chem. 272*, 8071–8076.
15. Cullingford, T.E., Bhakoo, K., Peuchen, S., Dolphin, C.T., Patel, R., and Clark, J.B. (1998) Distribution of mRNAs Encoding the Peroxisome Proliferator-Activated Receptor α, β, and γ and the Retinoid X Receptor α, β, and γ in Rat Central Nervous System, *J. Neurochem. 70*, 1366–1375.
16. Granneman, J., Skoff, R., and Yang, X. (1998) Member of the Peroxisome Proliferator-Activated Receptor Family of Transcription Factors Is Differentially Expressed by Oligodendrocytes, *J. Neurosci. Res. 51*, 563–573.
17. Kremarik-Bouillaud, P., Schohn, H., and Dauça, M. (2000) Regional Distribution of PPAR Beta in the Cerebellum of the Rat, *J. Chem. Neuroanat. 19*, 225–232.
18. Gregoire, F.M., Smas, C.M., and Sul, H.S. (1998) Understanding Adipocyte Differentiation, *Physiol. Rev. 78*, 783–809.
19. Rosen, E.D., Sarraf, P., Troy, A.E., Bradwin, G., Moore, K., Milstone, D.S., Spiegelman, B.M., and Mortensen, R.M. (1999) PPAR Is Required for the Differentiation of Adipose Tissue *in vivo* and *in vitro, Mol. Cell 4*, 611–617.
20. Bassu-Modak, S., Braissant, O., Escher, P., Desvergne, B., Honegger, P., and Wahli, W. (1999) Peroxisome Proliferator-Activated Receptor Beta Regulates Acyl-CoA Synthetase 2 in Reaggregated Rat Brain Cell Cultures, *J. Biol. Chem. 274*, 35881–35888.
21. Bastie, C., Luquet, S., Holst, D., Jehl-Petri, C., and Grimaldi, P.A. (2000) Alterations of Peroxisome Proliferator-Activated PPAR (δ) Activity Affect Fatty Acid-Controlled Adipose Differentiation, *J. Biol. Chem. 275*, 38768–38773.
22. Peters, J.M., Lee, S.S., Li, W., Ward, J.M., Gavrilova, O., Everett, C., Reitman, M.L., Hudson, L.D., and González, F.J. (2000) Growth, Adipose, Brain, and Skin Alterations Resulting from Targeted Disruption of the Mouse Peroxisome Proliferator-Activated Receptor Beta (Delta), *Mol. Cell. Biol. 20,* 5119–5128.
23. He, T.C., Chan, T.A., Vogelstein, B., and Kinzler, K.W. (1999) PPAR Delta Is an APC-Regulated Target of Nonsteroidal Antiinflammatory Drugs, *Cell 29,* 335–345.
24. Mano, H., Kimura, C., Fujisawa, Y., Kameda, T., Watanabe-Mano, M., Kaneko, H., Kaneda, T., Hakeda, Y., and Kumegawa, M. (2000) Cloning and Function of Rabbit Peroxisome Proliferator-Activated Receptor δ/β in Mature Osteoclasts, *J. Biol. Chem. 275*, 8126–8132.
25. Oliver, W.R., Shenk, J.L., Snaith, M.R., Russell, C.S., Plunket, K.D., Bodkin, N.L., Lewis, M.C., Winegar, D.A., Sznaidman, M.L., Lambert, M.H., *et al.* (2001) A Selective Peroxisome Proliferator-Activated Receptor δ Agonist Promotes Reverse Cholesterol Transport, *Proc. Natl. Acad. Sci. USA 98,* 5306–5311.
26. Forman, B.H., Chen, J., and Evans, R.M. (1997) Hypolipidemic Drugs, Polyunsaturated Fatty Acids, and Eicosanoids Are Ligands for Peroxisome Proliferator Activated Receptor α and γ, *Proc. Natl. Acad. Sci. USA 94*, 4312–4317.
27. Yu, K., Bayona, W., Kallen, C.B., Harding, H.P., Ravera, C.P., McMahon, G., Brown, M., and Lazar, M.A. (1995) Differential Activation of Peroxisome Proliferator-Activated Receptors by Eicosanoids, *J. Biol Chem. 270*, 23975–23983.
28. Vidal-Puig, A., Jiménez-Liñán, M., Lowell, B.B., Hamann, A., Hu, E., Spiegelman, B., Flier, J.S., and Moller, D.E. (1996) Regulation of PPARγ Gene Expression by Nutrition and Obesity in Rodents, *J. Clin. Invest. 97*, 2553–2561.
29. Fajas, L., Schoonjans, K., Gelman, L., Kim, J.B., Najib, J., Martin, G., Fruchart, J.C., Briggs, M., Spiegelman, B.M., and Auwerx, J. (1999) Regulation of Peroxisome Proliferator-Activated Receptor Gamma Expression by Adipocyte Differentiation and Determination Factor 1/Sterol Regulatory Element Binding Protein 1: Implications for Adipocyte Differentiation and Metabolism, *Mol. Cell. Biol. 19*, 5495–5503.
30. Hashimoto, T., Cook, W.S., Qi, C., Yeldandi, A.V., Reddy, J.K., and Rao, M.S. (2000) Defect in Peroxisome Proliferator-Activated Receptor α-Inducible Fatty Acid Oxidation Determine the Severity of Hepatic Steatosis in Response to Fasting, *J. Biol. Chem. 275*, 28918–28928.
31. Kersten, S., Seydoux, J., Peters, J.M., González, F.J., Desvergne, B., and Wahli, W. (1999) Peroxisome Proliferator Activated Receptor α Mediates the Adaptive Response to Fasting, *J. Clin. Invest. 103*, 1489–1498.
32. Kersten, S., Mandard, S., Tan, N.S., Escher, P., Metzger, D., Chambon, P., González, F.J., Desvergne, B., and Wahli, W. (2000) Characterization of the Fasting-Induced Adipose Factor FIAF, a Novel Peroxisome Proliferator-Activated Receptor Target Gene, *J. Biol. Chem. 275*, 28488–28493.
33. Reeves, P.G., Neilsen, F.H., and Fahey, G.C. (1993) Committee Report on the AIN-93 Purified Rodent Diet, *J. Nutr. 123*, 1939–1951.
34. Greiner, R.S., Moriguchi, T., Hutton, A., Slotnick, B.M., and Salem, N., Jr. (1999) Rats with Low Levels of Brain Docosahexaenoic Acid Show Impaired Performance in Olfactory-Based and Spatial Learning Tasks, *Lipids 34* (Suppl.), S239–S243.
35. Bligh, E.G., and Dyer, W.J. (1959) A Rapid Method of Total Lipid Extraction and Purification, *Can. J. Biochem. Physiol. 37*, 911–917.
36. Morrison, W.R., and Smith, L.M. (1961) Preparation of Fatty Acid Methyl Esters and Dimethylacetals from Lipids with Boron Fluoride-Methanol, *J. Lipid Res. 35*, 600–608.
37. Salem, N., Jr., Reyzer, M., and Karanian, J. (1996) Losses of Arachidonic Acid in Rat Liver After Alcohol Inhalation, *Lipids 31*, S153–S156.
38. Salem, N., Jr. (1989) Omega-3 Fatty Acids: Molecular and Biochemical Aspects, in *New Protective Roles of Selected Nutrients in Human Nutrition* (Spiller, G., and Scala, J., eds.), pp. 109–228, Alan R. Liss, New York.
39. Hamilton, J., Greiner, R., Salem, N., and Kim, H. (2000) n-3 Fatty Acid Deficiency Decreases Phosphatidylserine Accumulation Selectively in Neuronal Tissues, *Lipids 35*, 863–869.
40. Rolland, V., Dugail, I., Le Liepvre, X., and Lavau, M. (1995) Evidence of Increased Glyceraldehyde-3-phosphate Dehydrogenase and Fatty Acid Synthetase Promoter Activities in Transiently Transfected Adipocytes from Genetically Obese Rats, *J. Biol. Chem. 270,* 1102–1106.
41. Weisinger, G., Gavish, M., Mazurika, C., and Zinder, O. (1999) Transcription of Actin, Cyclophilin and Glyceraldehyde Phosphate Dehydrogenase Genes: Tissue- and Treatment-Specificity, *Biochim. Biophys. Acta 1446*, 225–232.
42. Zhong, H., and Simons, J.W. (1999) Direct Comparison of GAPDH, β-Actin, Cyclophilin, and 28S rRNA as Internal Standards for Quantifying RNA Levels Under Hypoxia, *Biochem. Biophys. Res. Commun. 259*, 523–526.

43. Okuno, M., Kajiwara, K., Imai, S., Kobayashi, T.Y., Honma, N., Maki, T., Suruga, K., Goda, T., Takase, S., Muto, Y., and Moriwaki, H. (1997) Perilla Oil Prevents the Excessive Growth of Visceral Adipose Tissue in Rats by Down-Regulating Adipocyte Differentiation, *J. Nutr. 127,* 1752–1757.
44. Bonilla, S., Redonnet, A., Noel-Suberville, C., Pallet, V., Garcin, H., and Higueret, P. (2000) High-Fat Diets Affect the Expression of Nuclear Retinoic Acid Receptor in Rat Liver, *Br. J. Nutr. 83*, 665–671.
45. Ouali, F., Djouadi, F., Merlet-Bénichou, C., and Bastin J. (1998) Dietary Lipids Regulate β-Oxidation Enzyme Gene Expression in the Developing Rat Kidney, *Am. J. Physiol. 275 (Renal Physiol. 44),* F777–F784.
46. Nisoli, E., Carruba, M.O., Tonello, C., Macor, C., Federspil, G., and Vettor, R. (2000) Induction of Fatty Acid Translocase/CD36, Peroxisome Proliferation-Activated Receptor-Gamma2, Leptin, Uncoupling Proteins 2 and 3, and Tumor Necrosis Factor-Alpha Gene Expression in Human Subcutaneous Fat by Lipid Infusion, *Diabetes 49*, 319–324.
47. Makrides, M., Neumann, M., Simmer, K., Pater, J., and Gibson, R. (1995) Are Long-Chain Polyunsaturated Fatty Acids Essential Nutrients in Infancy? *Lancet 345*, 1463.
48. Fernstrom, J.D. (1999) Effects of Dietary Polyunsaturated Fatty Acids on Neuronal Function, *Lipids 34*, 161–169.
49. Bush, R.A., Armand, M., Remé, C.E., and Williams, T.P. (1994) Dietary Deficiency of n-3 Fatty Acid Alters Rhodopsin Content and Function in the Rat Retina, *Invest. Ophthalmol. Vis. Sci. 35*, 91–100.
50. Rotstein, N., Politi, L.E., and Aveldaño, M.I. (1998) Docosahexaenoic Acid Promotes Differentiation of Developing Photoreceptors in Culture, *Invest. Ophthalmol. Vis. Sci. 39,* 2750–2758.
51. Braissant, O., and Wahli, W. (1998) Differential Expression of Peroxisome Proliferator Activated Receptor-α, -β and -γ During Rat Embryonic Development, *Endocrinology 139,* 2748–2754.
52. Foot, M., Cruz, T.F., and Clandinin, M.T. (1982) Influence of Dietary Fat on the Lipid Composition of Rat Brain Synaptosomal and Microsomal Membranes, *Biochem. J. 208*, 631–640.
53. Bourre, J.M., Pascal, D., Durand, G., Masson, M., Dumont, O., and Piciotti, M. (1984) Alterations in the Fatty Acid Composition of Rat Brain Cells (neurons, astrocytes and oligodendrocytes) and of Subcellular Fractions (myelin synaptosomes) Induced by a Diet Devoid of n-3 Fatty Acids, *J. Neurochem. 43*, 342–348.
54. Yamamoto, N., Saitoh, M., Moriuchi, A., Nomura, M., and Okuyama, H. (1987) Effect of Dietary α-Linolenate/Linoleate Balance on Brain Lipid Composition and Learning Ability of Rats, *J. Lipid Res. 28*, 144–151.
55. Martínez, M. (1992) Abnormal Profiles of Polyunsaturated Fatty Acids in the Brain, Liver, Kidney and Retina of Patients with Peroxisomal Disorders, *Brain Res. 583*, 171–182.
56. Infante, J.P., and Huszagh, V.A. (2000) Secondary Carnitine Deficiency and Impaired Docosahexaenoic (22:6n-3) Acid Synthesis: A Common Denominator in the Pathophysiology of Disease of Oxidative Phosphorylation and β-Oxidation, *FEBS Lett. 468*, 1–5.
57. Moriguchi, T., Greiner, R.S., and Salem N., Jr. (2000) Behavioral Deficits Associated with Dietary Induction of Decreased Brain Docosahexaenoic Acid Concentration, *J. Neurochem. 75*, 2563–2573.
58. Greiner, R.S., Moriguchi, T., Hutton, A., Slotnick, B.M., and Salem, N., Jr. (2001) Olfactory Discrimination Deficits in n-3 Fatty Acid Deficient Rats, *Physiol. Behav. 72*, 379–385.
59. Mata de Urquiza, M., Liu, S., Sjöberg, M., Zetterström, R.H, Griffiths, W., Sjövall, J., and Perlmann, T. (2000) Docosahexaenoic Acid, a Ligand for the Retinoid X Receptor in Mouse Brain, *Science 290*, 2140–2144.

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