Vitamin A Deficiency Enhances Docosahexaenoic and Osbond Acids in Liver of Rats Fed an α-Linolenic Acid-Adequate Diet

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ABSTRACT: The synthesis of docosahexaenoic (DHA, 22:6n-3) and Osbond acid (OA, 22:5n-6) is regulated by the heterodimer of peroxisome proliferator-activated receptor and retinoid X receptor (RXR). 9-*Cis* retinoic acid, a metabolite of vitamin A, is the most potent ligand of RXR. We tested whether vitamin A deficiency impairs DHA and OA synthesis in rats fed a vitamin A- and α-linolenic acid (ALA)-sufficient (VASALAS), vitamin A-sufficient and ALA-deficient (VASALAD), vitamin A–deficient and ALA-sufficient (VADALAS), or vitamin A- and ALA-deficient (VADALAD) diet. After 7 wk of feeding, liver and colon choline (CPG) and ethanolamine (EPG) phosphoglyceride FA were analyzed. The VADALAS compared with the VASALAS rats had elevated levels of both DHA ($P < 0.05$) and OA ($P < 0.005$) in liver CPG and EPG. In contrast, the VADALAD group had a lower DHA ($P < 0.01$) and higher OA ($P < 0.005$) level in CPG and EPG of both tissues than their VASALAD counterparts. ALA deficiency reduced DHA and enhanced OA levels in liver and colon CPG and EPG in both the vitamin A-sufficient (VASALAS vs. VASALAD) and -deficient (VADALAS vs. VADALAD) rats ($P < 0.005$). The study demonstrates that ALA deficiency reduced DHA and enhanced OA levels in tissue membranes, and dietary vitamin A deficiency has a profound effect on membrane DHA and OA in rat tissues. Both vitamin A and DHA are involved in a myriad of vital physiological functions pertaining to growth and development and health. Hence, there is a need for a further study to unravel the mechanism by which vitamin A influences membrane DHA and OA.

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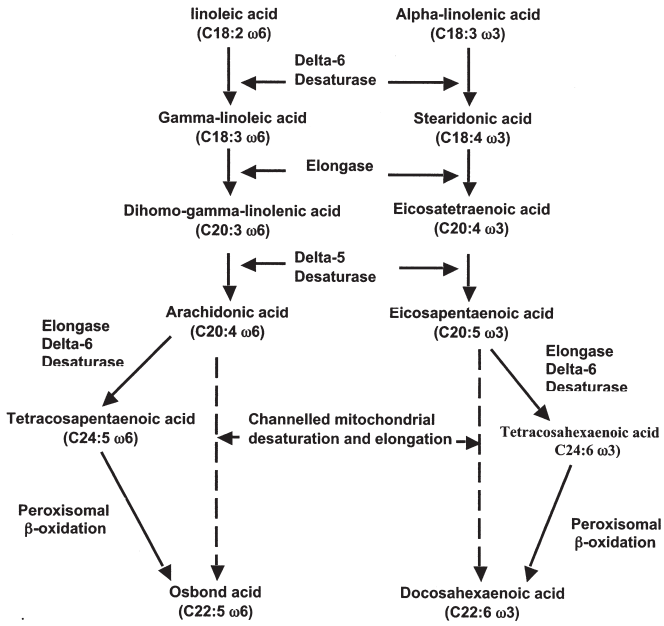
DHA (20:6n-3) is a major constituent of neural and retinal membrane lipids (1,2). In addition, it is present in appreciable proportions in other cell and subcellular membranes (3,4).

Membrane DHA is obtained either preformed from animal products, particularly from fish, shellfish, and fish oil, or from α-linolenic acid (ALA, 18:3n-3) by *de novo* synthesis. It is generally accepted that the synthesis of DHA from ALA involves a series of elongation and desaturation steps in endoplasmic reticulum followed by a final single step β-oxidation reaction in the peroxisomes (5–7) (Scheme 1). Osbond acid (OA, 22:5n-6) is also thought to be synthesized from its precursor FA, linoleic acid (LA, 18:2n-6), by the same pathway, following elongation, desaturation, and β-oxidation reactions.

The activation of peroxisome proliferator-activated (PPAR) and retinoid X (RXR) receptors is imperative for the proliferation of peroxisomes and activation of the key enzymes involved in peroxisomal β-oxidation. On activation by a specific ligand, PPAR form a heterodimer complex (PPAR-RXR) with activated RXR and subsequently bind to specific DNA sequences and initiate inducible transcriptional activity (8–11). The heterodimer PPAR-RXR also has been shown to up-regulate the activity of Δ6 and Δ5 desaturases (12), enzymes that are vital for the synthesis of the long-chain n-6 and n-3 FA, including DHA and OA.

PPAR are members of the intracellular type II nuclear hormone receptor superfamily. Three subtypes have been identified, $PPAR_{\alpha}$, $PPAR_{\beta}/PPAR_{\delta}$, and $PPAR_{\gamma}$ (13). They are acti-



SCHEME 1

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Abbreviations: ALA, α-linolenic acid; CPG, choline phosphoglyceride; EPG, ethanolamine phosphoglyceride; OA, Osbond acid (= docosapentaenoic acid); PPAR, peroxisome proliferator-activated receptor; RXR, retinoid X receptor; VADALAD, vitamin A- and ALA-deficient; VADALAS, vitamin A-deficient and ALA-sufficient; VASALAD, vitamin A-sufficient and ALA-deficient; VASALAS, vitamin A- and ALA-sufficient.

TABLE 1
Gross Nutrient Composition of the Vitamin A-Sufficient (VAS) and -Deficient (VAD) Diets

Ingredients	VAD (g/kg diet)	VAS (g/kg diet)
Casein, vitamin-free	200.0	200.0
DL-Methionine	3.0	3.0
Sucrose	437.98	437.98
Corn starch	200.0	200.0
Oils	90.0	90.0
Cellulose (fiber)	30.0	30.0
Mineral mix, AIN-76 (170915)	35.0	35.0
Calcium carbonate, $CaCO_3$	4.0	4.0
Vitamin A (IU)	0	19825
Vitamin mix, Teklad (40060)	10.0	10.0
Ethoxyquin (antioxidant)	0.016	0.016

TABLE 2
Percent FA Composition of the α-Linolenic Acid (ALA)-Deficient (ALAD) and -Sufficient (ALAS) Diets

FA	ALAD (% of total FA)	ALAS (% of total FA)
14:0	1.12	0.976
16:0	21.85	19.44
18:0	2.31	2.49
18:1n-9	18.81	19.25
18:2n-6	53.09	49.21
18:3n-3	0.31	6.82
18:2n-6/18:3n-3	170:1	7:1

vated by polyunsaturated, conjugated, and branched FA, eicosanoids, and synthetic ligands such as fibrates (14). In contrast to PPAR, RXR is activated primarily by 9-*cis* retinoic acid, which is a metabolite of vitamin A (8,9), and by phytanic acid with a low affinity (15).

Previously (16), we showed that vitamin A deficiency enhances OA and reduces DHA levels in liver and colon choline (CPG) and ethanolamine (EPG) phosphoglycerides in rats fed a diet deficient in n-3 and sufficient in n-6 FA. The finding was rather intriguing since vitamin A deficiency would have been expected to reduce the levels of both DHA and OA owing to a reduction of activation of RXR and consequently impaired peroxisomal proliferation. In the current study, we have investigated the effect of vitamin A deficiency on levels of DHA and OA in liver and colon phosphoglycerides in rats fed a diet sufficient or deficient in n-3 FA.

EXPERIMENTAL PROCEDURES

Animals. Pathogen-free, weanling male Wistar rats (n = 24) were obtained from the Harlan laboratory, The Weizmann Institute of Science, Rehovot, Israel. They were housed in metal cages in a room with controlled temperature (22 ± 2°C), RH (65 ± 5%), and light (0800–2000 h).

Diets. Twenty-four rats were randomly distributed into four groups and fed one of the following diets: vitamin A- and α-linolenic acid (ALA)-deficient (VADALAD, n = 6), vitamin A-deficient and ALA-sufficient (VADALAS, n = 6), vitamin A-sufficient and ALA-deficient (VASALAD, n = 6), or vitamin A- and ALA-sufficient (VASALAS, n = 6). The diet, which was obtained from ICN Nutritional Biochemicals (Costa Mesa, CA) (cat. no. 960220) contained 9% fat, 20% protein, and 60% carbohydrate (Tables 1 and 2). With the exceptions of vitamin A and n-6 and n-3 FA, the four diets had optimal and comparable amounts of vitamins and trace elements. The linoleic acid/ALA ratio of the ALA-sufficient diet was 7:1 and that of the ALA-deficient, 170:1. The rats had free access to their respective diets and water. Their food consumption was monitored daily, and they were weighed every other day.

After they were fed for 7 wk, at which time the liver vitamin A concentration of the deficient group was lower than 5 μg/g liver, the rats were sacrificed by decapitation and colon and liver tissues were removed for analyses. All procedures were conducted with full compliance to the guidelines of the Ethics Committee and Policy of Animal Care and Use of the Hebrew University.

Analysis of lipids and FA. A method modified from Folch *et al.* (17) was used to extract liver and colon total lipids. The tissues were homogenized in chloroform and methanol (2:1 vol/vol) containing 0.01% BHT as an antioxidant under N_2. Phosphoglyceride classes were separated by TLC on silica gel plates (Merck KGaA, Darmstadt, Germany) by the use of these developing solvents: Chloroform, methanol, and water (60:30:4 by vol) containing 0.01% BHT. CPG and EPG bands were detected by spraying with a methanolic solution of 2,7-dichlorofluorescein (0.01% wt/vol) and identified by the use of authentic standards.

FAME were prepared by heating the CPG and EPG with 5 mL of 15% acetyl chloride in methanol in a sealed vial at 70°C for 3 h under N_2. FAME were separated by a gas–liquid chromatograph (HRGC MEGA-2 Series; Fisons Instruments, Milan,Italy) fitted with a BP-20 capillary column (30 m × 0.32 mm i.d., 0.25 μm film; SGE Ltd., Milton Keynes, United Kingdom). Hydrogen was used as a carrier gas. It has a broad minimum van Deemter profile, which is vital for optimal performance, as compared with helium and nitrogen. In addition, it has faster diffusion and lower viscosity than the latter gases. The injector, oven, and detector temperatures were 235, 210, and 260°C. The FAME were identified by comparison of retention times with authentic standards and interpretation of ECL values. Peak areas were quantified by computer software (EZChrom Chromatography Data System; Scientific Software, Inc., San Ramon, CA).

Analysis of liver vitamin A. The liver specimen, 100 mg, was thoroughly minced, flushed with nitrogen, and saponified with a 50% ethanolic potassium hydroxide (BDH Chemicals, Poole, United Kingdom) for 25 min at 60°C. The saponified sample was cooled, diluted with de-ionized water, and neutralized with hydrochloric acid (BDH Chemicals). Extraction of vitamin A was carried out with 2 mL of hexane for 5 min on a Rotamixer Shaker (Baird and Tatlock, Ramford, Essex, United Kingdom). After centrifugation at 800 × g for 15 min, the upper organic layer containing vitamin A was transferred to a brown glass tube and subsequently evaporated to dryness on a water bath at 37°C under a stream of nitrogen. The re-

TABLE 3
Food Consumption, Weight Gain, Liver Vitamin A Concentration, and Somatic Index[a]

	VADALAS (n = 6)	VASALAS (n = 6)	VADALAD (n = 6)	VASALAD (n = 6)
Food consumption (g/d)	13.6 ± 1.1	17.7 ± 1.1	13.5 ± 0.9	18.6 ± 1.4
Weight gain (g/wk)	32.9 ± 5.2	41.2 ± 5.2	32.1 ± 4.4	41.5 ± 7.8
Liver vitamin A (μg/g liver)	<5	271.1	<5	296.4
Liver somatic index (liver/body weight)	3.4 ± 0.4	3.3 ± 0.3	3.4 ± 0.3	3.7 ± 0.6

[a]VADALAD, vitamin A- and ALA-deficient; VADALAS, vitamin A-deficient and ALA-sufficient; VASALAD, vitamin A-sufficient and ALA-deficient; VASALAS, vitamin A- and ALA-sufficient.

sulting vitamin A residue was redissolved in 100 μL methanol (BDH Chemicals) and a 25-μL aliquot taken for analysis. The entire extraction procedure was carried out under subdued light.

The extracted vitamin A was separated by HPLC (Agilent 1100 series; Agilent Technologies, Waldbronn, Germany) connected to a 250 × 4.6 mm and a 5 μ SphereClone ODS reversed-phase column (Phenomenex, Macclesfield, Cheshire, United Kingdom) and detected at 325 nm with a multiple wavelength diode array detector (Agilent 1100 series). It was eluted with acetonitrile/dichloromethane/methanol (75:15:10). Retinyl acetate was used as an internal standard. Vitamin A concentration was computed from a linear graph of retinol external standards. ChemStation data system version A10.01 (Agilent Technologies) was used for peak area measurement and quantification.

Data analyses. The data are expressed as mean ± SD. The Kruskal–Wallis one-way ANOVA nonparametric method was used to compare liver and colon FA levels of the four dietary groups. Differences between two dietary groups were analyzed by the Mann–Whitney U nonparametric test. All the statistical analyses were performed by the use of SPSS software for Windows, Release 10 (SPSS, Chicago, IL).

RESULTS

Food consumption and weight gain. The food consumption of the VADALAD and VADALAS groups was lower ($P < 0.05$) than that of the VASALAD and VASALAS groups (Table 3). No difference in food consumption was found between VADALAD and VADALAS, and between VASALAS and VASALAD, respectively.

Consistent with change of the food consumption, the weight gain of the VADALAD and VADALAS groups was lower ($P < 0.005$) than that of the VASALAD and VASALAS groups (Table 3). No difference in weight gain was observed between VADALAD and VADALAS, and between VASALAS and VASALAD, respectively. Moreover, no difference in liver somatic index was found among VADALAS, VASALAS, VADALAD, and VASALAD groups.

TABLE 4
Liver Choline Phosphoglyceride FA (%) of the Rats Fed an ALA-Adequate or -Deficient Diet With or Without Vitamin A[a]

FA	VADALAS (n = 6)	VASALAS (n = 6)	VADALAD (n = 6)	VASALAD (n = 6)
16:0	26.68 ± 1.57^{b,c}	20.42 ± 1.33^{a,c,d}	24.67 ± 0.86^{a,b}	25.19 ± 1.98^{b}
18:0	16.82 ± 2.46^{d}	15.35 ± 2.38^{d}	16.32 ± 1.37^{d}	20.85 ± 1.57^{a,b,c}
Σ Saturates	43.77 ± 1.74^{b,c,d}	36.15 ± 1.10^{a,c,d}	41.30 ± 1.14^{a,b,d}	46.31 ± 1.10^{a,b,c}
18:1n-9	5.00 ± 1.06^{b,d}	6.61 ± 0.89^{a,d}	5.89 ± 0.34^{d}	3.60 ± 0.70^{a,b,c}
18:1n-9 +18:1n-7	7.44 ± 1.75^{b}	11.31 ± 1.78^{a,c,d}	8.56 ± 1.87^{b,d}	5.80 ± 0.74^{b,c}
Σ Monoenes	8.74 ± 1.27^{b,d}	13.08 ± 2.46^{a,c,d}	9.44 ± 2.10^{b,d}	6.68 ± 0.93^{a,b,c}
18:2n-6	16.01 ± 2.80^{b}	21.43 ± 2.09^{a,c,d}	15.86 ± 2.60^{b}	14.56 ± 1.37^{b}
18:3n-6	0.15 ± 0.02^{b,c}	0.35 ± 0.06^{a,c,d}	0.42 ± 0.04^{a,b,d}	0.19 ± 0.05^{b,c}
20:3n-6	0.46 ± 0.08^{d}	0.56 ± 0.16^{c,d}	0.37 ± 0.06^{b,d}	0.19 ± 0.05^{a,b,c}
20:4n-6	19.42 ± 2.14^{d}	21.76 ± 1.78^{d}	21.45 ± 1.47^{d}	26.27 ± 1.68^{a,b,c}
22:4n-6	0.24 ± 0.04^{b,c}	0.13 ± 0.03^{a,c,d}	0.97 ± 0.20^{a,b,d}	0.25 ± 0.03^{b,c}
22:5n-6	0.55 ± 0.07^{b,c,d}	0.06 ± 0.05^{a,c,d}	7.01 ± 1.10^{a,b,d}	1.80 ± 0.41^{a,b,c}
Σ n-6	37.40 ± 1.32^{b,c,d}	44.95 ± 2.03^{a}	46.65 ± 1.79^{a,d}	43.52 ± 1.24^{a,c}
18:3n-3	0.35 ± 0.19^{b}	0.81 ± 0.27^{a}	—^{b}	—
20:5n-3	0.05 ± 0.03^{b}	0.47 ± 0.10^{a}	—	—
22:5n-3	0.72 ± 0.15^{b,c,d}	0.48 ± 0.09^{a,c,d}	0.10 ± 0.03^{a,b}	0.12 ± 0.01^{a,b}
22:6n-3	6.01 ± 0.88^{b,c,d}	2.69 ± 0.75^{a,c,d}	0.68 ± 0.09^{a,b,d}	1.65 ± 0.25^{a,b,c}
Σ n-3	7.05 ± 0.69^{b,c,d}	4.45 ± 0.56^{a,c,d}	0.80 ± 0.09^{a,b,d}	1.83 ± 0.24^{a,b,c}

[a]A superscript letter denotes a significant difference from the corresponding group at $P < 0.05$ level. For abbreviations see Tables 2 and 3 .
[b]—, trace.

TABLE 5
Liver Ethanolamine Phosphoglyceride FA (%) of Rats Fed Adequate or Deficient ALA Diets With or Without Vitamin A[a]

FA	VADALAS ($n = 6$)	VASALAS ($n = 6$)	VADALAD ($n = 6$)	VASALAD ($n = 6$)
16:0	22.32 ± 0.69[b,c,d]	16.83 ± 0.86[a]	18.61 ± 1.72[a,d]	16.75 ± 1.08[a,c]
18:0	24.45 ± 1.07[b,c,d]	21.90 ± 1.49[a,d]	22.60 ± 2.18[a,d]	28.85 ± 1.98[a,b,c]
Σ Saturates	47.09 ± 0.83[b,c]	38.83 ± 1.95[a,c,d]	41.27 ± 1.20[a,b,d]	45.72 ± 2.37[b,c]
18:1n-9	2.88 ± 0.46[d]	3.27 ± 0.33[d]	3.03 ± 0.21[d]	1.92 ± 0.17[a,b,c]
18:1n-9 + 18:1n-7	4.17 ± 0.59[b,d]	6.59 ± 1.16[a,c,d]	4.33 ± 0.47[b,d]	3.13 ± 0.35[a,b,c]
Σ Monoenes	4.44 ± 0.58[b]	7.09 ± 0.97[a,c,d]	4.65 ± 0.58[b,d]	3.35 ± 0.42[b,c]
18:2n-6	5.38 ± 0.98[b,d]	7.88 ± 1.22[a,c]	5.73 ± 1.69[b,d]	7.79 ± 1.16[a,c]
18:3n-6	0.05 ± 0.01[b,c,d]	0.12 ± 0.05[a,c]	0.28 ± 0.02[a,b,d]	0.10 ± 0.03[a,c]
20:3n-6	0.25 ± 0.03[b]	0.46 ± 0.14[a,c,d]	0.29 ± 0.08[b]	0.25 ± 0.08[b]
20:4n-6	20.44 ± 0.78[b,d]	27.14 ± 2.02[a,c]	21.77 ± 1.55[b,d]	28.51 ± 1.46[a,c]
22:4n-6	0.78 ± 0.11[b,c]	0.55 ± 0.05[a,c,d]	2.56 ± 0.44[a,b,d]	0.97 ± 0.14[b,c]
22:5n-6	1.20 ± 0.14[b,c,d]	0.27 ± 0.06[a,c,d]	16.84 ± 1.52[a,b,d]	4.40 ± 0.61[a,b,c]
Σ n-6	28.26 ± 0.26[b,c,d]	36.83 ± 2.41[a,c,d]	47.81 ± 0.9[a,b,d]	42.23 ± 2.13[a,b,c]
18:3n-3	0.10 ± 0.04	0.13 ± 0.07	—[b]	—
20:5n-3	0.08 ± 0.04[b]	0.55 ± 0.21[a]	—	—
22:5n-3	1.42 ± 0.31[c,d]	1.40 ± 0.30[b,c,d]	0.20 ± 0.06[a,b,d]	0.43 ± 0.13[a,b,c]
22:6n-3	14.08 ± 1.20[b,c,d]	10.98 ± 1.93[a,c,d]	1.74 ± 0.20[a,b,d]	4.44 ± 0.49[a,b,c]
Σ n-3	15.50 ± 1.13[b,c,d]	13.05 ± 1.63[a,c,d]	1.94 ± 0.20[a,b,d]	4.88 ± 0.48[a,b,c]

[a,b]For superscripts and abbreviations see Tables 3 and 4.

Liver FA. (i) CPG. The FA composition of liver CPG is presented in Table 4. The mean DHA level in CPG of the VADALAS group was significantly higher than that of the VASALAS ($P < 0.05$), VASALAD ($P < 0.005$), and VADALAD ($P < 0.005$) groups. In addition, the VADALAD rats had a lower level of DHA in CPG compared with the VASALAD ($P < 0.05$) and VASALAS ($P < 0.005$) groups. Of the two vitamin A-sufficient groups, VASALAS and VASALD, the former had a higher DHA level ($P < 0.01$).

As was expected, the proportion of OA, which is a biochemical marker of n-3 FA deficiencies, was increased by ALA deficiency both in the vitamin A-sufficient (VASALAD vs. VASALAS, $P < 0.005$) and -deficient (VADALAD vs. VADALAS, $P < 0.005$) rats. Surprisingly, vitamin A deficiency enhanced the level of OA in the ALA-sufficient (VADALAS vs. VASALAS; $P < 0.005$) and ALA-deficient (VADALAD vs. VASALAD; $P < 0.005$) groups.

(ii) EPG. The mean liver EPG FA composition is given in Table 5. Consistent with the manifestations of n-3 FA insufficiencies, ALA deficiency decreased DHA level in both the vitamin A-sufficient (VASALAD vs. VASALAS, $P < 0.005$) and -deficient (VADALAD vs. VADALAS, $P < 0.005$) groups. The decrease in DHA was associated with a concomitant increase in OA (VASALAD vs. VASALAS, $P < 0.005$) and (VADALAD *vs* VADALAS, $P < 0.005$). As in CPG, the EPG of vitamin A-deficient rats regardless of their ALA status had a significantly elevated proportion of OA.

Colon FA. (i) CPG. Table 6 shows colon CPG FA of the four groups of rats. The VADALAS animals had a higher level of DHA than those of the VASALAS ($P < 0.05$), VADALAD ($P < 0.01$), and VASALAD ($P < 0.005$) rats. In contrast to the VADALAS, the VADALAD rats had reduced DHA compared with the VASALAD rats ($P < 0.01$). Vitamin A deficiency increased the level of OA in the ALA-deficient, VADALAD, ($P < 0.05$) but not in the ALA sufficient, VADALAS, rats.

(ii) EPG. Percent FA composition of colon EPG is given in Table 7. ALA deficiency significantly reduced the level of DHA in the vitamin A-sufficient (VASALAD vs. VASALAS, $P < 0.005$) and -deficient (VADALAD vs. VADALAS, $P < 0.005$) rats. Vitamin A deficiency reduced DHA in the ALA-deficient (VADALAD vs. VASALAD) but not in the ALA-sufficient (VADALAS vs. VASALAS, $P > 0.05$) rats. The level of OA significantly increased in the ALA-deficient compared with the ALA-sufficient groups (VADALAD vs. VADALAS, $P < 0.005$; VASALAD vs. VASALAS, $P < 0.005$). Although vitamin A deficiency in both the VADALAS and VADALAD groups was associated with an increase in OA, the difference with their corresponding vitamin A-sufficient counterparts did not reach a stastically significant level (VADALAS vs. VASALAS, VADALAD vs. VASALAD, $P = 0.078$).

DISCUSSION

Consistent with our previous findings in rats (18) and chicks (19), the vitamin A-deficient groups, regardless of the ALA content of their diet, consumed less food compared with their vitamin A-sufficient counterparts. The reduction of food intake and consequent lower weight gain of the vitamin A-deficient animals were most likely due to vitamin A deficiency-induced atrophy of the gustatory apparatus (20,21) and a loss of taste (20–22). The increase in OA and the concomitant reduction of DHA in the ALA-deficient rats, regardless of vitamin A status, suggest that dietary ALA and LA may be the primary sources of membrane DHA and OA, respectively. These findings are analogous to some reports about n-3 FA deficiencies (23,24).

This partial investigation of the effect of vitamin A defi-

TABLE 6
Colon Choline Phosphoglyceride FA (%) of Rats Fed ALA-Adequate or -Deficient Diet With or Without Vitamin A[a]

FA	VADALAS (n = 6)	VASALAS (n = 6)	VADALAD (n = 6)	VASALAD (n = 6)
16:0	31.17 ± 3.20	32.75 ± 2.71	32.31 ± 3.03	28.37 ± 3.50
18:0	10.97 ± 1.83	10.91 ± 0.99	11.51 ± 1.38	11.55 ± 1.76[c]
Σ Saturates	43.37 ± 2.37	44.86 ± 2.20[d]	44.68 ± 2.45[d]	40.43 ± 1.96[b,c]
18:1n-9	10.27 ± 1.47	10.41 ± 1.84	10.16 ± 1.44[d]	12.05 ± 0.53[c]
18:1n-9 + 18:1n-7	15.03 ± 2.06	15.26 ± 2.22	14.68 ± 1.59[d]	17.08 ± 0.68[c]
Σ Monoenes	17.23 ± 2.05	17.82 ± 2.53	16.43 ± 1.67[d]	18.94 ± 0.92[c]
18:2n-6	13.29 ± 2.05	14.02 ± 1.46	12.14 ± 2.20[d]	15.47 ± 2.23[c]
18:3n-6	0.18 ± 0.04[d]	0.14 ± 0.04	0.17 ± 0.04[d]	0.11 ± 0.03[a,c]
20:3n-6	2.19 ± 0.22[b,d]	1.66 ± 0.33[a]	1.94 ± 0.40	1.14 ± 0.16[a]
20:4n-6	12.38 ± 0.74[c,d]	11.43 ± 1.11[c,d]	14.17 ± 0.92[a,b]	15.16 ± 2.13[a,b]
22:4n-6	1.01 ± 0.13[d]	0.99 ± 0.06[d]	1.28 ± 0.26	1.44 ± 0.16[a,b]
22:5n-6	0.16 ± 0.04[c,d]	0.11 ± 0.04[c,d]	0.74 ± 0.16[a,b,d]	0.48 ± 0.04[a,b,c]
Σ n-6	30.18 ± 1.73[d]	29.29 ± 1.46[d]	31.39 ± 1.86[d]	35.32 ± 2.63[a,b,c]
18:3n-3	0.29 ± 0.08[b]	0.54 ± 0.11[a]	—[b]	—
20:5n-3	0.19 ± 0.05	0.25 ± 0.06	—	—
22:5n-3	0.29 ± 0.02[c,d]	0.33 ± 0.07[c,d]	0.05 ± 0.02[a,b]	0.11 ± 0.07[a,b]
22:6n-3	1.09 ± 0.26[b,c,d]	0.70 ± 0.16[a,c,d]	0.35 ± 0.06[a,b,d]	0.46 ± 0.06[a,b,c]
Σ n-3	1.86 ± 0.22[c,d]	1.82 ± 0.23[c,d]	0.44 ± 0.07[a,b,d]	0.67 ± 0.18[a,b,c]

[a,b]For superscripts and abbreviations see Tables 3 and 4.

ciency on FA composition in liver membrane lipids of rats fed an ALA-deficient diet, consistent with our previous study (16), demonstrated that vitamin A deficiency leads to a reduction in DHA and a concomitant increase in OA in liver CPG and EPG. The reduction in DHA was predicted since vitamin A deficiency was expected to impair peroxisomal proliferation and inhibit the activity of acyl-CoA oxidase owing to an insufficiency of 9-*cis* retinoic acid, which is the potent ligand of RXR. However, the vitamin A deficiency-induced increase in OA was unexpected and rather intriguing. Both DHA and OA are thought to be synthesized by a common shared microsomal-peroxisomal pathway involving chain elongation and desaturation reactions in the endoplasmic reticulum followed by a final one-step β-oxidation reaction in the peroxisomes (5,25). Consequently, an impairment of peroxisomal proliferation would have been expected to lead to a reduction in both OA and DHA. The contrasting effects of vitamin A and ALA deficiency on the proportions of DHA and OA in liver CPG and EPG seemed to lend credence to the proposition that the two FA are synthesized by independent pathways involving n-6- and n-3-specific enzymes (6,7) or to the suggestion that the desaturation of n-6 and n-3 involves two distinct Δ6 desaturases (26). Indeed, Infante *et al.* (27) reported that the straight-chain acyl-CoA oxidase knockout mouse had decreased DHA and increased threefold OA in liver. Our enhancement of OA and reduction of DHA levels in VADALAD rats, similar to their findings, can be explained by hypothesizing that DHA and OA are synthesized by a carnitine-dependent multifunctional mitochondrial synthase residing in the outer mitochondrial membrane, and beyond this, that there is another redundant microsomal pathway that is only suitable for OA synthesis (6,7). In contrast to the rats fed the VADALAD diet, which had reduced DHA and enhanced OA levels, the levels of both DHA and OA were enhanced in the rats that were fed VADALAS diet. Paradoxically, this finding is consistent with the conventional microsomal-peroxisomal and additional carnitine-dependent multifunctional mitochondrial pathways (5–7,26). These effects of vitamin A deficiency on membrane DHA and OA may have been mediated by enhanced peroxisomal proliferation and the consequential activation of peroxisomal enzymes, including acyl-CoA oxidase, activity of Δ6 and Δ5 desaturases, or incorporation. The current wisdom is that peroxisomal proliferation is mediated by PPAR-RXR complex after the activation of RXR by 9-*cis* retinoic acid, and it is difficult to envisage how this could be induced under vitamin A deficiency. However, the studies that reported that 9-*cis* retinoic acid is a potent ligand of RXR were conducted *in vitro* (28–30). Consequently, it is possible that the finding may not be portable to an *in vivo* physiological environment. Indeed, Lawrence *et al.* (31) have reported significantly higher induction of peroxisomal β-oxidation in vitamin A-deficient than -sufficient rats that were treated by the potent peroxisomal proliferator, nafenopin. Similarly, in a communication by Sohlenus *et al.* (32), the vitamin A-deficient mice untreated by the peroxisomal proliferator had about 40% higher volume and number of liver peroxisomes compared with their sufficient counterparts; the authors did not discuss this finding. Interestingly, Werner and DeLuca (33) were unable to detect 9-*cis* retinoic acid in tissues of vitamin A-deficient rats that were given a physiological amount of radioactive all-*trans* retinol. It is plausible that vitamin A deficiency may enhance the synthesis of OA and/or DHA through the induction of peroxisomal proliferation and the activation of β-oxidation without the involvement of 9-*cis* retinoic acid. On the other hand, both DHA and OA can be synthesized in another carnitine-dependent multifunctional mitochondrial pathway (6,7,27). There is evidence that DHA was still the predom-

TABLE 7
Colon Ethanolamine Phosphoglyceride FA (%) of Rats Fed ALA-Adequate or -Deficient Diet With or Without Vitamin A[a]

FA	VADALAS (n = 6)	VASALAS (n = 6)	VADALAD (n = 6)	VASALAD (n = 6)
16:0	5.45 ± 0.63	6.02 ± 0.57[d]	5.52 ± 0.50[d]	4.81 ± 0.41[b,c]
18:0	11.64 ± 1.07	11.61 ± 0.56	11.45 ± 1.10	11.31 ± 0.68
Σ Saturates	17.35 ± 1.40	17.91 ± 0.31[c,d]	16.98 ± 0.93[b]	16.25 ± 0.79[b]
18:1n-9	5.96 ± 0.62	6.40 ± 0.71	5.71 ± 0.30	5.78 ± 0.55
18:1n-9 + 18:1n-7	7.30 ± 0.69	7.99 ± 0.80	7.24 ± 0.37	7.19 ± 0.61
Σ Monoenes	8.10 ± 0.86	8.95 ± 0.97[c]	7.91 ± 0.58[b]	8.06 ± 0.71
18:2n-6	4.91 ± 0.87	5.36 ± 0.86	6.32 ± 1.61[d]	4.72 ± 0.77[c]
18:3n-6	0.07 ± 0.02[c,d]	0.06 ± 0.02	0.04 ± 0.01[a]	0.05 ± 0.01[a]
20:3n-6	2.01 ± 0.26[d]	1.79 ± 0.43	2.12 ± 0.33[d]	1.38 ± 0.41[a,c]
20:4n-6	30.44 ± 1.67	28.17 ± 1.70[c,d]	32.02 ± 1.18[b]	33.08 ± 2.64[b]
22:4n-6	4.27 ± 0.48[b,c,d]	4.82 ± 0.29[a,d]	5.41 ± 0.52[a,d]	6.63 ± 0.54[a,b,c]
22:5n-6	0.74 ± 0.13[c,d]	0.58 ± 0.08[c,d]	3.44 ± 0.64[a,b]	2.86 ± 0.54[a,b]
Σ n-6	42.68 ± 2.21[c,d]	41.23 ± 1.48[c,d]	49.09 ± 1.74[a,b]	49.06 ± 2.44[a,b]
18:3n-3	0.12 ± 0.04[b]	0.36 ± 0.11[a]	—[b]	—
20:5n-3	0.55 ± 0.12	0.58 ± 0.07	—	—
22:5n-3	0.96 ± 0.12[b,c,d]	1.32 ± 0.18[a,c,d]	0.08 ± 0.01[a,b,d]	0.32 ± 0.06[a,b,c]
22:6n-3	3.30 ± 0.64[c,d]	3.26 ± 0.41[c,d]	0.69 ± 0.10[a,b,d]	1.72 ± 0.31[a,b,c]
Σ n-3	4.91 ± 0.64[c,d]	5.52 ± 0.41[c,d]	0.79 ± 0.11[a,b,d]	2.09 ± 0.36[a,b,c]

[a,b]For superscripts and abbreviations see Tables 3 and 4.

inant FA in straight-chain acyl-CoA oxidase null mice, and its amount was comparable to that in their corresponding wild-type animals (27). The characteristics of these multifunctional pathways are that the enzyme-bound products are recycled back as substrates until the desired final product is formed. In these recycling reactions, alternative *cis*-desaturations and elongation of the enzyme-bound acyl intermediates would introduce the methylene-interrupted double-bond structure of the final products. The enhanced DHA and OA suggest that a termination reaction catalyzed by an acyl carrier protein-connected carnitine acyltransferase might be preferential for the release of DHA or OA from enzyme in vitamin A-deficient rats.

Although vitamin A deficiency has been shown to enhance the activity of Δ5 (34,35) and Δ9 (36) desaturases, such influence was not apparent in this study. This difference could be a reflection of the rat strain (37) or diet used.

From the present study, it is evident that dietary vitamin A deficiency has a profound effect on membrane DHA and OA in rat tissues. It increases the proportions of OA consistently regardless of the level of dietary ALA. In contrast, the effect on DHA is dependent on dietary ALA level; it is reduced when dietary ALA is limiting and is enhanced when ALA is adequate. Both vitamin A and DHA are involved in a myriad of vital physiological functions pertaining to growth and development and health. Hence there is a need for a further study to unravel the mechanism by which vitamin A influences membrane DHA and its n-6 counterpart, OA.

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