Levels of Polyunsaturated Fatty Acids in Tissues from Zinc-Deficient Rats Fed a Linseed Oil Diet

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The effect of zinc deficiency on the levels of n-6 and n-3 polyunsaturated fatty acids (PUFA) in lipids from tissues of rats fed a diet containing linseed oil was investigated. Rats were fed either a control diet (25 mg Zn/kg) or a zinc-deficient diet (0.8 mg Zn/kg) for 10 d. To avoid energy and nutrient deficiency, 11.6 g of diet per day was administered by gastric tube. At the end of the experiment, rats fed the zinc-deficient diet had drastically reduced plasma zinc concentration and alkaline phosphatase activity consistent with severe zinc deficiency in these rats. Zinc-deficient rats had higher levels of n-3 PUFA, in particular eicosapentaenoic acid (EPA), and lower levels of n-6 PUFA, in particular linoleic acid, in liver and plasma phosphatidylcholine (PC) and in erythrocyte membrane total lipids than did control rats. By contrast, the levels of n-3 PUFA in PC from testes and heart, and in phosphatidylethanolamine (PE) from liver, testes and heart, were only slightly different between zinc-deficient and control rats. The study suggests that desaturation of α-linolenic acid is not inhibited by zinc deficiency, and that in zinc-deficient rats, n-3 PUFA preferentially incorporated into phospholipids at the expense of n-6 PUFA, especially EPA into PC. The study also shows that the effect of zinc deficiency on PUFA levels is different for PC and PE in rat tissues.

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Zinc deficiency has been shown to accentuate essential fatty acid (EFA) deficiency in rats, and an interaction between zinc and EFA metabolism has been proposed (1). A number of studies on the role of zinc in EFA metabolism have been undertaken to examine the effects of zinc deficiency on the fatty acid composition of phospholipids, neutral lipids and total lipids in various tissues (2–10). In some of these studies, zinc deficiency was shown to elevate linoleic acid levels and to lower arachidonic acid levels in rat tissue lipids (3–5,9), and it was thus concluded that zinc plays a role in the desaturation of linoleic acid. This would also be consistent with reports on lowered *in vitro* Δ5 and Δ6 desaturase activities in several tissues of zinc-deficient rats (3,4,11). By contrast, other investigators have reported that arachidonic acid levels were not lowered (6,7), or even elevated (8), in tissues of zinc-deficient rats. The latter studies suggested that zinc would not play a significant role in fatty acid desaturation.

In previous studies interest mainly focused in the effects of zinc on the desaturation of linoleic acid, and thus in most of these experiments rats were fed diets containing oils rich in linoleic acid, such as corn oil (2–6,9) or safflower oil (7). However, fatty acid desaturases do not only catalyze desaturation of fatty acids of the n-6 series, but also catalyze desaturation of n-3 fatty acids. Yet, to our knowledge, the effect of zinc deficiency on the desaturation of fatty acid of the n-3 series has not been investigated. In the present study, rats were fed a zinc-deficient diet containing linseed oil which contains nearly 60% α-linolenic acid, and the levels of n-3 very long chain polyunsaturated fatty acids (PUFA) were examined in various tissue lipids to monitor the effect of zinc deficiency on the desaturation of α-linolenic acid. Thus, the present study should help in clarifying whether zinc deficiency impairs desaturation or not.

It is a common difficulty when studying that rats after a few days on a zinc-deficient diet reduce food intake (12,13). Thus, the effect of zinc deficiency is confounded by undernourishment in experiments in which rats are expected to voluntarily consume zinc-deficient diets (14–17). Reduced food intake is also known to affect desaturation of PUFA (7). In the present study, rats were therefore force-fed by gastric tube to control food intake. Force-feeding has been shown to be a practical approach when studying the effect of short-term zinc deficiency without having results confounded by insufficient nutrient intake (14–18). In a previous study (19) rats were force-fed either a zinc-deficient diet with coconut oil, or a zinc-deficient diet with fish oil. In that study, zinc-deficient rats fed the coconut oil diet developed a fatty liver whereas those fed the fish oil diet did not. This finding suggested that the effect of zinc deficiency on liver lipid levels depends on the dietary fat. Thus, the effect of zinc deficiency on liver lipid levels in rats which were force-fed a diet containing linseed oil was also investigated.

MATERIALS AND METHODS

Animals, diets and tube feeding. Twenty-five male SPF Sprague-Dawley rats weighing 123 ± 6 g (Savo GmbH, Kisslegg, Germany) were divided into two groups. The control group consisted of 12 rats; the depletion group consisted of 15 rats because of the higher risk of mortality. The rats were housed individually in Macrolon cages. A daily 12-h light/dark cycle and a temperature of 23°C and 60% relative humidity were maintained.

All rats were force-fed a semisynthetic diet by gastric tube four times a day (0800, 1300, 1800, 2300) as earlier described in detail (17). The composition of the basic experimental diet is shown in Table 1. The depletion diet contained 0.8 mg Zn/kg, the control diet was supplemented with zinc sulfate to give a zinc concentration of 25 mg/kg. The composition of the linseed oil was as follows (in g/100 g fatty acids): palmitic acid (16:0), 5.3; stearic acid (18:0), 3.7; oleic acid (18:1), 19.7; linoleic acid (18:2n-6), 14.5; and α-linolenic acid (18:3n-3), 56.8; other fatty acids were present only in traces

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Abbreviations: DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EFA, essential fatty acids; EPA, eicosapentaenoic acid; HPLC, high-performance liquid chromatography; MUFA, monounsaturated fatty acids; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PUFA polyunsaturated fatty acids; SFA, saturated fatty acids.

TABLE 1

Composition of the Experimental Diet

Ingredient	Amount (g/kg)
Casein	200
Corn starch	328
Sugar	300
Fiber (cellulose)	30
Linseed oil	80
Mineral mixture[a]	40
Vitamin mixture[b]	20
DL-Methionine	2

[a]Minerals per kg diet: $Na_2HPO_4 \cdot 2\ H_2O$, 8.80 g; KH_2PO_4, 8.20 g; KCl, 6.00 g; $MgCl_2 \cdot 6\ H_2O$, 3.40 g; $CaCO_3$, 13.6 g; $FeSO_4 \cdot 7\ H_2O$ 248.8 mg; $CuSO_4 \cdot 7\ H_2O$, 47.2 mg; $MnSO_4 \cdot 5\ H_2O$, 123.1 mg; KI, 9.0 mg; $NiSO_4 \cdot 6\ H_2O$, 4.48 mg; $NaMoO_4 \cdot 2\ H_2O$, 0.50 mg; $SnCl_2 \cdot 2H_2O$, 0.57 mg; $Na_2SeO_3 \cdot 5\ H_2O$, 0.67 mg; $CrCl_3 \cdot 6\ H_2O$, 0.51 mg; NH_4VO_3, 0.23 mg; $Na_2SiO_3 \cdot 5\ H_2O$, 1.51 mg.
[b]Vitamins per kg diet: 1.7 mg all-*trans* retinol; 7.5 µg cholecalciferol; 150 mg all-*rac*-α-tocopherol; 5 mg menadione sodium bisulfite; 5 mg thiamin · HCl; 10 mg riboflavine; 6 mg pyridoxine · HCl; 50 mg Ca pantothenate; 20 mg nicotinic acid; 1000 mg choline chloride; 0.2 mg folic acid; 0.025 mg cyanocobalamin; sugar to 20 g.

(<0.1 g/100 g fatty acids). Diet slurries were freshly prepared before each feeding by mixing the dry dietary components (92 g) with linseed oil (8 g) and 60 mL of doubly distilled water (with or without zinc sulfate). Immediately before feeding, the slurry was heated in a glass bottle at 50°C for a few minutes. The gastric tube consisted of a 5-mL syringe connected to a slide catheter. During tube feeding, the rats were conscious and held by hand. The catheter was inserted into the stomach of the rats, and the slurry was slowly injected. To avoid contamination, zinc-deficient rats were always fed before the control rats. Each rat received 4 mL of slurry per feeding (equal to 11.6 g of food per day). The rats had free access to drinking water (doubly distilled water, supplemented with 0.14 g/L sodium chloride to adjust osmolarity to that of tap water).

After 8 d, the rats fed the depletion diet showed symptoms of zinc deficiency such as sparse and coarse hair, skin lesions around the mouth, eyes and paws, ataxy, and lethargy; the rats appeared severely ill. At days 9 and 10 of the experiment, two rats fed the zinc-deficient diet died. Therefore, the experiment had to be terminated. At day 11 of the experiment 12 h after the last feeding, all rats were killed by decapitation after light diethyl ether anesthesia. Blood was collected from the neck into heparinized tubes, and liver, heart, testes and the mesentery of the small intestine were excised. Plasma and tissue samples were stored at −80°C until analyzed.

Lipid analyses. Lipids of liver, heart, testes, erythrocyte membranes, mesentery of small intestine, and plasma were extracted with *n*-hexane/isopropanol (3:2, vol/vol; containing butylated hydroxytoluene as antioxidant) according to Hara and Radin (20). Prior to extraction, erythrocytes were washed three times with sodium chloride solution (9 g/L), and hemolyzed. Erythrocyte membrane fragments were washed three times with hypotonic Tris buffer, pH 7.4 (21).

Phospholipids of the extract were separated by high-performance liquid chromatography (HPLC) (22) using a Merck-Hitachi (Darmstadt, Germany) system consisting of a gradient pump (L-6200), a diode array (L-3000), a 25 cm x 0.4 cm (internal diameter) Si 60 (5 µm) cartridge (LiChroCART, Merck), an integrator (D-2000), and a fraction collector (Model 201; Gilson, Villiers-le-Bel, France). The gradient system consisted of the mobile phases (i) acetonitrile, (ii) acetonitrile/85% phosphoric acid (99.8:0.2, vol/vol), and (iii) methanol/85% phosphoric acid (99.8:0.2, vol/vol). This method allowed base line separation of all major phospholipid classes. In the present study, phosphatidylcholine (PC) includes all subclasses of choline glycerophospholipids and phosphatidylethanolamine (PE) all ethanolamine glycerophospholipids. All lipids to be analyzed for their fatty acid composition were transmethylated with boron trifluoride/methanol (100 g/L) reagent (23). Fatty acid methyl esters were separated by capillary gas chromatography (Sichromat 2-8; Siemens, Karlsruhe, Germany) using a programmed temperature vaporizing injection system, a flame-ionization detector and a 50 m CP-Sil 88 WCOT capillary column (Chrompack, Middleburg, The Netherlands). Fractions were identified by comparing their retention times with those of individual pure standards, and were quantified relative to heptadecanoic acid methyl ester used as internal standard (24).

For measurement of liver total cholesterol and triglycerides, extracted lipids were dissolved in Triton X-100 as described by De Hoff *et al.* (25). Total cholesterol and triglycerides were determined using enzymatic reagent kits obtained from Boehringer (Mannheim, Germany). Concentrations of liver phospholipids were calculated by the amount and the mean molecular mass of its bound fatty acids after separation of phospholipids by HPLC and gas chromatographic fatty acid analysis (22).

Zinc analyses and activity of alkaline phosphatase. The zinc concentration of blood plasma was measured directly by aspirating a dilute solution (1:5, vol/vol) into a flame of an atomic spectrophotometer (model 5100; Perkin-Elmer, Überlingen, Germany). The activity of alkaline phosphatase (E.C. 3.1.3.1) in plasma was measured using commercial kit reagents (Boehringer) and an auto analyzer (Hitachi, Model 704; Boehringer).

Data analysis. Treatment effects were evaluated by analysis of variance using Minitab (release 7.2) software (MINITAB, Inc., State College, PA).

RESULTS

Growth and zinc status. The initial body weight was 122.5 ± 6.7 g (mean ± SD) for the control rats and 123.2 ± 5.6 g for the rats fed the zinc-deficient diet. During the first eight days of the experiment, the weight gain of the rats fed the two diets was similar; the body weight at day 8 was 154.4 ± 7.2 g for the control rats and 152.1 ± 4.7 g for the zinc-deficient rats. Thereafter, the zinc-deficient rats ceased to grow, whereas the control rats continued their weight gain. The final body weight at day 11 was 169.5 ± 6.6 g for the control rats and 152.0 ± 6.6 g for the zinc-deficient rats ($P < 0.05$). The zinc concentration in plasma was 15.3 ± 2.0 µmol/L in the control rats and 4.4 ± 0.6 µmol/L in the zinc-deficient rats. The

TABLE 2

Fatty Acid Composition of Phosphatidylcholine from Liver and Plasma of Control and Zinc-Deficient Rats[a]

Fatty Acid	Liver		Plasma	
	Control (mol/100 mol)	Zinc-deficient (mol/100 mol)	Control (mol/100 mol)	Zinc-deficient (mol/100 mol)
Σ Saturated	44.44 ± 1.06	45.46 ± 1.93	45.58 ± 0.98[b]	47.97 ± 2.14[c]
Σ Monounsaturated	10.84 ± 0.74[b]	9.67 ± 0.90[c]	10.24 ± 0.78[b]	8.86 ± 0.80[c]
18:2n-6	14.06 ± 1.13[b]	10.30 ± 1.98[c]	19.12 ± 1.13[b]	14.93 ± 2.54[c]
Metabolites				
18:3n-6	0.16 ± 0.03	0.18 ± 0.03	0.10 ± 0.03	0.13 ± 0.04
20:3n-6	0.91 ± 0.10[b]	0.62 ± 0.14[c]	1.05 ± 0.14[b]	0.66 ± 0.11[c]
20:4n-6	10.86 ± 0.53	10.40 ± 0.92	10.10 ± 0.60	9.69 ± 1.13
22:4n-6	0.09 ± 0.04	0.09 ± 0.03	0.10 ± 0.03	0.08 ± 0.02
22:5n-6	0.15 ± 0.05	0.13 ± 0.03	0.21 ± 0.08	0.20 ± 0.06
18:3n-3	1.81 ± 0.25[b]	2.61 ± 0.36[c]	1.64 ± 0.30[b]	2.66 ± 0.75[c]
Metabolites				
20:5n-3	7.46 ± 0.74[b]	11.85 ± 1.21[c]	4.91 ± 0.54[b]	9.69 ± 1.13[c]
22:5n-3	2.19 ± 0.46[b]	1.82 ± 0.23[c]	2.07 ± 0.42[b]	1.64 ± 0.25[c]
22:6n-3	5.87 ± 0.73	5.90 ± 0.86	4.31 ± 0.46	4.09 ± 0.82
Σ (n-6)	26.52 ± 1.06[b]	21.91 ± 2.66[c]	31.02 ± 1.04[b]	25.92 ± 1.97[c]
Σ (n-3)	17.84 ± 1.46[b]	22.70 ± 1.38[c]	13.16 ± 0.91[b]	17.21 ± 1.47[c]
Σ (n-6)/Σ(n-3)	1.50 ± 0.16[b]	0.97 ± 0.17[c]	2.37 ± 0.19[b]	1.52 ± 0.20[c]

[a]Mean ± SD (n = 12 for control group; n = 13 for zinc-deficient group). Means were compared by pairs (Zn^+ vs. Zn^-) within one tissue. Values with different superscripts (b, c) are significantly different ($P < 0.05$).

activity of alkaline phosphatase in plasma was 397 ± 71 Units/L in the control rats and 163 ± 48 Units/L in the zinc-deficient rats.

Fatty acid composition of lipids. The fatty acid composition of PC from liver and plasma is shown in Table 2. The effect of zinc deficiency on fatty acid composition was similar in liver and plasma PC. Total monounsaturated fatty acids (MUFA), linoleic acid (18:2n-6), dihomo-γ-linolenic acid (20:3n-6), docosapentaenoic acid (DPA, 22:5n-3) and total n-6 PUFA were lower in liver and plasma PC of zinc-deficient rats; α-linolenic acid (18:3n-3), eicosapentaenoic acid (EPA, 20:5n-3) and total n-3 PUFA were elevated in liver and plasma PC of zinc-deficient rats. In plasma PC, total saturated fatty acids

TABLE 3

Fatty Acid Composition of Phosphatidylcholine from Testes and Heart of Control and Zinc-Deficient Rats[a]

Fatty acid	Testes		Heart	
	Control (mol/100 mol)	Zinc-deficient (mol/100 mol)	Control (mol/100 mol)	Zinc-deficient (mol/100 mol)
Σ Saturated	47.70 ± 0.39	47.88 ± 1.40	41.16 ± 0.93	41.02 ± 0.85
Σ Monounsaturated	18.32 ± 0.40[b]	19.01 ± 0.48[c]	10.14 ± 0.61	10.07 ± 0.79
18:2n-6	4.79 ± 0.27	4.60 ± 0.45	11.84 ± 1.56[b]	13.61 ± 1.74[c]
Metabolites				
18:3n-6	0.18 ± 0.07	0.16 ± 0.06	0.05 ± 0.02	0.05 ± 0.01
20:3n-6	1.31 ± 0.11	1.34 ± 0.12	0.49 ± 0.07	0.45 ± 0.04
20:4n-6	12.23 ± 0.33[b]	11.80 ± 0.52[c]	20.56 ± 0.83[b]	18.30 ± 0.91[c]
22:4n-6	0.85 ± 0.05	0.82 ± 0.06	0.43 ± 0.04	0.44 ± 0.03
22:5n-6	10.86 ± 0.40	10.47 ± 0.69	0.31 ± 0.07	0.35 ± 0.10
18:3n-3	0.14 ± 0.05	0.15 ± 0.04	1.36 ± 0.18[b]	1.64 ± 0.22[c]
Metabolites				
20:5n-3	0.41 ± 0.05[b]	0.56 ± 0.10[c]	1.81 ± 0.20	1.93 ± 0.20
22:5n-3	0.14 ± 0.01[b]	0.16 ± 0.02[c]	4.22 ± 0.48	4.23 ± 0.55
22:6n-3	1.86 ± 0.15	1.82 ± 0.26	6.61 ± 0.56	6.97 ± 0.97
Σ (n-6)	30.59 ± 0.43[b]	29.60 ± 1.18[c]	33.95 ± 1.15	33.43 ± 1.30
Σ (n-3)	2.60 ± 0.18	2.74 ± 0.29	14.22 ± 0.74	15.00 ± 1.24
Σ (n-6)/Σ (n-3)	11.84 ± 0.88[b]	10.89 ± 1.06[c]	2.40 ± 0.16	2.25 ± 0.27

[a]Mean ± SD (n = 12 for control group; n = 13 for zinc-deficient group). Means were compared by pairs (Zn^+ vs. Zn^-) within one tissue. Values with different superscripts (b,c) are significantly different ($P < 0.05$).

TABLE 4

Fatty Acid Composition of Phosphatidylethanolamine from Liver, Testes and Heart of Control and Zinc-Deficient Rats[a]

	Liver		Testes		Heart	
Fatty acid	Control (mol/100 mol)	Zinc-deficient (mol/100 mol)	Control (mol/100 mol)	Zinc-deficient (mol/100 mol)	Control (mol/100 mol)	Zinc-deficient (mol/100 mol)
Σ Saturated	45.27 ± 0.78[b]	46.35 ± 1.16[c]	32.34 ± 0.60	32.08 ± 0.76	42.70 ± 1.07	42.98 ± 0.82
Σ Monounsaturated	4.93 ± 0.54	5.19 ± 0.74	10.43 ± 0.82[b]	11.23 ± 0.78[c]	6.28 ± 0.59	6.27 ± 0.72
18:2n-6	5.75 ± 0.62[b]	4.52 ± 0.99[c]	3.44 ± 0.23[b]	2.97 ± 0.28[c]	6.39 ± 0.79	7.04 ± 0.84
Metabolites						
18:3n-6	0.06 ± 0.01[b]	0.07 ± 0.01[c]	—	—	—	—
20:3n-6	0.45 ± 0.05[b]	0.33 ± 0.09[c]	0.77 ± 0.07[b]	0.70 ± 0.08[c]	0.26 ± 0.03	0.26 ± 0.04
20:4n-6	11.41 ± 0.77[b]	11.55 ± 0.73[c]	21.63 ± 0.67	21.38 ± 1.12	15.84 ± 0.91	15.48 ± 1.23
22:4n-6	0.09 ± 0.02	0.09 ± 0.01	2.58 ± 0.17[b]	2.45 ± 0.06[c]	0.53 ± 0.17	0.56 ± 0.07
22:5n-6	0.14 ± 0.07	0.18 ± 0.05	21.68 ± 0.63	21.57 ± 0.82	1.07 ± 0.12	1.07 ± 0.10
18:3n-3	1.18 ± 0.20[b]	2.63 ± 0.50[c]	0.15 ± 0.03	0.15 ± 0.02	1.30 ± 0.18[b]	1.47 ± 0.21[c]
Metabolites						
20:5n-3	10.97 ± 0.68	11.53 ± 1.23	0.70 ± 0.08[b]	0.85 ± 0.10[c]	1.24 ± 0.17	1.23 ± 0.17
22:5n-3	4.29 ± 0.75[b]	3.49 ± 0.48[c]	0.44 ± 0.06	0.46 ± 0.10	4.48 ± 0.51	4.61 ± 0.52
22:6n-3	14.54 ± 1.30[b]	13.27 ± 1.51[c]	4.40 ± 0.40	4.75 ± 0.47	18.91 ± 1.67	18.15 ± 2.17
Σ (n-6)	18.08 ± 1.15[b]	16.85 ± 1.26[c]	50.37 ± 1.20[b]	49.35 ± 1.11[c]	24.30 ± 1.30	24.57 ± 2.01
Σ (n-3)	31.34 ± 1.42	31.30 ± 1.36	5.69 ± 0.43[b]	6.21 ± 0.50[c]	26.04 ± 1.45	25.56 ± 1.95
Σ (n-6)/Σ (n-3)	0.58 ± 0.06	0.54 ± 0.05	8.90 ± 0.71[b]	8.00 ± 0.70[c]	0.94 ± 0.09	0.97 ± 0.15

[a]Mean ± SD (n = 12 for control group; n = 13 for zinc-deficient group). Means were compared by pairs (Zn^+ vs. Zn^-) within one tissue. Values with different superscripts (b, c) are significantly different ($P < 0.05$).

(SFA) were also higher in zinc-deficient rats than in control rats, and zinc-deficient rats had a decreased total n-6/n-3 PUFA ratio in liver and plasma PC.

The fatty acid composition of PC from testes and heart is shown in Table 3. The effects of zinc deficiency on the fatty acid composition of testes and heart PC were less pronounced than the effects on the fatty acid composition of liver and plasma PC. Total MUFA, EPA (20:5n-3) and DPA (22:5n-3) were significantly elevated in testes PC of zinc-deficient rats, whereas arachidonic acid (20:4n-6) and total n-6 PUFA were lower. The total n-6/n-3 PUFA ratio in testes PC was lower in zinc-deficient rats than in control rats. The only effect of zinc deficiency on heart PC seen was an elevation of linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3).

The fatty acid composition of PE from liver, testes and

TABLE 5

Fatty Acid Composition of Total Lipids from Erythrocyte Membranes and Mesentery of Small Intestine of Control and Zinc-Deficient Rats[a]

	Erythrocyte membranes		Mesentery of small intestine	
Fatty acid	Control (mol/100 mol)	Zinc-deficient (mol/100 mol)	Control (mol/100 mol)	Zinc-deficient (mol/100 mol)
Σ Saturated	48.79 ± 0.97	48.82 ± 1.32	34.12 ± 1.74	34.50 ± 0.91
Σ Monounsaturated	13.79 ± 1.41	13.98 ± 1.07	34.94 ± 1.15	35.78 ± 1.62
18:2n-6	8.81 ± 0.72[b]	7.77 ± 0.83[c]	13.48 ± 0.77	12.96 ± 1.06
Metabolites				
18:3n-6	0.07 ± 0.02[b]	0.09 ± 0.02[c]	0.06 ± 0.01	0.08 ± 0.03
20:3n-6	0.46 ± 0.04	0.40 ± 0.10	0.10 ± 0.03	0.10 ± 0.02
20:4n-6	14.64 ± 1.07	14.66 ± 1.11	0.24 ± 0.09	0.24 ± 0.04
22:4n-6	0.80 ± 0.09	0.83 ± 0.08	—	—
22:5n-6	0.52 ± 0.04	0.53 ± 0.05	—	—
18:3n-3	1.66 ± 0.14[b]	1.92 ± 0.20[c]	16.00 ± 1.19	15.14 ± 1.48
Metabolites				
20:5n-3	2.84 ± 0.34[b]	3.24 ± 0.27[c]	0.20 ± 0.07	0.24 ± 0.04
22:5n-3	3.28 ± 0.28	3.21 ± 0.35	0.27 ± 0.07	0.30 ± 0.03
22:6n-3	3.03 ± 0.26	3.04 ± 0.29	0.16 ± 0.07	0.19 ± 0.04
Σ (n-6)	25.62 ± 1.23	24.63 ± 1.88	14.10 ± 0.83	13.62 ± 1.09
Σ (n-3)	11.03 ± 0.67	11.64 ± 0.72	16.84 ± 1.35	16.10 ± 1.49
Σ (n-6)/Σ (n-3)	2.33 ± 0.11[b]	2.12 ± 0.18[c]	0.84 ± 0.10	0.86 ± 0.13

[a]Mean ± SD (n = 12 for control group; n = 13 for zinc-deficient group). Means were compared by pairs (Zn^+ vs. Zn^-) within one tissue. Values with different superscripts (b, c) are significantly different ($P < 0.05$).

TABLE 6

Concentrations of Lipids in Liver of Control and Zinc-Deficient Rats[a]

Lipid	Control (mg/g liver)	Zinc-deficient (mg/g liver)
Triglycerides	29.1 ± 9.5	28.1 ± 13.4
Total cholesterol	2.49 ± 0.53[b]	2.01 ± 0.42[c]
Phosphatidylcholine	12.1 ± 1.2[b]	13.6 ± 1.1[c]
Phosphatidylethanolamine	7.37 ± 0.79	7.06 ± 1.02

[a]Mean ± SD (n = 12 for control group; n = 13 for zinc-deficient group). Values with different superscripts (b, c) are significantly different ($P < 0.05$).

heart is shown in Table 4. In general, the effects of zinc deficiency on PE were less pronounced than those on PC. Total SFA, γ-linolenic acid (18:3n-6) and α-linolenic acid (18:3n-3) were elevated in liver PE of zinc-deficient rats, whereas linoleic (18:2n-6), dihomo-γ-linolenic acid (20:3n-6), docosatetraenoic acid (22:4n-6) and total n-6 PUFA were lower. Testes PE from zinc-deficient rats contained higher levels of total MUFA, EPA (20:5n-3) and total n-3 PUFA and lower levels of linoleic acid (18:2n-6), dihomo-γ-linolenic acid (20:3n-6), docosatetraenoic acid (22:4n-6) and total n-6 PUFA than that from control rats. Zinc-deficient rats also had a lower total n-6/n-3 PUFA ratio for testes PE. In heart PE, the only change seen due to zinc deficiency was a slight elevation of α-linolenic acid.

The fatty acid composition of total lipids from erythrocyte membranes and the mesentery of small intestine is shown in Table 5. In erythrocyte membrane total lipids of zinc-deficient rats, linoleic acid (18:2n-6) was lower, but total n-3 PUFA and, in particular, α-linolenic acid (18:3n-3) and EPA (20:5n-3) were elevated. Zinc-deficient rats also had a lower total n-6/n-3 PUFA ratio for erythrocyte membrane lipids. In contrast, zinc deficiency did not change the fatty acid composition of total lipids from mesentery of small intestine.

Liver lipids levels. Control rats and zinc-deficient rats had similar liver triglyceride and PE concentrations (Table 6). By contrast, total cholesterol was lower in zinc-deficient rats than in control rats, and PC was higher in zinc-deficient rats than in control rats.

DISCUSSION

The present study was undertaken to investigate the effect of zinc deficiency on the fatty acid patterns of rats fed a diet rich in α-linolenic acid. For this purpose, a rat model was used that permits induction of a severe zinc deficiency without having the observed effects due to zinc deficiency distorted by changes due to energy and nutrient intake. The disadvantage of the model is that it can only be used for short-term studies because rats force-fed a diet containing extremely low zinc concentrations cannot survive beyond 10 or 12 d. In zinc-deficient rats that consumed the diet *ad libitum,* plasma alkaline phosphatase activity, which is an approximate indicator of the zinc state (26,27), was greatly reduced already after 2 to 4 d on a zinc-deficient diet (28,29). This means that a zinc-deficient state existed only for approximately 6 to 8 d. Although the model we used may be physiologically less relevant, it is useful to study the effects of very severe zinc deficiency. In physiologically more relevant models, the effects of zinc deficiency on fatty acid metabolism are typically being investigated using moderately zinc-deficient rats (9), which typically consume adequate quantities of food. However, effects due to severe zinc deficiency might remain hidden when using these models.

In the present study the effect of zinc deficiency on fatty acid metabolism was examined by following its effect on the fatty acid composition of PC and PE. These two phospholipid classes represent approximately 80% of the total phospholipids in rat liver, heart and testes and approximately 90% of total phospholipids in rat plasma (30), and most of the PUFA is associated with there phospholipid fractions (31). In general, fatty acid compositions can be modified by a variety of processes (31), and changes in fatty acid compositions alone do not allow specific conclusions to be drawn about any of the individual processes. Nevertheless, the observation that the levels of EPA in liver phospholipids were unchanged or even elevated suggests that zinc deficiency does not affect Δ5 and Δ6 desaturation of α-linolenic acid, at least not within the time frame of the present study. This disagrees with the results of some earlier studies which showed that zinc deficiency inhibited Δ5 and Δ6 desaturation (3–5,9,11) in rats fed diets containing oils rich in n-6 fatty acids. In the present study, the effects of zinc deficiency on the fatty acid composition of phospholipids were more pronounced in liver and plasma than in other tissues investigated. This might be due to the higher turnover rates of phospholipids in liver than in other tissues (32).

An interesting observation in the present study was that zinc-deficient rats had elevated levels of EPA in PC but not in PE. This could indicate that zinc deficiency may alter the incorporation of EPA into PC. Increased levels of EPA in PC were recently also observed in zinc-deficient rats which had been force-fed a fish oil diet (19). By contrast, the levels of α-linolenic acid were higher in both PC and PE. However, in this and recent studies (18,19), independent of the dietary fat, the levels of linoleic acid were reduced in rats which had been force-fed with zinc-deficient diets. This is in contrast to results obtained on rats that consumed a zinc-deficient diet *ad libitum* (4,5,9,33). The lower levels of linoleic acid we observed in the present study may be due to increased oxidation. The increased levels of linoleic acid which have been reported in the literature were suggested to be due to reduced desaturation (4,5,9,33).

The level of arachidonic acid, which is often used as a measure of desaturation (5,9,33), was not changed by zinc deficiency in either PC or PE. However, it should be noted that feeding a diet rich in α-linolenic acid is known to suppress the desaturation of linoleic acid (34) which is reflected by lower levels of arachidonic acid compared with rats fed diets containing fats with lower levels of n-3 fatty acids (3–9,33).

In a previous study, rats which were force-fed a zinc-deficient diet based predominately on coconut oil developed fatty livers, whereas rats fed a zinc-deficient diet based predominately on fish oil did not (19). In the present study, total lipid and triglyceride levels in liver were not different between control and zinc-deficient rats. This suggests that linseed oil, like fish oil, can prevent the formation of fatty liver which has been observed in rats force-fed zinc-deficient diets based on coconut oil.

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