PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 177, 441–446 (1984)

Essential Fatty Acids in Tissue Phospholipids and Triglycerides of the Zinc-Deficient Rat (41970)

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Abstract. This study addressed the possibility that zinc deficiency has different effects on the fatty acid composition of triglyceride compared to total phospholipid. Male weanling Sprague–Dawley rats were maintained for 6 weeks on a semisynthetic diet deficient in zinc (3 mg/kg zinc). Control rats (40 mg/kg zinc) were pair-fed. Lipid fractionation and fatty acid analysis were by thin-layer and gas chromatography, respectively. In zinc-deficient rats, the percentage of linoleic acid was increased or that of arachidonic acid was decreased in total phospholipids of plasma, liver, and testis, and in skin total lipids. Saturated and monounsaturated fatty acids were increased in the triglyceride of liver but decreased in the triglyceride of epididymal fat of zinc deficient rats. Essential fatty acids, as a proportion of total fatty acids, were decreased in triglyceride of liver but increased in triglyceride of epididymal fat of zinc-deficient rats. Our fatty acid data from tissue total phospholipids therefore support the concept that linoleic acid desaturation is impaired in zinc deficiency.

An interaction of zinc with essential fatty acids (EFA) in various species has been recognized for some time. In 1958, Hanson demonstrated that supplemental zinc was more effective at curing parakeratosis when fed to pigs in the presence of added corn oil (1). Similarly, the parakeratosis-like effects of experimental zinc deficiency in pigs were shown to be less severe when the diet was supplemented with corn oil (2). The onset of symptoms of EFA deficiency in rats was more rapid when the diet contained low zinc and high calcium but was less rapid when the diet contained high zinc and low calcium (3). More recently, evidence from analysis of fatty acids in tissue lipids has been presented which has confirmed that zinc deficiency in the rat is associated with changes in EFA composition of total lipid extracts from various tissues (4–7). To date, analysis of the composition of 20 and 22 carbon EFA in specific lipid fractions of tissues from zinc-deficient animals is lacking. We have therefore analyzed the fatty acid composition of total phospholipids and triglycerides from various tissues of zinc-deficient rats with a view to providing data about the effects of zinc deficiency on long chain EFA.

Our data confirm that the EFA composition of tissue lipids is abnormal in zinc deficiency, and support the previous suggestion that one of the effects of zinc deficiency is to inhibit desaturation of linoleic acid (18:2n6). In addition, zinc dificiency appears to have opposite effects on the fatty acid composition of liver and epididymal fat triglycerides.

Methods. *Animals and diets.* Weanling male Sprague–Dawley rats (starting weight, 50–60 g, Charles River, Canada) were housed in three groups of 10 rats each in polypropylene cages. The cages had stainless-steel tops and raised stainless-steel wire floors to prevent coprophagy. The rats were fed a pelleted, semisynthetic diet for 6 weeks which contained 3 mg/kg zinc (zinc deficient—ZD) or contained 40 mg/kg zinc (control—CT). The diet (Table I) was freely available to the ZD and *ad libitum*-fed rats. CT rats were individually pair-fed to the ZD rats on a daily basis. Distilled deionized water was also freely available.

After the 6-week period, the rats were starved overnight and anesthetized with ether. Blood was drawn from the abdominal aorta into EDTA-anticoagulated plastic syringes. Blood plasma was separated after centrifugation and frozen for analysis. Liver, testes, and epididymal fat were removed, washed in ice-cold saline, and frozen for lipid analysis.

Zinc analysis. The concentration of zinc in the rat plasma was analyzed by atomic absorption spectrophotometry (Instrumentation Laboratories 457). Samples were aspi-

0037-9727/84 $1.50

TABLE I. COMPOSITION OF THE DIET (g/kg)

Egg white solids, spray-dried	200.0	*p*-Aminobenzoic acid	0.110
Dextrose, monohydrate	664.2	Ascorbic acid	0.991
Corn oil	100.0	Vitamin B12	0.00003
$CaHPO_4$	19.8	Calcium pantothenate	0.066
KCl	2.29	Choline (dihydrogen citrate)	1.43
NaCl	0.78	Folic acid	0.002
$MgSO_4$	2.48	Inositol	0.110
$MnSO_4 \cdot H_2O$	0.12	Menadione	0.05
$FeSO_4 \cdot 7H_2O$	0.17	Niacin	0.01
KIO_3	0.0004	Pyridoxine HCl	0.022
Na_2SeO_3	0.0002	Riboflavin	0.022
$CrK(SO_4)_2 \cdot 12H_2O$	0.0193	Thiamin HCl	0.022
$CuSO_4$	0.013	Dry vitamin A palmitate (500,000 U/g)	0.04
$ZnCO_3$	0.080*	Dry vitamin D2 (500,000 U/g)	0.004
Biotin	0.004	Dry vitamin E acetate (500 U/g)	0.24

rated directly following dilution at 1:5 with deionized distilled water. Polypropylene tubes were used exclusively to avoid contamination of the samples. Standard solutions were prepared in distilled deionized water.

Lipid extraction and fatty acid analysis. Tissue total lipids were extracted by the method of Folch *et al.* (8). Triglyceride (TG) and total phospholipid (tPL) were separated from the total lipid extract by thin-layer chromatography (9). Total fatty acids were extracted from skin of the paws after saponification. Fatty acids from tPL of plasma, liver, and testes, from TG of liver and epididymal fat, and from total lipid of skin were transesterified as previously described (9, 10). Fatty acid methyl esters were analyzed by gas chromatography (Hewlett Packard 5880) operated under the conditions previously specified (9).

Statistics. Comparisons between unpaired data were made using Student's *t* test (one tailed).

Results. *General.* In both the pair-fed CT and ZD groups, final body weights were significantly lower after 6 weeks than the weight of rats fed the same diet *ad libitum*. Although the CT rats weighed more than the ZD rats, the difference was not significant. Testes, thymus, and epididymal fat pad weights were lower in ZD rats, but liver and adrenal weights did not differ between ZD and CT rats.

Plasma zinc analysis. The concentration of zinc (mean ± SD) in the plasma of the ZD rats was significantly lower than in the CT rats (38 ± 7 vs 107 ± 9, μg/100 ml, mean ± SD).

Tissue lipid fatty acid composition. In *ad libitum*-fed compared to pair-fed control rats, 18:2n6 was significantly lower ($P < 0.01$) and 20:4n6 was significantly higher ($P < 0.01$) in both plasma and liver tPL. Thus, pair-feeding caused an increase in the ratio of 18:2n6/20:4n6 in both plasma (0.7 to 1.4, $P < 0.01$) and liver (0.3 to 0.5, $P < 0.01$). Effects of pair-feeding on other fatty acids were not significantly different. All the following data refer to differences between the pair-fed and ZD groups.

Among the EFA in plasma tPL, arachidonic acid (20:4n6) was significantly decreased in the ZD rats (Table II). Both palmitoleic (16:1n7) and oleic (18:1n9) acids were increased in plasma tPL of ZD rats.

In the liver tPL of ZD rats, 18:2n6 and γ-linolenic acid (18:3n6) were increased but 20:4n6 was decreased. α-Linolenic acid (18:3n3) and docosapentaenoic acid, n3 (22:5n3), were also increased, but their respective desaturase products, eicosapentaenoic acid (20:5n3) and docosahexaenoic acid (22:6n3), were decreased in ZD rats (Table II).

Compared to the CT group, 16:0 was decreased but 18:0 and 18:1n9 were significantly increased in the testes of ZD rats (Table II). 18:2n6 and docosatetraenoic acid (22:4n6) were increased but 22:5n6 was decreased in the testes of ZD rats compared to

TABLE II. FATTY ACID COMPOSITION (mg/100 g) OF PLASMA, LIVER, AND TESTES TOTAL PHOSPHOLIPIDS AND SKIN TOTAL LIPIDS FROM ZINC-DEFICIENT (ZD) AND PAIR-FED CONTROL (CT) RATS FED THEIR RESPECTIVE DIETS FOR 6 WEEKS

		Plasma	Liver	Testes	Skin
16:0	ZD	25.7 ± 3.9	19.0 ± 1.7	30.0 ± 2.0	17.8 ± 0.7
	CT	27.3 ± 1.7	18.2 ± 0.4	32.9 ± 1.8**	18.1 ± 0.8
16:1n9	ZD	1.2 ± 0.5	1.5 ± 0.6	0.8 ± 0.1	5.8 ± 0.9
	CT	0.8 ± 0.1**	1.0 ± 0.3	0.7 ± 0.04	6.1 ± 1.0
18:0	ZD	10.8 ± 1.4	16.1 ± 2.6	6.9 ± 0.8	7.3 ± 1.1
	CT	11.9 ± 1.5	17.6 ± 1.4	6.3 ± 0.03*	8.0 ± 0.8
18:1n9	ZD	7.9 ± 1.1	7.3 ± 0.8	13.1 ± 1.2	22.1 ± 2.1
	CT	6.8 ± 0.6*	6.1 ± 0.8	10.4 ± 0.4**	21.0 ± 1.1
18:2n6	ZD	31.6 ± 4.6	17.6 ± 2.1	4.8 ± 0.3	26.0 ± 3.1
	CT	29.5 ± 2.6	15.8 ± 1.7*	4.4 ± 0.2*	21.6 ± 2.0**
18:3n6[a]	ZD	TR[b]	1.0 ± 0.3	0.1 ± 0.03	1.0 ± 0.1
	CT	TR	0.6 ± 0.1**	0.1 ± 0.1	1.1 ± 0.1
20:3n6	ZD	0.5 ± 0.1	1.0 ± 0.3	1.4 ± 0.1	1.5 ± 0.3
	CT	0.5 ± 0.1	0.9 ± 0.3	1.4 ± 0.1	2.4 ± 0.4**
20:4n6	ZD	16.8 ± 4.0	26.2 ± 1.1	17.1 ± 1.2	6.7 ± 1.4
	CT	20.9 ± 2.0*	29.4 ± 1.3**	17.0 ± 0.6	7.9 ± 0.9*
22:4n6	ZD	0.5 ± 0.2	1.0 ± 0.2	2.1 ± 0.5	1.2 ± 0.3
	CT	0.4 ± 0.2	0.9 ± 0.1	1.7 ± 0.1*	2.2 ± 0.6**
22:5n6	ZD	1.1 ± 0.6	2.5 ± 0.5	16.1 ± 2.3	0.4 ± 0.1
	CT	1.1 ± 0.3	2.9 ± 0.8	18.6 ± 0.9**	0.4 ± 0.1
18:3n3	ZD	TR	0.3 ± 0.1	0.2 ± 0.02	2.6 ± 0.6
	CT	TR	0.2 ± 0.1*	0.1 ± 0.01**	3.4 ± 0.8**
20:5n3	ZD	TR	0.1 ± 0.04	0.2 ± 0.03	TR
	CT	TR	0.3 ± 0.1**	0.1 ± 0.01**	TR
22:5n3	ZD	TR	0.5 ± 0.1	0.8 ± 0.1	TR
	CT	TR	0.4 ± 0.1*	0.7 ± 0.1	TR
22:6n3	ZD	1.9 ± 0.5	3.9 ± 0.7	1.2 ± 0.2	TR
	CT	1.4 ± 0.3	4.1 ± 0.5	1.0 ± 0.1*	TR

Note. Each value represents the mean ± SD of 10 samples.
[a] 18:3n6 has the same retention time as 20:0.
[b] Only trace amounts (<0.1%) detected.
* $P < 0.05$; ** $P < 0.01$ compared between ZD and CT.

the CT rats. The n3 EFA, 18:3n3, 22:5n3, and 22:6n3 were also increased in the testes tPL of ZD rats.

18:2n6 was significantly increased but 20:3n6, 20:4n6, 22:4n6, and 18:3n3 were significantly decreased in skin total lipids of ZD rats (Table II).

The fatty acid composition of liver and epididymal fat TG was changed in generally opposite directions in ZD compared to CT rats: non-EFA (16:0, 16:1n7, and 18:1n9) were increased in the liver TG but decreased in the epididymal fat TG; n6 EFA (except 22:4n6 and 22:5n3) were decreased in liver TG but increased in epididymal fat TG; and n3 EFA (except 18:3n3) were decreased in liver TG but increased in epididymal fat TG (Table III).

Discussion. Our data on the effects of zinc deficiency on growth and tissue weights confirm what has previously been reported; when CT rats are pair-fed to those receiving a ZD

TABLE III. FATTY ACID COMPOSITION (mg/100 g) OF LIVER AND EPIDIDYMAL FAT TRIGLYCERIDES FROM ZINC DEFICIENT (ZD) AND PAIR-FED CONTROL (CT) RATS ON THEIR RESPECTIVE DIETS FOR 6 WEEKS

		Liver	Epididymal fat
16:0	ZD	23.2 ± 4.1	18.9 ± 1.1
	CT	21.6 ± 2.1	22.9 ± 1.9**
16:1n7	ZD	3.4 ± 2.6	3.7 ± 0.6
	CT	1.9 ± 0.5	5.8 ± 0.7**
18:0	ZD	2.0 ± 0.5	3.9 ± 0.5
	CT	3.4 ± 1.2**	3.8 ± 0.6
18:1n9	ZD	26.6 ± 5.1	28.1 ± 1.2
	CT	22.7 ± 2.6*	28.8 ± 1.2
18:2n6	ZD	32.3 ± 8.0	41.2 ± 2.4
	CT	35.0 ± 3.0	35.4 ± 2.2**
18:3n6[a]	ZD	1.8 ± 0.4	0.5 ± 0.1
	CT	1.8 ± 0.7	0.3 ± 0.1**
20:3n6	ZD	0.3 ± 0.2	0.2 ± 0.1
	CT	0.5 ± 0.1*	0.2 ± 0.1
20:4n6	ZD	4.5 ± 3.1	0.7 ± 0.1
	CT	8.5 ± 2.8**	0.4 ± 0.1**
22:4n6	ZD	0.8 ± 0.4	—
	CT	TR[b]**	—
22:5n6	ZD	0.2 ± 0.1	—
	CT	TR**	—
18:3n3	ZD	0.8 ± 0.2	1.1 ± 0.04
	CT	0.8 ± 0.1	0.9 ± 0.1**
22:5n3	ZD	0.2 ± 0.1	—
	CT	0.8 ± 0.2**	—
22:6n3	ZD	0.3 ± 0.3	—
	CT	1.2 ± 0.4**	—

Note. Each value represents the mean ± SD of 10 samples.

[a] 18:3n6 has the same retention time as 20:0.

[b] Only trace amounts (<0.1%) detected.

* $P < 0.05$; ** $P < 0.01$ compared between ZD and CT for both tissues.

diet, final body weights after 4–6 weeks are not significantly different but epididymal fat and thymus are significantly decreased in the ZD rats (11).

Tissue fatty acid composition in zinc-deficient rats has previously been reported (4, 5, 7, 12–14). However, except for the detailed analysis of zinc deficiency effects on brain lipids (14), these studies have only reported the analysis of tissue total lipids. The data presented in this paper indicate that changes in fatty acid composition induced by zinc deficiency are not the same in tPL and TG (Tables II, III). In tPL, the effects of zinc deficiency were generally consistent with those previously reported for tissue total lipids; in plasma, liver, and testes tPL, either 20:4n6 was significantly lower or 18:2n6 was significantly higher in the ZD rats. The result was that in each of these tissues, the ratio of 18:2n6/20:4n6 was significantly higher in the ZD rats. However, both 18:2n6 and 20:4n6 were decreased in liver TG (18:2n6, ns) but were increased in epididymal fat TG.

The changes in fatty acid composition in the tissue tPL presumably reflect altered desaturation of EFA; when the ratio of 18:2n6/20:4n6 is increased in tissue tPL, activity of either the Δ^6- or Δ^5-desaturase or both is considered to be decreased (15). *In vitro* assays of liver microsomal Δ^6- and Δ^5-desaturases support the view that activity of these enzymes is decreased in zinc deficiency (12, 16, 17). *In vivo* evidence also suggests that zinc deficiency causes decreased desaturation of 18:2n6; unlike control rats in which 18:2n6, when added to the diet, does not accumulate in tissue tPL, zinc deficient rats accumulate significant amounts of 18:2n6 (7). The effect of zinc deficiency on microsomal desaturation of 18:2n6 may be tissue specific; in liver (12, 16, 17), testes (17), and pregnant uterus (18), 18:2n6 desaturation is decreased but in mammary tissue from lactating rats it has been shown to be increased threefold (19). Perhaps as a result of the generalized decrease in the activity of the EFA desaturases in zinc deficient rats, the activity of the Δ^9-desaturase appeared to be increased based on the lower 18:0/18:1n9 ratio in plasma, liver, testes, and skin of the ZD rats (Table II). This, too, has been suggested by *in vitro* assay (6).

The effect on fatty acid composition of pair-feeding compared to *ad libitum* feeding was significant for 18:2n6 and 20:4n6. The increased ratio of 18:2n6/20:4n6 in plasma and liver (results in text) suggests that semi-starvation causes decreased activity of the Δ^6- and Δ^5-desaturases. It has been recently reported that zinc deficiency has no effect on tissue lipid composition when the effect of pair-feeding is discounted (20). Our data indicate that if the diet is not totally devoid

of zinc (3 mg/kg zinc was used in this study), zinc deficiency has a significant effect on tissue EFA greater than that of pair-feeding alone.

Increased 18:3n6 has been reported in the plasma lipids from children with acrodermatis enteropathica, leading to the suggestion that zinc deficiency may affect the chain elongation rather than desaturation of EFA (21). As with reduced desaturase activity, this would also cause lower levels of 18:3n6 products, e.g., 20:3n6 and possibly 20:4n6. Our data do not completely eliminate this possibility; in fact, 18:3n6 was increased in the liver tPL of the ZD rats. However, since 16:0 is chain elongated to 18:0, and 18:0 was not significantly lower (except in epididymal fat TG), nor 16:0 significantly higher in any tissue lipids measured in our ZD rats, the defect in EFA metabolism in ZD rats seems more likely to be centered at the desaturases.

Analysis of the composition of 22 carbon n6 and n3 EFA in the tPL of liver and testes suggests that zinc deficiency may also affect the Δ^4-desaturase; the 22:5n3/22:6n3 ratio in liver tPL and the 22:4n6/22:5n6 ratio in liver and testis tPL (Table II) were increased in zinc-deficient rats. Since the fatty acid data are only of percentage composition, too much emphasis should not be placed on fatty acid ratios. Nevertheless, in this case, *in vitro* desaturase assays have confirmed the previously proposed effect of zinc deficiency on the EFA desaturases (11). Perhaps as a result of the decreased activity of the EFA desaturases in zinc deficiency, the activity of the Δ^9-desaturase appears to be increased based on the lower 18:0/18:1n9 ratio in plasma, liver, testes, and skin of the ZD rats. This, too, has been suggested by *in vitro* assay (6).

The fatty acid composition of liver TG was, in general, affected by zinc deficiency in a manner opposite to that in the epididymal fat TG (Table III). These differences presumably reflect effects of zinc deficiency on tissue fatty acid metabolism distinct from the EFA desaturases. As in the case of pyridoxine deficiency (22), zinc deficiency may have effects on the hepatic or lipoprotein lipases, perhaps increasing the former and decreasing the latter. Such an effect of zinc deficiency on these enzymes would influence turnover of EFA between tissue PL and TG as previously reported (23, 24) and thereby account for the distinct difference in the effect of zinc deficiency on TG EFA composition in the liver and epididymal fat. The fact that both 18:2n6 and 20:4n6 increased in the liver TG and both decreased in epididymal fat TG suggests that for an indication of possible effects of zinc deficiency on EFA desaturases, more reliable information is obtained from phospholipid rather than TG fatty acids.

S.C.C. thanks the National Research Council of Canada for an Industrial Research Fellowship during the tenure of this research. N. Morse, M. A. Ryan, V. Kyte, and S. Sheffield provided excellent technical assistance.

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Received March 16, 1984. P.S.E.B.M. 1984, Vol. 177.
Accepted July 31, 1984.