British Journal of Nutrition (1988), **59**, 273–278

Evidence that adverse effects of zinc deficiency on essential fatty acid composition in rats are independent of food intake

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(*Received 4 September 1987 – Accepted 9 November 1987*)

1. Young male rats were fed on diets containing 3·4, 36 or 411 mg zinc/kg for 10 weeks in order to determine whether effects of Zn deficiency on plasma and liver essential fatty acid composition could be distinguished from those of reduced protein and energy intake.

2. Fatty acid analysis revealed that a Zn intake of 3·4 mg/kg (plasma Zn 0·80 *v.* 1·97 mmol/l in controls fed on 36 mg Zn/kg) resulted in a significant increase in the linoleic acid:arachidonic acid ratio in both plasma and liver phospholipids in comparison with rats fed on 36 or 411 mg Zn/kg.

3. Zn supplementation (411 mg/kg) decreased the linoleic acid:arachidonic acid ratio in plasma phosphatidylserine compared with that of the controls.

4. The previously reported increase in arachidonic acid (mol %) in liver triacylglycerol of Zn-deficient rats was shown to be a function of a reduced liver triacylglycerol pool size; quantitatively, triacylglycerol content of arachidonic acid in the liver was not significantly affected by Zn intake.

The interaction between zinc and essential fatty acid (EFA) metabolism has been the subject of considerable research. Initially, studies involving supplementation of Zn-deficient humans or rats with EFA added to the diet, or the exacerbation of EFA deficiency by Zn deficiency, suggested a role of Zn in EFA metabolism (Cunnane, 1982). Fatty acid values from total lipid extracts of tissues indirectly confirmed earlier work (Bieri & Prival, 1966; Bettger *et al.* 1979; Odutunga, 1982; Huang *et al.* 1982; Krieger *et al.* 1986). More recently, studies of the interaction of Zn in EFA metabolism have focused on the Δ6- and Δ5-desaturases which control synthesis of arachidonic acid (20:4*n*−6). Reports describing in vitro assay of desaturase activity in both *ad lib.*-fed and pair-fed controls have demonstrated impaired synthesis of arachidonic acid in Zn-deficient rats (Clejan *et al.* 1982; Ayala & Brenner, 1983; Fields & Kelleher, 1983; Tsai *et al.* 1983).

In spite of this evidence, the experimental conditions used have left unresolved the effects of altered appetite and food intake induced by feeding a diet depleted of Zn. The inhibitory effect of Zn deficiency on food intake is maximal in the young rat 2–3 weeks post-weaning. If rats at this age are fed on a diet with no added Zn (final Zn concentration 0·5–1·0 mg Zn/kg diet), pair-feeding indicates that reduced food intake has a significant effect on EFA composition and metabolism regardless of Zn intake (Kramer *et al.* 1984). However, if the diet is not totally devoid of Zn (3 mg Zn/kg diet), the effects of reduced food intake (pair-feeding) and Zn deficiency are clearly distinguishable (Cunnane *et al.* 1984).

The recent report of Fogerty *et al.* (1985) has added to the debate by demonstrating that in adult rats, arachidonic acid (mol %) was increased in the extract of the total or neutral lipids of liver. Their report supports previous observations (Huang *et al.* 1982; Cunnane *et al.* 1984) suggesting that the effect of Zn deficiency on the proportional content of arachidonic acid in liver lipids depends on whether phospholipids or neutral lipids are being assayed. The present study seeks to address these issues and to investigate whether (a) Zn deficiency affects EFA composition independently of food intake, (b) Zn supplementation has effects consistent with a role of Zn in EFA metabolism, (c) it is important to distinguish

between phospholipids and neutral lipids in relating effects on fatty acid composition to nutrient imbalances. These points require clarification if a significant role of Zn in EFA metabolism is to be established.

METHODS

Thirty weanling male Sprague-Drawley rats (mean weight 50 (SE 2) g) were housed individually in polypropylene cages with stainless-steel gridded wire floors and cage tops (Nalgene). They were fed on a semi-synthetic diet (Teklad, Madison, WI) in which the Zn content was formulated to be 40 mg/kg. The diet contained (g/kg):200 spray-dried egg white, 505 maize starch, 100 maize oil, 150 cellulose, 35 AIN-76 mineral mix and 10 AIN-76A vitamin mix as recommended by the American Institute of Nutrition (1977). At 10 d post-weaning (mean body-weight 95 (SE 4) g), the rats were divided into three groups of ten rats each and were given the same diet in which the Zn content was formulated to be 4 mg/kg (Zn(−)), 40 mg/kg (control) or 400 mg/kg (Zn(+)). The measured Zn content of these diets was 3·4, 36 and 411 mg/kg respectively. Deionized, distilled water was available *ad lib.* from acid-washed polycarbonate bottles with stainless-steel sippers (Nalgene). The rats were weighed weekly and food intake was measured daily. After 10 weeks on the diets, the rats were anaesthetized under diethyl ether and blood was drawn from the abdominal aorta into EDTA-anticoagulated syringes, centrifuged and the plasma frozen. Liver was also removed and frozen.

Lipid analysis was conducted as described previously (Cunnane *et al.* 1984). Total lipids of plasma and liver were extracted with chloroform–methanol (2:1, v/v) containing 0·2 g butylated hydroxytoluene/l as antioxidant. Total phospholipids and triacylglycerols (liver only) were separated by thin-layer chromatography using standard methods (Cunnane *et al.* 1984). These lipid fractions were *trans*-esterified with boron trifluoride–methanol and the composition of the fatty acid methyl esters was determined by gas–liquid chromatography under the conditions previously specified (Cunnane *et al.* 1984). Total phospholipids were quantified by the ammonium ferrothiocyanate method (Stewart, 1980) and triacylglycerols were quantified by gas–liquid chromatography using triheptadecanoin as the internal standard. The Zn content of the diet and plasma was assayed by atomic absorption spectrophotometry (Instrumentation Laboratories 457) under conditions of minimal contamination.

Statistical analyses were done using one-way analysis of variance followed by Duncan's Multiple Range Test.

RESULTS

The weight gained by the Zn(−) rats was 43% lower than that gained by either the controls or the Zn(+) group ($P < 0·01$). Absolute food intake was not different between groups but food conversion efficiency (g weight gain/g food intake) was only 65% of control values in the Zn(−) group ($P < 0·01$, Table 1). Liver weight (absolute and relative to body-weight) was significantly lower in the Zn(−) group compared with either the controls or Zn(+) group. Plasma Zn level in the Zn(−) group was 40% of that in the controls ($P < 0·01$) and was 34% higher than control values in the Zn(+) group (not significant).

The main fatty acids relevant to the role of Zn in EFA metabolism are linoleic acid (18:2$n-6$), dihomo-γ-linolenic acid (20:3$n-6$) and arachidonic acid (20:4$n-6$) in the phospholipid fractions. The values describing phospholipid fatty acid composition are therefore limited to these fatty acids (Table 2 and 3). In liver, triglyceride levels of the non-EFA, palmitic acid (16:0), palmitoleic acid (16:1$n-7$) and oleic acid (18:1$n-9$), are also shown (Table 3).

Table 1. *Effects of altered zinc intake on body-weight, food intake, liver weight and plasma Zn concentrations of rats*

(The Zn(−) diet contained 3·4 mg Zn/kg, the control diet 36 mg Zn/kg and the Zn(+) diet 411 mg Zn/kg. Values are means with their standard errors for ten rats/group)

Group...	Zn(−)		Control		Zn(+)	
	Mean	SEM	Mean	SEM	Mean	SEM
Final body-wt (g)	281[a]	11	425[b]	17	451[b]	9
Change in body-wt (g)	193[a]	10	339[b]	7	350[b]	20
Food intake (g/d)	10·2	2·0	12·4	1·6	9·9	1·7
Body-wt gain/food intake (g/g)	0·26[a]	0·04	0·40[b]	0·05	0·51[b]	0·05
Liver wt (g)	8·2[a]	0·5	15·4[b]	0·7	13·7[b]	1·0
Liver:body-wt (g/kg)	29[a]	0·2	36[b]	0·2	30[b]	0·3
Plasma Zn (mmol/l)	0·80[a]	0·20	1·97[b]	0·26	2·65[b]	0·29

[a, b] Values in horizontal rows with different superscript letters were significantly different (one-way ANOVA and Duncan's Multiple Range Test): $P < 0·01$.

Table 2. *Effect of altered zinc intake on the fatty acid composition (mol%) of two plasma phospholipid subclasses and plasma concentration of phosphatidylcholine and phosphatidylserine*

(The Zn(−) diet contained 3·4 mg Zn/kg, the control diet 36 mg Zn/kg and the Zn(+) diet 411 mg Zn/kg. Values are means with their standard errors for ten rats/group)

Group...	Zn(−)		Control		Zn(+)	
	Mean	SEM	Mean	SEM	Mean	SEM
Phosphatidylcholine						
18:2*n*−6	31·1[a]	1·7	23·4[b]	2·5	22·1[b]	1·9
20:3*n*−6	0·7[a]	0·1	1·3[b]	0·2	0·5[a]	0·1
20:4*n*−6	20·1[a]	2·0	25·7[a]	2·3	30·4[b]	1·9
18:2*n*−6/20:4*n*−6	1·55[a]	0·20	0·94[b]	0·12	0·72[b]	0·06
mmol/l	7·3	1·7	9·1	1·3	8·4	1·5
Phosphatidylserine						
18:2*n*−6	20·6	2·0	21·0	1·3	17·5	1·1
20:3*n*−6	0·4[a]	0·05	1·1[b]	0·1	0·3[a]	0·04
20:4*n*−6	14·1[a]	0·8	17·5[a]	0·6	19·7[b]	0·3
18:2*n*−6/20:4*n*−6	1·44[a]	0·11	1·21[a]	0·90	0·90[b]	0·06
mmol/l	4·0	0·4	5·8	1·2	5.0	0·7

[a, b] Values in horizontal rows with different superscript letters were significantly different (one-way ANOVA and Duncan's Multiple Range Test): $P < 0·01$.

The fatty acid composition of the two main phospholipid subclasses of plasma is shown in Table 2. The proportional content of linoleic acid was significantly higher in the phosphatidylcholine subclass of the Zn(−) group than in the other groups but did not differ significantly between groups in the phosphatidylserine fraction. In each of the plasma phospholipid subclasses studied, dihomo-γ-linolenic acid was significantly higher in the controls than in the other groups whereas arachidonic acid was lowest in the Zn(−) group (not significant compared with controls) and highest in the Zn(+) group ($P < 0·01$, Table

Table 3. *Phospholipid and triacylglycerol fatty acid composition (mol%) and total phospholipid and triacylglycerol content of liver in rats fed on different amounts of dietary zinc*

(The Zn(−) diet contained 3·4 mg Zn/kg, the control diet 36 mg Zn/kg and the Zn(+) diet 411 mg Zn/kg. Values are means with their standard errors for ten rats/group)

Group...	Zn(−)		Control		Zn(+)	
	Mean	SEM	Mean	SEM	Mean	SEM
Phospholipid:						
18:2*n*−6	17·1[a]	0·6	13·8[b]	0·5	12·2[b]	0·7
20:3*n*−6	0·9[a]	0·1	0·7[a]	0·1	1·6[b]	0·2
20:4*n*−6	31·7	0·2	32·9	0·6	31·0	0·8
18:2*n*−6/20:4*n*−6	0·54[a]	0·02	0·42[b]	0·02	0·39[b]	0·03
mg/g	19·1	0·8	17·6	0·3	16·7	0·5
Triacylglycerol:						
16:0	18·2[a]	2·6	30·0[b]	1·3	24·7[c]	1·4
16:1*n*−7	2·3[a]	0·4	3·6[a]	0·7	10·5[b]	1·8
18:1*n*−9	13·1[a]	1·1	25·8[b]	1·4	16·7[a]	1·7
18:2*n*−6	51·3[a]	3·0	26·4[b]	3·4	39·9[c]	2·2
20:4*n*−6	6·8[a]	1·5	1·7[b]	0·4	6·3[a]	1·4
mg/g	1·4[a]	0·6	6·5[b]	1·4	1·3[a]	0·4

[a, b, c] Values in horizontal rows with different superscript letters were significantly different (one-way ANOVA and Duncan's Multiple Range Test): $P < 0·01$.

2). In the phosphatidylcholine subclass the ratio of linoleic acid:arachidonic acid was higher in the Zn(−) group compared with the controls ($P < 0·01$); in the phosphatidylserine subclass the ratio was lower in the Zn(+) group compared with the controls ($P < 0·01$).

In liver total phospholipid, linoleic acid was highest in the Zn(−) group, dihomo-γ-linoleic acid was highest in the Zn(+) group and arachidonic acid was not different between groups. The linoleic acid:arachidonic acid ratio was higher in the Zn(−) group than in the controls or Zn(+) group (Table 3). The fatty acid composition of liver triacylglycerol is also shown in Table 3. In the liver triacylgylcerol of the Zn(−) rats, proportional contents of palmitic acid and oleic acid were significantly lower whereas linoleic acid and arachidonic acid were significantly higher than those in the controls. In the liver triacylglycerol of the Zn(−) rats, proportional contents of palmitoleic acid, linoleic acid and arachidonic acid were higher than those in the controls but palmitic acid and oleic acid were lower. Total triacylglycerol was significantly lower in the Zn(−) and Zn(+) groups compared with controls.

DISCUSSION

The present study was designed to eliminate the variable of protein–energy intake while assessing the effects of dietary Zn deficiency on EFA composition in the rat. This was done by feeding approximately 3 mg Zn/kg diet to rats which were at least 10 d beyond weaning age. It was reasoned that evidence suggesting a role of Zn in EFA metabolism would be strengthened if an effect of Zn supplementation opposite to that of Zn deficiency were observed. Since one-tenth the adequate Zn intake (35–40 mg/kg) was chosen for Zn depletion, ten times the adequate intake was used for Zn supplementation, an amount which does not adversely affect weight gain in rats (Table 1 and Cunnane, 1985).

The present observations indicate that a moderate degree of Zn depletion (3·4 mg Zn/kg

diet given from 10 d post-weaning for 10 weeks) does not significantly impair food intake in rats (Table 1) but does significantly alter EFA composition in plasma and liver phospholipids (Tables 2 and 3). The EFA changes in the Zn-deficient rats resulted in an increase in the ratio of linoleic acid:arachidonic acid (higher linoleic acid or lower arachidonic acid depending on the phospholipid subclass; Tables 2 and 3) which is consistent with the majority of the previous experimental and clinical findings indicating an impairment of arachidonic acid synthesis during Zn deficiency.

The association between Zn and EFA does not depend only on the study of Zn deficiency. The present study included a group given ten times the amount of Zn given to the controls. The proportional content of arachidonic acid in plasma phosphatidylserine was significantly increased and the ratio, linoleic acid:arachidonic acid significantly decreased in the Zn(+) group compared with the controls (Table 2). These results are consistent with those previously reported showing higher arachidonic acid contents in liver phospholipids of Zn-supplemented rats (Cunnane, 1985). They are also consistent with the low levels of arachidonic acid seen in human serum phospholipids of patients with both acquired and inherited Zn deficiency in whom Zn supplementation significantly raises the serum content of arachidonic acid (Koletzko *et al.* 1984; Krieger *et al.* 1986; Cunnane & Krieger, 1988).

The explanation for the reported increase in the neutral lipid content (mol %) of arachidonic acid (Huang *et al.* 1982; Cunnane *et al.* 1984; Cunnane, 1985; Fogerty *et al.* 1985) lies in the effect of the triacylglycerol pool size on its proportional composition of arachidonic acid. The proportional composition of arachidonic acid in plasma or liver triacylglycerol of various species has been shown to vary inversely with the triacylglycerol pool size (Cunnane *et al.* 1986). Hence when the liver triaclyglycerol pool size was decreased, e.g. the 75% decrease in liver triaclyglycerol seen in the Zn(−) and Zn(+) groups (Table 3), the proportional composition (mol %) of arachidonic acid was significantly increased. The proportional increase in arachidonic acid in liver triaclyglycerol was not a function of increased synthesis of arachidonic acid but rather was a function of the triaclyglycerol pool size being reduced. In fact, the quantitative content of arachidonic acid in the liver triaclyglycerol was similar for the three groups in the present study: Zn(−) 95, control 111, Zn(+) 82 μg/g. Therefore, in using differences in proportional fatty acid composition to approximate changes in EFA metabolism, it is essential to isolate the phospholipid (the total pool size of which rarely fluctuates more than 20%) as has been recommended previously (Hill *et al.* 1982).

REFERENCES

American Institute of Nutrition (1977). *Journal of Nutrition* **107**, 1340–1348.
Ayala, S. & Brenner, R. R. (1983). *Acta Physiologica Latinoamericana* **33**, 193–204.
Bettger, W. J., Reeves, P. G., Moscatelli, E. A., Reynolds, G. & O'Dell, B. L. (1979). *Journal of Nutrition* **109**, 480–488.
Bieri, J. & Prival, E. (1966). *Journal of Nutrition* **89**, 55–61.
Clejan, S., Castro-Magana, M., Collipp, P. J., Jonas, E. & Maddaiah, V. T. (1982). *Lipids* **17**, 129–135.
Cunnane, S. C. (1982). *Progress in Lipid Research* **21**, 73–90.
Cunnane, S. C. (1985). In *Trace Elements in Man and Animals*, vol. 5, pp. 70–75 [C. F. Mills, I. Bremner and J. K. Chesters, editors]. Slough: Commonwealth Agricultural Bureaux.
Cunnane, S. C., Huang, Y.-S. & Manku, M. S. (1986). *Biochimica Biophysica Acta* **876**, 183–186.
Cunnane, S. C. & Krieger, I. (1988). *Journal of the American College of Nutrition* (In the Press).
Cunnane, S. C., Manku, M. S. & Horrobin, D. F. (1984). *Proceedings of the Society for Experimental Biology and Medicine* **177**, 141–146.
Fields, H. P. & Kelleher, J. (1983). *Proceedings of the Nutrition Society* **43**, 54A.
Fogerty, A. C., Ford, G. L., Dreosti, I. E. & Tinsley, I. J. (1985). *Nutrition Reports International* **32**, 1009–1020.
Hill, E. G., Johnson, S. B., Lawson, L. D., Mafouz, M. M. & Holman, R. T. (1982). *Proceedings of the National Academy of Sciences, USA* **79**, 953–957.

Huang, Y. S., Cunnane, S. C., Horrobin, D. F. & Davignon, J. (1982). *Atherosclerosis* **42**, 193–207.
Koletzko, B., Bretschneider, A. & Bremer, H. J. (1984). *European Journal of Pediatrics* **143**, 310–313.
Kramer, T. R., Briske-Anderson, M., Johnson, S. B. & Holman, R. T. (1984). *Journal of Nutrition* **114**, 1224–1230.
Krieger, I., Alpern, B. E. & Cunnane, S. C. (1986). *American Journal of Clinical Nutrition* **43**, 955–958.
Odutunga, A. A. (1982). *Clinical and Experimental Pharmacology and Physiology* **9**, 213–221.
Stewart, J. C. M. (1980). *Analytical Biochemistry* **104**, 10–14.
Tsai, S. L., Craig-Schmidt, M. C., Weete, J. D. & Keith, R. E. (1983). *Federation Proceedings* **42**, 823.

Printed in Great Britain