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Parenteral Linoleic and γ-Linolenic Acids Ameliorate the Gross Effects of Zinc Deficiency (40920)

S. C. CUNNANE AND D. F. HORROBIN

Clinical Research Institute, 110 Pine Avenue West, Montreal H2W 1R7, Canada

Abstract. Zinc deficiency and essential fatty acid deficiency in rats have gross effects which are very similar. Zinc, prostaglandin E_1, and dihomo-γ-linolenic acid (prostaglandin E_1 precursor) all have very similar effects on rat vascular smooth muscle. Zinc deficiency has no consistent effect on tissue zinc or zinc enzymes. These observations suggest that the mechanism by which zinc deficiency affects growth and metabolism may be via inhibition of prostaglandin synthesis from essential fatty acids. This hypothesis was examined using zinc deficient rats supplemented with oils containing various concentrations of essential fatty acids. Male Wistar rats were maintained on a zinc deficient diet for 5 weeks and supplemented daily with one of three oils containing different concentrations of essential fatty acids: olive oil (mainly oleic acid), safflower oil (mainly linoleic acid), or evening primrose oil (mainly linoleic and γ-linolenic acids). The olive oil treated rats did not benefit from this treatment in any respect. Dermal lesions were actually worse in this group than in the untreated zinc deficient rats. Safflower oil supplementation significantly inhibited the development of dermal lesions but was of only marginal benefit with respect to growth. Evening primrose oil supplementation also blocked the development of dermal lesions and restored growth to 50% of control. It is suggested that a primary defect of zinc deficiency is to inhibit essential fatty acid metabolism to prostaglandins either by blocking linoleic acid desaturation to γ-linolenic acid or by inhibiting mobilization of dihomo-γ-linolenic acid from tissue membrane stores.

Recent observations have suggested that a close relationship exists between zinc (Zn) and the metabolism of essential fatty acids (EFA) to prostaglandins (PGs). This suggestion is based on four distinct observations: (i) The gross effects of EFA and Zn deficiency (particularly growth, reproductive, and dermal) are qualitatively similar. (ii) Parenteral administration of EFA can partially ameliorate the gross effects of experimental dietary Zn deficiency (1–3). (iii) Although Zn is a component of many key enzymes, the gross features of Zn deficiency have never been clearly related to a substantial loss of Zn from any tissue (other than plasma) nor to a specific Zn enzyme defect. (iv) We have shown that Zn, dihomo-γ-linolenic acid (DGLA)—the precursor of the 1 series PGs, and PGE_1 have very similar effects on responses to vasoconstrictor agents in vascular smooth muscle which are quantitatively unlike those of PGE_2 or arachidonic acid—the precursor of the 2 series PGs (4). These observations suggest that Zn may have effects on PG synthesis from the EFA or on PG actions such that when present, it would increase the synthesis of PGs or, when deficient, would lead to a decrease in PG synthesis with typical EFA deficiency symptoms resulting.

We have further investigated the role of Zn in EFA metabolism by determining the effects of parenteral supplementation of Zn deficient rats using three different primarily unsaturated fatty acid sources: olive oil, safflower oil, and evening primrose oil. Owing to the different composition of these oils, we felt that a differential response by any one of the Zn deficient groups with fatty acid supplementation would indicate the most effective component fatty acid, be it oleic, linoleic, γ-linolenic acid, or a combination of these. We here present evidence that the combination of γ-linelenic and linoleic acids in the supplemental oil very significantly increased the weight gain, tissue weights, and dermal condition of Zn deficient rats. Linoleic acid (safflower oil) treatment almost completely corrected the dermal lesions but had little effect on growth or tissue weights. Non-EFA such as

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oleic acid (olive oil) exacerbated the effect of Zn deficiency.

Materials and methods. Animals. The 21-day-old male Wistar rats were housed in six groups of 7 rats each over a period of 5 weeks. The cages were solid bottomed and had tops made of stainless steel. The rats were weighed weekly and examined daily for dermal lesions. The lesions were assessed on a scale from 0.0 to 4.0 which indicated normal to severely lesioned skin, respectively, as has been previously described (5).

Diets. The rats were maintained for the duration of the experiment on the following dietary treatments: (1) Control—Purina rat chow plus tap water, both *ad libitum.* (ii) Zn deficient (ZnDEF)—Zn deficient diet plus deionized water, both *ad libitum.* (iii) Zn supplemented (ZnSUP)—Zn deficient diet, plus deionized water containing 25 μg/ml Zn ($ZnSO_4$ $7H_2O$, Baker). (iv) Zn deficient plus olive oil (ZnOLO)—Zn deficient diet and deionized water, both *ad libitum,* plus 250 μl/day olive oil injected subcutaneously. (v) Zn deficient plus safflower oil (ZnSFO)—Zn deficient diet and deionized water, both *ad libitum,* plus 250 μl/day safflower oil injected subcutaneously. (vi) Zn deficient plus evening primrose oil (ZnEPO)—Zn deficient diet and deionized water, both *ad libitum,* plus 250 μl/day evening primrose oil injected subcutaneously. The amount of food given to the Zn supplemented rats was equal in weight to the average amount of food eaten by the Zn deficient rats during the previous week, as calculated on a daily basis.

The Zn deficient diet (see Appendix), based on dried egg albumin, was supplied by Zeigler Brothers, Gardeners, Pennsylvania, and contained an average of 1.1 ppm Zn. The EPO was supplied by Agricultural Holdings, London, England. The SFO and OLO were obtained commercially. The oils contained an average of 1.7, 0.97, and 1.2 ppm Zn, respectively. Thus, for the duration of the experiment, the oil supplemented rats received a maximum of 15 μg Zn from this source. The oils had the following fatty acid compositions (in parentheses is the weight (mg) of each individual fatty acid injected each day): Olive oil—11% (26) saturated fats, 76% (182) oleic acid, and 7% (16) linoleic acid; safflower oil—8% (18) saturated fats, 15% (34) oleic acid, 72% (172) linoleic acid; evening primrose oil—7% (16) saturated fats, 11% (26) oleic acid, 73% (176) linoleic acid, and 10% (24) γ-linolenic acid (see Appendix).

Tissue zinc analysis. The rats were sacrificed by decapitation after 5 weeks on the diets at which time the blood and organs were analyzed for Zn content. All glassware and plastic test tubes used for the collection of blood and tissue samples were washed in 0.1 *N* HCl for 24 hr prior to use. Pituitary, adrenal, and liver samples were removed, wet weighed, dried to constant weight, dry weighed, and stored at −10° prior to analysis. Analysis of the Zn content of the serum, tissue samples, Zn deficient food, and oil supplements was performed using an atomic absorption spectrophotometer (Perkin−Elmer 403). The dried tissue samples were wet digested in 1.0 ml concentrated HNO_3 (certified grade reagent, Fisher) for 48 hr. The tissue acid solution was diluted 1:10 with deionized water and analyzed. The fatty acids were dissolved in a mixture of isoamyl acetate and methanol (1:1) and analyzed directly. Serum was diluted with deionized water (1:10) and analyzed directly.

Results. General. The rats in each group were assessed during the experiment by daily observations, weekly weighing, and at the end of the experiment by total weight gained with respect to controls and ZnSUP rats, organ weight−body weight ratios, dermal scores, and tissue Zn concentration. Statistical analysis was always done between the ZnDEF group and the others, using Student's *t* test. After 5 weeks on the Zn deficient diet, the ZnDEF rats exhibited severely retarded growth, hunched posture, keratosis of the paws, tail, and nasal, genital, and eye regions, moderate to extensive alopecia, and bleeding from the paws and nose. The ZnOLO rats were also afflicted with the described symptoms, but had more severe alopecia and postural defects. The ZnSFO rats had better posture, some improvement in the condition of the dermis, and had less alopecia. The effect of Zn deficiency as described above was not ob-

served in the ZnEPO rats. There were few signs of dermal lesions and no postural effects of Zn deficiency in these rats. Behaviorally they were very significantly improved over the other Zn deficient rats.

Growth and dermal scores. The weight change of all groups was not significantly different over the first week of the experiment (Fig. 1). Thereafter, the ZnDEF rats showed almost zero or negative growth (lost weight). The growth of the ZnOLO rats was not significantly different from that of the ZnDEF rats at any stage of the experiment (weight gain was 7% of control and 9% of the ZnSUP rats). Skin lesions were very severe in both these groups (Table IA). The growth of the ZnSFO rats was significantly improved ($P < 0.05$) compared to the ZnDEF rats but was still only 16% of control and 20% of the ZnSUP group (Table IB). The dermal scores of this group were considerably improved compared to the ZnDEF group and were comparable to those of the ZnEPO rats.

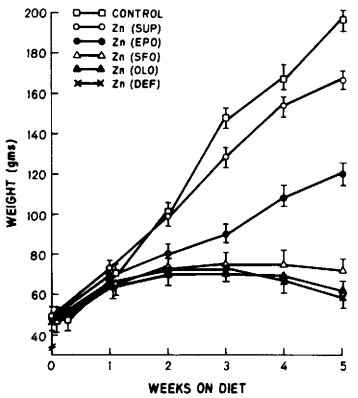

FIG. 1. Effect of zinc deficiency and supplementation with various oils on growth curves. Control—Purina fed; ZnSUP—Zn supplemented; ZnEPO—Zn deficient plus evening primrose oil; ZnSFO—Zn deficient plus safflower oil; ZnOLO—Zn deficient plus olive oil; and ZnDEF—Zn deficient, untreated. Growth of the ZnDEF, ZnOLO, ZnSFO, and ZnEPO groups was significantly less than control after 2 weeks ($P < 0.05$). Growth of the ZnSUP group was significantly less than control after 3 weeks ($P < 0.05$). Growth of the ZnDEF, ZnOLO, and ZnSFO groups was significantly less than that of the ZnEPO group after 4 weeks ($P < 0.01$) and very significantly less than control after 5 weeks on the respective diets ($P < 0.001$).

Supplementation with the EPO significantly improved ($P < 0.01$) the growth of the ZnEPO rats compared to the ZnDEF group. Weight gain of the ZnEPO group was 47% of control and 59% of the ZnSUP rats. There were significantly fewer lesions of the dermis in the ZnEPO rats, particularly on the paws. Zn supplementation reversed all the visible aspects of the effect of Zn deficiency and growth was 80% of control ($P < 0.05$).

Organ weights. The weights of liver, adrenal, thymus, epididymal fat, and testis of the six groups are shown in Table IC. Liver weight was not significantly different in any of the groups except for ZnEPO in which it was significantly elevated. Adrenal weights were significantly higher in the ZnDEF, ZnOLO, and ZnSFO rats compared to the other groups. Thymus and epididymal fat were significantly decreased in weight in the ZnDEF, ZnOLO, and ZnSFO rats compared to the others. Testis weights were significantly increased in the ZnDEF and ZnOLO groups.

Tissue zinc analysis. Zn levels of serum, pituitary, adrenal, and liver are shown in Table ID. Serum Zn concentration was very significantly depressed in all four Zn deficient groups compared to the controls. However, the level of Zn in the ZnEPO rats was significantly increased above that of the ZnDEF group. Serum Zn was also higher in the ZnSUP group than control values. Pituitary, adrenal, and liver Zn concentration were not affected by Zn deficiency, Zn supplementation, nor by the supplemental oils.

Discussion. The results of this experiment confirm previous observations (1, 2) that concurrent treatment of Zn deficient rats with parenteral EFA can prevent the dermal lesions and partially block the growth defect caused by Zn deficiency. The results also suggest that the presence of γ-linolenic acid in the evening primrose oil was a critical factor and indicate an important role for this acid.

The olive oil was low in EFA content (7% linoleic acid) and was completely ineffective in altering the onset of the Zn deficiency symptoms; in fact, it exacerbated the dermal and postural effects of Zn defi-

TABLE I. EFFECT OF ZINC DEFICIENCY AND SUPPLEMENTATION WITH VARIOUS OILS ON DERMAL SCORES, WEIGHT GAIN, ORGAN WEIGH AND SERUM AND TISSUE ZINC CONCENTRATION IN MALE WISTAR RATS

	ZnDEF[a]	Control[b]	ZnOLO[c]	ZnSFO[d]	ZnEPO[e]	ZnSU
Dermal scores						
aws[g]	2.8 ± 0.4	0.0***	3.0 ± 0.3	1.8 ± 0.1**	1.0 ± 0.1*	0.
ail	1.7 ± 0.2	0.0***	1.3 ± 0.2	0.5 ± 0.1*	1.2 ± 0.1**	0.
Final weight (g)	59 ± 3	196 ± 5***	61 ± 3	71 ± 4	118 ± 7**	166 ±
Weight gain	11 ± 3	148 ± 5***	13 ± 3	24 ± 4*	70 ± 7***	118 ±
Weight change (control 100%)	7	—	9	16	47	
Organ weights						
iver[h]	3.2 ± 0.9	3.5 ± 0.1	3.1 ± 0.7	3.0 ± 0.7	4.0 ± 0.3*	3.4 ±
drenal	17.8 ± 3.1	10.2 ± 0.5***	19.4 ± 4.1	16.2 ± 2.1	13.2 ± 2.9**	8.9 ±
hymus	0.11 ± 0.1	0.42 ± 0.1***	0.09 ± 0.0	0.14 ± 0.1	0.37 ± 0.0***	0.45 ±
pididymal fat	0.16 ± 0.1	0.38 ± 0.1***	0.19 ± 0.2	0.15 ± 0.1	0.37 ± 0.1**	0.41 ±
estis	0.89 ± 0.2	0.62 ± 0.1**	0.82 ± 0.2	0.73 ± 0.1[i]	0.69 ± 0.2*	0.61 ±
Tissue zinc						
erum[i]	30 ± 5	122 ± 4***	28 ± 5	32 ± 5	48 ± 6*	153 ±
ituitary[j]	27 ± 6	35 ± 6	31 ± 5	32 ± 8	33 ± 6	35 ±
drenal[j]	24 ± 3	23 ± 2	23 ± 2	22 ± 1	22 ± 3	25 ±
iver[j]	32 ± 2	34 ± 1	30 ± 1	32 ± 1	32 ± 2	36 ±

ote. Each value is the mean ± SEM for six samples per group.
Zn deficient–untreated.
Control.
Zn deficient–olive oil treated.
Zn deficient–safflower oil treated.
Zn deficient–evening primrose oil treated.
Zn supplemented.
0.0 to 4.0, normal to severe dermal lesions, respectively.
g/100 g body wt except for adrenal (mg/100 g body wt).
ug/100 ml serum.
ug/g tissue (wet wt).
* $P < 0.05$ compared to ZnDEF.
** $P < 0.01$ compared to ZnDEF.
*** $P < 0.001$ compared to ZnDEF.

ciency. This may have resulted from competition with EFA by the oleic acid present in large amounts (76%) in olive oil. Dietary olive oil has been shown to induce marginal EFA deficiency (6) which may also help account for its effect in worsening the sumptoms of Zn deficiency.

The safflower oil contained linoleic acid in similar amounts to the evening primrose oil but contained no γ-linolenic acid. The safflower oil was of only marginal benefit with respect to weight gain (9% greater than the ZnDEF group), although the total weight gained in this group was twice that of the ZnDEF group. Greater food intake by the ZnSFO and ZnEPO groups may also have contributed to their greater weight gain than the ZnDEF group.

The dermal scores of the ZnSFO group were markedly improved compared to the ZnDEF group and were comparable to those of the ZnEPO group suggesting a direct benefit of linoleic acid alone in overcoming the dermal lesions caused by Zn deficiency. Linoleic acid has been shown previously to have a direct beneficial effect on the skin in EFA deficiency (7).

Treatment of the Zn deficient rats with EPO shifted all the organ weights substantially toward the values in the control rats. This was particularly true of thymus and epididymal fat and contrasted with the lack of effect of safflower and olive oil. The much greater effect of evening primrose oil than the other oils on body and organ weights suggested that γ-linolenic acid was important in blocking the effect of Zn deficiency on growth and development. Linoleic acid is normally converted to γ-linolenic acid by an NADPH-dependent desaturase system. Zn has been shown to be important in the NADPH–NADP cycle and it is possible that an important effect of Zn deficiency was to inhibit this enzyme (8, 9). The results are also consistent with the possibility that Zn had an additional effect in mobilizing free DGLA from its esterified form in membrane stores but do not provide any direct evidence for this (4). Supplementation with the γ-linolenic acid would provide DGLA directly, bypassing the need for its mobilization.

This hypothesis is further supported by the finding that arachidonic acid levels in the plasma and dorsal skin of Zn deficient rats remain unchanged compared to controls (5). Similarly, in acrodermatitis enteropathica, in which severe deficiencies of both Zn and EFA are implicated, arachidonic acid in plasma has been shown not to be significantly different from control, nor was arachidonic acid of any clinical benefit when administered (10).

Zn measurement of tissues such as pituitary, adrenal, and liver confirmed that even severe Zn deficiency does not alter tissue Zn concentration (1, 11). The increase in serum Zn in the ZnEPO group suggests that there may have been endogenous mobilization of Zn, increased efficiency of Zn absorption, or Zn contamination due to coprophagy in this group. These suggestions cannot be substantiated at this stage. The extremely low Zn concentration in all of the supplemental oils precludes the possibility that significant exogenous Zn was available from this source. The elevated values for serum Zn in the Zn(EPO) group (48 μg/100 ml) were still well within the range normally reported for serum Zn in Zn deficiency.

Supplementation of female Zn deficient rats with evening primrose oil has been shown to improve their weight gain to within 70–80% of control (1). This difference with the males reported in the present study (40–50% of control) may be due to a lower EFA requirement of the females (12). A beneficial effect of corn oil (45% linoleic acid) on the growth of male rats has been reported elsewhere (5).

We conclude that the onset of the gross effects of Zn deficiency, particularly in proliferating tissues such as skin, can be inhibited by parenteral administration of EFA containing linoleic and γ-linolenic acids. In view of the fact that corn oil was present in the diets of the Zn deficient rats, it is significant that subcutaneous injections of the EFA-containing oils inhibited the onset of the Zn deficiency symptoms. A defect in fatty acid absorption in the Zn deficient rats is therefore a possibility.

APPENDIX

Composition of the Zinc Deficient Diet by Percentage

1. Egg white solids	20.0

2. Sucrose	31.0
3. Corn starch	31.2
4. Corn oil	10.0
5. Cellulose powder	3.0
6. Vitamin premix (see A)	0.5
7. Mineral premix (see B)	4.0
8. Choline chloride	0.3

A. Vitamin Premix (Murthy's)[a]

1. Vitamin A (palmitate)	30,000 IU
2. Vitamin D_2 (Calciferol)	4000 IU
3. Vitamin E (DL-α-tocopherol)	100 IU
4. Vitamin K	2 mg
5. Vitamin B_{12}	0.02 mg
6. Thiamin mononitrate	20.58 mg
7. Riboflavin	20.0 mg
8. Niacinamide	100 mg
9. D-Calcium pantothenate	60 mg
10. Pyridoxine hydrochloride	10 mg
11. Folic acid	0.5 mg
12. D-Biotin	1.0 mg
13. *i*-Inositol	333 mg

[a] Yield of vitamins/kg of diet.

B. Mineral Premix—100% (Bernhart and Tomarelli)

1. $CaCO_3$	2.10
2. $CaHPO_4$	73.5
3. $Cu_2C_6H_4O_7\ 5H_2O$	0.05
4. $Fe(C_6H_4O_7)5H_2O$	0.56
5. MgO	2.50
6. $MnH(C_6H_4O_7)$	0.84
7. KI	0.001
8. K_2HPO_4	8.10
9. K_2SO_4	6.80
10. NaCl	3.06
11. Na_2HPO_4	2.14
12. Citric acid	0.23

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