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Maternal Essential Fatty Acid Supplementation Increases Zinc Absorption in Neonatal Rats: Relevance to the Defect in Zinc Absorption in Acrodermatitis Enteropathica[30]

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Summary

Pregnant zinc deficient and zinc adequate rats were injected subcutaneously with evening primrose oil throughout gestation and for 3 days post partum. The nursing pups were injected intragastrically with zinc-65 on day 3 of life and sacrificed 4 h later. The % of the total injected zinc recovered in the carcass (minus the gut and gut contents) was significantly increased in those pups nursed by mothers injected with evening primrose oil, regardless of their dietary zinc intake. The fatty acid composition of the total lipid extract of the gut and gut contents of the neonates with increased zinc-65 absorption indicated that these pups had higher proportions of arachidonic acid and other metabolites of linoleic acid than did those with lower zinc-65 absorption. In other 3-day-old rat pups, intragastric injection of linoleic, gamma-linolenic or dihomo-gamma-linolenic acids along with the dose of zinc-65 very significantly increased zinc-65 absorption in a dose-related manner. Arachidonic acid however had no significant effect on zinc-65 absorption. Prostaglandin E_1 caused a significant increase in zinc-65 absorption but prostaglandin E_2 had no consistent effect. Indomethacin caused a dose-related inhibition of zinc-65 absorption.

Speculation

The increase in absorption of zinc-65 in neonatal rats suckled by mothers previously treated with evening primrose oil (81% essential fatty acids) is probably due to the transfer, in the breast milk, of increased amounts of the essential fatty acids–linoleic, gamma-linolenic, and dihomo-gamma-linolenic acid–and possibly prostaglandin E_1. It is suggested that this observation can account in part for the fact that human breast milk increases the absorption of zinc by human infants when compared with cow's milk formulae. The data would furthermore indicate that the onset of the symptoms of acrodermatitis enteropathica, which invariably occurs on weaning from human breast milk, may be exacerbated by the significantly lower essential fatty acid content of cow's milk or milk formulae compared to human breast milk. Essential fatty acids and possibly some of their metabolites such as prostaglandin E_1, which are present in human breast milk, may therefore be important for adequate zinc absorption in the neonatal period.

The lethal inherited childhood disease, acrodermatitis enteropathica (AE) has been identified as a disorder of zinc absorption (2) and responds with essentially complete remission to oral zinc supplementation (18). Before its identification as a disorder of zinc absorption AE was shown to cause defective essential fatty acid (EFA) metabolism (4, 26). The parent EFA, linoleic acid (18:2ω6), is metabolized via gamma-linolenic acid (18:3ω6) to dihomo-gamma-linolenic acid (20:3ω6) that in turn may be directly converted to 1 series prostaglandins including prostaglandin E_1 or maybe further metabolized to arachidonic acid (20:4ω6). Arachidonic acid is the precursor of the 2 series prostaglandins which include prostaglandin E_2 and $F_{2\alpha}$. Remission occurs in some infants for whom breast feeding is resumed (4) and has also been shown to occur in three infants administered an intravenous fat emulsion containing cottonseed oil (26); however, feeding infants with AE on cow's milk or milk formulae invariably worsens their condition. In fact it is often the transfer of the infant from breast milk to cow's milk that exacerbates the disease and allows the initial diagnosis of AE to be made.

The questions therefore arise: if AE is a disorder of zinc absorption, why do infants with AE respond positively to breast milk but not to cow's milk and why would there be an improvement after intravenous feeding of cottonseed oil? The zinc content of mature human breast milk is significantly less than in cow's milk (16, 24) although other reports have shown the two milks to have similiar zinc contents (7, 27), and there is no appreciable amount of zinc in cottonseed oil. Therefore the availability of dietary zinc must be affected by a factor common to human breast milk and cottonseed oil. EFA are present in amounts 6–8 times greater in human breast milk than in cow's milk (15) and one of the main EFA, linoleic acid, is present in cottonseed oil at 45% by weight. Enhanced zinc absorption due to the increased amounts of linoleic acid or its metabolites in breast milk or cottonseed oil may therefore be part of the mechanism by which children with AE respond favourably to these two preparations but not to cow's milk.

The possibility that EFA may alter zinc absorption in neonatal rats was therefore addressed in this study. The absorption of zinc-65 by rat pups suckled by mothers injected throughout gestation with evening primrose oil (EPO, 81% EFA) was compared to that of controls. The effect of maternal zinc deficiency on zinc-65 absorption by rat pups was also assessed for purposes of comparison. The fatty acid composition of the entire gut and gut contents of the rat pups in each group was measured to determine which EFA may have affected zinc-65 absorption. The effects of individual EFA, prostaglandins, and the prostaglandin synthesis inhibitor-indomethacin on zinc-65 absorption by neonatal rats were also studied in order to determine whether EFA or prostaglandins were responsible for differences in zinc-65 absorption.

MATERIALS AND METHODS

Second parity female hooded Lister rats of the Rowett strain were mated with males of the same strain. They were kept under 'barrier-maintained' conditions and were housed individually in polypropylene-stainless steel cages. From day 1 of gestation (the day on which conception plugs were found) the following experimental diets were offered: (1) control-untreated, 20 ppm zinc diet; (2) control-treated, 20 ppm zinc diet plus daily EPO injection; (3) chronic zinc deficient-untreated, 5 ppm zinc diet; (4) chronic zinc

deficient-treated, 5 ppm zinc diet plus daily EPO injection; (5) acute zinc deficient-untreated, 10 ppm zinc diet for 15 days followed by 0.5 ppm zinc diet for remainder of gestation; and (6) acute zinc deficient-treated, 10 ppm/0.5 ppm zinc diet as in (5) plus daily EPO injection.

The diets were semi-synthetic and were based on egg albumin, sucrose, arachis oil, inorganic salts, minerals and vitamins. They were prepared as previously described (28). The diets and deionized water were available *ad libitum*. The EPO was injected subcutaneously between the scapulae in graded amounts from 500 μl/kg/day on day 1 of gestation to 700 μl/kg/day during lactation. The EPO contained 1.7 μg/ml/zinc; an amount which provided no more than 5 μg of zinc to each rat so treated over the course of the experiment. Its fatty acid composition: linoleic acid (72%), gamma-linolenic acid (9%), oleic acid (10%), and other fatty acids (9%).

The rats were allowed to litter under natural conditions and the mothers nursed the surviving pups. On day 3 of lactation, the pups from each mother were given a direct transabdominal intragastric injection of zinc-65 (0.2 μCi in 100 ml saline; specific activity, 1 mCi/kg, Amersham) under light ether anesthesia and returned to their respective mothers. Four h after the injection they were sacrificed by prolonged ether anesthesia. The whole body radioactivity was measured and then the gut and gut contents were dissected from the carcass and the radioactivity in both compartments measured using a well-type gamma counter (Tracerlab, Hersham, England).

The entire gut and gut contents from stomach to large intestine of the neonates in each group were homogenized and saponified with ethanolic KOH. The non-saponified fats were extracted with ether and discarded. The remaining aqueous layer was acidified and the lipids extracted with ether. The methyl esters of fatty acids were prepared using diazo methane (CH_2N_2) and the total fatty acid composition of the lipids analyzed by gas chromatography (25).

To determine the possible effects of specific EFA, prostaglandins or indomethacin on zinc-65 absorption by neonatal rats, a further experiment was run using 3 day old rat pups divided into the following treatment groups: (1) linoleic acid; (2) gamma-linolenic acid; (3) dihomo-gamma-linolenic acid; (4) arachidonic acid; (5) prostaglandin E_1; (6) prostaglandin E_2; and (7) indomethacin. Each group was subdivided so that a minimum of three rat pups per group were controls for that group while the other rat pups in each group received the treatment at one of three concentrations (see the captions to Figures 1 and 2 for concentrations of EFA, prostaglandins, and indomethacin used). All the pups were injected with the zinc-65 as previously described. The EFA, prostaglandins or indomethacin were injected simultaneously with the zinc-65. The EFA and prostaglandins were initially dissolved in ethanol before being injected with the zinc-65 in the saline vehicle.

Statistical analysis of the data was done using Student's *t* test.

RESULTS

As shown in Table 1, zinc-65 absorption was significantly increased in the pups in the acute zinc deficient group compared to the untreated chronic zinc deficient and untreated control groups, an effect which has been reported elsewhere (21). In the surviving pups of mothers treated with EPO, zinc-65 absorption was significantly increased regardless of dietary zinc intake; in the chronic zinc deficient group, maternal EPO treatment increased zinc-65 absorption in the pups by 55% ($P < 0.05$) whereas in the controls, EPO treatment increased zinc-65 absorption by 34% ($P < 0.01$).

The fatty acid composition of the gut and gut contents of the neonates in each group is shown in Table 2. In the groups in which zinc absorption was increased (acute zinc deficient-untreated, chronic zinc deficient-EPO treated and control-EPO treated), arachidonic acid was increased above that found in the untreated controls. In addition, the ratio of linoleic acid to arachidonic acid was significantly lower in the three groups in which zinc absorption was enhanced. In the chronic zinc deficient group (5 ppm zinc diet) treated with EPO in which zinc absorption was increased by 55% compared to the untreated chronic zinc deficient group, there was an increase in palmitic acid, in the palmitic-palmitoleic acid ratio, and an increase in the 18:3 acids (ω3 and ω6). In the control group treated with EPO, in which zinc absorption was increased by 34% compared to the untreated controls, palmitic acid was decreased but arachidonic acid and the 20:3 acids (ω6 and ω9) were increased ($P < 0.05$). Although linoleic acid levels in the gut and gut contents of the various groups (whether zinc deficient, EPO treated or zinc deficient and EPO-treated) remained the same, the products of linoleic acid metabolism were elevated in those groups in which zinc absorption was also elevated.

Table 1. *% absorption of intragastrically injected zinc-65 (0.2 μCi in 100 μl saline; specific activity, 1 mCi/kg in neonatal rat pups from mothers maintained on zinc adequate (20 ppm zinc) or zinc deficient diets (5 ppm or 10–0.5 ppm zinc) and either untreated or injected concurrently with evening primrose oil (EPO).*

Dietary zinc content (ppm)	% absorption[1]	
	Untreated	EPO treated[2]
20	18.8 ± 1.4[4] (8)	25.1 ± 1.9[6] (11)
5	17.2 ± 4.8 (5)	26.7 ± 1.8[7] (12)
10–0.5[3]	32.3 ± 2.3[8] (3)	[5]

[1] Amount of radioactivity in carcass minus that in the gut and gut contents as a % of the total radioactivity recovered.

[2] Mothers in these groups were subcutaneously injected with EPO 500–700 μl/kg/day) throughout gestation.

[3] 10 ppm zinc diet for the first 15 days of gestation followed by 0.5 ppm zinc diet for the remainder.

[4] Mean ± S.E.; number of rat pups in each group is in brackets.

[5] 100% mortality of pups born to mothers in this group.

[6] $P < 0.01$ compared to the untreated 20 ppm group.

[7] $P < 0.05$ compared to the untreated 5 ppm group.

[8] $P < 0.05$ compared to all other groups.

The effects of increasing concentrations of the individual EFA on zinc-65 absorption are shown in Figure 1. Arachidonic acid had no significant effect on zinc-65 absorption, while linoleic and gamma-linolenic acids very significantly increased zinc-65 absorption ($P < 0.01$, at all concentrations). Dihomo-gamma-linolenic acid increased zinc-65 absorption at 10 ng and 1 μg ($P < 0.05$).

The effects of prostaglandins E_1, E_2, and indomethacin on zinc-65 absorption are shown in Figure 2. Indomethacin caused inhibition of zinc-65 absorption which reached 100% in the rat pups injected with 100 μg indomethacin. Prostaglandin E_1 caused a small but significant increase in zinc absorption but prostaglandin E_2 had no consistent effect.

DISCUSSION

These results demonstrate that subcutaneous EPO injection into pregnant rats throughout gestation significantly increases zinc absorption in neonatal rats irrespective of the maternal dietary zinc intake. This suggests that the mechanism regulating zinc absorption in neonates is responsive not only to changes in zinc intake by the mother, but even where zinc availability is adequate, is also responsive to increased maternal EFA intake. The results are consistent with a previous report in which supplemental EFA were shown to increase plasma zinc levels in rats (8).

The data on fatty acid composition of the combined gut and gut contents suggest that the increased maternal EFA intake affected zinc absorption by increasing the availability of the

Table 2. *Total lipid fatty acid composition of combined gut wall and gut contents from 3-day-old rat pups*

	Dietary zinc content (ppm)				
Fatty acids	10/0.5 (3)	5 (5)	5-EPO[4] (8)	20 (8)	20-EPO[4] (8)
16:0	6.1 ± 1.0[3]	6.6 ± 0.8	7.7 ± 1.3	9.5 ± 1.4[5]	6.5 ± 0.4[9]
16:1ω7	5.3 ± 0.4	6.1 ± 0.8	5.8 ± 1.3	5.4 ± 1.5	7.0 ± 0.4[5]
18:0	1.5 ± 0.2	2.1 ± 0.2	2.3 ± 0.7	2.4 ± 0.2[6]	2.5 ± 0.3[5]
18:1ω9	35.9 ± 4.2	38.6 ± 2.6	34.2 ± 3.6	36.5 ± 3.1	34.4 ± 1.8
18:2ω6	26.2 ± 0.8	26.2 ± 0.5	25.2 ± 0.6	26.4 ± 1.4	25.5 ± 0.2
18:3[1]	3.4 ± 0.3	3.0 ± 0.2	4.7 ± 0.6[5, 7]	3.5 ± 0.4	4.0 ± 0.8
20:3[2]	2.8 ± 0.4	3.1 ± 0.4	4.0 ± 1.5	2.8 ± 0.4	4.4 ± 0.5[5, 9]
20:4ω6	14.2 ± 1.7	11.0 ± 0.9[5]	12.5 ± 0.6	9.3 ± 0.7[6, 8]	13.6 ± 1.3[9]
20:5ω3	4.5 ± 0.7	3.3 ± 1.2	3.5 ± 1.2	2.4 ± 0.5[a]	2.5 ± 0.6[5]
Fatty acid ratios					
16:0/16:1ω7	1.15 ± 0.09	1.09 ± 0.2	1.37 ± 0.14[7]	1.98 ± 0.40[5]	0.95 ± 0.07[9]
18:0/18:1ω9	0.04 ± 0.01	0.05 ± 0.01	0.07 ± 0.02	0.07 ± 0.01[5]	0.08 ± 0.01[5]
18:2ω6/20:4ω6	1.97 ± 0.41	2.41 ± 0.20	2.02 ± 0.05[7]	2.88 ± 0.25[8]	1.91 ± 0.19[9]

[1] ω3 and ω6 acids.
[2] ω6 and ω9 acids.
[3] Means ± S.E.
[4] EPO-evening primrose oil-treated groups.
[5] $P < 0.05$ with respect to 10/0.5 ppm zinc group.
[6] $P < 0.01$ with respect to 10/0.5 ppm zinc group.
[7] $P < 0.05$ between the 5 ppm zinc and 5 ppm zinc-EPO groups.
[8] $P < 0.05$ between the 20 ppm zinc and 5 ppm zinc-EPO groups.
[9] $P < 0.05$ between the 20 ppm zinc and 20 ppm zinc-EPO groups.

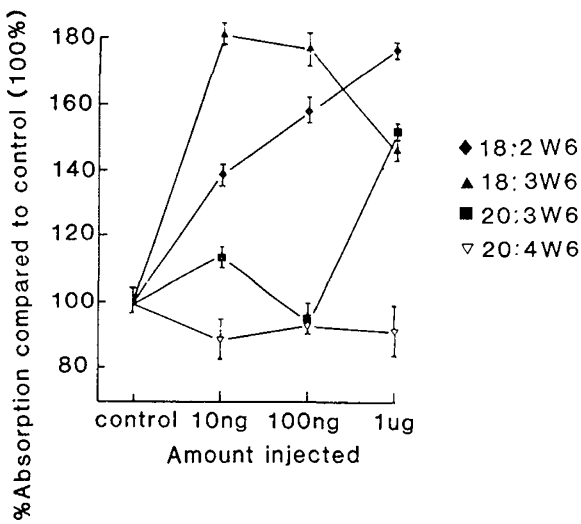

Fig. 1. The effect of individual essential fatty acids (EFA) on the absorption of zinc-65 by neonatal rats. Each EFA was dissolved initially in ethanol and subsequently injected intragastrically at each of three concentrations concurrently with the zinc-65, both of which were dissolved in 100 μl of the saline carrier. Each point represents the mean ± S.E. of injections into four rat pups. The mean absorption of zinc-65 by control groups for each treatment group is shown as 100% and the relative absorption differences in the EFA treated groups are shown as percentages of the control values. Linoleic acid (18:2ω6) and gamma-linolenic acid (18:3ω6) significantly increased zinc-65 absorption at all three concentrations tested ($P < 0.01$). Dihomo-gamma-linolenic acid (20:3ω6) increased zinc-65 absorption at 10 ng ($P < 0.05$) and 1 μg ($P < 0.01$) but had no effect at 100 ng. Arachidonic acid (20:4ω6) had no significant effect on zinc-65 absorption.

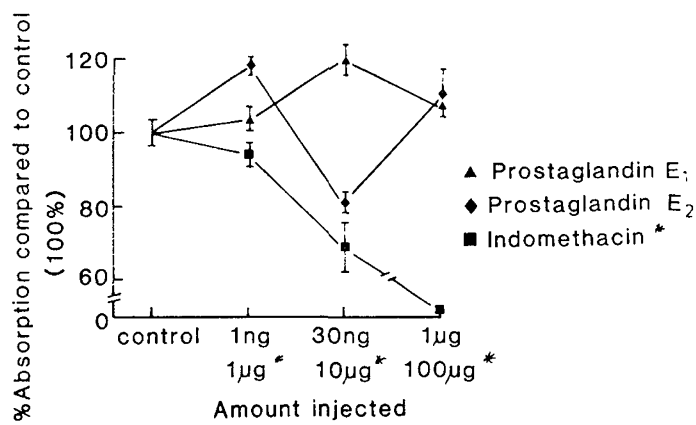

Fig. 2. The effect of prostaglandins E_1 and E_2 and indomethacin on absorption of zinc-65 by neonatal rats. The prostaglandins were dissolved initially in ethanol and subsequently injected intragastrically at each of three concentrations concurrently with the zinc-65, both of which were dissolved in 100 μl of the saline carrier. Each point represents the mean ± S.E. of injections into four rat pups. The mean absorption of zinc-65 by control groups for each treatment group is shown as 100%. The relative absorption differences in the treated groups are shown as percentages of these control values. Prostaglandin E_1 significantly increased zinc-65 absorption at 30 ng and 1 μg ($P < 0.05$) but had no significant effect at 1 ng. Prostaglandin E_2 increased zinc-65 absorption at 1 ng and 1 μg but decreased it at 30 ng ($P < 0.05$). Indomethacin caused a progressive decrease in zinc-65 absorption which was significant at 10 μg and 100 μg ($P < 0.01$).

linoleic acid metabolites, gamma-linolenic acid, dihomo-gamma linolenic acid, and arachidonic acid. This is supported by the finding that the ratio of linoleic to arachidonic acid was decreased in those neonates in which zinc absorption was increased, *e.g.*, the metabolites of linoleic acid but not linoleic acid itself were responsible for the effect of EPO injection.

The effects of injection of specific EFA on zinc-65 absorption (Fig. 1) suggest that linoleic acid itself may also contribute to zinc absorption without necessarily being metabolized to gamma-linolenic, dihomo-gamma-linolenic, or arachidonic acids; however, arachidonic acid had no significant effect on zinc-65 absorption

by the neonatal rats used in these studies. Linoleic acid was not elevated in the gut total fatty acids of the rat pups in which zinc absorption was increased even though it greatly enhanced zinc-65 absorption and conversely arachidonic acid was elevated but by itself had no significant effect on zinc-65 absorption; however, these findings are not necessarily inconsistent because the amount of injected linoleic acid which affected zinc-65 absorption (10 ng–1 μg) would not have increased the total measurable tissue content of linoleic acid. Also, the majority of linoleic acid in the total lipid fatty acid composition of the gut and gut contents would not be available to influence zinc absorption. It is also interesting to note that arachidonic acid infusion intravenously in an infant with AE had no beneficial effect on the infants condition (4). This correlates with the lack of effect of arachidonic acid on zinc-65 absorption presented here.

The mechanism by which the EFA affected zinc absorption is not known. Dietary EFA restriction has been shown to inhibit vitamin D-dependent calcium absorption in rats (13), which combined with the present results, suggests that EFA may affect cation absorption in general possibly by affecting the intestinal brush-border lipid composition thereby altering membrane permeability. Evidence has recently been presented which suggests that the defect in EFA metabolism caused by zinc deficiency (5, 8) may be an increase in the rate of Δ^6 and Δ^9 desaturation of long chain polyunsaturated fatty acids (6, 9, 10). This defect tends to result in an accumulation of linoleic acid metabolites in the tissues of zinc deficient animals (3, 10, 14). Hence the increase in zinc-65 absorption in zinc deficient rats is consistent with the known effect of zinc deficiency on EFA metabolism and suggests a feedback mechanism by which zinc absorption may be increased by EFA in zinc deficient rats.

The inhibitory effect of indomethacin on zinc-65 absorption (Fig. 2) is in agreement with previous observations on the effects of inhibitors of prostaglandin synthesis on zinc absorption (11, 21, 23). Prostaglandin E_2 has been reported to either increase (23) or decrease (21) zinc absorption. The data in Fig. 2 suggest that if less than 1 μg is injected into the stomach of a rat pup weighing 4–6 g, there is no consistent effect of prostaglandin E_2 on zinc absorption; however prostaglandin E_1 did cause a small but significant increase in zinc-65 absorption (Fig. 2). Whether or not prostaglandins affect the absorption of zinc by forming binding complexes as suggested elsewhere (23) is debatable. It seems more likely that prostaglandins would influence zinc absorption by altering factors such as blood flow to the gut, gut motility or a variety of factors influencing cation absorption in general. Prostaglandin E_1 is vasodilatory at the concentrations used in this experiment (17) and it increased zinc-65 absorption whereas both prostaglandin E_2 and $F_{2\alpha}$ have vasoconstrictor effects (17) and both these prostaglandins have been shown to inhibit zinc-65 absorption (21, 22).

If zinc absorption can be influenced by enhanced maternal transfer of EFA to the neonate as has been demonstrated here, this finding has direct repercussions in human nutrition. In particular, it provides a mechanism for the well known but poorly understood fact that zinc availability to human infants is significantly greater when the infant is fed human breast milk compared to cow's milk (5, 12, 16, 27). Human breast milk contains 6–8 times as much linoleic acid, dihomo-gamma-linolenic acid, and arachidonic acid as cow's milk (15, 20). Therefore the increase in zinc availability when an infant is breast fed may be due to the greater amount of EFA present in breast milk, a situation analogous to what is reported here. In infants in which zinc absorption may already be compromised, *e.g.*, in AE, weaning from breast milk to cow's milk or other milk formulae would exacerbate the defect in zinc absorption resulting in full development of the symptoms as has been previously reported (4, 26).

Recent evidence has been presented suggesting that the ability of human breast milk to enhance zinc absorption, particularly in AE, is not necesarily due to a specific low molecular weight zinc ligand (19). If this is so, it would appear that it is not the absence of a specific ligand, which so critically affects zinc absorption in AE, but rather a defect in the zinc uptake mechanism itself. In the absence of adequate amounts of cofactors including the EFA, such a defect would presumably block zinc absorption even in the presence of an adequate amount of zinc or zinc ligands.

Although other factors are undoubtedly present in human breast milk which influence zinc absorption (1), the effect of cottonseed oil in AE and the effect of EPO and specific EFA on zinc absorption in the rat suggest that linoleic acid and its metabolites have a signficant function in regulating zinc absorption from milk in both humans and the rat.

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