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Gestational Zinc Deficiency Amplifies the Regulation of Metallothionein Induction in Adult Mice (42702)

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Abstract. To determine if prenatal zinc deficiency has a persistent effect on metallothionein (MT) regulation, Swiss–Webster mice were mated and fed a diet containing either control (100 μg Zn/g) or low levels of zinc (5 μg Zn/g) from Day 7 of gestation to parturition. After birth all mice were given the control diet. Liver zinc and MT levels were 50% lower in newborn pups from dams fed the low zinc diets than in control pups. In control pups, liver zinc and MT concentrations were relatively stable during the first week of postnatal life. In contrast, in pups prenatally deprived of zinc, liver levels of zinc and MT increased such that by Day 3 of postnatal life, the levels were not significantly different from controls. At Day 56, serum IgM concentrations were significantly lower in the low zinc offspring. Liver zinc concentrations in the two groups of mice were similar at Day 70 postnatal, and in both groups liver MT levels were below detection limits. However, when Day 70 mice were given zinc injections to stimulate MT synthesis, the prenatally zinc deprived offspring showed markedly higher liver MT levels than did control mice given similar injections, despite similar liver zinc concentrations in the two groups. These results show that prenatal zinc deficiency has pronounced effects on postnatal MT metabolism which can persist into adulthood.

Gestational zinc deficiency in mice during the last two-thirds of pregnancy can have a detrimental influence on postnatal immune function, despite postnatal zinc repletion of the animals. For example, a gestational diet only moderately low in zinc (5 μg Zn/g) has been shown to result in abnormal serum immunoglobulin profiles in the offspring up to 6 months of age, and to reduce plaque-forming cell response to immunization with sheep red blood cells compared with those of controls (1, 2). The significance of these results is underscored by the observation that some of these effects are multigenerational, with the serum IgM and plaque-forming cell abnormalities in the prenatally zinc-deprived group persisting through three generations (1, 2).

We reasoned that although the immune system may be particularly susceptible to prenatal zinc deprivation, similar effects could be occurring in other zinc regulated systems. In particular, we were interested in whether zinc deficiency during gestation would alter the postnatal expression of metallothionein (MT), a protein involved in cellular zinc metabolism. Metallothioneins are a family of proteins of low molecular weight which have a high binding capacity for divalent metals (3). The synthesis of MT is regulated in part by metals such as zinc and copper and it has been suggested that MTs may serve as storage proteins for these essential trace elements (3–6). In many species, including the mouse, there is a rapid increase during the latter part of gestation in the synthesis of MT in fetal liver, which may be induced by the fetal accumulation of zinc and copper during this period (7–9). In addition to their putative storage function, MTs have also been implicated in the transfer of metals to apoproteins (3, 10). In the present study, we tested the hypothesis that prenatal zinc deficiency would produce a down regulation in the postnatal expression of MT that would persist into adulthood, resulting in abnormal postnatal zinc metabolism. This hypothesis would thus provide a possible explanation for the transgenerational effects of zinc deficiency previously observed in the immune system.

Materials and Methods. Following mating, young adult Swiss–Webster mice were fed a control purified diet (100 μg Zn/g) *ad*

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libitum until Day 7 of pregnancy when they were divided into two groups and fed either the control diet or a purified diet of the same composition containing a low concentration of zinc (5 μg Zn/g). The detailed composition of the diet has been published (2). The choice of these dietary levels of zinc was based on our previous studies of the effects of prenatal zinc deficiency on postnatal immune function (1, 2). Because zinc deficiency is known to cause inanition, which itself can stimulate MT synthesis (11, 12), a third group of restricted intake controls were fed the control diet in the same amounts eaten by the low zinc group. During the period of zinc deprivation, all mice were maintained under conditions designed to reduce zinc contamination (1, 2). At parturition (Day 0 of postnatal life) all dams and offspring were fed the control diet, which they consumed until the end of the experiment.

The concentrations of zinc, copper, iron, and MT in liver and kidney were assessed in offspring on Day 0 (the day of birth), Days 1, 3, and 5 and at 10 weeks of age. Tissue trace mineral concentrations were determined by flame atomic absorption spectrophotometry following wet ashing with concentrated nitric acid as described by Clegg *et al.* (13). Copper and iron concentrations were determined in addition to zinc to ascertain if any changes observed in tissue zinc were specific. MT was measured using the hemoglobin–cadmium saturation method of Onosaka and Cherian (14). At 8 weeks of age, blood was obtained by orbital sinus puncture and plasma IgM was measured using single radial immunodiffusion (15). At 10 weeks of age, basal levels of liver MT and zinc were measured in each group. Induced levels of liver and kidney MT were also measured by giving two injections of zinc (5 mg zinc/kg body weight), 32 and 8 hr before killing. The level of zinc used to induce MT synthesis in the adult mice and the timing of the injections were based on previous reports (3, 16). All data were analyzed using one-way analysis of variance with Duncan's multiple range test (17). All data are shown as means ± SE.

Results. The Day 0 neonates from dams in the prenatally zinc deprived group had significantly lower liver concentrations of zinc and MT compared to the two control groups (Table I). The levels of liver MT of control pups did not change significantly from Day 0 to Day 5, whereas liver MT concentrations of low zinc pups increased, reaching control levels by Day 3 (Table I). The zinc concentrations of the livers correlated with MT levels in all groups, remaining stable in controls while increasing in low zinc pups.

Liver copper and iron concentrations were not different among the groups, and did not change during the first week, indicating that marginal zinc deficiency did not alter metabolism of other elements (Table I).

TABLE I. THE EFFECT OF PRENATAL DIET ON LIVER MT, Zn, Cu, AND Fe LEVELS DURING THE FIRST 5 DAYS POSTNATAL

		Day 0 (μg/g)	Day 1 (μg/g)	Day 3 (μg/g)	Day 5 (μg/g)
MT	Control	567 ± 49[a]	733 ± 43[a]	601 ± 26[a]	664 ± 37[a]
	Low zinc	186 ± 36[b]	353 ± 26[b]	627 ± 35[a]	479 ± 25[b]
	Restricted	698 ± 54[a]	634 ± 74[a]	715 ± 155[a]	469 ± 70[b]
Zn	Control	57.6 ± 4.8[a]	45.6 ± 10.7[a,b]	54.4 ± 4.6[a,b]	61.9 ± 4.2[a]
	Low zinc	29.8 ± 3.0[b]	27.4 ± 8.3[b]	60.3 ± 2.8[b]	58.3 ± 4.5[a,b]
	Restricted	58.6 ± 4.8[a]	60.1 ± 6.3[a,c]	72.4 ± 8.9[a,c]	48.0 ± 3.1[b,c]
Cu	Control	28.3 ± 5.6[a]	30.1 ± 4.6[a]	33.0 ± 6.3[a]	28.8 ± 3.6[a]
	Low zinc	32.0 ± 3.1[a]	35.8 ± 0.2[a]	32.2 ± 2.6[a]	29.6 ± 4.3[a]
	Restricted	26.3 ± 2.1[a]	30.9 ± 3.6[a]	36.7 ± 4.8[a]	25.7 ± 5.5[a]
Fe	Control	137.4 ± 20.6[a]	196.5 ± 45.8[a]	86.8 ± 23.3[a]	68.7 ± 16.1[a]
	Low zinc	131.5 ± 27.9[a]	167.5 ± 73.7[a]	164.3 ± 26.3[b]	102.3 ± 26.6[a]
	Restricted	158.7 ± 23.8[a]	178.3 ± 45.8[a]	175.1 ± 20.3[a,b]	70.3 ± 19.2[a]

Note. Values are means ± SE. In each column, values not sharing a common superscript are statistically different at $P < 0.05$. Each mean represents an n from at least three different litters.

TABLE II. PLASMA IgM LEVELS OF 8-WEEK-OLD MICE

Prenatal diet	(*n*)	IgM (mg/100 ml)
Control, *ad lib.*	6	8.82 ± 1.56^a
Prenatally zinc-deprived	6	2.45 ± 1.71^b
Control, restricted	6	7.19 ± 2.93^a

Note. Values are shown as means ± SE. Means not sharing a common superscript are significantly different at $P < 0.05$.

Kidney MT levels were not detectable in the newborn pups, although kidney zinc concentrations were lower in the zinc-deficient group than in controls on Day 0 (results not shown).

Consistent with our previous findings, 8-week-old mice from the low zinc groups had low levels of plasma IgM (Table II) (1). At 10 weeks of age, basal liver zinc levels were not different among the groups, with mean concentrations of 43 ± 5, 34 ± 3, and 33 ± 6 μg/g in the control, prenatally zinc deprived, and restricted fed mice, respectively. Basal MT levels in the 10-week-old mice were too low to be measured by the hemoglobin–cadmium method, in all three groups. After the zinc injections, the liver zinc concentrations were also not different among the groups; however, the 10-week-old offspring of dams fed the low zinc diet had significantly higher levels of induced liver MT than did the control offspring (Fig. 1). Basal kidney zinc concentrations were similar in the three groups, 33 ± 2, 28 ± 3, and 30 ± 4 μg/g in the control, prenatally zinc deprived, and restricted fed mice, respectively. In contrast to liver, an amplification of MT was not observed in the kidneys of the prenatally zinc deprived 10-week-old mice compared to zinc-injected controls, suggesting some tissue specificity in this response (Fig. 2).

Discussion. Liver MT was low at birth in the prenatally low zinc pups, implying that MT production during development is partly determined by zinc intake. Liver MT was induced rapidly in the prenatally low zinc neonatal pups by the zinc repletion of the dams, as has also been seen in rat pups (18).

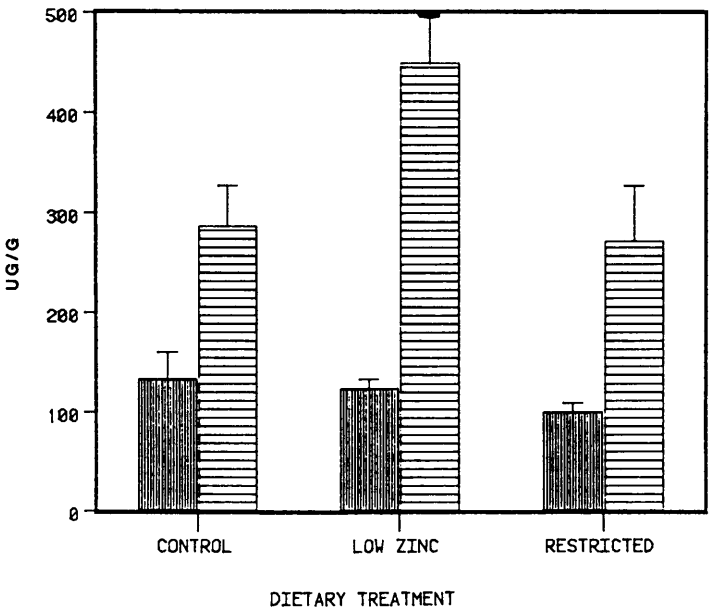

FIG. 1. Liver zinc (▥) and liver metallothionein (□) concentrations of 70-day-old mice from dams fed diets containing adequate zinc (100 μg/g diet) *ad libitum* or restricted intake, or marginal zinc (5 μg/g diet), during the last two-thirds of pregnancy. After parturition, all groups were fed the diet containing 100 μg zinc/g diet. Values are shown as means ± SE. A minimum of eight mice was used in each group. The liver MT concentration of the low zinc group was significantly higher than that of controls ($P < 0.05$).

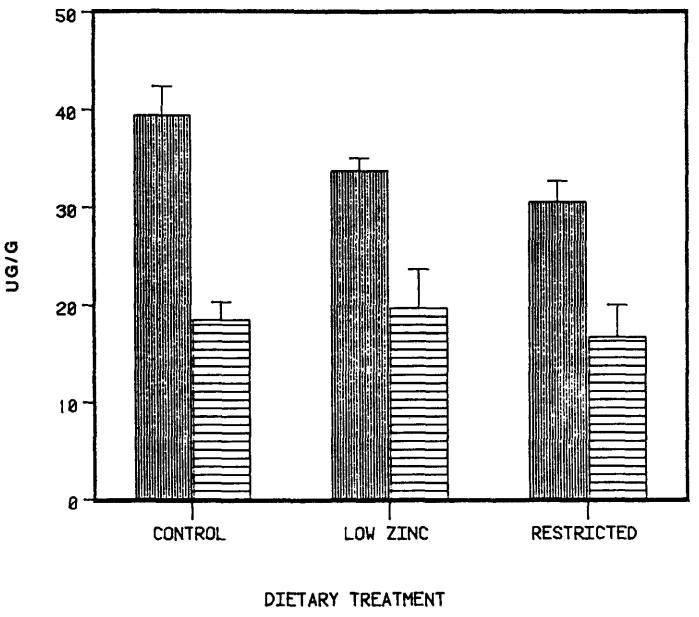

FIG. 2. Kidney zinc (▥) and kidney metallothionein (▤) concentrations of 70-day-old mice from dams fed diets containing adequate (100 μg zinc/g diet) *ad libitum* or restricted intake, or marginal (5 μg zinc/g diet) zinc during the last two-thirds of pregnancy. After parturition, all groups were fed the diet with adequate zinc. Values are shown as means ± SE. A minimum of eight mice was used in each group. There were no significant differences in kidney zinc or MT among the groups.

The observation of increased concentrations of liver MT in 10-week-old mice exposed to zinc deficiency *in utero* is consistent with our hypothesis that prenatal zinc deficiency can alter the adult expression of some proteins involved in zinc metabolism. However, in contrast to our initial hypothesis, MT production in the adult animal was not down-regulated as we predicted, but rather the prenatal zinc deficiency resulted in an alteration of MT production such that in response to the zinc stimulus the synthesis of the protein was increased.

One possible explanation for this alteration is MT gene amplification. This has been shown to occur in cadmium-resistant cell lines such as the Chinese hamster ovary and W7 mouse thymoma cells, which produce higher amounts of MT than nonresistant cells. The MT genes in these cell lines were found to be hypomethylated, which correlates with an increased transcription of the gene (4, 19). This has also been shown *in vivo* using a drug (5-azacytidine) which blocks methylation and results in higher MT hepatic concentrations in rats (20). Another example of amplification is found in the mouse Friend leukemia cell line and appears to be due to breaks in the DNA that result in aneuploidy and autonomously replicating MT DNA (21). Zinc is necessary for normal chromosome structure and deficiency during critical periods of development may lead to chromosomal abnormalities (22).

MT amplification has also been shown recently in lethal milk mutant mice. This mutation results in early zinc deficiency during suckling in pups. Cultured hepatocytes from adult lethal milk mutant mice have higher MT concentrations in both noninduced and zinc-induced cells than do hepatocytes from wild-type mice (23). The authors proposed that the induced MT was due primarily to changes in zinc metabolism which secondarily affect MT synthesis. An alternative explanation based on our findings is that zinc deficiency during prenatal development results in higher levels of MT in the adults.

Further studies are needed to determine if MT turnover has been altered by prenatal zinc deprivation, and whether these changes have any bearing on the immune defects also

seen in this moderate zinc deficiency. It is also important to know whether zinc metabolism and homeostasis have been altered by this change in MT metabolism. Knowledge of the mechanisms underlying altered expression of MT in prenatally zinc-deficient animals is critical to understanding the defects observed in cellular immune function in humans and animals subjected to zinc deficiency.

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