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Effect of the Severity of Maternal Zinc Deficiency on Pregnancy Outcome and Infant Zinc Status in Rhesus Monkeys

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ABSTRACT. To investigate the effects of the severity of maternal zinc deficiency on early development, rhesus monkeys were fed diets that were either moderately zinc-deficient (MZD) (2 μg Zn/g) or marginal in zinc (M) (4 μg Zn/g throughout pregnancy and lactation. Dams in the MZD group developed overt signs of zinc deficiency. Compared with control dams fed diets adequate in zinc (C) (50 or 100 μg Zn/g), both M and MZD dams showed low mitogen response. Pregnancy outcome was similar in all groups, and infants were considered healthy at delivery. From birth until d 30, infants were closely monitored for signs of zinc deficiency. On d 30, infants were killed and tissues were analyzed for several parameters reported to be affected by zinc status. MZD infants tended to have lower plasma zinc concentrations than C infants, although the difference was only significant at d 14. M infants tended to have lower plasma zinc concentrations than C infants. Mitogen response was lower in MZD and M infants than in C infants. However, mitogen responses were similar in MZD and M infants. Liver zinc concentrations were similar among the three groups of infants; however, zinc and metallothionein concentrations in (10 000 × *g*) liver supernatant fractions were lower in the MZD and M groups than in the C group. ^{65}Zn absorption/retention was higher in MZD and M mothers and infants than in C mothers and infants; there were no marked differences between MZD and M mothers or infants. In contrast to whole-body absorption, ^{65}Zn uptake/retention by isolated hepatocytes was similar among the three infant groups. Plasma metallothionein concentrations were higher in the MZD mothers during the 1st, 2nd, and 3rd trimester than in the M or C mothers and higher than C mothers on d 30 postpartum; plasma metallothionein concentrations were similar among the three groups of infants. These results demonstrate that feeding a diet containing 2 μg Zn/g to rhesus monkeys during pregnancy and lactation results in marked signs of zinc deficiency, whereas feeding a diet containing 4 μg Zn/g results in only subtle signs. (*Pediatr Res* 33: 233–241, 1993)

Abbreviations

PHA, phytohemagglutinin
Con A, concanavalin A
PWM, pokeweed mitogen
MZD, moderately zinc-deficient diet
M, marginal zinc diet
C, control zinc diet
CRPRC, California Regional Primate Research Center

There is now considerable evidence that maternal nutritional status can be a critical predictor of embryonic and fetal development. For example, during the last several years, a number of studies support the thesis that maternal zinc status can be a predictor of pregnancy outcome in human populations (1–5). Teratogenicity of maternal zinc deficiency in several other species, such as rodents and sheep (5, 6) is well documented. However, in contrast to studies with experimental animal models in which the extent of the induced zinc deficiency is often severe, human populations are more likely to develop conditions of marginal zinc deficiency. For this reason, our group has been studying the effects of marginal zinc deficiency on pregnancy outcome and infant development in rhesus monkeys. We have shown that marginal maternal zinc deficiency, induced by feeding diets containing 4 μg Zn/g during pregnancy and lactation, can result in a syndrome characterized by growth retardation, delayed bone growth and mineralization, delayed puberty, decreased taste acuity, behavioral lethargy, and immune dysfunction (7–13). The above studies have provided evidence for the hypothesis that marginal zinc deficiency represents a reproductive risk for primates; however, severe developmental anomalies have not been observed in the offspring of the marginally zinc-deficient monkeys. One of our research objectives entails the identification of the biochemical lesions underlying zinc deficiency–induced abnormal development. Therefore, we have now evaluated the effect of a more severe maternal zinc deficiency, induced by feeding a diet that contained 2 μg Zn/g on development in our rhesus monkey model. Data from this group of monkeys were contrasted with data obtained from animals fed the 4-μg Zn/g diet, and to control animals fed a 50- or 100-μg Zn/g diet. Some of the data for the 4-μg Zn/g and 100-μg Zn/g groups have been previously published (13, 14); however, they are repeated here to illustrate the dose-responsive nature of maternal zinc deficiency. Because it has been suggested that maternal iron supplementation during pregnancy may negatively affect the mother's zinc status (15–18) and such supplementation is common during pregnancy, we also evaluated the influence of maternal iron supplementation on maternal and infant zinc status.

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MATERIALS AND METHODS

Animals and Housing. Female rhesus monkeys (*Macaca mulatta*) 4 to 10 y of age were obtained from a breeding colony of healthy, multiparous monkeys maintained at the CRPRC at the University of California, Davis. Animals were housed individually in stainless steel cages in a temperature- (21 to 29°C) and light-controlled room (14 h light/10 h dark) with 25 to 35 animals/room. Deionized distilled water was provided *ad libitum* via an automatic water system. Food was given in stainless steel containers to minimize zinc contamination and spillage of the purified diet.

Diet and Breeding. Mating was accomplished by transferring the monkey to a cage of a proven breeder for a 2-h period on 2 alternate d around the estimated time of ovulation as determined from menstrual cycles. Mating was confirmed by a vaginal swab for sperm immediately after the mating period. Pregnancy was determined at 25 to 35 d of gestation with an RIA for monkey chorionic gonadotropin and/or ultrasound imaging examination. All animals determined to be nonpregnant were remated the next month. Experimental diets were initiated 2 wk before the initial mating. Dams in the control group were fed purified control diets that contained 100 μg Zn/g in y 1 and 50 μg Zn/g in y 2. The reduction in the zinc concentration of the control diet was done in response to previous concerns that the 100-μg Zn/g diet might represent a zinc-supplemented diet. There were no significant differences between the two sets of controls; thus, their data have been combined for the current study. Dams in the M and MZD groups were fed the same basal diet but with 4 μg Zn/g or 2 μg Zn/g, respectively. The composition of the diet is shown in Table 1. To investigate the effect of maternal iron supplements on maternal and infant zinc status, half of the dams in each dietary group were given daily iron supplements (4 mg ferrous sulfate/kg body weight) that were injected into a cookie that the monkeys found palatable. Nonsupplemented dams were given cookies injected with saline (placebo).

Diet Intake and Health Surveillance. Stool consistency and general health were monitored daily. Dams were weighed on d 0, 45, 90, 135, and 150 of pregnancy, on the day of delivery, and on d 30 of lactation. Infants were weighed at birth and on postnatal d 10 to 14 and 30. A subset of the dams in the MZD group developed signs of severe zinc deficiency during pregnancy. Based on the concern by CRPRC veterinarians that the health of some mothers was at risk, a subset of the animals (three) with the most pronounced signs of zinc deficiency was given a zinc supplement of 200 μg/d for 1 to 5 d. Data for the three dams that received the supplement were similar to those obtained for the unsupplemented animals; thus, the data for these two subgroups of the MZD group have been pooled.

Table 1. *Diet composition*

Ingredient	Amount (g/kg diet)
Spray-dried egg white	250.0
Cellulose	70.0
Sucrose	554.72
Corn oil	80.00
Choline bitartrate	3.6
Banana flavoring	2.0
Mineral mixture*	37.56
Vitamin mixture†	2.12

* Mineral mixture provided (g/kg diet): $CaCO_3$, 11.16; $MgSO_4$, 2.40; $CaHPO_4$, 6.31; K_2HPO_4, 12.96; $FeSO_4 \cdot 7H_2O$, 0.89; NaCl, 2.30; $MnSO_4 \cdot H_2O$, 0.123; KI, 0.0024; Na_2SeO_3, 0.00016; NaF, 0.0009; $CuCO_3$, 0.017; MgO, 1.39; $C_6H_9CrO_6$, 0.0018. The above diet was adjusted to contain 2, 4, 50, or 100 μg Zn/g by the addition of $ZnCO_3$.

† Vitamin mixture provided (g/kg diet): taurine, 0.5; inositol, 0.005; ascorbic acid, 1.0; calcium pantothenate, 0.025; thiamin hydrochloride, 0.03; pyridoxine hydrochloride, 0.01; niacinamide, 0.25; menadione sodium bisulfite, 0.0802; riboflavin, 0.005; para-aminobenzoic acid, 0.01; folic acid, 0.005; biotin, 0.004; vitamin A palmitate, 0.03 (500 000 U/g); vitamin B_{12}, 0.06 (0.1%); vitamin E acetate, 0.1 (500 U/g): vitamin D_3, 0.005 (400 000 U/g).

Experimental Procedures. Maternal. A total of 38 dams were successfully mated. The number of successfully mated animals per group was as follows: control + placebo, $n = 10$; control + iron supplement, $n = 4$; M + placebo, $n = 5$; M + iron supplement, $n = 5$; MZD + placebo, $n = 9$; MZD + iron supplement, $n = 6$.

At d 0, 45, 90, 135, and 150 of gestation, at delivery, and at d 10 to 14 and 30 postpartum, fasting blood samples were obtained from each of the dams for *1*) complete blood counts; *2*) serum calcium, phosphorus, creatinine, glucose, uric acid, protein, albumin, and triglyceride concentrations and γ-glutamyl transferase and alkaline phosphatase activities; *3*) plasma metallothionein levels; *4*) plasma zinc, copper, and iron levels; and *5*) peripheral blood lymphocyte responsiveness to PHA, Con A, and PWM. Fasting blood samples (heparinized and nonheparinized) were obtained between 1000 and 1130 h from the cephalic vein of unanesthetized monkeys. Blood was centrifuged at 1 500 × *g* for 10 min, and the plasma or serum was removed using plastic pipettes. Samples for analysis of trace elements were drawn using plastic syringes. Milk samples were collected by gentle hand-stripping of the teats into plastic vials after injection of the dams with oxytocin (2 IU) on d 1 to 3, 10, and 30 of lactation; milk was analyzed for zinc, copper, and iron concentrations. All samples were frozen immediately after removal and stored at −70°C in plastic vials.

Infants. Blood samples from the femoral vein were collected from the infants on postnatal d 10 to 14 and 30 and analyzed for the same parameters as those described for the dams. On postnatal d 30, the infants were killed by an overdose of pentobarbital. The liver, kidneys, thymus, adrenals, and spleen were quickly removed and weighed. Aliquots of liver were frozen at −70°C until analyzed for zinc, copper, iron, and metallothionein concentrations. Tissue copper and iron concentrations were measured, inasmuch as they can be affected by zinc deficiency (19, 20). One lobe of the liver was minced and incubated in collagenase solution for isolation of hepatocytes for ^{65}Zn uptake studies according to the method of Darwish *et al.* (21), as modified by Keen *et al.* (13).

^{65}Zn retention studies. ^{65}Zn retention was determined in the dams on gestational d 104 and 149 during lactation on postpartum d 28. Infant ^{65}Zn retention was determined on d 28. Dams were fasted overnight, and infant monkeys were separated from their mothers for a 4-h period before intubation of the radiolabeled diets. Each monkey was fed, via a nasogastric tube, ^{65}Zn-labeled infant formula (Enfamil; Mead Johnson, Evansville, IN) containing 2 μCi of ^{65}Zn per feeding. The radiolabel was allowed to equilibrate for 2 h before intubation. Intubation volume was 2 mL for infants and 5 mL for adults. Before each intubation, monkeys were counted for background or residual counts. The animals were counted for 5 min immediately after intubation and again at 14 d after dosing to determine isotope retention (14). The monkeys were counted in a whole-body counter that consisted of two 4 × 8-inch sodium iodide crystals (Harshaw/Filtrol, Solon, OH) interfaced with a multichannel analyzer (ND-66; Nuclear Data Co., Schaumberg, IL).

Immune response. Immunologic assays of the animals were done using a mitogen test battery (PHA, Con A, PWM) for peripheral lymphocytes as previously described (7). An optimal concentration of mitogen was used to elicit lymphocyte proliferation; stimulation was expressed relative to background.

Metallothionein analysis. Hepatic metallothionein concentrations were analyzed by the cadmium saturation method of Onosaka and Cherian (22). Samples were homogenized in 0.25 M sucrose and centrifuged at 10 000 × *g*. Aliquots of the supernatant were added to a 10 μg/mL cadmium solution to allow maximum binding of cadmium to metallothionein. Excess cad-

mium was removed by the addition of Hb and subsequent heat precipitation. Concentrations of metallothionein were determined by analyzing for cadmium concentration, using flame atomic absorption spectrophotometry, and assuming a ratio of 6 mol of cadmium to 1 mol of metallothionein.

Plasma metallothionein was determined using rabbit anti-rat metallothionein (RJV 4–12; generously provided by Dr. Justine Garvey, California Institute of Technology, Pasadena, CA) in a soluble competitive binding assay (23). Cross-reactivity of the antibody with purified metallothionein from rhesus monkey liver was demonstrated. The primary antibody was pelleted with protein A (Pansorbin, Calbiochem Corp., La Jolla, CA). ^{125}I-metallothionein was used as the tracer, and 0.1 ng was routinely the limit of detection. The intraassay coefficient of variation was less than 15% and the interassay coefficient of variation was less than 5%.

Mineral analysis. Plasma (1 mL), milk (1 mL), and liver (~0.3 g) samples were wet-ashed with 2 mL of 16 N nitric acid (Baker's Instra-analyzed, J. T. Baker Co., Philipsburg, NJ), evaporated and diluted with 0.1 N nitric acid as described by Clegg *et al.* (24). In addition to intact liver samples, livers were homogenized (as described above for metallothionein determination), and aliquots of the supernate and pellet were wet-ashed to provide preliminary information on the subcellular distribution of metals analyzed. Trace element concentrations were determined by flame atomic absorption spectrophotometry (model 551, Thermo-Jarrel Ash, Wilmington, MA). Certified reference solutions (1000 μg metal/mL, Fisher Scientific, Santa Clara, CA) were used to generate standard curves for each element. A sample of NBS bovine liver (SRM 1577, US Department of Commerce, National Bureau of Standards, Washington, DC) was analyzed to ensure accuracy and reproducibility of the elemental analysis.

Data analysis. Data were initially analyzed by multiple-factor analysis of variance using iron treatment and zinc treatments as variables. Iron supplementation had no effect on the outcome variables studied in any of the zinc groups; thus, the iron-supplemented and placebo groups were pooled within their respective dietary zinc groups. Similarly, sex was not a significant factor for the infant variables examined; therefore, data for males and females were combined. After combination of subgroups, data were analyzed using one-way analysis of variance. Fisher's least-significance difference test was used to examine for differences between group means. A p value ≤ 0.05 was considered significant. Data are shown as mean ± SEM.

Assurance of compliance with animal codes. All procedures conformed to the guidelines of the Animal Welfare Act and the *Guide for the Care and Use of Laboratory Animals* of the National Research Council (25). The CRPRC is fully accredited by American Association for Accreditation for Laboratory Animal Care. Individual protocols were approved by the campus veterinarian under the auspices of the Animal Care and Resources Committee of the University of California, Davis.

RESULTS

Iron Supplementation. Regardless of the dietary zinc group, iron supplementation was not found to affect the outcome variables investigated. For this reason, data for the iron supplemented and placebo subgroups within each dietary zinc group were combined to facilitate data presentation and interpretation.

Maternal health and pregnancy outcome. Signs of pronounced zinc deficiency were not observed in any of the mothers from the M group. A higher proportion (four of 15) of dams in the MZD group were characterized by dermatitis compared with dams in the C (one of 14) and MZD (zero of 10) groups; the dermatitis primarily occurred during the 3rd trimester. There were no significant differences in food intake among C, M, and MZD dams during the last trimester. One dam in the MZD group had markedly lower food intake throughout gestation, which became more pronounced during the last trimester; her plasma zinc concentrations were consistently 40 to 60% lower than those of the other MZD dams and her infant was stillborn. The same dam also had a severe case of dermatitis. Thirty percent (six of 20) of the C, 28% (four of 14) of the M, and 6% (one of 16) of the MZD dams were determined not pregnant 25 d after breeding despite three monthly matings. In pregnant dams, total pregnancy loss (abortion and stillbirth) was 21% (three of 14) in C dams, 20% (two of 10) in M dams, and 33% (five of 15) in MZD dams. The two abortions in the C group occurred on d 43 and 44 of gestation, the two abortions in the M group occurred on days 60 and 143, and the three abortions in the MZD group occurred on days 45, 45, and 50. Length of gestation was not influenced by dietary zinc. No gross malformations were noted in the neonates.

With regard to those dams who had successful pregnancies, maternal weight gain during gestation (d 0 to 150) was nearly 50% lower in MZD dams compared with C and M dams (1.47 ± 0.16, 1.30 ± 0.25, and 0.75 ± 0.32 kg in C, M, and MZD groups, respectively; $p < 0.05$ for C *versus* MZD). Weight gain from d 0 to d 90 of gestation was similar among the groups (0.58 ± 0.09, 0.41 ± 0.17, and 0.36 ± 0.29 kg in C, M, and MZD groups, respectively). Dams fed the MZD diet were characterized by very poor weight gain from d 90 to d 150 of gestation, being significantly lower ($p < 0.05$) than in C and M dams (0.89 ± 0.14, 0.90 ± 0.15, and 0.11 ± 0.26 kg in C, M, and MZD groups, respectively).

Plasma zinc concentrations were consistently lower ($p < 0.05$) throughout gestation in MZD dams than in C or M dams; during pregnancy plasma zinc concentrations decreased in the MZD group, whereas they were relatively constant in C and M dams (Table 2). Plasma zinc concentrations of dams fed the C and M diets were similar at all time points. During lactation, MZD dams had significantly ($p < 0.05$) lower plasma zinc concentrations than C dams and tended to have lower concentrations than

Table 2. *Influence of maternal zinc intake on maternal plasma zinc, copper, and iron concentrations**

	Group	n	Zinc (μmol/L)	Copper (μmol/L)	Iron (μmol/L)
Gestation day					
0	C	9	10.10 ± 0.76	19.99 ± 2.68	22.02 ± 4.30
	M	2	12.85 ± 2.75	17.63 ± 1.42	27.04 ± 1.25
	MZD	13	8.72 ± 0.92	16.37 ± 1.57	18.44 ± 2.15
45	C	12	11.47 ± 0.61^{a,b}	20.93 ± 1.57	20.59 ± 2.15
	M	7	14.69 ± 1.99^{a}	16.05 ± 1.42	38.86†
	MZD	14	10.10 ± 1.07^{b}	19.83 ± 1.26	21.49 ± 1.43
90	C	12	13.61 ± 1.07^{a}	24.39 ± 1.42	26.14 ± 5.73
	M	7	12.70 ± 1.53^{a}	20.46 ± 1.89	31.87 ± 4.48
	MZD	12	7.65 ± 0.76^{b}	25.34 ± 1.57	22.02 ± 2.15
135	C	12	11.63 ± 0.76^{a}	28.49 ± 2.52	23.46 ± 1.97
	M	7	11.78 ± 1.38^{a}	23.76 ± 1.10	27.93 ± 1.97
	MZD	10	7.50 ± 0.76^{b}	30.37 ± 2.83	22.38 ± 2.69
150	C	10	11.47 ± 0.76^{a}	31.95 ± 1.73	23.46 ± 4.12
	M	4	10.56 ± 0.76^{a}	30.06 ± 1.42	26.68 ± 5.01
	MZD	9	5.66 ± 0.61^{b}	26.91 ± 1.26	27.22 ± 5.19
Term	C	12	10.25 ± 0.76^{a}	35.88 ± 2.05	23.99 ± 1.25
	M	7	11.47 ± 0.92^{a}	30.37 ± 2.99	21.67 ± 2.33
	MZD	12	5.66 ± 0.61^{b}	32.89 ± 2.20	21.67 ± 1.97
Lactation day					
14	C	10	14.99 ± 0.92^{a}	15.11 ± 1.42	22.56 ± 1.97
	M	7	11.93 ± 1.22^{a,b}	12.91 ± 2.52	25.78 ± 3.58
	MZD	9	9.33 ± 1.38^{b}	17.31 ± 1.26	24.89 ± 2.51
30	C	10	13.31 ± 0.92^{a}	11.65 ± 0.94^{a}	18.98 ± 2.69
	M	7	10.86 ± 0.61^{a,b}	9.60 ± 1.10^{a}	21.49 ± 1.61
	MZD	9	8.57 ± 1.38^{b}	17.94 ± 1.73^{b}	19.88 ± 1.79

* Data are shown as mean ± SEM. Means within a column at each gestation/lactation day not sharing a common superscript are significantly different from each other.

† Only one sample was available for this analysis.

M dams. With the exception of higher plasma copper concentrations in the MZD group than in C and M dams at d 30 of lactation, plasma copper and iron concentrations were similar among the groups at all time points; plasma copper concentrations in all three groups increased during the course of pregnancy. The MZD dams tended to have low plasma albumin concentrations, low plasma alkaline phosphatase activities, and high plasma triglycerides throughout gestation and lactation compared with C and M dams (Table 3). There were no consistent differences among the groups in serum calcium, phosphorus, creatinine, glucose, uric acid, or protein concentrations, or γ-glutamyl transferase activity (Table 3). Hematologic indices were similar among the groups at all time points (data not shown).

Plasma metallothionein concentrations were higher in MZD dams than in C and M dams (Table 4). Means were consistently higher in MZD dams throughout pregnancy and lactation compared with C and M dams and were statistically higher at d 45, 90, and 135 of gestation and at d 30 of lactation.

Peripheral lymphocyte response to mitogens varied widely among the animals, although overall, dams fed the low-zinc diets were characterized by a reduced responsitivity to the mitogens (Table 5). Compared with C dams, dams in the M group showed a low responsiveness to PHA on d 45 and 90 and to Con A on d 45, 90, and 135 of gestation and at term. Compared with controls, dams in the MZD group showed a reduced responsiveness to PHA on d 0 and 90 of gestation and at d 30 of lactation and to Con A on d 0 and 90 of gestation and at d 30 of lactation. Responsiveness to PWM was similar in C and M dams at all time periods and was lower in MZD dams than in C dams on d 90 of gestation and at d 30 of lactation.

Milk zinc concentrations were consistently lower in MZD dams than in C dams throughout the 30 d of lactation, and M

Table 3. *Influence of maternal zinc intake on maternal and infant serum chemistry**

Day	Group	*n*	Alkaline phosphatase (μkat/L)	Albumin (g/L)	Triglycerides (mmol/L)	Calcium (mmol/L)	Phosphorus (mmol/L)
Dams							
45	C	11	2.60 ± 0.30	39.7 ± 1.2	0.306 ± 0.047[a]	2.45 ± 0.04[a]	1.24 ± 0.15
	M	8	2.68 ± 0.18	38.0 ± 1.0	0.295 ± 0.080[a]	2.48 ± 0.03[a]	1.19 ± 0.11
	MZD	13	2.08 ± 0.20	38.3 ± 1.3	0.543 ± 0.052[b]	2.34 ± 0.02[b]	1.30 ± 0.07
90	C	11	2.08 ± 0.15[a]	34.4 ± 0.9	0.552 ± 0.071[a]	2.40 ± 0.07	1.38 ± 0.11[a,b]
	M	8	2.75 ± 0.28[b]	34.9 ± 2.1	0.386 ± 0.050[a]	2.50 ± 0.08	1.68 ± 0.15[a]
	MZD	12	1.60 ± 0.22[a]	32.5 ± 1.4	0.920 ± 0.145[b]	2.29 ± 0.03	1.19 ± 0.09[b]
135	C	10	2.80 ± 0.35[a]	35.3 ± 0.9[a]	0.671 ± 0.100	2.32 ± 0.05	1.20 ± 0.09[a]
	M	8	2.60 ± 0.37[a]	34.5 ± 1.3[a]	0.615 ± 0.122	2.38 ± 0.03	1.58 ± 0.10[b]
	MZD	11	1.92 ± 0.23[b]	31.2 ± 0.9[b]	0.964 ± 0.130	2.26 ± 0.03	1.26 ± 0.09[a]
Term	C	11	2.98 ± 0.32	29.4 ± 0.8	0.725 ± 0.327	2.32 ± 0.03	1.29 ± 0.11
	M	8	3.37 ± 0.42	29.1 ± 1.2	0.597 ± 0.113	2.42 ± 0.06	1.25 ± 0.10
	MZD	11	2.57 ± 0.40	27.2 ± 1.1	0.769 ± 0.147	2.31 ± 0.03	1.35 ± 0.10
30 postpartum	C	10	4.98 ± 0.42[a]	40.3 ± 0.7[a]	0.474 ± 0.085[a]	2.46 ± 0.04	1.56 ± 0.12
	M	8	4.82 ± 0.42[a]	39.6 ± 0.6[a]	0.531 ± 0.107[a]	2.51 ± 0.04	1.31 ± 0.12
	MZD	11	3.12 ± 0.53[b]	36.8 ± 1.3[b]	0.907 ± 0.247[b]	2.42 ± 0.08	1.33 ± 0.13
Infants							
10–14	C	10	18.80 ± 1.45	31.6 ± 1.8	0.979 ± 0.189	2.41 ± 0.10	2.20 ± 0.08
	M	8	21.74 ± 2.68	29.8 ± 1.5	0.690 ± 0.122	2.61 ± 0.09	2.13 ± 0.16
	MZD	9	16.72 ± 2.02	31.0 ± 0.6	0.913 ± 0.112	2.45 ± 0.03	2.22 ± 0.10

Day	Group	*n*	Creatinine (μmol/L)	Glucose (mmol/L)	Protein (g/L)	GGT (μkat/L)	Uric acid (μmol/L)
Dams							
45	C	11	69.1 ± 6.7	3.17 ± 0.21	73.1 ± 2.1	0.812 ± 0.077	7.02 ± 1.96
	M	8	72.9 ± 2.7	3.10 ± 0.17	72.5 ± 1.6	0.815 ± 0.058	7.44 ± 2.44
	MZD	13	80.3 ± 2.7	3.40 ± 0.09	74.5 ± 1.4	0.670 ± 0.047	11.90 ± 2.02
90	C	11	67.5 ± 4.3	3.24 ± 0.018[a]	66.1 ± 2.7	0.757 ± 0.055	8.62 ± 1.84
	M	8	65.2 ± 2.8	2.51 ± 0.12[b]	69.1 ± 2.7	0.909 ± 0.087	12.67 ± 2.62
	MZD	12	75.4 ± 3.0	2.99 ± 0.14[a,b]	70.4 ± 1.3	0.725 ± 0.080	12.37 ± 2.50
135	C	10	73.4 ± 4.4	3.50 ± 0.24	73.7 ± 1.8	0.648 ± 0.052	4.76 ± 1.49
	M	8	70.7 ± 2.4	4.30 ± 0.84	75.8 ± 3.0	0.835 ± 0.065	6.66 ± 3.27
	MZD	11	77.8 ± 2.9	3.67 ± 0.29	74.3 ± 1.8	0.670 ± 0.062	9.22 ± 1.67
165	C	11	81.2 ± 5.6	4.16 ± 0.31	64.9 ± 1.1[a]	0.530 ± 0.043	2.18 ± 0.89
	M	8	69.7 ± 3.5	3.61 ± 0.25	67.6 ± 2.4[a,b]	0.608 ± 0.053	5.23 ± 1.78
	MZD	11	82.7 ± 3.8	3.92 ± 0.28	71.6 ± 1.2[b]	0.557 ± 0.043	3.81 ± 0.89
30 postpartum	C	10	82.2 ± 3.3[a,b]	3.58 ± 0.11	73.2 ± 0.8	0.700 ± 0.042[a,b]	2.97 ± 1.01
	M	8	72.9 ± 3.6[a]	3.49 ± 0.19	74.0 ± 1.4	0.758 ± 0.053[a]	5.17 ± 2.08
	MZD	11	86.5 ± 3.8[b]	3.96 ± 0.21	76.2 ± 1.9	0.580 ± 0.047[b]	5.95 ± 2.62
Infants							
10–14	C	10	62.8 ± 4.2	5.96 ± 0.47	48.7 ± 1.9	1.50 ± 0.13	4.76 ± 1.49
	M	8	57.5 ± 3.7	7.11 ± 0.74	48.6 ± 1.8	1.79 ± 0.16	5.95 ± 1.61
	MZD	9	60.9 ± 1.77	5.31 ± 0.35	48.0 ± 1.1	1.50 ± 0.12	5.29 ± 1.84

* Data are shown as mean ± SEM. Means within columns at each gestation/lactation day not sharing a common superscript are statistically significant from each other ($p < 0.05$). GGT, γ-glutamyl transferase.

Table 4. *Influence of maternal zinc intake on plasma metallothionein concentrations (nmol/L)**

Day	*n*	Group C	Group M	Group MZD
Dam				
45	8/5/11	17.0 ± 1.8[a]	19.2 ± 3.2[a]	30.9 ± 3.4[b]
90	7/5/13	13.0 ± 2.3[a]	22.7 ± 3.8[a]	35.7 ± 3.7[b]
135	8/7/13	21.3 ± 3.6[a]	21.9 ± 1.4[a]	52.0 ± 11.1[b]
Term	7/5/13	26.2 ± 9.5	15.1 ± 3.1	48.3 ± 15.0
14 postpartum	4/3/8	32.8 ± 5.2	27.9 ± 1.0	37.0 ± 4.9
30 postpartum	7/3/11	18.7 ± 2.5[a]	30.0 ± 6.4[a,b]	50.9 ± 11.3[b]
Infant				
30	7/6/9	10.8 ± 3.2	11.5 ± 5.8	7.6 ± 1.3

* Data are shown as mean ± SEM. Means within rows not sharing a common superscript are statistically different from each other ($p < 0.05$).

Table 5. *Influence of maternal zinc intake on peripheral blood lymphocyte response to the mitogens Con A, PHA, and PWM in dams and d-30 infants**

Day	Group	*n*	Con A†	PHA	PWM
Dams					
0	C	12	39 ± 4[a]	41 ± 6[a]	9.7 ± 1.4
	M	7	37 ± 6[a]	39 ± 3[a]	9.9 ± 1.3
	MZD	16	23 ± 3[b]	16 ± 2[b]	7.9 ± 1.2
45	C	12	47 ± 3[a]	55 ± 7[a]	11.9 ± 1.4
	M	7	14 ± 3[b]	20 ± 5[b]	9.8 ± 2.3
	MZD	15	43 ± 8[a]	51 ± 10[a,b]	12.6 ± 1.6
90	C	12	77 ± 6[a]	87 ± 9[a]	20.6 ± 2.4[a]
	M	7	43 ± 3[b]	51 ± 4[b]	18.0 ± 2.1[a,b]
	MZD	13	48 ± 11[b]	36 ± 8[b]	11.1 ± 2.7[b]
135	C	12	44 ± 5[a]	30 ± 4	10.0 ± 1.7
	M	7	16 ± 3[b]	43 ± 3	10.3 ± 2.8
	MZD	12	29 ± 9[a,b]	48 ± 12	7.7 ± 1.8
Term	C	12	39 ± 6[a]	37 ± 5	10.1 ± 1.8
	M	7	15 ± 2[b]	36 ± 6	7.8 ± 0.9
	MZD	11	32 ± 8[a,b]	39 ± 12	6.1 ± 1.7
30 post-partum	C	11	31 ± 5[a]	49 ± 6[a]	9.1 ± 1.3[a]
	M	7	31 ± 6[a,b]	37 ± 8[a]	8.9 ± 2.3[a,b]
	MZD	10	17 ± 2[b]	18 ± 3[b]	4.8 ± 0.8[b]
Infant					
15	C	10	48 ± 8	45 ± 6[a]	22 ± 6
	M	7	42 ± 13	53 ± 14[a]	15 ± 5
	MZD	8	27 ± 10	19 ± 5[b]	11 ± 5

* Data are shown as the mean ± SEM. Means within columns at each gestation/lactation day not sharing a common superscript are statistically different from each other ($p < 0.05$).

† Data are shown as the mean stimulation index.

dams had lower early milk (d 1–3) zinc concentrations than C dams. The differences among means, however, were not statistically significant due to large variations among the mothers. Dams in the MZD group were characterized by high milk iron concentrations compared with both M and C dams. Compared with C dams, dams in the MZD group had low milk copper concentrations on d 1 to 3 but high milk copper concentrations on d 30 (Table 6).

Dams in both the M and MZD groups retained a nearly 2-fold higher percentage of ^{65}Zn from a radiolabeled meal during both gestation and lactation than C dams (Table 7). There were no significant differences between M and D dams in ^{65}Zn retention.

Infant outcome. Body weight at birth and d 30 and weight gain between the two time points were similar among the groups

Table 6. *Influence of maternal zinc intake on milk zinc, copper, and iron concentrations**

Day	Group	*n*	Zinc (μmol/L)	Copper (μmol/L)	Iron (μmol/L)
1–3	C	8	65.78 ± 16.22	52.09 ± 9.44[a]	13.25 ± 4.30[a]
	M	5	43.29 ± 9.33	48.32 ± 4.09[a,b]	10.39 ± 4.66[a]
	MZD	10	43.29 ± 9.64	30.22 ± 2.99[b]	30.08 ± 3.22[b]
10–14	C	10	24.02 ± 2.75	15.11 ± 2.20	7.70 ± 3.58[a]
	M	6	26.92 ± 7.19	18.57 ± 2.36	8.42 ± 4.48[a]
	MZD	9	20.96 ± 4.74	12.28 ± 3.93	37.78 ± 5.37[b]
30	C	10	23.56 ± 1.99	2.36 ± 0.79[a]	7.88 ± 2.86[a]
	M	7	23.10 ± 1.99	2.68 ± 0.63[a]	6.80 ± 0.72[a]
	MZD	9	16.06 ± 7.34	9.92 ± 3.93[b]	32.95 ± 4.30[b]

* Data are shown as the mean ± SEM. Means within columns at each lactation day not sharing a common superscript are statistically different from each other ($p < 0.05$).

Table 7. *Influence of maternal zinc intake on ^{65}Zn retention from a labeled meal in dams and d-28 infants**

Day	Group	*n*	% Retention
Dam			
104	C	11	43 ± 5[a]
	M	9	76 ± 3[b]
	MZD	12	68 ± 5[b]
149	C	12	37 ± 6[a]
	M	7	65 ± 7[b]
	MZD	11	68 ± 6[b]
28 postpartum	C	9	37 ± 4[a]
	M	7	72 ± 10[b]
	MZD	8	69 ± 10[b]
Infant			
28	C	10	42 ± 5
	M	7	65 ± 11
	MZD	8	80 ± 18

* Data are shown as mean ± SEM. Means within columns at each gestation/lactation day not sharing a common superscript are statistically different from each other ($p < 0.05$).

(Table 8). However, infant birth weight as a percentage of maternal body weight at d 0 of gestation was significantly lower in M and MZD infants compared with C infants (Table 8). There were no significant differences in organ weights among the infants, with the exceptions of a higher weight and higher percentage of body weight for adrenals in M infants compared with MZD and C infants, respectively, a lower percentage of body weight for kidneys in MZD compared with M infants, and a higher percentage of body weight for spleen in MZD compared with C infants (Table 8). Plasma zinc concentrations tended to be lower at birth in MZD infants compared with C and M infants ($p = 0.07$); the MZD infants had less plasma zinc than C infants on d 14 ($p < 0.05$) (Table 9). However, by d 30 postpartum, plasma zinc concentrations were similar among the groups. Infants born to dams fed the M diet had plasma zinc concentrations that were intermediate between values for the C and MZD infants. Plasma copper and iron concentrations were similar in infants from the three groups at all time points (Table 9). Serum metallothionein concentrations were similar among the infants (Table 4).

There were no group differences in red blood cell number, Hb concentration, hematocrit, mean cell Hb, mean cell Hb concentration, or mean cell volume (data not shown). Concentrations of serum calcium, phosphorus, creatinine, glucose, uric acid, protein, albumin, globulin, triglycerides, and activities of γ-glutamyl transferase and alkaline phosphatase were similar among the groups (Table 3).

As with the dams, there was considerable variation among the

Table 8. *Influence of maternal zinc intake on infant weight and growth and tissue size on postnatal day 30**

	C	M	MZD
Birth			
wt (g)	481 ± 17	443 ± 21	451 ± 16
% Dam wt† at d 0 gestation	9.40 ± 0.38[a]	8.05 ± 0.38[b]	8.08 ± 0.44[b]
D 30			
wt (g)	598 ± 31	579 ± 40	544 ± 26
Wt gain (g)	117 ± 23	137 ± 30	93 ± 26
Liver (g)	17.6 ± 1.3	16.2 ± 0.9	15.9 ± 0.8
% Body wt	2.87 ± 0.12	2.85 ± 0.16	2.82 ± 0.68
Kidney (g)	3.18 ± 0.21	3.31 ± 0.34	2.72 ± 0.14
% Body wt	0.53 ± 0.02[a,b]	0.57 ± 0.04[a]	0.47 ± 0.01[b]
Thymus (g)	2.59 ± 0.29	2.81 ± 0.57	2.24 ± 0.20
% Body wt	0.43 ± 0.04	0.45 ± 0.08	0.40 ± 0.03
Spleen (g)	1.05 ± 0.09	1.11 ± 0.13	1.18 ± 0.07
% Body wt	0.17 ± 0.01[a]	0.19 ± 0.01[a,b]	0.21 ± 0.01[b]
Adrenals (g)	0.37 ± 0.02[a,b]	0.45 ± 0.06[a]	0.32 ± 0.03[b]
% Body wt	0.062 ± 0.002[a]	0.077 ± 0.007[b]	0.057 ± 0.003[a]

* Data are shown as mean ± SEM.

† Means within a row not sharing a common superscript are significantly different from each other ($p < 0.05$).

Table 9. *Influence of maternal zinc intake on infant plasma zinc, copper, and iron concentrations**

Day	Group	*n*	Zinc (μmol/L)	Copper (μmol/L)	Iron (μmol/L)
0	C	9	12.54 ± 1.07	10.39 ± 1.42	36.35 ± 6.27
	M	7	10.10 ± 0.92	8.97 ± 1.42	50.32 ± 19.70
	MZD	5	9.03 ± 1.22	8.18 ± 1.10	32.95†
14	C	9	14.07 ± 1.22[a]	14.79 ± 1.26	47.09 ± 7.70
	M	5	10.88 ± 1.22[a]	13.69 ± 2.20	27.40 ± 8.59
	MZD	8	7.04 ± 0.76[b]	13.06 ± 0.92	28.47 ± 2.51
30	C	10	12.24 ± 0.76	13.22 ± 0.79	26.32 ± 2.86
	M	7	11.17 ± 0.76	14.95 ± 3.31	21.31 ± 3.04
	MZD	9	10.56 ± 0.76	11.96 ± 0.47	24.71 ± 1.79

* Data are shown as mean ± SEM. Means within a column at each lactation day not sharing a common superscript are statistically different from each other ($p < 0.05$).

† Only one sample was available for this analysis.

infants' responsiveness to the mitogens tested. Infants of mothers in the MZD group showed a 44 to 58% reduced responsiveness to Con A, PHA, and PWM compared with C infants, although only the difference in the response to PHA was statistically different (Table 5). Infants of M dams showed a 32% reduced responsiveness to PWM compared with C infants; this difference, however, was not statistically significant.

Liver zinc concentrations at d 30 were similar among the groups (Table 10). Analysis of the subcellular fractions indicated that infants of mothers fed either of the low-zinc diets tended to have lower 10 000 × *g* supernatant zinc concentrations compared with C infants, whereas pellet zinc concentrations were similar. Mean hepatic metallothionein concentrations were 35% lower in M infants and 61% lower in MZD infants than in C infants. However, due to the large variation among the values, the differences were not statistically significant. Despite differences in liver zinc concentration, ^{65}Zn uptake and retention by isolated hepatocytes was similar among the three groups (data not shown).

Infants from MZD dams retained a higher percentage of ^{65}Zn from a radiolabel meal compared with C and M infants (90 and 23% higher, respectively); M infants had a 55% higher retention of ^{65}Zn than C infants (Table 7). Due to the large variation in ^{65}Zn retention among the infants, these values were not statistically different.

Regression analysis. To further investigate the influence of infant zinc status on infant growth and development, data from the C, M, and MZD groups were pooled for regression analysis. Significant positive correlations were observed between liver metallothionein and liver zinc concentrations ($r = 0.81$, $p < 0.01$) and liver supernatant zinc concentrations ($r = 0.96$, $p < 0.01$). Significant negative correlations were observed between infant weight gain and liver zinc concentrations ($r = -0.59$, $p < 0.01$) and liver supernatant zinc concentrations ($r = -0.64$, $p < 0.01$). Similar to liver zinc, infant plasma zinc concentration at d 30 was significantly correlated with infant weight gain ($r = -0.41$, $p < 0.05$). Regression analysis did not reveal any significant associations between growth and tissue copper or iron levels.

^{65}Zn retention was positively correlated to plasma zinc concentrations in the dams at d 149 to 150 for the controls ($r = 0.60$, $p = 0.069$) and for the M and MZD dams combined ($r = 0.67$, $p < 0.05$). At d 24 to 28 of lactation, ^{65}Zn retention in the mothers was negatively correlated to plasma zinc concentrations for the groups combined ($r = -0.41$, $p < 0.05$). In the infants, there was a lack of correlation in the ^{65}Zn retained to plasma zinc concentrations ($r = -0.246$, $p = 0.247$). Infant growth rate from birth to d 30 was correlated with ^{65}Zn retention in the control group ($r = 0.686$, $p < 0.05$) but not in the M and MZD groups ($r = 0.068$, $p = 0.81$).

DISCUSSION

In addition to evaluating the effects of feeding variable levels of dietary zinc on pregnancy outcome in the rhesus monkey, a goal of this study was to provide additional information on the effects of iron supplementation on maternal and infant zinc status. Several investigators have reported that the consumption of iron supplements can reduce zinc absorption and/or alter an individual's zinc status (14–18); however, this is not a consistent finding (26, 27). In the 1990 report on nutrition and pregnancy issued by the US National Academy of Sciences (28), it was recommended that pregnant women consuming iron supplements in excess of 30 mg/d should take a zinc supplement of 15 mg/d. Earlier, we reported that iron supplements did not affect the zinc status of rhesus monkey dams or their infants when fed diets containing either 100 or 4 μg Zn/g (13, 14). The results from the current study strengthen the observation that even in a condition of clear zinc deficiency, an interaction between iron and zinc is not evident when the iron supplements are provided in a complex food item.

The results obtained in the current study support the idea that purified diets containing 4 μg Zn/g and 2 μg Zn/g represent "marginal" and "moderately deficient" zinc diets, respectively, for the pregnant or lactating rhesus monkey. As previously reported (13), maternal plasma zinc concentrations were not markedly affected by the level of zinc deprivation induced by the marginal zinc diet. However, maternal plasma zinc concentrations were influenced by the moderately zinc-deficient diet. The average maternal plasma zinc concentration in the MZD group was lower than the control zinc groups at all time points tested, with the difference between them being statistically significant 75% of the time. It is interesting to note that in the MZD group the lowest plasma zinc concentrations occurred between midgestation and delivery, indicating the extent of the zinc deficiency in the MZD group was most severe during late gestation, when peak fetal growth was occurring. We have shown that fetal body movements, as assessed by ultrasound, were altered in the MZD group compared with controls but not in the M group (29), providing further evidence that the 2-μg Zn/g group was more seriously affected than the 4-μg Zn/g group. This is consistent with the observation that four mothers in the MZD group developed severe dermatitis during the 3rd trimester. After d 90 of pregnancy, mothers in the MZD group were also

Table 10. *Influence of maternal dietary zinc intake on liver zinc, copper, iron, and metallothionein concentrations in d-30 infants**

Liver	Group	*n*	Zinc (nmol/g)	Copper (nmol/g)	Iron (nmol/g)	MT (nmol/g)
Whole tissue	C	10	395 ± 34	326 ± 66	5139 ± 537[a,b]	
	M	7	356 ± 34	255 ± 47	3832 ± 824[b]	
	MZD	10	304 ± 43	403 ± 66	7109 ± 949[a]	
10 000 × *g* supernate	C	11	288 ± 52	154 ± 32	3707 ± 483	34.3 ± 13.9
	M	7	194 ± 28	76 ± 8	2310 ± 645	22.3 ± 9.1
	MZD	9	196 ± 29	85 ± 14	3921 ± 627	13.5 ± 6.4
10 000 × *g* pellet	C	11	132 ± 17	184 ± 33	1701 ± 161	
	M	7	124 ± 8	132 ± 16	1522 ± 394	
	MZD	9	107 ± 8	143 ± 32	1970 ± 304	

* Data are shown as mean ± SEM. Means within a column not sharing a common superscript are statistically different from each other ($p < 0.05$). MT, metallothionein.

characterized by a marked reduction in weight gain compared with control and M mothers. Outcome of pregnancy was notably poorer in MZD mothers with dermatitis; one of these animals had a stillbirth and another delivered the smallest infant in the study (355 g).

Given the above, a surprising observation in the current study was that maternal ^{65}Zn retention at d 149 was similar in the M and MZD groups, with both groups having a retention about twice that of controls. One interpretation of the above is that in both zinc-deprived groups there were similar enhancements in zinc absorption and similar reductions in zinc excretion. Thus, there may be a limit to the homeostatic mechanisms up-regulating zinc absorption. Apparently, only in the M group were these changes adequate to allow for normal rates of maternal weight gain. It is interesting to note that the calculated retention of ^{65}Zn between the d-104 and d-149 absorption tests was 85 ± 3, 87 ± 2, and 83 ± 2% in the C, M, and MZD dams, respectively. Thus, the turnover of endogenous zinc pools was similar among the three groups of mothers.

The finding that maternal plasma zinc concentrations in the MZD group increased after delivery suggests that *1*) maternal loss of zinc via milk was less than the flux of zinc into the fetus, *2*) there was significant mobilization of tissue zinc occurring during the lactation period, and/or *3*) dietary zinc absorption was increased after delivery. The last explanation seems the least likely, given that ^{65}Zn retention data for all of the groups were similar before and after delivery. However, the first two possibilities are supported by the observation that the retention of ^{65}Zn between the second and third ^{65}Zn absorption trials (calculated as described above as the percentage change in ^{65}Zn retention between gestation d 149 and lactation d 14) was 69 ± 2, 77 ± 2, and 75 ± 3% in the C, M, and MZD dams, respectively.

Similar to the mothers, infant plasma zinc concentrations were lower in the MZD group than in the C and M groups at all time points tested, although this difference was only statistically significant at d 14. In the infants, plasma zinc concentrations in the M group were intermediate between the C and MZD groups, a finding that was not observed in the mothers. Thus, the measurement of maternal plasma zinc concentrations was of some predictive value for the MZD group but was of limited value for the M group. Presumably, the low plasma zinc concentrations in the MZD infants reflected a combination of poor zinc status at birth as well as a lower dietary intake of zinc via milk. Consistent with previous data, milk copper concentrations decreased sharply between d 1 to 3 and d 30 (30) in all groups. It is interesting to note that milk iron concentrations were consistently higher in the MZD group than in the M or C groups. Whether the higher milk iron concentrations in the MZD group reflect the occurrence of a zinc-iron interaction within mammary tissue remains to be ascertained. In contrast to zinc, plasma copper and iron concentrations were similar among the three groups of infants. Thus, the observed differences in milk concentrations of these elements among the groups were not reflected in infant plasma concentrations.

There was a negative correlation between infant plasma zinc concentrations and infant ^{65}Zn retention in the control ($r = -0.257$, $p = 0.50$) and low-zinc ($r = -0.202$, $p = 0.47$) groups. However, although there was a strong positive correlation between ^{65}Zn retention and weight gain in the control infants ($r = 0.686$, $p = 0.028$), this was not true for the low-zinc groups ($r = 0.068$, $p = 0.808$). One interpretation of the above is that a higher proportion of absorbed zinc is going to growth processes in controls than in the low-zinc infants. Presumably, this would occur if the "deficient" animal was incorporating zinc into sites (such as zinc-requiring enzymes, "zinc finger-dependent" binding sites, etc.) that need to be saturated before the use of zinc for growth. This suggestion is in keeping with the idea that zinc is a regulator of the growth process.

Consistent with previous findings (9, 13), mitogen responsitivity was lower in MZD and M mothers and infants than in controls; however, there was not a consistent difference between the M and MZD groups. This observation was somewhat surprising, as it was predicted that the immune system would be more severely compromised in MZD animals than in the M animals. These results suggest that the sensitivity of the rhesus monkey's immune system to zinc deficiency is such that a significant proportion of the immune changes already occurred in the MZD animals. That the immune system is affected by even marginal zinc deficiency is well established (31–35) and underscores the potential value of assessing mitogen sensitivity as an early marker for zinc status. Regardless of the explanation for the lack of differences in mitogen sensitivity between the M and MZD animals, our results emphasize the potential risk for zinc deficiency-associated reductions in immunocompetency in both the mother and infant.

Whereas maternal serum calcium chemistry data was similar in C and M groups, mothers in the MZD group were characterized by low albumin concentrations, low alkaline phosphatase activity, and high triglyceride concentrations compared with controls. Serum alkaline phosphatase activity and albumin concentrations have been reported to be influenced by zinc deficiency in humans in the direction observed in this study (36–38). The reduction in alkaline phosphatase activity can be ascribed to the fact that alkaline phosphatase is a zinc metalloenzyme; however, it should be stressed that the activity of this enzyme is typically only affected in cases of severe zinc deficiency (39). The mechanisms underlying the observed changes in albumin and triglyceride concentrations have not been identified. It has been suggested that reductions in circulating albumin concentrations may reflect an overall reduction in protein synthesis in zinc-deficient subjects (38), although this is an area of debate. In contrast to our data for rhesus monkeys, rodent models of zinc deficiency are often associated with a reduction in circulating triglyceride concentrations (40). At present, we do not have an explanation for this species difference. In contrast to the mothers, there were no group differences among the infants for serum alkaline phosphatase activity or albumin or triglyceride concentrations. One interpretation of the above is that the severity of

the zinc deficiency was more pronounced in the mothers than in the infants, and this would be consistent with the mobilization of hepatic zinc stores accumulated by the fetus during late gestation.

As part of our blood chemistry panel, we evaluated the influence of dietary zinc on serum metallothionein concentrations. Based on the reported observation of low plasma metallothionein concentrations in rats (41) and low erythrocyte metallothionein concentrations in humans (42) fed zinc-deficient diets, we predicted that serum metallothionein concentrations would be a useful predictor of zinc status for the rhesus monkey. However, in contrast to our expectation, we observed high concentrations of plasma metallothionein in MZD mothers compared with C and M mothers. One interpretation of this result is that when the mothers in the MZD group developed signs of zinc deficiency (severe dermatitis) it resulted in an inflammatory condition that in part stimulated maternal hepatic metallothionein synthesis, possibly via tumor necrosis factor α, IL-1, γ-interferon, and/or glucocorticoids. It is well documented that the synthesis of metallothionein in liver can be stimulated by many of the cytokines that are elaborated during inflammation (43, 44). We suggest that an inflammation-released increase in maternal liver metallothionein levels in the MZD group was reflected by an increase in serum metallothionein concentrations. If this scenario is correct, it suggests that the measurement of serum metallothionein concentrations may be of limited diagnostic value in clinical situations with regard to identification of zinc deficiency in monkeys or humans who have overt pathologies. This observation is supported by the observation of higher plasma metallothionein concentrations in C dams at birth and d 14 of lactation compared with pregnancy or d 30 of lactation. Peripelvic and uterovaginal trauma during delivery and uterine involution should result in cytokine production, which would contribute to observed differences in plasma metallothionein at these times. If the proposed acute phase response did occur in MZD mothers, the increase in newly synthesized hepatic metallothionein would sequester zinc and further depress serum zinc concentrations, thus reducing the amount of zinc available to the fetus. The above would be consistent with reports that the developmental toxicity associated with some teratogenic agents may, in part, be due to an induced embryonic/fetal zinc deficiency secondary to a redistribution of zinc from maternal plasma to maternal liver occurring as a consequence of the above type of acute phase response (1, 45).

The absence of any signs of anemia in the MZD group is consistent with our recent report of normal hematologic indices in mothers and infants in the MZD group (13). As discussed in that article (13), anemia was previously reported to be a consequence of zinc deficiency in rhesus monkeys (7, 8); however, in our opinion, the anemia in those reports was most likely due to a multinutrient deficiency, as the diets were also low in copper. The data from the current study indicate that, in monkeys, effects of zinc deficiency on hematologic parameters primarily are manifested only with very severe deficiencies, or when zinc deficiency acts synergistically with other nutrient deficiencies.

One objective of the current study was to extend previous investigations on the influence of zinc deficiency on hepatic trace mineral concentrations and metabolism in the monkey. We reported previously that although hepatic zinc and metallothionein concentrations were reduced in adult nonpregnant rhesus monkeys fed a zinc-deficient diet for 15 mo (46) (Note: The zinc-"deficient" animals were not characterized by dermatitis at the time of biopsy), d-30 infant liver zinc and metallothionein concentrations were similar in infants from mothers fed control and marginal zinc diets (13). Consistent with the above, in the current study, liver zinc concentrations were similar in infants from C mothers and MZD mothers; however, liver metallothionein concentrations and zinc concentrations in $10\,000 \times g$ liver supernatants were lower in MZD infants compared with controls. These data, along with the low plasma zinc concentrations in infants in the MZD group support the idea that these infants were zinc-deficient at the time of necropsy. The observation of a strong inverse correlation between d 30 liver supernatant zinc concentrations and infant weight gain between d 0 and d 30 ($r = -0.639$, $p < 0.01$) is consistent with the idea that this pool of zinc contributed to the growth process.

Similar to the maternal data, ^{65}Zn retention was markedly higher in infants from M and C mothers (80, 65, and 42%, respectively). Increases in zinc absorption in zinc-deficient rodents (47, 48) and humans (49–51) have also been documented. However, in contrast to the marked differences in whole-body ^{65}Zn retention, liver supernatant zinc concentrations, and metallothionein concentrations, the infant hepatocyte ^{65}Zn uptake *in vitro* was similar among the three groups. This observation argues against an up-regulation of hepatic zinc uptake in the zinc-deficient animal.

A critical observation from the current study is the lack of gross malformations occurring in MZD infants. Given the very low concentration of zinc in the MZD diet (2 μg/g), this suggests that, for human populations, it is unlikely that the consumption of a diet low in zinc will by itself typically result in the induction of severe malformations such as spina bifida. However, this does not rule out the possibility that some individuals may have a genetic background that increases their susceptibility to the teratogenic potential of zinc deficiency. For example, an increased frequency of severe birth defects is recognized as a risk in women with acrodermatitis enteropathica unless they receive zinc supplements (52, 53). Similarly, there may be a synergistic interaction between maternal zinc deficiency and other developmental insults that increases the risk for some malformations above that which would occur with either zinc deficiency or the developmental insult alone (1). In theory, the synergism could result from either the zinc deficiency increasing the susceptibility of embryonic cells to another insult or from the insult noted acting to further reduce zinc transport to the embryo. An example of the first situation would be if embryonic zinc deficiency resulted in a compromised antioxidant defense system, which would increase the susceptibility of the embryo to factors such as ionizing radiation. An example of the second would be concomitant exposure to cigarette smoke and a marginal zinc diet, because cigarette smoke contains cadmium, which concentrates in the placenta and reduces zinc flux into the embryo (54, 55). Future investigations on the above types of interactions should provide valuable information on how maternal nutritional status can influence pregnancy outcome in human populations.

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