Placental Copper Transport in Rats: Effects of Elevated Dietary Zinc on Fetal Copper, Iron and Metallothionein

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ABSTRACT We hypothesized that the competition between zinc (Zn) and copper (Cu) during fetal accretion of copper could be discriminated at either the dam-to-placenta or placenta-to-fetus stage. This premise was tested by feeding dams a high Zn diet (1000 mg/kg, HZn) during the second half of gestation. One day before delivery, dams were anesthetized, fetuses removed and both maternal and fetal tissues and plasma obtained and assayed. Other rats were fed a normal Zn concentration diet (32.4 mg/kg, ND) throughout pregnancy. There were significantly lower fetal liver Cu concentrations and greater plasma Fe concentrations, but not plasma Cu concentrations or liver Fe concentrations in the HZn group. Both dam and fetal liver concentrations were greater in the HZn than in the ND group. Plasma Cu levels were lower in the HZn-fed than in the ND-fed dams. Placental tissue from the HZn litters had a greater concentration of Zn and Fe than did the ND group, whereas no effect was noted for Cu concentration. Metallothionein (MT) levels were elevated in dam livers and placenta in the HZn group, but there were no differences in fetal liver MT. The dynamic assessment of placental transport was conducted by injecting 2.5 mg/kg Cu acetate intravenously into dams of both groups. Sequential samplings of dam and fetal blood and placentas were taken from 0 to 60 min. After the Cu bolus, there was a consistently higher plasma Cu concentration in the HZn than in the ND dams, but no alteration in the concentration of Cu in the placenta or fetal plasma. This study indicates that placental Cu uptake is not affected by a high Zn diet in the dam. In addition, the greater Zn concentration in the placenta of HZn than in ND litters results in abnormal fetal Cu, Fe and Zn concentrations, suggesting that an imbalanced maternal mineral consumption is deleterious to normal divalent metal accretion. J. Nutr. 128: 1037–1041, 1998.

KEY WORDS: • high zinc diet • placental transport • copper • iron • rats

Interactions between chemically related mineral elements during gestation are affected by the uphill gradient from mother-to-fetus, known to exist with Zn and Fe (Graham et al. 1994), and the downhill gradient characteristic of placental Cu transport in humans (Kundu et al. 1981, Nagel et al. 1986, Takacs et al. 1984, Yasodhara et al. 1991) and rodents (McAr- dle and Erlich 1991, Romeu et al. 1986). Dietary imbalances in the dam may affect the fetus before any metabolic abnormality could be detected in the mother because of the relatively greater length of exposure of the fetus to this stress. Such an imbalance could specifically affect fetal Cu.

Zn, Cu and Fe are essential mineral elements that exhibit important interactions and possible inhibition of each other’s transport and bioavailability (Brewer et al. 1985, Hurley et al. 1983, Reinstein et al. 1984). Excessive intake of one of these elements, beyond its normal presence in the diet, may result in an overt deficiency of another element. In particular, with the indiscriminate use of high dose over-the-counter Zn preparations, it is possible to observe deleterious effects. In humans, excessive Zn intake may result in acute or chronic intoxications manifested as lethargy, confusion, abdominal cramps, vomiting or fever (Calesmick and Dinan 1988, Chobanian 1981). The interaction between Zn and Cu can result in various manifestations of Cu deficiency, including macrocytic anemia and neutropenia (Botash et al. 1992, Gyorffy and Chan 1992, Hoffman et al. 1988). However, Zn-Cu competition has beneficially exploited for the treatment of Wilson’s disease as a means to prevent Cu accumulation (Brewer et al. 1990).

It has been shown that, in rats, a high Zn maternal diet resulted in a depressed fetal liver Cu concentration only when the dam was Cu deficient. However, the role of the placenta in the dynamics of Cu transport was not clearly elucidated (Reinstein et al. 1984). We designed this study to determine the extent to which a high Zn diet, as defined in the text, with normal Cu intake, fed to pregnant rats would affect Cu and Fe transport from dam to placenta and placenta to fetus, as well as the mineral status of the fetus by the end of gestation. This information may be relevant to the formulation and use of mineral supplements during pregnancy.

MATERIALS AND METHODS

Animals and diets. Sprague-Dawley females (200–250 g, Zivic-Miller, Pittsburgh, PA) were mated in our animal facility. Day 0 was
Experimental design. Assessment of a high Zn diet on fetoplacental mineral status. The study evaluated the effects of a high Zn diet fed to the dams on their mineral nutritional status and that of their fetuses, as well as on the mineral composition of the placenta. One day before delivery, the dams were anesthetized with ketamine/xylazine (87:13 g/L, dose 1.0 mL/kg, Fort Dodge Laboratories, Fort Dodge, IA). Dams were laparotomized, fetuses removed and blood from the abdominal aorta was obtained to exsanguinate the rat. The dam’s liver was promptly removed. Placentas and fetuses were weighed and blood collected from a neck incision after severing the spinal cord. Dam and fetal livers and placentas were frozen immediately and analyzed within 1 wk. This protocol was approved by the Institutional Animal Care and Utilization Committee.

Dynamic assessment of placental Cu transport. This approach compared plasma Cu concentration in the dams and fetuses of both diet groups after an intravenous Cu challenge. The anatomy of the uterus enabled the removal of one or more fetuses at a time without interrupting the circulation of the other fetuses (Rosso 1975, Wapnin and Dierks-Ventling 1971, Wapnin et al. 1996). Other dams fed either the HZn or the ND diet during the second half of gestation were given, under anesthesia, an injection of Cu acetate via the femoral vein at a dose of 39.5 μmol/kg Cu (2.5 mg/kg) in ~0.5 mL. Fetuses and their placentas were removed from an abdominal incision at 0, 10, 20, 40 and 60 min. Dam blood was also sampled at the same time via the cavernous sinus. At the end of 60 min, the dams were killed by exsanguination from the abdominal aorta. Rats that showed respiratory distress were killed before the last collection time point. Fetal and maternal blood were centrifuged at 600 × g for 10 min and plasma collected for chemical analysis.

Analytical methods. Cu, Zn and Fe were assayed by atomic absorption spectrophotometry (Spectra AA10, Varian Instruments, Sunnyvale, CA). Samples of fetal and dam livers, and placentas were treated in 15.5 mol/L nitric acid at room temperature until digested, diluted to 10 mL and filtered through ash-free filter paper under pressure before analysis. Hematocrit (Hct) of fetuses and dams were also obtained from heparinized blood collections. Metallothionein (MT) was assayed by the Cd-heme method (Onosaka and Cherian 1982).

Statistical analysis. Data were analyzed by the Mann-Whitney test (Zar 1984) by using a computer program (Buchan 1994); data are presented in tables and figures as means ± SEM. Plasma Cu concentrations after a Cu bolus were analyzed by two-way main effects (diet and time) ANOVA. The threshold of significance was α = 0.05.

RESULTS

One day before expected delivery, dam weights were significantly lower in those fed the HZn diet, with no differences in fetal weight between the groups (Table 1). However, litter size was smaller in the HZn dietary treatment group. There was also no difference in the Hct of dams or fetuses between the two groups. MT in the liver of the HZn diet–fed dams was elevated, but the differences in fetal livers were not significant.

Dam and fetal plasma Cu concentrations 1 d before the end of gestation are shown in Figure 1A. The HZn diet resulted in lower dam plasma Cu levels compared with the ND group, but no differences were observed between the two fetal groups. Dam plasma Zn was significantly higher in the HZn group, with no differences in fetal plasma Zn levels (Fig. 1B). Dam plasma Fe concentrations did not differ between groups (Fig. 1C), but fetal plasma Fe was higher in the HZn than in the ND group.

Liver Cu concentrations in dams and fetuses are presented in Figure 2A. No differences were found in dam liver Cu. In contrast, fetuses of HZn dams had lower hepatic Cu than fetuses of ND dams. The relative deficit in fetal Cu concentration was clearly seen when expressed as the ratio of fetal to dam liver Cu, which was significantly lower in the HZn group than in the ND group (HZn, 2.83 ± 0.24, n = 9 vs. ND, 4.56 ± 0.51, n = 6; P = 0.0076). Both dam and fetal liver Zn concentrations were elevated in the HZn diet group (Fig. 2B). In contrast, the dietary treatment did not affect either dam or fetal liver Fe concentration (Fig. 2C).

Placental weights were not affected by maternal diet (Table 2). The HZn dietary treatment produced no effect on placental Cu concentration, whereas both Zn and Fe were significantly elevated in the HZn group compared with the ND group. Similarly, placental MT in the HZn litters was also higher than in the ND rats.

After the administration of a Cu acetate bolus of 39.5 μmol/kg, the HZn diet–fed dams maintained generally higher plasma Cu concentrations than the ND group in the period between 10 and 60 min after injection (Fig. 3) (two-way ANOVA for time and diet, P = 0.0267). At 60 min, circulating Cu was significantly higher in the HZn dams than in the ND group. The injection of Cu to the dams resulted in no differ-

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1 Abbreviations used: Hct, hemocrit; HZn, high zinc diet; MT, metallothionein; ND, normal zinc diet.

| Table 1 | Effects of a high Zn (HZn) compared with a normal Zn diet (ND) on weight, hematocrit and liver metallothionein (MT) of rat dams and fetuses† |
|---------|-----------------|-----------------|-----------------|-----------------|-----------------|
|         | HZn diet        | ND diet         | HZn diet        | ND diet         |
| WEIGHT, g | 394.5 ± 7.1*    | 427.0 ± 10.0    | 3.76 ± 0.06    | 3.62 ± 0.10    |
| n        | 19              | 9               | 19*             | 92              |
| LITTER SIZE, n | —               | —               | 12.6 ± 0.4**   | 14.7 ± 0.7     |
| n        | —               | —               | 19              | 9               |
| HEMATOCRIT | 0.40 ± 0.01    | 0.40 ± 0.02     | 0.43 ± 0.02    | 0.39 ± 0.01    |
| n        | 18              | 6               | 19              | 9               |
| LIVER MT, nmol/g | 6.81 ± 1.53** | 1.01 ± 0.56     | 67.7 ± 6.4     | 57.9 ± 4.3     |
| n        | 8               | 6               | 8               | 8               |

† Values are means ± SEM. * P < 0.05; ** P < 0.02 vs. ND diet. Each fetal weight is based on litter average.
the HZn diet, with its consequent high maternal plasma Zn, made such differences disappear, presumably as a result of saturation of Zn transport mechanisms from placenta to fetus. Also of interest is the almost threefold difference in hepatic Cu concentration between fetuses and dams, in spite of a marked downhill gradient from dam-to-fetus in the plasma. Furthermore, the fetus/dam hepatic Cu ratio in the HZn group was significantly lower than that of the ND group, indicating that the fetuses of the HZn dams had an impaired capacity to store Cu in their livers, or that Cu transported to the fetus had diminished.

Although fetal hepatic Fe was unaffected by the maternal diet, HZn fetuses had higher plasma Fe, which might be related to the elevated placental Fe concentration and consequent downhill gradient. In contrast, the elevated placental Zn of the HZn group did not reflect proportional changes in fetal plasma Zn, but was consistent with the increased hepatic Zn concentration, suggesting that the accretion process takes place over a relatively extended period. The lack of differences of Cu concentration in fetal plasma or placenta between the two groups at any time point (not shown). We also found no differences in dam liver Cu or Fe 60 min after the Cu bolus.

**FIGURE 1** Plasma Cu (A), Zn (B) and Fe (C) concentrations of rat dams fed either a normal diet (ND) or high Zn diet (HZn) and their fetuses. Values are means ± SEM. *P < 0.05; ***P < 0.001 vs. the ND diet. The number of animals were as follows: ND dams, 9; HZn dam, 19; ND fetuses, 9 litters; HZn fetuses, 19 litters. Fetuses of the same litter were pooled for these determinations.

**FIGURE 2** Liver Cu (A), Zn (B) and Fe (C) concentrations of rat dams fed either a normal diet (ND) or high Zn diet (HZn) and their fetuses. Values are means ± SEM. *P < 0.05; **P < 0.01; ***P < 0.001 vs. the ND group. The number of animals were as follows: ND dams, 6; HZn dams, 9; ND fetuses, 12 litters; HZn fetuses, 19 litters.

**DISCUSSION**

*Effects of HZn on the fetus.* The major finding in this study was that a dietary excess of Zn during the second half of the gestation produced substantial changes in fetal liver mineral concentrations. The well-known Cu-Zn interaction was clearly manifested in fetal liver as a decrease of Cu in the presence of the unexpectedly greater hepatic Zn accumulation in the HZn fetuses. These occurred in spite of indistinguishable fetal plasma Cu and Zn concentrations at the time of removal of the fetuses. In the ND group, the expected uphill plasma Zn gradient between dam and fetus was observed. However,
Comparison of maternal high Zn (HZn) to a normal Zn diet (ND) on the placental weight, mineral and metallothionein (MT) concentration in rats

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<tr>
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<th>HZn diet</th>
<th>ND diet</th>
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<tr>
<td>Placental weight, g</td>
<td>0.515 ± 0.014</td>
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<tr>
<td>n</td>
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<td>9^2</td>
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<td>Cu, nmol/g tissue</td>
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<td>32.8 ± 3.4</td>
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<td>Zn, nmol/g tissue</td>
<td>332 ± 21.4***</td>
<td>215 ± 6.4</td>
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<tr>
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<td>19</td>
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<tr>
<td>Fe, nmol/g tissue</td>
<td>1820 ± 3***</td>
<td>1500 ± 61</td>
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<td>9</td>
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<tr>
<td>MT, nmol/g</td>
<td>0.76 ± 0.18*</td>
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1 Values are means ± SEM. * P < 0.05; *** P < 0.001 vs. ND diet.
2 Each placental weight is based on litter average.

LITERATURE CITED


