Maternal Iron and Zinc Supplementation during Pregnancy Affects Body Weight and Iron Status in Rat Pups at Weaning

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Abstract

Pregnant women worldwide are frequently iron (Fe) and zinc (Zn) deficient. Therefore, cosupplementation with Fe and Zn during pregnancy is common. Although Fe supplementation programs are successful, studies suggest that Zn supplementation negatively affects maternal Fe metabolism. However, little is known about the effects of maternal Fe or Zn supplementation on Fe metabolism in the offspring. We developed a rat model to investigate if Fe and/or Zn supplementation during pregnancy affects regulation of nonheme Fe absorption and Fe status in offspring and if these effects are dependent upon maternal Fe and Zn status at conception. Control (C; fed a Fe- and Zn-adequate diet; 75 and 25 μg/g, respectively) or Fe- and Zn-deficient (D; fed a Fe- and Zn-deficient diet; 12 and 10 μg/g, respectively) rats were supplemented with Fe (27 mg/wk), Zn (4.5 mg/wk), Fe+Zn (27 mg Fe, 4.5 mg Zn/wk), or placebo throughout pregnancy. At postnatal d 21, body weight (BW), hemoglobin (Hb), hematocrit (Hct), liver and intestine Fe concentration, liver hepcidin, and intestine Fe transporter expression were determined in pups. Zn supplementation of C dams decreased pup BW (P < 0.0001), whereas it increased pup BW in D dams (P < 0.0001). Zn supplementation of C dams did not affect Hb and Hct in pups but increased the liver Fe concentration (P = 0.0002). However, Zn supplementation of D dams decreased hepcidin expression in their offspring (P < 0.0001). In C dams, Fe and Fe+Zn supplementation decreased ferroportin levels in pup intestine compared with pups from unsupplemented dams (P < 0.05). In conclusion, Zn supplementation of dams with adequate Fe and Zn status increases offspring liver Fe concentration and postnatally compromises BW. Therefore, potential adverse effects of Zn supplementation should be evaluated.

Introduction

Iron (Fe) and zinc (Zn) deficiency are 2 of the most common nutritional disorders in the world (1,2). There is increasing evidence that concurrent Fe and Zn deficiencies exist in many populations, resulting primarily from low meat and high dietary phytate intakes (3). Pregnant women are at especially high risk of Fe and Zn deficiency because of markedly increased Fe and Zn requirements during pregnancy (4,5). To meet these increased demands for fetal growth, pregnant women are often supplemented with Fe and/or Zn. Studies have shown that Fe supplementation during pregnancy increases maternal Fe stores (6,7), but few studies have followed the Fe status of their infants after birth. Similarly, Zn supplementation increases the maternal serum Zn concentration (8,9) and infant birth weight and head circumference (9). As a result and due to the fact that Fe and Zn deficiencies often coexist in many populations, combined supplementation of Fe and Zn is often prescribed. However, several studies suggest that cosupplementation of Fe and Zn results in negative interactions of one micronutrient on the status of the other (10,11). For example, at high molar ratios, Fe supplementation decreases Zn absorption (12) and, similarly, Zn supplementation decreases nonheme Fe absorption in humans (13). Studies in developing countries have investigated the effect of Zn supplementation on Fe status in pregnant women and infants. An adverse influence of Zn supplementation on indices of Fe status was found among pregnant Peruvian women who were randomized to receive Fe or Zn supplementation (14). Though there was no difference in percent Fe absorption, the serum Fe concentration was lower among women who received Zn supplementation compared with unsupplemented women. This suggests that cosupplementation of Fe and Zn during pregnancy may interfere with maternal Fe metabolism and subsequently affect fetal and ultimately infant Fe metabolism.

Few human intervention trials have been specifically designed to investigate the effects of Fe, Zn, or combined Fe and Zn...
supplementation during pregnancy on Fe status and/or offspring development. Previously, our group showed that molecular mechanisms regulating Fe absorption during infancy are developmentally regulated and that the ability to homeostatically regulate Fe absorption does not develop until late infancy (15). Whether maternal codeficiencies of Fe and Zn in combination with Fe and/or Zn supplementation affect the development of Fe regulatory mechanisms or have any consequence for the offspring’s Fe status is not known.

Iron absorption mainly occurs in the duodenum, where nonheme Fe is taken up by epithelial cells via divalent metal transporter 1 (DMT1). Expression of DMT1 is reduced when Fe-enriched diets are fed but increases when Fe intake is restricted (16). Inside the enterocyte, Fe can be stored as ferritin or exported through the basolateral membrane into systemic circulation. Fe export into circulation is mediated by ferroportin (FPN), which is abundantly expressed in the basolateral membrane of polarized enterocytes (17,18). Iron efflux from the enterocyte also requires the action of a membrane-bound ferrooxidase, hephaestin, which is a multicooper oxidase (19). FPN and hephaestin colocalize on the basolateral membrane of enterocytes (20). Intestinal Fe absorption is regulated in response to Fe stores and erythropoietic demand (21) via hepcidin, a key regulator of Fe absorption (22,23). Hepcidin is synthesized by hepatocytes and targets the FPN of enterocytes (24) to reduce intestinal Fe absorption. Cell culture studies show that hepcidin binding to FPN initiates a rapid phosphorylation of 1 of 2 adjacent tyrosine residues of the cytosolic domain of FPN (25). After phosphorylation, the hepcidin-FPN complex is internalized through clathrin-coated pits. Subsequently, the phosphates are removed and FPN becomes ubiquitinated, which directs the protein for degradation. Hepcidin synthesis is induced by Fe loading and is suppressed by anemia. Urinary hepcidin levels correlate with Fe status in human pregnancy (26). However, it is not known whether maternal Fe and Zn deficiency and/or supplementation during pregnancy affects the expression of hepcidin in infants and thereby influences Fe status and absorption.

Weaning is a critical period when the risk of Fe deficiency is increased due to a higher Fe requirement for growth and the depletion of Fe stores (27). In this study, we developed a rat model to explore the effects of maternal Fe and/or Zn supplementation during pregnancy on Fe status and the ability to regulate Fe metabolism in the offspring at weaning.

**Materials and Methods**

**Rats.** This study was approved by the Animal Research Services at the University of California, Davis, which is accredited by the American Association for the Accreditation of Laboratory Animal Care. Female virgin Sprague-Dawley 7- to 8-wk-old rats weighing 180–200 g were purchased from Charles River. During the acclimation period (72 h), rats consumed ad libitum commercial rodent diet (LabDiet) and deionized water. Rats were housed in polycarbonate cages under constant conditions (temperature, 22°C; humidity, 65%; 12-h-light/-dark cycle). After acclimation, rats were randomly divided into 2 groups and consumed ad libitum the experimental diets, which differed in only Fe and Zn concentrations.

**Experimental diets.** The protein base of the experimental diets was egg albumin. Vitamins and minerals (except Fe and Zn) were added according to AIN guidelines for laboratory animals (AIN93G) (28). Mineral mix without Fe or Zn was purchased from MP Biomedicals. To achieve the desired levels of Fe and Zn, FeSO$_4$·7H$_2$O (Sigma) and ZnCl$_2$ (J. T. Baker) were added.

The control group (C; n = 18) received a diet containing 75 μg Fe and 25 μg Zn/kg diet throughout the study. The Fe- and Zn-deficient group (D; n = 18) received a diet containing 12 μg Fe and 10 μg Zn/kg diet throughout the study, which was intended to produce moderate Fe and Zn deficiency similar to that observed in human populations, i.e. no overt signs of deficiency such as reduced food intake, hair loss, etc., but lower Fe and Zn status. The rats consumed 25 g/d during pregnancy and lactation. Thus, the control diet provided ~1125–1875 μg Fe/d and 375–625 μg Zn/d and the deficient diet ~180–300 μg Fe/d and 150–250 μg Zn/d. Rats were fed the experimental diet for 4 wk, and then were bred and pregnancy was confirmed by the presence of a vaginal plug. Rats from each diet group were subdivided into 4 groups (Supplemental Fig. 1) that received supplements of Fe (9 mg), Zn (1.5 mg), or Fe (9 mg) and Zn (1.5 mg) mixed in grape jelly (placebo; Welch’s). The Fe and Zn content of the grape jelly was measured by atomic absorption spectrophotometry and found to be negligible. Supplementation was given 3 times/wk throughout pregnancy. The amounts of supplemental Fe and Zn given to each rat correspond to the amount of supplemental Fe and Zn that pregnant women in developing countries receive (5,6). The body weight (BW) and number of offspring born were considered when calculating the supplement doses.

**Sample collection.** On gestation day (GD) 20, dams from both diet groups (n ≥ 6/group; C and D) were killed by CO$_2$ asphyxiation. Maternal hemoglobin (Hb), hematocrit (Hct), plasma Zn, liver, small intestine and femur Fe and Zn concentrations, and fetal liver Fe and Zn concentrations were measured as described below to first verify that maternal and fetal Fe and Zn status were affected by our dietary paradigm. In a subsequent study, rats were allowed to give birth and litters were culled to 10 pups/litter for the following measurements. The number of females born were considered when calculating the supplement doses. Females born to both diet groups were killed on postnatal day 1.0 mL and diluted with ultra-pure water. Plasma (120 μL) was digested in 1.0 mL of 1 mol/L HNO$_3$ for 48 h. Samples were wet-ashed to a volume of ~1.0 mL and diluted with ultra-pure water. Plasma (120 μL) was digested in 1.0 mL of 1 mol/L HNO$_3$ for 48 h. Samples were analyzed by flame atomic absorption spectrophotometry (Smith-Heifjie 4000, Thermo Jarrell Ash).

**Mineral analysis.** Tissue and plasma Fe and Zn concentrations were measured as previously described (29). Briefly, ~0.2 g of tissues were digested in acid-washed vials with 3.0 mL of 16 mol/L HNO$_3$ for 48 h. Samples were wet-ashed to a volume of ~1.0 mL and diluted with ultra-pure water. Plasma (120 μL) was digested in 1.0 mL of 1 mol/L HNO$_3$ for 48 h. Samples were analyzed by flame atomic absorption spectrophotometry (Smith-Heifjie 4000, Thermo Jarrell Ash).

**RNA isolation and cdNA synthesis.** Total RNA was isolated from small intestine preserved in RNALater by using Trizol (Invitrogen) according to the manufacturer’s instructions. After isolation, RNA was quantified by spectrophotometry and the integrity was assessed by examination of 28S and 18S bands in 2% agarose gel electrophoresis. cDNA was synthesized from 1.0 μg of total RNA using TaqMan reverse transcription kit (Applied Biosystems) in 50 μL reaction mixture following the manufacturer’s instructions. The reaction mixture was incubated at 25°C for 10 min, then at 48°C for 30 min and heated to 95°C for 5 min. The cDNA products were stored at −20°C until used for real-time semiquantitative PCR.

**Real-time semiquantitative PCR.** An ABI 7900 HT real-time thermo-cycler (Applied Biosystems) and ABI Prism software version 2.1 (Applied
Bioseps) were used for semiquantitative real-time PCR amplification. Gene-specific primers for DMT1, FPN, hephaestin, ferritin, and GAPDH were designed by using Primer Express software (Applied Biosystems) (Supplemental Table 1) and obtained from Qiagen. The primers were designed only to span intronic sequences to avoid coamplification of genomic DNA. Reactions were performed in 25-µL final volumes with 1.5 µL of cDNA by using the SYBR Green detection system (Applied Biosystems). The following protocol was used for thermocycling: 50°C for 2 min; 95°C for 10 min; and 40 cycles of 95°C for 15 s, 60°C for 1 min, and 95°C for 15 s. The linearity of the dissociation curves was analyzed and the mean cycle time of the linear part of the curve was designated as Ct. Each sample was analyzed in duplicate and normalized to GAPDH using the following equation: \( \Delta C_t = C_t_{\text{transporter}} - C_t_{\text{GAPDH}} \). The fold-change in Fe transporter expression in response to supplementation was calculated using the following equation: \( 2^{\Delta C_{\text{GENE}}} \), where \( \Delta C_{\text{GENE}} = \Delta C_t_{\text{transporter}} - \Delta C_t_{\text{control}} \). Values represent fold of control (set to 1.0) ± SD.

Western-blot analysis. Small intestine (~0.4 g) was homogenized in HEPES-EDTA buffer containing protease inhibitor as previously described (30). The homogenate was centrifuged at 15000 × g for 15 min at 4°C. The protein concentration was determined by the Bradford assay (31). Total cellular proteins (100 µg) were separated by SDS-PAGE, transferred to nitrocellulose membrane, and incubated with primary and secondary antibody as previously described (32,33). Bands were visualized by autoradiography by using SuperSignal Femto Chemiluminescent Detection System (Pierce). The relative amount of immunoreactive proteins on Western blots was quantified in arbitrary units by densitometry using the Chemidoc Gel Quantification System (Bio-Rad). The band intensities were normalized within and between gels using respective bands from pups born to unsupplemented dams.

Statistical analysis. Results are expressed as mean ± SD. Statistical analysis was performed using SAS (version 9.2, SAS Institute). Data were checked for normality and skewed data (liver hepcidin expression) were log-transformed before analysis. Data from dams at GD20 were analyzed by t test and the data from pups were analyzed by mixed model considering the random effect of dam. When the interaction was significant, differences between different supplementation were considered significant at \( P < 0.05 \).

Results

Maternal and fetal Fe and Zn status at GD20. D dams had lower Hb and Hct levels and lower Fe concentrations in liver, femur, and intestine compared with C dams (Table 1). Liver Fe concentration was also lower in fetuses from D dams compared with those from C dams, verifying that both dams and their offspring were Fe deficient. The maternal plasma Zn concentration was lower in D dams than in C dams. The fetal liver Zn concentration did not differ between the groups, illustrating that the maternal Zn deficiency was indeed mild, as intended.

Offspring BW at PN21. Pups from D dams had higher BW compared with those from C dams (Fig. 1). Among the pups from C dams, maternal +Fe increased, whereas +Zn and +Fe+Zn decreased BW compared with those from unsupplemented dams. In contrast, among the pups from D dams, maternal +Fe had no effect, whereas +Zn and +Fe+Zn increased BW compared with pups from unsupplemented dams.

Offspring Fe status at PN21. To determine whether maternal Fe and Zn supplementation during pregnancy affects the Fe status in the offspring, we measured Hb, Hct, and liver and intestine Fe concentrations at weaning (Table 2). As expected, offspring born to C dams had higher Hb and Hct levels compared with those from D dams. Among offspring born to C dams, Fe supplementation (+Fe) had no effect on Hb and Hct levels; however, +Fe increased Hb and Hct levels in the offspring of D dams. Thus, Fe supplementation during pregnancy did not affect offspring Fe status when maternal Fe and Zn status was normal, but it improved offspring Fe status when dams were Fe and Zn deficient. Zn supplementation (+Zn) had no main effect on offspring Hb and Hct levels; however, +Zn significantly reduced the positive effect of Fe supplementation on Fe status, illustrating a negative effect of maternal Zn supplementation on offspring Fe status.

Overall, offspring from D dams had a lower liver Fe concentration compared with those from C dams (Table 2). In offspring from C dams, the liver Fe concentration was higher if the dams were supplemented with Zn compared with pups from dams supplemented with Fe or Fe+Zn. In contrast, in offspring from D dams, Fe, Zn, and Fe+Zn supplementation during pregnancy did not affect the offspring liver Fe concentration. Thus, Zn supplementation during pregnancy increased offspring liver Fe concentration when maternal Fe and Zn status was normal.

**TABLE 1** Effects of maternal dietary Fe and Zn concentrations on maternal and fetal Fe and Zn status at GD20 in placebo supplemented dams

<table>
<thead>
<tr>
<th>Variable</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Hb, g/L  | 127 ± 5 | 117 ± 3*
| Hct      | 0.36 ± 0.01 | 0.34 ± 0.01* |
| Liver Fe, µmol/g | 1.58 ± 0.38 | 1.07 ± 0.18* |
| Intestine Fe, µmol/g | 0.64 ± 0.20 | 0.20 ± 0.05* |
| Femur Fe, µmol/g | 0.68 ± 0.18 | 0.47 ± 0.05* |
| Plasma Zn, µmol/L | 12.2 ± 3.1 | 6.11 ± 1.5* |
| Liver Zn, µmol/g | 0.35 ± 0.03 | 0.33 ± 0.01 |
| Intestine Zn, µmol/g | 0.34 ± 0.02 | 0.30 ± 0.01* |
| Fetal |   |   |
| Liver Fe, µmol/g | 4.73 ± 0.79 | 2.85 ± 0.58* |
| Liver Zn, µmol/g | 1.23 ± 0.17 | 1.10 ± 0.15 |

*Values are means ± SD, n = 6. *Different from C, \( P < 0.05 \).

1. The Fe and Zn concentrations were 75 and 25 µg/g diet, respectively.
2. The Fe and Zn concentrations were 12 and 10 µg/g diet, respectively.

3. The Fe and Zn concentrations were 12 and 10 µg/g diet, respectively.
TABLE 2  Effect of maternal diet and Fe and Zn supplementation during pregnancy on pup Fe status indicators at PN21

<table>
<thead>
<tr>
<th>Variable</th>
<th>Dietary group</th>
<th>Placebo</th>
<th>Fe</th>
<th>Zn</th>
<th>Fe + Zn</th>
<th>P-value; 3-way interaction</th>
<th>Significance; 3-way interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Diet × Fe</td>
<td>Zn Diet × Fe</td>
</tr>
<tr>
<td>Hb, g/L</td>
<td>C</td>
<td>94 ± 6</td>
<td>99 ± 16</td>
<td>100 ± 5</td>
<td>97 ± 9</td>
<td>0.38</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>56 ± 11</td>
<td>91 ± 4</td>
<td>63 ± 5</td>
<td>80 ± 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hct</td>
<td>C</td>
<td>0.31 ± 0.02</td>
<td>0.33 ± 0.05</td>
<td>0.32 ± 0.01</td>
<td>0.32 ± 0.02</td>
<td>0.62</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>0.19 ± 0.01</td>
<td>0.29 ± 0.02</td>
<td>0.20 ± 0.01</td>
<td>0.26 ± 0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver Fe, μmol/g</td>
<td>C</td>
<td>0.66 ± 0.11</td>
<td>0.75 ± 0.14</td>
<td>1.09 ± 0.18</td>
<td>0.77 ± 0.20</td>
<td>0.08</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>0.36 ± 0.06</td>
<td>0.56 ± 0.18</td>
<td>0.36 ± 0.07</td>
<td>0.40 ± 0.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestine Fe, μmol/g</td>
<td>C</td>
<td>0.25 ± 0.08</td>
<td>0.42 ± 0.07</td>
<td>0.32 ± 0.06</td>
<td>0.39 ± 0.13</td>
<td>0.49</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>0.07 ± 0.03</td>
<td>0.07 ± 0.03</td>
<td>0.06 ± 0.03</td>
<td>0.08 ± 0.05</td>
<td></td>
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</table>

1 Values are means ± SD, n = 8. Labeled means without a common letter differ, P < 0.05.
2 *P < 0.05.

Offspring from C dams had higher intestinal Fe concentrations compared with offspring from D dams (Table 2). The intestinal Fe concentration of offspring was higher if dams were not previously deficient but were given Fe supplementation (C dams +Fe); however, this effect was not observed if the dams were previously Fe and Zn deficient (D dams +Fe). Maternal Zn supplementation did not affect intestinal Fe concentration in the offspring.

Offspring liver hepcidin expression at PN21. Liver hepcidin mRNA expression in the offspring from D dams was only ~2% of that of the offspring from C dams (P < 0.0001) (Fig. 2). Among offspring from C dams, +Fe and +Zn during pregnancy had no effect on the offspring’s liver hepcidin expression. In contrast, in offspring from D dams, +Fe increased liver hepcidin expression, whereas +Zn decreased the expression. Thus, Fe supplementation during pregnancy increased liver hepcidin expression in offspring when maternal Fe and Zn status was low but had no effect on hepcidin expression when maternal Fe and Zn status was normal.

DMT1, FPN, hephaestin, and ferritin expression in offspring intestine at PN21. Overall, offspring from C dams had lower expression levels of DMT1 than those from D dams (P < 0.0001) (Fig. 3). Among offspring from C dams, supplementation of Fe and/or Zn during pregnancy had no effect on DMT1 mRNA expression. However, in offspring from D dams, +Fe decreased DMT1 mRNA expression compared with offspring from unsupplemented dams. Zn supplementation, with or without Fe supplementation, did not affect DMT1 expression in pups from D dams compared with pups from unsupplemented dams.

Overall, pups from C dams had higher FPN mRNA expression than those from D dams. Among the pups from C dams, +Fe decreased FPN mRNA expression, but +Zn had no effect. However, in pups from D dams, Fe and Zn supplementation had no effect in offspring FPN expression.

Offspring from D dams had lower hephaestin expression than offspring from C dams (P < 0.0001). There was no effect of maternal supplementation on hephaestin expression in pups from C or D dams.

We detected no effect of maternal diet on ferritin expression. Among the pups from C dams, +Zn and +Fe+Zn increased ferritin mRNA expression compared with those from unsupplemented dams. Among the offspring from D dams, +Fe, +Zn, or Fe+Zn had no effect on ferritin mRNA expression in pup intestine. Thus, although there was no effect of maternal Fe and Zn supplementation on offspring intestinal ferritin expression when maternal Fe and Zn status was low, maternal Zn and Fe+Zn supplementation during pregnancy augmented the ferritin expression in the intestine of offspring when maternal Fe and Zn status was normal.

DMT1, FPN, and hephaestin protein levels in offspring intestine at PN21. DMT1, FPN, and hephaestin protein levels were assessed by immunoblot in the intestine of offspring from each diet group to evaluate the effects of maternal status and maternal Fe, Zn, or Fe+Zn supplementation during pregnancy on Fe transporter protein abundance (Table 3). Overall, Fe supplementation during pregnancy decreased DMT1 and FPN abundance in pup intestine. On the other hand, supplementation during pregnancy did not affect hephaestin abundance in the intestine of the offspring.

Discussion

Mild to moderate Fe and Zn deficiencies during pregnancy are much more common than severe deficiencies in humans. However, most animal models have utilized diets very low in Fe and/or Zn levels to model effects of Fe and Zn deficiencies on human health and disease. In this study, we developed a rat model of moderate Fe and Zn deficiency that did not severely affect pregnancy outcome or offspring viability. Importantly, there were no overt signs of deficiency such as reduced food intake, hair loss, etc., even though maternal Fe and Zn status and fetal Fe status were altered. The level of Fe and Zn supplementation in this study was structured to reflect levels of Fe and Zn...
supplementation during pregnancy in humans. In our model, Fe supplementation of Fe- and Zn-deficient dams during pregnancy modulated Fe status (Hb, Hct, and liver Fe concentration) and the Fe regulatory peptide hepcidin, which is consistent with previous reports (16,34–36). Importantly, Fe supplementation in nondeficient dams resulted in Fe accumulation in the small intestine, suggesting activation of protective mechanisms in the small intestine to limit nonheme Fe absorption when Fe is not needed (37). We therefore think that this is an appropriate model to study the effects of maternal Fe and Zn supplementation during pregnancy on postnatal Fe metabolism in offspring.

Similar to reports in humans, we found that offspring BW was affected by maternal supplementation during pregnancy and the effect depended on maternal Fe and Zn status. Iron-only supplementation increased BW in offspring from C (adequate in Fe and Zn) dams compared with those from unsupplemented dams, whereas there was no effect in the offspring from D (deficient in Fe and Zn) dams. Recently, 2 randomized controlled trials with nonanemic Fe-replete pregnant women consuming a diet presumably adequate in Fe and Zn (38,39) documented that Fe supplementation was associated with higher BW compared with newborns from unsupplemented mothers. In contrast, studies in developing countries, where the maternal diet is generally low in Fe and Zn, did not demonstrate positive effects of Fe supplementation during pregnancy on BW (40,41). Reports on the effects of Zn supplementation on BW are conflicting. In a placebo-controlled trial in presumably Zn-deficient (low plasma Zn concentration) African American women, prenatal Zn supplementation resulted in a significant increase in BW (9). However, in a series of randomized trials in Asia and Latin America, Zn supplementation had no effect on BW (42). These differences likely reflect the fact that the latter populations consisted of heterogeneous groups of women with both adequate and inadequate Zn status. This is consistent with our observations that both Zn and Fe+Zn supplementation in rats with a documented Fe and Zn deficiency increased offspring BW, whereas the opposite effect was observed in rats with adequate Fe and Zn nutrure. These data indicate that effects of maternal supplementation on BW may depend on maternal nutritional status.

As expected, Fe supplementation during pregnancy improved Fe status only in offspring from D dams, illustrating tight regulation of the mechanisms that regulate Fe homeostasis in the mother. Interestingly, Zn supplementation of C dams during pregnancy increased liver Fe concentration in their offspring, which was associated with decreased BW. It is currently unclear how Zn supplementation decreases BW. However, excess accumulation of Fe in the liver may compromise offspring growth by generating free radicals and inducing oxidative stress (21). Similarly, in humans, Fe supplementation of Fe-replete infants has been shown to negatively affect length (43) and weight gain (44,45).

In this study, we demonstrated that Zn supplementation during pregnancy had no effect on Fe status in the offspring from previously deficient dams. However, when given along with supplemental Fe, Zn reduced the effects of Fe supplementation on restoring Fe status, consistent with observations in human infants (46). It is known that Fe and Zn given together as a supplement can interact with each other and compete for absorption in the small intestine (12,13). Herein, our data suggest that the inhibitory effect of maternal Zn supplementation on nonheme Fe absorption has important ramifications with respect to Fe status in the developing offspring.

Fe absorption is regulated by hepcidin, which is synthesized by the liver and its expression parallels liver Fe concentration (47). It is a potent homeostatic regulator of Fe absorption and acts by inhibiting the efflux of Fe from the intestine to the circulation (24). We expected to find parallel changes in hepatic Fe concentration and hepcidin expression. In fact, in offspring from C dams, Fe supplementation during pregnancy did not affect the offspring liver Fe concentration and had no effect on hepcidin expression. However, in offspring from D dams, although Fe supplementation did not increase the offspring liver Fe concentration, it increased hepcidin mRNA expression. The implications of this finding remain to be elucidated. Interestingly, Zn supplementation increased the offspring liver Fe concentration, but it decreased hepcidin expression. This decreased hepcidin expression may allow release of more iron from the intestine to the systemic circulation.

DMT1 is involved in the uptake of Fe from the intestinal lumen into the enterocytes (48), whereas FPN is involved in the release of Fe to the systemic circulation. Fe supplementation
decreased DMT1 and FPN protein levels in the intestine. We also observed that Fe supplementation increased intestinal Fe concentration. While these data suggest that intestinal Fe stores would increase, we observed no increase in ferritin expression. Mechanisms by which Fe could build up in the cell without an increase in ferritin may include other proteins or low-molecular weight ligands within the cell that can bind the imported Fe (49).

We found that maternal Zn supplementation during pregnancy increased the liver Fe concentration in pups. With regard to absorption, Zn supplementation had no effect on DMT1, FPN, and hephaestin protein levels in the intestine. Although these protein levels remained similar in the offspring, we speculate that higher hephaestin activity may have increased Fe transfer into systemic circulation and thus increased liver Fe concentration. Recent studies in both in vivo and in vitro models suggest that hephaestin activity is a prime regulator of intestinal Fe absorption (50); however, we did not measure this activity. Interestingly, we found that Zn supplementation increased intestinal ferritin expression, which may lead to trapping of Fe in the intestine. This suggests that Fe status of infants may not be improved by Fe supplementation during pregnancy when Zn is added with supplemental Fe.

These findings may have implications when recommending Zn supplementation during pregnancy. In women with adequate Fe and Zn status, maternal Zn supplementation alone or in combination with Fe may compromise the BW and dysregulate Fe homeostasis mechanisms of infants during the postnatal period. In women with Fe- and Zn-deficient status, Zn supplementation may interfere with the role of Fe in improving Fe status by dysregulating the Fe homeostasis mechanism, particularly the expression of ferritin. More research is necessary to evaluate the potential adverse effects of Zn supplementation alone or in combination with Fe.

Acknowledgments

We thank Andrew G. Hall and Winyoo Chowanadisai for providing various technical help while conducting the study. We are also thankful to Janet M. Peerson for constructive help with statistical analyses. M.B.H., S.L.K., and B.L. designed the research; and M.B.H. conducted the research, analyzed data, and wrote the 1st draft of the paper. All authors read and approved the final manuscript.

Literature Cited


