Maternal Zinc Deficiency in Rats Affects Growth and Glucose Metabolism in the Offspring by Inducing Insulin Resistance Postnatally\textsuperscript{1,2}

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Abstract

Interactions among zinc (Zn), insulin, and glucose metabolism are complex. Maternal Zn deficiency affects maternal carbohydrate metabolism, but the mechanisms underlying changes in glucose homeostasis of offspring are not well understood. Rats consumed Zn-deficient (ZnD; 7 \(\mu\)g/g) or control (ZnC; 25 \(\mu\)g/g) diets ad libitum from 3 wk preconception to 21 d postparturition. Litters were culled to 7 pups/dam postnatally and pups were allowed to nurse their original mothers; after weaning, pups were fed nonpurified diet. Insulin and glucose tolerance tests were performed on the pups at wk 5 and 10. Although there was no difference in birth weight between groups, ZnD pups weighed significantly more than controls by d 10 (+5%) and 20 (+10%). Both blood glucose and serum insulin-like growth factor (IGF-1) concentrations at wk 3 were significantly higher in ZnD pups than in controls. Both male and female ZnD rats were less sensitive to insulin and glucose stimulation than controls at wk 5 and 10. At wk 15, serum leptin concentrations were higher in male ZnD rats than in controls. Phosphorylation of muscle Akt protein, an insulin receptor (IR) signaling intermediate, was lower in female ZnD rats than in controls at wk 15, but they did not differ in phosphorylation of IR. Maternal Zn deficiency resulted in greater serum IGF-1 concentrations and the excessive postnatal weight gain in their offspring as well as impaired subsequent glucose sensitivity. It was associated with gender-specific alterations in the serum leptin concentration and the insulin signaling pathway. These findings suggest that suboptimal maternal Zn status induces long-term changes in the offspring related to abnormal glucose tolerance. J. Nutr. 140: 1621–1627, 2010.

Introduction

Zinc (Zn) is an essential micronutrient and is critical for normal growth and development in humans and other mammals (1–3). Increased Zn requirements during periods of rapid growth (2), such as infancy, adolescence, pregnancy, and lactation, may also increase the risk for Zn deficiency. Zn deficiency is common in developing countries (4) due to inadequate dietary Zn intake and poor Zn bioavailability (2). It is estimated that pregnant women are at high risk for at least mild to moderate Zn deficiency in both developing and developed countries (5,6).

Hales et al. (7) proposed the “developmental origin of health and disease,” whereby inadequate nutrition during fetal life may result in permanent metabolic modifications and increased risk of diabetes and other diseases in adulthood. The prevalence of maternal Zn deficiency worldwide is difficult to estimate but is fairly prevalent. Thus, the potential effect of such deficiency on subsequent development of adult diseases such as diabetes in the offspring may be of great importance. To investigate the effect of maternal Zn deficiency on diabetes in the offspring, a study was designed to explore potential links between maternal Zn deficiency and subsequent insulin resistance in the offspring and to study mechanisms by which this phenomenon might occur.

Several hundred metalloproteins require Zn for diverse cellular functions, in which it either participates directly in chemical catalysis (enzymes) or is important for maintaining the structure and stability of proteins (8,9) [such as insulin and insulin receptor (IR)\textsuperscript{5}]. The interactions among Zn, insulin, and glucose homeostasis are complex, and Zn deficiency might induce a state of insulin deficiency by interfering with either insulin storage or activation. A connection between Zn metabolism and diabetes mellitus is demonstrated by consistent symptoms of hyperzincuria and hypozincemia in type 2 diabetic patients (10,11). In a previous study conducted in weanling rats (12), we found that severe postnatal Zn deficiency in pups induced hyperglycemia,
and mild Zn deficiency also altered glucose metabolism. Studies have shown that transient mild maternally high Zn deficiency during the last week of gestation affects maternal carbohydrate metabolism (13). Zn is also known to have insulin-mimetic effects (14), and increased phosphorylation of PI3K/Akt with Zn exposure has been demonstrated (15), suggesting that Zn deficiency might stimulate a state of insulin resistance through modulating IR signaling cascades. However, the actual effects of maternal Zn deficiency during pregnancy are postnatal glucose homeostasis and the mechanisms behind these effects are not well understood.

Transient mild maternal Zn deficiency during the last week of gestation has been shown to stimulate growth as well as increase the fetal protein/DNA ratio (13). Insulin-like growth factor (IGF-1) was found to participate in the stimulation of DNA and protein synthesis, which is mediated through its receptor signaling pathway (16). The IGF-1 receptor requires Zn for optimal configuration due to a molecular structure similar to the IR. Leptin and adiponectin are mainly produced by adipose tissue and play important roles in energy balance. Studies have shown that rats fed a marginally Zn-deficient diet (3 mg/kg) had decreased leptin expression, independent of changes in food intake (17), and severe Zn deficiency suppressed only circulating leptin concentrations but also leptin secretion by rat adipocytes (18). In the present study, we investigated the effects of maternal Zn deficiency induced throughout pregnancy and lactation on postnatal growth, carbohydrate metabolism/insulin signaling cascades, and hormone concentrations in the offspring.

Materials and Methods

Rats. The study was conducted under the auspices of Animal Resource Services of the University of California, Davis, which is accredited by the American Association for the Accreditation of Laboratory Animal Care. Virgin female Sprague-Dawley rats (n = 10; 7–8 wk) were obtained from Charles River. Rats were housed in solid plastic hanging cages under constant conditions (temperature, 22°C; humidity, 65%) with a 12-h dark-light cycle and were allowed to consume food ad libitum. After a 3-d acclimatization period, rats were randomly assigned to 1 of the 2 experimental diets. The Zn-deficient pregnant rat model has been developed previously in our laboratory (19). The Zn-adapted control group (ZnC; n = 6) was fed a semipurified diet containing 25 mg Zn/kg of diet while the other group (ZnD; n = 6) received a diet marginally deficient in Zn (7 mg Zn/kg of diet) (12,20). Rats were fed these diets from 3 wk preconception to 21 d postparturition. On postnatal d 2, pups were culled to 7 pups/litter and nursed by their respective dams. Weight of pups was monitored and one-half of the remaining pups were killed at wk 3 after 4 h of food deprivation. The remaining pups were separated by gender, weaned, and fed a standard nonpurified rodent diet (LabDiet 5001, PMI). The proximate composition of this cereal-based diet is 28.5% protein, 13.5% lipid, and 58.0% carbohydrate. For signaling experiments, the rats were injected intraperitoneally with 8 units/kg of insulin (Humulin R, Eli Lilly & Co.) after 12–14 h of food deprivation. The rats were killed 10 min after injection and tissues were removed and snap-frozen.

Insulin and glucose tolerance tests. An insulin tolerance test (ITT) was performed after 4 h of food deprivation (between 1300 and 1600 h). Rats (n = 9 rats of each gender/group) were briefly removed from their cages for tail blood sampling and glucose concentrations were measured with a glucose meter (Easycheck, Home Aide Diagnostics). After measuring fasting glucose, rats were injected intraperitoneally with insulin. Dosages of 0.7 or 0.8 units/kg of Humulin R were used at wk 5 or 10, respectively. In this test, blood glucose from tail blood was sampled at 0, 10, 20, 30, 45, 60, and 90 min after insulin injection. After a 3-d acclimatization period, glucose tolerance test (GTT) experiments were performed on the same rats after 12–15 h of food deprivation. Rats were injected intraperitoneally with 2 g/kg of glucose solution (Sigma) and blood for glucose and insulin analyses was sampled at the same time points as ITT after glucose injection. The insulinogenic index (IGI) was used to estimate insulin secretion ability and insulin reserves in the pancreas and the following equation was used for GTT calculations (21):

\[
IGI = \frac{\text{Ins}_{10} - \text{Ins}_0}{\text{Glc}_{10} - \text{Glc}_0},
\]

where \(\text{Ins}_0\) and \(\text{Glc}_0\) are the basal insulin and glucose concentrations at 0 min (mU/L, mmol/L), respectively, and \(\text{Ins}_{10}\) and \(\text{Glc}_{10}\) are the insulin and glucose concentrations 10 or 30 min after glucose injection.

Mineral analysis. Zn concentrations of diets and tissues were analyzed by flame atomic absorbance spectrometry as previously described (22) using a SOLAAR M series atomic absorbance spectrometer (Thermo Electron). Samples (−0.1 g) were wet ashed with 3 mL mineral analysis grade 16 mol/L nitric acid (Fisher Scientific).

IGF-1, insulin, leptin, and adiponectin assays. Serum insulin and leptin concentrations were measured with commercial RIA kits (Sensi-tive rat insulin RIA kit and rat leptin RIA kit, Linco Research). The ELISA kit for rat adiponectin (Rat adiponectin ELISA kit) was from Millipore. Serum IGF-1 concentrations were measured with a commercial kit (IGF-1 high sensitive ELISA kit; IDS).

Antibodies. Rabbit polyclonal antibody against IR was purchased from Santa Cruz Biotechnology. Rabbit polyclonal phosphospecific antibodies against Akt1/2/3 and ERK1/2 were from Santa Cruz Biotechnology and Cell Signaling Technology, respectively. Monoclonal anti-phosphotyrosine (4G10) antibody was from Upstate Biotechnology, Inc.

Immunoprecipitation and immunoblotting. Tissues were homogenized in a modified radioimmune precipitation assay buffer (50 mmol/L Tris-Cl, pH 7.5, 150 mmol/L NaCl, 1% Nonidet P-40, 0.5% deoxycholate, 0.1% SDS, and 5 mmol/L EDTA) containing 2 mmol/L sodium orthovanadate and a protease inhibitor cocktail (Sigma). The lysates were clarified by centrifugation at 16,000 × g for 30 min and protein concentrations were measured using the Bradford assay (Bio-Rad). For immunoprecipitation, lysates were incubated with appropriate antibodies at 4°C for 3 h to overnight. Immune complexes were collected on protein G-Sepharose beads, washed extensively, resolved by SDS-PAGE, transferred onto nitrocellulose membranes at 350 mA for 60 min, and blocked in 10 mmol/L Tris-Cl, pH 7.4, 150 mmol/L NaCl, 0.05% Tween 20 with 5% bovine serum albumin. After incubation with appropriate primary and secondary antibodies (used at the concentrations recommended by their suppliers), specific bands were visualized with Super Signal Femto Chemiluminescent Reagent (Pierce) and molecular masses of visualized proteins were assessed relative to molecular weight markers (GE Healthcare).

Data analysis. Data are expressed as means ± SD. Student’s t test was performed to determine gender effects and data were pooled when there were no differences between males and females. Student’s t test was also performed to determine significant differences between ZnD and ZnC groups. Statistical analysis was conducted using GraphPad Prism 3.02 and significance was accepted at P < 0.05.

Results

Food intake of the female dams fed ZnC or ZnD diets for 9 wk did not differ. Weights of dams before pregnancy (ZnC, 233.5 ± 12.6 g; ZnD, 247.6 ± 13.2 g; n = 5–6) and after lactation (ZnC, 264.0 ± 21.8 g; ZnD, 269.6 ± 19.1 g; n = 5–6) did not differ, and there was no difference in litter size between the ZnC and ZnD (13.91 ± 1.92 pups/litter; n = 11) groups.

Growth of pups. Birth weight did not differ between the ZnC and ZnD groups or between males and females (5.9 ± 0.8 g; n =
TABLE 1  Effects of mild maternal Zn deficiency in rats on serum and tissue Zn in male and female offspring1

<table>
<thead>
<tr>
<th>Age</th>
<th>d 2</th>
<th>wk 3</th>
<th>ZnC</th>
<th>ZnD</th>
<th>ZnC</th>
<th>ZnD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Zn, μmol/L</td>
<td>62.9 ± 4.3</td>
<td>59.5 ± 8.5</td>
<td>25.1 ± 3.2</td>
<td>23.9 ± 2.9</td>
<td></td>
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</tr>
<tr>
<td>Liver Zn, μmol/g</td>
<td>2.08 ± 0.26</td>
<td>1.31 ± 0.20*</td>
<td>0.56 ± 0.08</td>
<td>0.50 ± 0.08*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total liver Zn, μmol</td>
<td>2</td>
<td>2</td>
<td>0.72 ± 0.11</td>
<td>0.79 ± 0.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muscle Zn, μmol/g</td>
<td>2</td>
<td>2</td>
<td>0.20 ± 0.02</td>
<td>0.17 ± 0.03*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Data are means ± SD, n = 20. *Different from ZnC, P < 0.05.
2 Not measured.

132). The weight of pups at d 10 and 20 did not differ between genders, but pups from ZnD dams weighed more than ZnC pups by postnatal d 10 (+5%; ZnC, 20.6 ± 2.2 g; ZnD, 21.7 ± 1.8 g; n = 35–42; P < 0.05) and d 20 (+10%; ZnC, 41.7 ± 2.7 g; ZnD, 44.3 ± 3.2 g; n = 35–42; P < 0.05). Rats consumed ad libitum nonpurified rodent diet after weaning and male rats in the ZnC (154.6 ± 12.3 g) and ZnD (157.0 ± 10.4 g) groups weighed more than female ZnC (133.8 ± 17.7 g) and ZnD (130.6 ± 9.6 g) rats at wk 5 (P < 0.05; n = 7–9). However, body weight did not differ between dietary groups at wk 5, 10, and 15.

Zn status of pups. Serum Zn concentrations at d 2 and wk 3 did not differ between dietary groups, but liver and muscle Zn concentrations were lower in ZnD pups compared with ZnC pups (Table 1). Interestingly, the proportion of liver to total body weight was significantly higher in ZnD pups compared with ZnC pups at wk 3 (data not shown), which resulted in no difference in total liver Zn content (Table 1). Although serum Zn concentrations of female ZnD rats did not differ from ZnC rats at wk 15 (data not shown), male ZnD rats had higher serum Zn concentrations (20.8 ± 2.0 μmol/L; P < 0.05) than ZnC rats (18.2 ± 2.3 μmol/L). Pancreas and muscle Zn concentrations and total liver Zn contents did not differ between dietary groups at wk 15 (data not shown).

Blood glucose and serum hormone concentrations. ZnD pups had higher basal blood glucose concentrations than ZnC pups, but basal serum insulin concentrations did not differ at wk 3 (Table 2). At wk 15, basal glucose concentrations in male, but not female, ZnD rats tended to be higher than in ZnC rats (P = 0.07; Table 2). Insulin concentrations did not differ between dietary groups in either males or females at 15 wk of age (Table 2).

To explore mechanisms behind the effects of maternal Zn deficiency on pup postnatal growth, serum IGFI-1 concentrations were measured in pups at wk 3. Serum IGFI-1 concentrations of ZnD rat pups (62.7 ± 6.8 nmol/L; n = 9) were higher than in ZnC pups (49.5 ± 6.6 nmol/L; n = 12; P < 0.05). Serum adiponectin concentrations did not differ between ZnD and ZnC pups at 3 or 15 wk of age (Table 2). Serum leptin concentrations were greater in male ZnD rats at wk 15 than in ZnC rats, but there were no differences in female rats or in either gender at 3 wk of age (Table 2).

Insulin sensitivity. Basal glucose and insulin concentrations did not differ between dietary groups at wk 5 and 10 in female or male rats. During the ITT, female ZnD rats exhibited a significantly smaller decrease in blood glucose concentrations at 10 and 20 min than ZnC rats at both wk 5 (Fig. 1B) and 10 (Fig. 1D), and male ZnD rats had a significantly higher fold of basal blood glucose concentrations than ZnC rats only at 10 and 60 min at wk 10 (Fig. 1C). During the GTT, male ZnD rats had significantly greater blood glucose concentrations at 20 and 30 min than ZnC rats at wk 5 (Fig. 2A). At wk 10, male ZnD rats had significantly higher blood glucose concentrations at 20, 30, 45, and 60 min than ZnC rats (Fig. 2C) and female ZnD rats had a significantly higher blood glucose concentration at 30 min than ZnC rats at wk 10 (Fig. 2D).

IGI, the insulin secretion ability index, was calculated using glucose and insulin concentrations from the GTT. At 10 min, but not at 30 min, the IGI was 29% lower in male ZnD rats than in controls (Fig. 3A). Females did not differ at either time (Fig. 3B).

Insulin signaling in muscle. Total IR, Akt1/2/3, and ERK1/2 phosphorylation did not differ between dietary groups in muscle of male rats at wk 15 (data not shown). Phosphorylation of Akt1/2/3 in female ZnD rats was significantly lower than in ZnC rats, but phosphorylation of IR and ERK1/2 did not differ (Fig. 4A,B).

Discussion

Increased Zn requirements during pregnancy and infancy are suspected to increase the risk for Zn deficiency among the young. Remarkably, it has been estimated that ~80% of pregnant women worldwide consume less than the recommended dietary allowance for Zn (6). To investigate the effects of mild maternal Zn deficiency on early signs of diabetes in the offspring, the present study was designed to provide some links between moderate maternal Zn deficiency and abnormalities in postnatal growth and insulin sensitivity and to explore mechanisms by which these might occur.

TABLE 2  Effects of mild maternal Zn deficiency in rats on blood glucose and serum insulin, IGFI-1, leptin, and adiponectin concentrations at 3 and 15 wk in the offspring1

<table>
<thead>
<tr>
<th>Age</th>
<th>ZnC</th>
<th>ZnD</th>
</tr>
</thead>
<tbody>
<tr>
<td>wk 3</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>Blood glucose, mmol/L</td>
<td>8.0 ± 1.3</td>
<td>8.8 ± 0.7*</td>
</tr>
<tr>
<td>Insulin, pmol/L</td>
<td>76 ± 9</td>
<td>81 ± 13</td>
</tr>
<tr>
<td>Leptin, nmol/L</td>
<td>0.10 ± 0.01</td>
<td>0.11 ± 0.02</td>
</tr>
<tr>
<td>Adiponectin, nmol/L</td>
<td>32.3 ± 8.4</td>
<td>29.2 ± 4.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age</th>
<th>ZnC</th>
<th>ZnD</th>
<th>ZnC</th>
<th>ZnD</th>
</tr>
</thead>
<tbody>
<tr>
<td>wk 15</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Blood glucose, mmol/L</td>
<td>5.7 ± 0.5</td>
<td>6.1 ± 0.4</td>
<td>6.2 ± 0.8</td>
<td>6.4 ± 0.4</td>
</tr>
<tr>
<td>Insulin, pmol/L</td>
<td>301 ± 143</td>
<td>320 ± 167</td>
<td>288 ± 189</td>
<td>289 ± 143</td>
</tr>
<tr>
<td>Leptin, nmol/L</td>
<td>0.15 ± 0.05</td>
<td>0.36 ± 0.14*</td>
<td>0.13 ± 0.04</td>
<td>0.14 ± 0.02</td>
</tr>
<tr>
<td>Adiponectin, nmol/L</td>
<td>35.2 ± 15.5</td>
<td>39.3 ± 12.5</td>
<td>40.6 ± 16.6</td>
<td>51.1 ± 18.1</td>
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</tbody>
</table>

1 Data are means ± SD. *Different from ZnC, P < 0.05.
Maternal Zn deficiency increased IGF-1 concentration associated with postnatal overgrowth of pups. In the present study, litter size and birth weight did not differ between ZnD and ZnC rats, confirming previous results by others using models of mild Zn deficiency (10 mg Zn/kg of diet) during pregnancy (23) or preconception (24,25). These results suggest that there are no adverse effects of mild to moderate maternal Zn deficiency before and/or during pregnancy on perinatal growth. Mildly Zn-deficient rats had higher Zn turnover during pregnancy, which can be interpreted as an attempt to maintain a supply of Zn from the maternal placenta to the growing fetus (24). The restricted fetal growth typical of severely Zn-deficient (1 mg Zn/kg of diet) rats was not found in our moderately Zn-deficient (7 mg Zn/kg of diet) rats, suggesting that regulation of maternal Zn homeostasis might be able to spare Zn to meet the Zn requirements for fetal growth in a state of moderate Zn availability. In contrast, although moderately Zn-deficient (5.8 mg Zn/kg of diet) rats had no depression of neonatal growth during lactation, body weights of these suckling pups were slightly higher than ZnC rats at d 17 postpartum (26). Our ZnD pups had significantly higher postnatal weights than ZnC pups at both d 10 and 20, in combination with elevated serum IGF-1 concentrations, suggesting that elevated IGF-1 concentrations in newborns of Zn-deficient dams might be closely associated with increased postnatal growth.

**FIGURE 1** Changes in blood glucose concentrations during an ITT in ZnC and ZnD male (A,C) and female (B,D) rat pups at wk 5 (A,B) and 10 (C,D) of age. Values are means ± SD, n = 6–8. *Different from ZnC at that time, P < 0.05.

**FIGURE 2** Changes in blood glucose concentrations during a GTT in ZnC and ZnD male (A,C) and female (B,D) rat pups at wk 5 (A,B) and 10 (C,D) of age. Values are means ± SD, n = 5–9. *Different from ZnC at that time, P < 0.05.
Maternal Zn deficiency induced a long-term effect on insulin resistance in the offspring. The “developmental origin of health and disease” hypothesis proposed by Hales and Barker (7) links poor fetal and infant growth with subsequent development of diabetes and cardiovascular disease (7). An association between prenatal exposure to famine and abnormal glucose tolerance in adults has been shown in humans (27). It is suggested that programming caused by early malnutrition may result in long-term or lifetime metabolic modifications, including insulin resistance and diabetes. Maternal mineral deficiency has been found to be associated with impaired glucose metabolism in rats (28,29). However, a specific link between pre- and postnatal exposure to Zn deficiency and glucose metabolism is missing. In the present study, rat pups from mildly Zn-deficient dams had impaired glucose tolerance, suggesting that early exposure to Zn deficiency might be related to the development of insulin resistance and/or diabetes in later life.

Maternal Zn deficiency decreased phosphorylation of insulin signaling cascades in female pups. Zn has insulinomimetic effects, including stimulation of lipogenesis and glucose transport via activation of IR and intracellular second messenger signaling pathways, such as mitogen-activated protein kinase and protein kinase B (9,14). Phosphorylation of Akt is activated in cells exposed to Zn and in Zn-deficient mice given Zn (30,31), suggesting that Zn participates in activation of the Akt signaling pathway. We found significantly lower phosphorylation of total muscular Akt in female rats from mildly Zn-deficient dams compared with controls after insulin stimulation, suggesting that early exposure to Zn deficiency might depress Akt signaling cascades in female rats, which could result in impaired glucose metabolism in adulthood. However, further work is required to elucidate the regulatory mechanism of Akt signaling cascades in the development of insulin resistance during maternal Zn deficiency.

Maternal Zn deficiency was associated with alterations in insulin secretion and decreased leptin concentrations in male rats. Leptin functions in the central nervous system by reducing food intake and increasing energy expenditure (32). Multiple other peripheral actions of leptin also have been suggested with the identification of the leptin receptor-mediated signaling pathway, which includes suppression of biosynthesis of preproinsulin and inhibition of insulin secretion from its storage vesicles in pancreatic β-cells (33). Inhibition of insulin secretion via leptin has been demonstrated in human and rodent pancreatic islets and β-cell lines (34-36). In our study, insulin resistance, increased leptin concentrations, and a reduction in pancreatic insulin reserves were observed in male offspring of Zn-deficient dams and there was also a positive correlation between glucose concentration at 20 min during GTT and leptin concentration in these rats (r² = 0.83; P = 0.01; data not shown). These findings suggest that Zn deficiency induced changes in both IR sensitivity and leptin-mediated inhibition of insulin biosynthesis and secretion.

In addition, leptin resistance has been defined from observations in obese patients and in animal models in which increased leptin concentrations seem to exert fewer effects in the hypothalamus than in pancreas (37). In turn, leptin resistance has been found to be associated with insulin resistance (38). Circulating leptin concentrations are positively correlated with the degree of adiposity (39), and we found increased leptin concentrations in male rats born to ZnD dams by postnatal wk 15. Although there were no significant differences in weight gain between groups at wk 15, the percentage of the gain that was fat was not measured. Increased leptin concentrations in pups of ZnD dams may result from excess fat deposition, but the actual cause and effect relationship between leptin and insulin resistance is still unknown. Taken together, our data demonstrate that mild maternal Zn deficiency is associated with elevated leptin concentrations and insulin resistance in male offspring in adulthood, but the underlying mechanisms still need be explored.

Maternal Zn deficiency impairs glucose metabolism in male offspring. Epidemiologic studies have shown that men
have a higher risk of developing diabetes than women (40,41), but the mechanism underlying this gender difference is unclear. Although we observed similar effects of maternal Zn deficiency on insulin resistance in male and female rats, different mechanisms appear to be involved. A sex-specific effect of Zn deprivation on growth has been found in other studies (42,43), and data also suggest that males are more susceptible to nutritional deprivation than females (44). We observed elevated leptin concentrations only in males born to ZnD dams. Leptin expression and secretion are regulated by sex hormones, including androgenic and estrogenic hormones (45), suggesting that gender-specific regulation of leptin expression might participate in the development of insulin resistance in offspring of Zn-deficient dams. Furthermore, Hamdi et al. (46) have shown that marginally Zn-deficient rats have significantly lower serum testosterone concentrations without differences in follicle-stimulating hormone and luteinizing hormone compared with controls, indicating that interactions between Zn deficiency and serum leptin and testosterone concentrations might contribute to the gender-specific development of insulin resistance.

In conclusion, mild maternal Zn deficiency resulted in greater serum IGF-1 concentrations and the excessive postnatal weight gain in their offspring and impaired subsequent glucose sensitivity. It was associated with gender-specific alterations in serum leptin concentrations and the insulin-signaling pathway. These findings strongly suggest that suboptimal maternal Zn status induces long-term, gender-specific changes related to abnormal glucose metabolism in the offspring.

Acknowledgments
All authors contributed to the design of the study, and M.J. conducted the animal experiments and data analysis. The manuscript was written by M.J. with significant input by the other authors. All authors had primary responsibility for final content. All authors read and approved the final version of the manuscript.

Literature Cited