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Redistribution of tissue zinc pools during lactation and dyshomeostasis during marginal zinc deficiency in mice

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Abstract

Zinc (Zn) requirements are increased during lactation. Increased demand is partially met through increased Zn absorption from the diet. It is estimated that 60 to 80% of women of reproductive age are at risk for Zn deficiency due to low intake of bioavailable Zn and increased demands during pregnancy and lactation. How Zn is redistributed within the body to meet the demands of lactation, and how Zn deficiency affects this process, is not understood. Female C57bl/6J mice were fed a control (ZA; 30 mg Zn/kg) or a marginally Zn deficient (ZD; 15 mg Zn/kg) diet for 30 d prior to mating through mid-lactation and compared with nulliparous mice fed the same diets. While stomach and plasma Zn concentration increased during lactation in mice fed ZA, mice fed ZD had lower stomach Zn concentration and abrogated plasma Zn levels during lactation. Additionally, femur Zn decreased during lactation in mice fed ZA, while mice fed ZD did not experience this decrease. Furthermore, red blood cell, pancreas, muscle and mammary gland Zn concentration increased, and liver and adrenal gland Zn decreased during lactation, independent of diet, while kidney Zn concentration increased only in mice fed ZD. Finally, maternal Zn deficiency significantly increased the liver Zn concentration in offspring but decreased weight gain and survival. This study provides novel insight into how Zn is redistributed to meet the increased metabolic demands of lactation and how marginal Zn deficiency interferes with these homeostatic adjustments.

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Conflict of interest
The authors declare no conflicts of interest.

Keywords

lactation; zinc deficiency; zinc pool; redistribution

Introduction

Dramatic metabolic and micronutrient shifts occur during lactation. In particular, the demand for zinc (Zn) is doubled during lactation (~4 mg Zn/d) [1], to support milk production and provide ~1–3 mg Zn/d for secretion into milk. Zn demands are partially met by increased fractional Zn absorption [2]. It has been proposed that Zn pools in trabecular bone may be drawn upon to partially meet these homeostatic adjustments as well [3–5]. However, lactation appears to have a limited effect on endogenous Zn loss from pancreatic enzyme secretion or renal Zn conservation [6]. How the body redistributes Zn pools to meet the increased demand during lactation is not understood.

Numerous populations worldwide consume considerably less Zn than is recommended to achieve optimal nutrition status including approximately 60–80% of women of reproductive age [7–9]. However, numerous studies have shown that Zn intake does not influence the Zn concentration of breast milk in women [10, 11]. To explain how Zn levels in milk are preserved in the presence of systemic Zn deficiency, two hypotheses, for which there is some evidence, are that Zn in the diet is taken up more efficiently [2] and conservation of endogenous Zn loss in populations consuming a zinc deficient diet [12]; however, these observations do not mathematically compensate for the increased demand for Zn during lactation. These considerations suggest that increased Zn absorption alone cannot maintain milk Zn concentration. An additional alternative hypothesis is that dietary deficiency in Zn leads to redistribution of Zn from tissues within the body, prioritizing delivery of Zn to the infant while depleting reserves in other organs of the mother. Herein, we utilized C57bl/6J mice to assess the changes in tissue Zn distribution that normally occur during lactation and determined effects of a marginally Zn deficient diet. The studies reported here are the first to illustrate how tissue Zn stores in a number of organs, some unsuspected, are redistributed to meet enhanced metabolic demands during lactation.

Methods

Animals

This study was approved by the IACUC Committee at the Pennsylvania State University, which is accredited by the American Association for the Accreditation of Laboratory Animal Care. Female C57BL/6 mice (5 wks of age) were obtained commercially (Charles River, Wilmington, MA) and housed individually in polycarbonate cages. Mice were maintained on a 12 h light/dark cycle under controlled temperature and humidity. Mice were fed a commercially available purified casein-based diet based on AIN93 (MP Biomedicals, Solon, OH) containing 30 mg Zn/kg (ZA) or a diet reduced only in Zn (15 mg/kg, ZD) [13] throughout the study. The Zn content of the diet was verified by atomic absorption spectrophotometry.

Lactating mice—Nulliparous mice were fed ZA or ZD (n=15 mice/diet) for 30 d prior to conception then bred and allowed to deliver naturally. Litter outcomes (litter size and weight) were assessed at birth and mid-lactation (lactation day, LD 8) for dams that gave birth (n=7, ZA; n=4, ZD). On LD 8, dams were removed from offspring for 2 h to control for any effects of suckling on tissue Zn distribution. Lactating dams were then anesthetized using isoflurane and subcutaneously injected with 4U oxytocin to stimulate milk ejection as previously described [14]. Milk Zn concentration was reported previously [13]. Dams were subsequently euthanized by CO_2 asphyxiation.

Non-lactating mice—Nulliparous mice (n = 6 mice/diet) were fed the same diets for the same period of time for comparison. Nulliparous mice were euthanized by CO_2 asphyxiation.

Blood was drawn via cardiac puncture with heparinized syringes and was subsequently centrifuged at 1,500 x g for 10 minutes at 4°C to separate blood plasma and erythrocytes, which were stored at −80°C until analysis. The liver, femur, spleen, muscle, kidneys, mammary gland, small intestine, stomach, pancreas, and adrenal glands were removed and snap frozen on dry ice and stored at −80°C until analysis.

Zn Analysis

The Zn concentration of plasma, packed erythrocytes, and tissues was determined by atomic absorption (AA) spectrophotometry as previously described [13]. Briefly, wet organs were digested in 69% nitric acid for ~4 days, boiled until sample volume decreased to approximately 0.5 mL, and subsequently diluted with double distilled water to appropriate volumes. Accuracy of the method was established by analysis of NBS Standard Reference 1577 Bovine Liver (National Bureau of Standards). Plasma samples were diluted 1:5 in 0.1N nitric acid for 1 week and measured.

Leptin

Plasma leptin was measured using a commercially available ELISA kit (Mouse Leptin ELISA Kit, RAB0334) as directed (Sigma-Aldrich, St. Louis, MO). Plasma samples used to measure plasma leptin concentration were isolated as described above.

Hemoglobin analysis

Blood samples were processed as previously described [13] to isolate the plasma fraction. After the plasma was removed, the hemoglobin concentration in packed red blood cells was analyzed using a Hemoglobin Detection Kit (Arbor Assays, Ann Arbor, MI).

Statistical Analysis

Data are expressed as mean ± SD and analyzed by *2*-way ANOVA to test for an interaction between diet and phenotype (liver, femur, spleen, muscle, kidney, mammary gland, intestine, plasma, stomach, pancreas, adrenal gland, erythrocytes, leptin) or Student's t-test (offspring liver Zn concentration and growth) after ensuring homogeneity of sample variance by determining the number of studentized residuals in each observation using SAS, version 9.3 (SAS Institute; Cary, NC). Two-way ANOVA analysis was conducted using

GraphPad Prism 5.0 (San Diego, CA) and significance was demonstrated at $p < 0.1$ for an interaction effect, or $p < 0.05$ for an effect of independent variables (Table 1). Mouse survival was analyzed using Kaplan-Meier survival curve analysis.

Results

Absorption/Digestion (stomach, intestine, pancreas)

During lactation, Zn was redistributed into organs responsible for the digestion and absorption of nutrients (Fig 1; Table 1). There was a significant interaction between phenotype and diet on stomach Zn concentration ($p<0.05$) such that in mice fed a ZA diet, stomach Zn concentration increased by 18% during lactation, while in mice fed a ZD diet stomach Zn concentration decreased (Fig 1A; Table 1). There was no effect of phenotype or diet on small intestine Zn concentration (Fig 1B, Table 1). There was a significant increase in pancreas Zn concentration during lactation (~50%; $p<0.0001$; Fig 1C; Table 1); however, no significant effect of diet was observed.

Circulation (red blood cell, plasma Zn, plasma leptin)

During lactation, Zn was redistributed into compartments responsible for systemic circulation and the exchangeable Zn pool (Fig 2; Table 1). Erythrocyte Zn concentration significantly increased by 27% during lactation ($p<0.01$; Fig 2A; Table 1). There was a significant interaction between phenotype and diet on plasma Zn concentration ($p<0.1$) such that in mice fed a ZA diet, plasma Zn concentration increased during lactation, while in mice fed a ZD diet plasma Zn concentration did not change (Fig 2B; Table 1). There was a significant effect of both phenotype ($p<0.01$) and diet ($p<0.01$) on plasma leptin levels. Plasma leptin decreased by ~49% during lactation in mice consuming a ZA diet. Additionally, plasma leptin decreased by ~59% in nulliparous mice consuming a ZD diet but ~89% in lactating mice consuming a ZD diet compared to nulliparous mice consuming a ZA diet. Furthermore, plasma leptin decreased ~72% in lactating mice consuming a ZD diet compared to nulliparous mice consuming a ZD diet (Fig 2C; Table 1).

Nutrient Processing and Storage Organs (muscle, liver, femur)

During lactation, Zn was redistributed in nutrient processing organs and organs that could potentially participate in Zn mobilization to meet enhanced Zn requirements (Fig 3; Table 1). Muscle Zn concentration significantly increased by 37% during lactation but was not affected by diet (Fig 3A; Table 1). Additionally, liver Zn concentration significantly decreased by 7% during lactation in mice fed a ZA diet (Fig 3B; Table 1) and decreased significantly more (18%) in lactating mice fed a ZD diet ($p<0.01$). Finally, there was a significant interaction between phenotype and diet on femur Zn concentration ($p<0.05$) such that in mice fed a ZA diet, femur Zn concentration decreased by 27% during lactation, while in mice fed a ZD diet, femur Zn concentration, which was lower initially, increased slightly (Fig 3C; Table 1).

Excretion (kidney)

There was no significant effect of phenotype on kidney Zn concentration. However, kidney Zn concentration was significantly higher (~4%, nulliparous; ~9%, lactating) in mice fed a ZD diet compared with mice fed a ZA diet ($p<0.01$; Fig 4; Table 1).

Regulation organs (spleen, adrenal glands)

There was no effect of phenotype or diet on spleen Zn concentration (Fig 5A; Table 1). During lactation there was a decrease in adrenal gland Zn concentration (~50%; $p<0.01$; Fig 5B; Table 1); however, no significant effect of diet was observed.

Nutrient transfer (mammary gland, offspring liver)

During lactation mammary gland Zn concentration significantly increased (Fig 6A, $p<0.0001$). We previously showed that mice fed a ZD diet had 20% lower milk Zn concentration than mice fed a ZA diet [13]. In contrast, the liver Zn concentration of offspring from dams fed a ZD diet was significantly higher (15%) compared with offspring from dams fed a ZA diet ($p<0.05$; Fig 6B).

Offspring outcomes

To investigate effects of alterations in Zn redistribution on offspring health, we recorded several metrics including offspring weight gain and survival to mid-lactation. Offspring from dams fed a ZD diet gained significantly less weight (68%) compared with offspring from dams fed a ZA diet ($p<0.05$; Fig 7A). Additionally, offspring from dams fed a ZD diet had significantly reduced survival compared with offspring from dams fed a ZA diet ($p<0.01$; Fig 7B).

Discussion

Numerous homeostatic adjustments occur during lactation, including shifts in hormone balance. Major metabolic adaptations in fatty acid uptake and utilization [15], thermogenesis [16], and gluconeogenesis [17, 18] occur, and small intestine [19] and mammary gland mass increase to absorb and transfer nutrients to the developing offspring. Many of these homeostatic adjustments are Zn-dependent [20–22]. Here we utilized a marginal Zn restriction paradigm which more closely represents suboptimal Zn intake in women, as opposed to a severe Zn restriction model that is usually employed. We showed that during lactation, Zn is redistributed in numerous tissues to meet these physiological demands and that Zn redistribution is compromised by suboptimal Zn intake. Although mice were not substantially Zn deficient, decreased milk production and Zn concentration [13] and reduced offspring weight gain and survival illustrate profound consequences of marginal Zn intake during times of enhanced demand such as pregnancy and the early neonatal period. While circulating leptin levels decline during lactation to drive hyperphagia [23], this is the first report that marginal Zn deficiency reduced leptin levels, which were further abrogated during lactation. Additional studies to understand effects of Zn deficiency on the hypothalamic-pituitary axis, food intake and milk production are warranted.

A novel finding was that Zn concentration in tissues required for nutrient digestion increased during lactation. In the stomach, this may be needed to meet increased requirements for Zn-dependent proteins during lactation such as gastrin [24] [25], perhaps driven by increased expression of Zip11 [26, 27]. Alternatively, Zn is physiologically coupled to acid secretion [28] which is consistent with increased stomach acid secretion during lactation [29]. Consistent with the need to increase the digestion, absorption and assimilation of nutrients, we show for the first time that pancreatic Zn concentration increases by ~50% during lactation. Substantial Zn (~1–2 mg Zn/day), along with enzymes such as peptidases, amylases, and lipases necessary for the digestion of nutrients, is secreted from pancreatic acinar cells in zymogen granules [30, 31]. The vesicular Zn transporter ZnT2 imports Zn into zymogen granules presumably to provide Zn for pancreatic enzymes [30]. Regulation of ZnT2 through STAT5 activation [32] [30] suggests a regulatory role for the lactogenic hormone prolactin [32] in pancreatic Zn accumulation during lactation. However, we did not differentiate between exocrine and endocrine pancreas in our study, thus it is possible that Zn accumulated in β-cell granules as well. Increased pancreatic Zn content during lactation could reflect enhanced insulin activity during lactation, which is critical in nutrient channeling to the mammary gland for milk biosynthesis [33].

In humans, approximately 4–6% of bone mass is lost during a 6 month lactation period, which could contribute up to 20% of the Zn secreted into milk [5]. Consistent with this theory, we observed a 21% decrease in bone Zn concentration by mid-lactation in mice. We speculate that this occurs through increased osteoclast activity similar to the demineralization of bone to provide calcium for milk [34, 35]. Importantly, our study revealed that Zn mobilization was either impaired in mice consuming a Zn deficient diet, or Zn accrual in bone did not occur prior to/during pregnancy. This suggests that women who consume a Zn deficient diet may have suboptimal storage of bone Zn to draw upon during lactation, potentially compromising a main source of Zn for secretion into milk.

Previous reports speculated that increased *SLC39A4* (ZIP4) expression in the liver increases liver Zn concentration during lactation [36]; however, we found that liver Zn concentration is actually lower during lactation, which we speculate reflects normal metabolic adaptations that occur. For example, lactation decreases hepatic lipogenesis [21], suggesting a shift in Zn-dependent enzyme activities related to hepatic lipid metabolism to support milk production [37]. Alternatively, Zn may be mobilized to increase the rate of gluconeogenesis during lactation [20]. Moreover, in marginally Zn deficient mice liver Zn concentration decreased by a further 10% suggesting that the liver may serve as a Zn reservoir that is drawn upon to meet enhanced needs. This substantial decrease in liver Zn concentration may alter the activity of Zn-dependent liver enzymes [38] and fatty acid utilization [15]. Further studies are needed to understand the role of Zn redistribution in these key metabolic tissues during lactation.

An intriguing finding was that adrenal gland Zn concentration decreased by ~50% during lactation. Recent evidence suggests that Zn efflux via ZnT8 may facilitate this decrease [39], potentially mediating the synthesis and/or secretion of corticosteroids and catecholamines that are critical for mammary gland function and lactation [40, 41]. Further studies are required to understand the role of Zn in regulating adrenal function.

Studies in lactating women establish that decreased Zn excretion may partially meet enhanced requirements [6]. While we did not find that kidney Zn concentration increased in lactating mice fed a Zn adequate diet, mice fed a marginal Zn diet had increased Zn retention in the kidney. Thus we speculate that women evaluated in these reports may have actually been sub-clinically Zn deficient. Alternatively, the lack of Zn retention in the kidney may reflect optimal Zn reabsorption during lactation in Zn adequate mice, while Zn retention in the kidney in Zn deficient mice may suggest defects in this process. Several studies have identified Zn transporters expressed in the kidney that may play a role in Zn retention. Zip8 is localized to the apical membrane of proximal tubules of the kidney [42], directly implicating Zip8 function in the reuptake of Zn from the lumen of the proximal tubules. Further studies are required to better understand the role of Zn reabsorption in maintaining Zn homeostasis.

In conclusion, our observational investigation into Zn deficiency-induced physiological perturbations of normal homeostatic adjustments in tissue Zn pool distribution during the phenotypic transition to a lactating state indicate that consuming a moderately Zn deficient diet has numerous effects on Zn metabolism and suggests that the physiological processes that are regulated by Zn may be compromised during periods of enhanced demand.

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Abbreviations

AA	Atomic Absorption
Zn	Zinc

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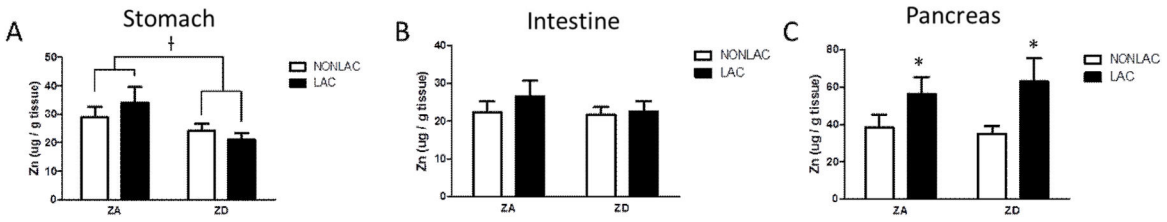

Figure 1.

A marginally Zn deficient diet perturbed the normal homeostatic Zn redistribution that occurs during lactation in tissues responsible for digestion and absorption. Data represent mean tissue Zn concentration ± SD. A) A significant interaction between diet and phenotype was detected ($p<0.05$). Additionally, a significant effect of diet was detected ($p<0.01$) such that stomach Zn concentration was significantly lower in all mice fed a Zn deficient (ZD) diet (lactating (LAC), n=4; nulliparous (NONLAC), n=6) compared to all mice fed a Zn adequate (ZA) diet (LAC, n = 7; NONLAC, n=6). B) Small intestine Zn concentration was not affected by phenotype or diet. C) Pancreas Zn concentration was significantly higher in both lactating mice fed ZA (n=7) and ZD (n=4) compared to nulliparous mice fed either a ZA or ZD diet (n=6/diet, *$p<0.0001$).

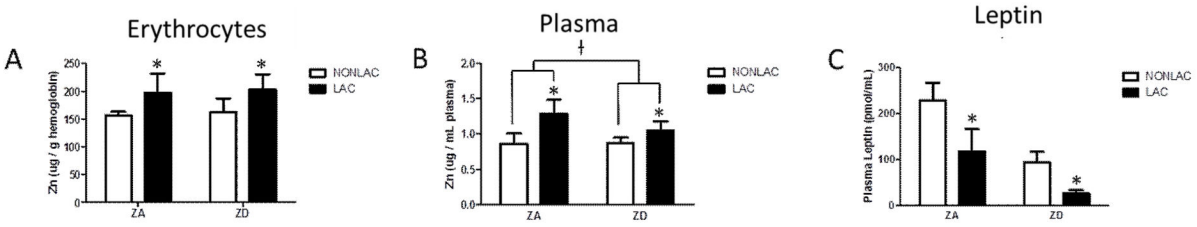

Figure 2.

A marginally Zn deficient diet perturbed the normal homeostatic Zn redistribution that occurs during lactation in circulating Zn pools and plasma leptin concentration. (A) Data represent mean erythrocyte Zn concentration ± SD. Erythrocyte Zn concentration was significantly higher in both lactating (LAC) mice fed ZA (n=7) and ZD (n=4) compared to nulliparous (NONLAC) mice fed either a ZA or ZD diet (n=6/diet, *$p<0.01$). (B) Data represent mean plasma Zn concentration ± SD. A significant interaction between diet and phenotype was detected ($p<0.1$). Plasma Zn concentration was significantly higher in both lactating mice fed ZA (n=7) and ZD (n=4) compared to nulliparous mice fed either a ZA or ZD diet (n=6/diet, *$p<0.05$). (C) Data represent mean plasma leptin concentration ± SD. Plasma leptin concentration was significantly lower in both lactating mice fed ZA (n=7) and ZD (n=4) compared to nulliparous mice (n=6/diet, *$p<0.001$) fed either a ZA or ZD diet, and was significantly lower in all mice consuming a Zn-deficient diet compared to all mice consuming a Zn adequate diet ($p<0.001$).

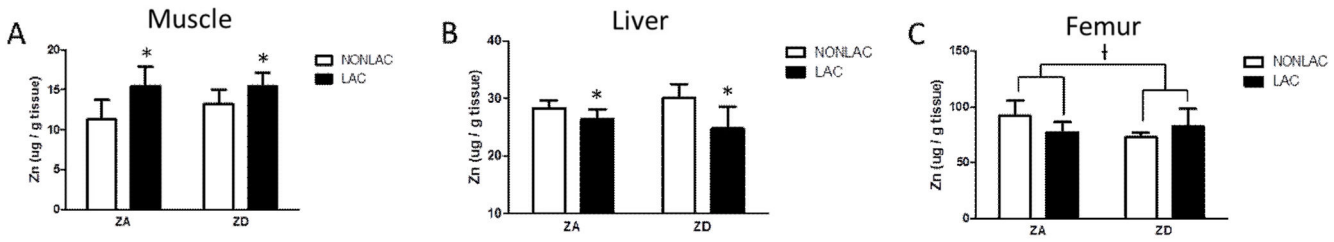

Figure 3.

A marginally Zn deficient diet perturbed the normal homeostatic Zn redistribution that occurs during lactation in tissues responsible for storage and nutrient processing. Data represent mean tissue Zn concentration ± SD. A) Muscle Zn concentration was significantly higher in both lactating (LAC) mice fed ZA (n=7) and ZD (n=4) compared to nulliparous (NONLAC) mice fed either a ZA or ZD diet (n=6/diet, *$p<0.01$). B) Liver Zn concentration was significantly lower in both lactating mice fed ZA (n=7) and ZD (n=4) compared to nulliparous mice fed either a ZA or ZD diet (n=6/diet, *$p<0.01$). C) A significant interaction between diet and phenotype on femur Zn concentration was detected ($p<0.05$).

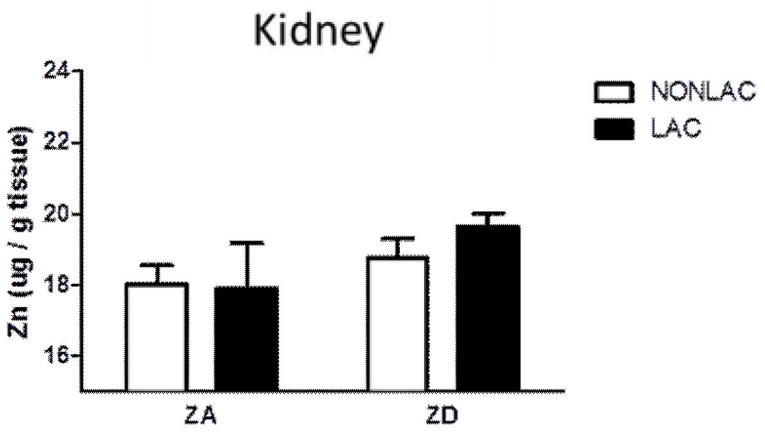

Figure 4.

Effects of lactation and a marginal Zn diet on the Zn concentration in tissues critical for regulation. Data represent mean kidney Zn concentration ± SD. There was no effect of phenotype on kidney Zn concentration. Kidney Zn concentration was significantly higher in all mice consuming a Zn deficient (ZD) diet (lactating (LAC), n=4; nulliparous (NONLAC), n=6) compared to all mice consuming a Zn adequate (ZA) diet ($p<0.01$; LAC, n=7; NONLAC, n=6).

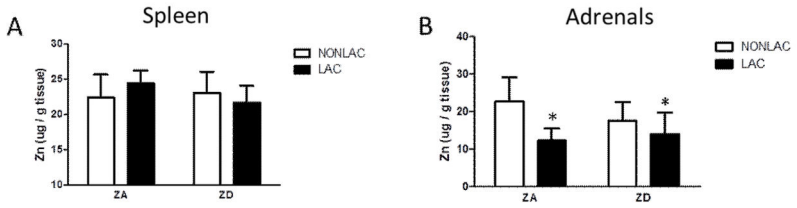

Figure 5.

Effects of lactation and a marginal Zn diet on the Zn concentration in tissues critical for regulation. Data represent mean tissue Zn concentration ± SD. A) There was no effect of phenotype or diet on spleen Zn concentration. B) Adrenal gland Zn concentration was significantly lower in both lactating (LAC) mice fed ZA (n=7) and ZD (n=4) compared to nulliparous (NONLAC) mice fed either a ZA or ZD diet (n=6/diet, *$p<0.01$).

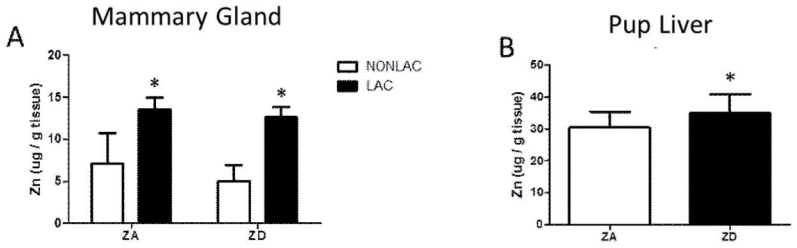

Figure 6.

Lactation and a marginal Zn diet caused significant changes in Zn concentration in tissues associated with nutrient transfer to the offspring. Data represent mean tissue Zn concentration ± SD. (A) Mammary gland Zn concentration was significantly higher in both lactating (LAC) mice fed a Zn adequate (ZA) and Zn deficient (ZD) diet compared to nulliparous (NONLAC) mice fed either a ZA or ZD diet (n=6/diet, *$p<0.001$). (B) Zn concentration in the liver of offspring from mice fed ZA was significantly higher compared with the liver of offspring from mice fed a ZD diet (n = 20 offspring/group, *$p<0.05$).

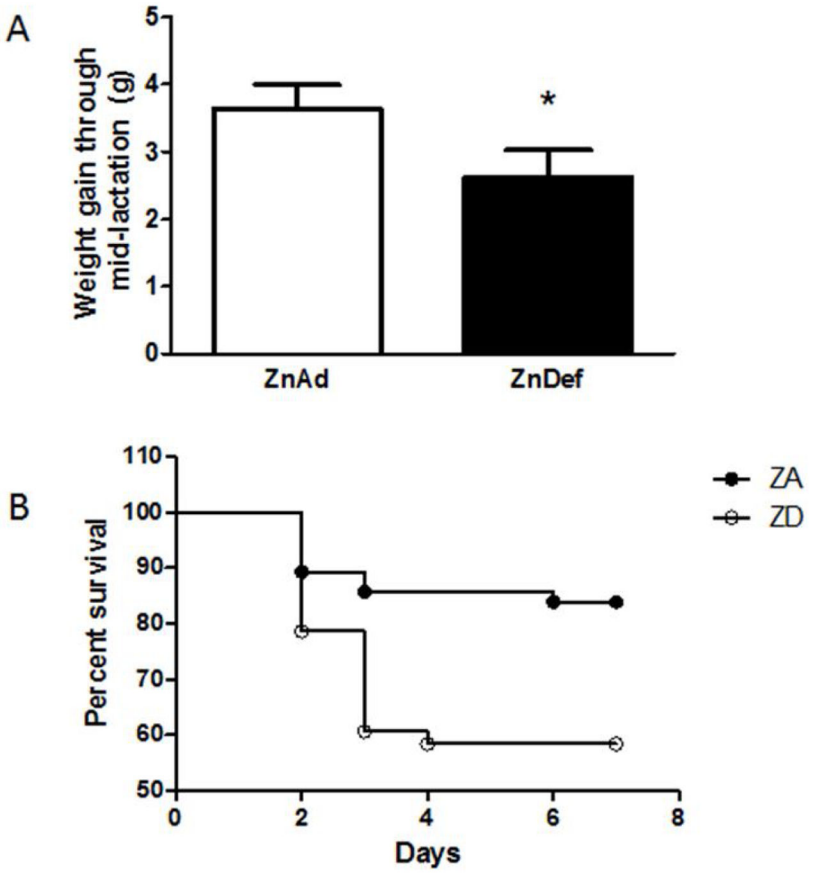

Figure 7.

A marginally Zn deficient maternal diet reduces indices of offspring health. (A) Data represent mean offspring weight gain ± S.D of offspring from dams fed a Zn deficient diet (ZD, n=5 litters) compared with offspring from dams fed a Zn adequate (ZA, n=6 litters) diet (*$p<0.01$). (B) Kaplan-Meier analysis indicates that there was a significant effect of maternal Zn intake on offspring survival, $p < 0.01$.

Table 1

Zinc is redistributed between organs in response to lactation, and consuming a marginally zinc deficient diet perturbs this redistribution. A p-value < 0.05 for effects of independent variables or <0.1 for an interaction effect represents a significant effect of each independent variable as determined by 2-way ANOVA. NS = Not Significant.

Function	Tissue	Phenotype	Diet	Interaction
Absorption/Digestion	Stomach	NS	< 0.01	< 0.05
	Small Int.	NS	NS	NS
	Pancreas	< 0.01	NS	NS
Systemic Circulation	Erythrocytes	< 0.01	NS	NS
	Plasma	< 0.01	NS	< 0.1
Nutrient Processing and Storage	Muscle	< 0.01	NS	NS
	Liver	< 0.01	NS	NS
	Femur	NS	NS	< 0.05
Excretion	Kidney	NS	< 0.01	NS
Regulatory Organs	Spleen	NS	NS	NS
	Adrenal Gland	< 0.01	NS	NS
Nutrient Transfer	Mammary Gland	< 0.01	NS	NS