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Nutrition

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 60–80% 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 days 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.

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

Abbreviations: AA, atomic absorption; Zn, zinc.

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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 weeks 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 days 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 $1500 \times g$ for 10 min 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
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.

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	Erythocytes	<0.01	NS	NS
	Plasma	<0.01	NS	<0.01
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

NS: not significant.