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Zinc deficiency suppresses plasma leptin concentrations in rats

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*Leptin concentrations during zinc deficiency were measured. Leptin is produced by adipose tissue and has potent affects on body weight and food intake regulation. Zinc deficiency results in anorexia, but the cause for this anorexia is not well understood. Aberrant regulation during zinc deficiency of leptin expression or secretion could be a factor in this anorexia. Two groups of Sprague-Dawley male rats were provided AIN-93-based diets made either adequate or deficient in zinc (+Zn, −Zn; 30 or 1 mg Zn/kg diet) and a third group (PF) was provided the +Zn diet at the reduced levels consumed by −Zn rats. In Study 1, +Zn, −Zn, and PF rats (*n = *12 ea.) were fed using a 4-hr meal-feeding protocol for 4, 9, and 28 days. Leptin concentrations in −Zn rats were lower than both +Zn and PF groups (*P < *0.05) on Days 9 and 28. In Study 2, 24 rats were divided into −Zn (*n = *6), +Zn (*n = *6), and PF (*n = *12) groups. On day 21, six PF rats were provided a meal (PF-fed: PF-F); the other six PF rats were not offered this final meal (PF-restricted: PF-R). Plasma leptin concentrations were again lowest in the −Zn group. Reduced leptin levels during zinc deficiency suggest that leptin is responding normally, signaling low body fat levels during zinc deficiency. It seems that leptin is not a dominant factor in the development of zinc deficiency-induced anorexia.* (J. Nutr. Biochem. 9:47–51, 1998) © *Elsevier Science Inc. 1998*

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Introduction

Studies presented over the past 2 years indicate that leptin plays a role in the homeostatic system defending normal body weight.[1–3] Circulating leptin levels reflect body fat stores, and one of the targets of circulating leptin may be a receptor in the brain.[4] This receptor, when activated by leptin binding, is hypothesized to function in part by down-regulating the production of hypothalamic, appetite-regulating neuropeptide Y (NPY).[5,6] Hypothalamic NPY synthesis and secretion is thought to be regulated by leptin and other hormones or signals.[7,8] On a molar basis, NPY is the most potent stimulant of appetite ever identified when considering agents administered either orally, peripherally, or centrally.[9–11] During fasting, circulating leptin levels are reduced, whereas secretion, cellular peptide content, and mRNA levels for NPY are all increased.[12–14] Thus, the discovery of leptin has defined a "leptin-NPY-appetite" axis, which is one component of a complex appetite regulation system.

In rodents, experimental zinc deficiency results in loss of appetite.[15,16] The cause of this anorexia is not well understood; the best hypothesis at present suggests alterations in brain amino acid metabolism and neurotransmitter concentrations are important.[16] Recently, higher hypothalamic NPY levels have been reported during zinc deficiency-induced anorexia.[17,18] This finding suggests that a paradox

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exists during zinc deficiency: NPY levels are high, yet appetite is low.

The present study was undertaken to quantitate circulating leptin levels during zinc deficiency. Down-regulated leptin levels in zinc deficiency would be consistent with elevated levels of NPY, suggesting that an "NPY paradox" or resistance to NPY exists during zinc deficiency. However, up-regulated leptin levels during zinc deficiency would provide a possible mechanism by which zinc deficiency produces anorexia: by reducing hypothalamic NPY levels. Other peptides have been found that are elevated during zinc deficiency,[19] so it is conceivable that zinc deficiency could increase levels of the regulatory factor leptin. In either case, determining if leptin levels are increased or decreased during zinc deficiency is important to confirm the regulation of the appetite control system during zinc deficiency.

Methods and materials

Animals and diets

All animal protocols were approved by the University of Illinois Laboratory Animal Care Advisory Committee. Two separate studies were completed. Male Sprague-Dawley outbred rats obtained from a commercial supplier (Harlan, Indianapolis, IN USA) were individually housed in suspended stainless steel cages on a 12/12-hr light cycle. Diet containers were weighed daily and spillage was collected to determine daily intake. Ultrapure water was provided in zinc-free bottles. Zinc deficiency was considered to be established when a reduction in intake occurred along with the 3- to 4-day cycle of intake that is characteristic of zinc deficiency.[20] Femur samples were collected to assay bone zinc content using atomic absorption spectrophotometry.[21] Femurs were pooled within groups in Study 1 and assayed individually in Study 2. Diets were based on the AIN-93 recommendations for rodents[22] with spray-dried egg white as the source of protein, extra biotin supplemented at 0.2 mg/kg diet, and phosphorus concentration increased to accommodate the change from casein to egg white as recommended. Zinc concentration of the diets was verified at 1 or 30 mg Zn/kg diet (−Zn or +Zn, respectively) by atomic absorption spectrophotometry.

In Study 1, 36 rats weighing 65 to 70 g were randomly assigned to three groups: zinc-adequate (+Zn), zinc-deficient (−Zn), and a third group offered zinc-adequate diet, but provided at the reduced level of the average daily intake of the −Zn group ("pair-fed", PF). Because PF rats eventually become 'meal-feeders', consuming their daily allotment of diet within a few hours, all rats in Study 1 were trained for 1 week to meal feed on +Zn diet before beginning the study. Diet was provided at the onset of dark for 4 hr, at which time all diet containers were removed from cages until the onset of dark on the next day. After the training period, 12 rats each were assigned to +Zn, −Zn, and PF groups. For the duration of Study 1, each rat had access to its respective diet jar only for the first 4 hr of the dark cycle. This allowed control of total intake, dietary zinc, and diurnal intake patterns in one experiment. Immediately before the meal period of the 4th, 9th, and 28th day of the study, four rats from each group were killed after inhalation of carbon dioxide by decapitation. Trunk blood was collected for isolation of plasma.

Study 2 used 24 rats (85 to 90 g) in four groups. In this study, rats had access to diet 24 hr per day to contrast freely-feeding −Zn rats to the meal feeding design used in Study 1. Rats were maintained for 21 days in +Zn, −Zn, and two pair-fed groups ($n = 6$ each). One of the PF groups was provided 8 g of diet 4 hr before killing (pair fed and fed on final day: 'PF-F') and the second group of PF rats was not fed before killing (pair fed and food restricted on final day; 'PF-R'). Blood was collected by cardiac puncture into a heparinized syringe, plasma was obtained by centrifugation (10,000 × g for 15 min).

Radioimmunoassays

Leptin concentrations were measured by using a mouse leptin radioimmunoassay kit according to the supplier's recommended protocol (Linco Research, Inc., St. Charles, MO USA). The mouse anti-leptin antibody was validated by the manufacturer against purified recombinant rat leptin, exhibiting a cross reactivity to rat leptin of greater than 90%. The minimum level of detection for this assay was 0.2 ng/mL and the ED-50 was 2.4 ng/mL. Circulating insulin concentrations were measured using a rat insulin RIA kit (Linco Research, Inc.) as described previously.[23]

Carcass lipid determinations

Eviscerated rat carcasses were autoclaved and homogenized with a laboratory blender according to Mickelsen.[24] Dry-matter determinations of lipid content were made using a methanol-chloroform (1:4 v/v) extraction of a freeze-dried aliquot of the carcass homogenate.[25]

Glucose concentration determinations

Plasma glucose concentrations were quantified using a Hitachi model 911 automated clinical chemistry analyzer at the College of Veterinary Medicine clinical chemistry core facility at the University of Illinois, Urbana, IL USA, using the glucose/HK reagent system (Boehringer Mannheim, Indianapolis, IN USA).

Statistics

All analyses used $P < 0.05$ as the minimal criterion of statistical significance and values are reported as means ± SEM. For Study 1, main effects and diet versus day interactions were determined using a two-way ANOVA (SigmaStat, Sausalito, CA USA). The Fisher least significance difference test was performed to identify mean differences when significant main effects or interactions were observed. For Study 2, differences between treatments were determined using ANOVA as calculated by the SAS GLM program (SAS Version 6; SAS Institute, Cary, NC USA). Post hoc analyses used the Student-Newman Keul test and the Pearson correlation test.

Results

Study 1

Rats consuming −Zn diet reduced intake and exhibited the 3 to 4 day cycle of variable intake characteristic of zinc deficiency. Pooled femur Zn concentrations for +Zn, −Zn, and PF groups were (in mmol Zn/kg bone) 1.4, 1.3, and 1.4 on Day 4; 1.4, 1.0, and 1.4 on Day 9; and 1.5, 0.8, and 1.4 on Day 28; respectively.

Serum leptin concentrations and body weights are shown in *Figure 1*. The training period for meal feeding affected growth of rats, and Study 1 reflected lower growth rates for +Zn meal-fed rats than rats provided ad libitum access to diet as in Study 2. In Study 1, −Zn and PF rats ate less than +Zn rats ($P < 0.05$) from day 6 through day 28.

Serum leptin concentrations were highest in animals fed the +Zn diet (1.12 ± 0.17 ng/mL) followed by the PF

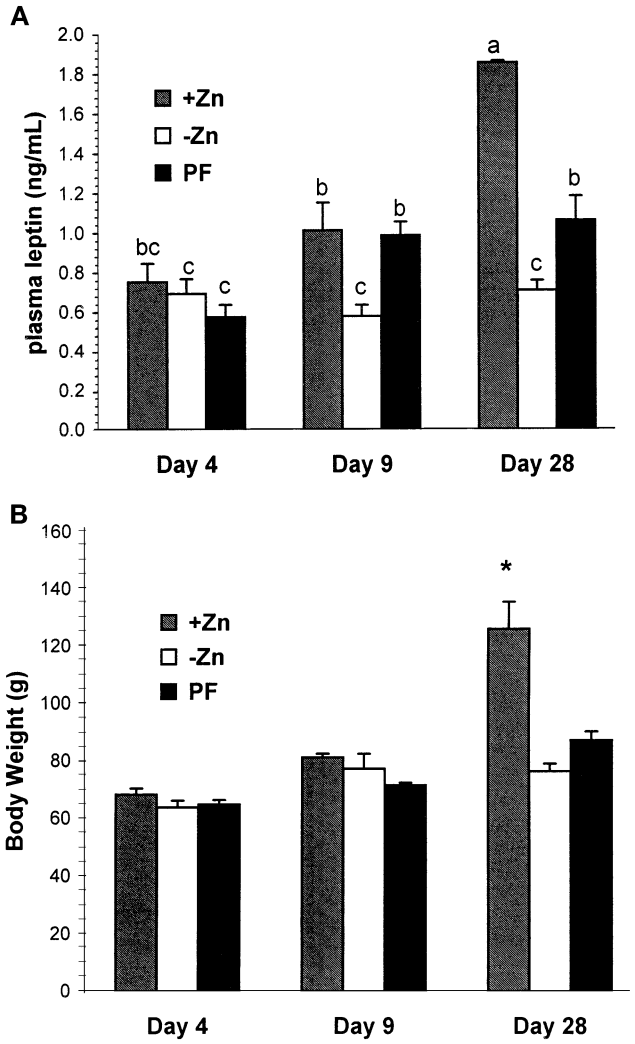

Figure 1 Serum leptin concentrations and body weights of rats fed zinc-adequate (+Zn); zinc-deficient (−Zn), or pair-fed (PF) diets on Days 4, 9, and 28 of dietary treatment ($n = 4$ each). Values represent means ± SEM with significant differences of $P < 0.05$ represented with different letters (A) or '*' (B) within the same graph.

(0.87 ± 0.08 ng/mL) and −Zn group (0.66 ± 0.04 ng/mL), all different, $P < 0.05$. Serum leptin concentrations did not differ on Day 4 (0.67 ± 0.05 ng/mL) and Day 9 (0.84 ± 0.08 ng/mL); these values were significantly lower than Day 28 (1.15 ± 0.15 ng/mL). There was no change in leptin concentrations for −Zn rats between Days 4 and 28; however, leptin concentrations increased in the PF between days 4 and 9 and in +Zn between Days 9 and 28. Significantly lower leptin concentrations were measured in −Zn versus both PF and +Zn rats ($P < 0.05$) on days 9 and 28.

Study 2

Growth, intake, cycling, and bone zinc values indicated a significant zinc deficiency had been established *(Table 1)*. Plasma leptin concentrations were reduced in −Zn rats (0.40 ± 0.01 ng/mL) compared with +Zn (0.58 ± 0.05 ng/mL) and pair-fed rats (0.62 ± .05 ng/mL), $P < 0.05$ *(Table 1)*. Carcass lipid was significantly lower ($P < 0.05$) in −Zn and PF than +Zn rats, insulin was significantly higher in PF-F rats than all others, while plasma glucose was unchanged by treatment *(Table 1)*.

Discussion

In Study 1, serum leptin concentrations were significantly lower in −Zn rats than +Zn or PF rats. Lower leptin concentrations in −Zn rats were associated with lower body weights and presumably less body fat than +Zn rats. When food intake was controlled by pair feeding rats +Zn diet at the level of intake of −Zn rats, lower leptin levels were observed in −Zn than in PF rats on days 9 and 28, despite equal body weights.

Determining that PF and +Zn rats have higher leptin levels eliminates possible speculation that zinc deficiency aberrantly increased leptin secretion and/or reduced leptin turnover in the blood, either of which could produce abnormally high circulating leptin concentrations in −Zn rats. To our knowledge, this is the first report indicating that leptin concentrations are altered by zinc deficiency. Abnor-

Table 1 Plasma leptin, insulin, glucose, body lipid, bone zinc, weight gain, and food intake in rats assigned to Zn deficient (−Zn), Zn adequate (+Zn), pairfed, fed on day 21 (PF-F), and pairfed and food restricted on day 21 (PF-R) diet groups for 21 days

Item	Treatment			
	+Zn	−Zn	PF-F	PF-R
Leptin (ng/ml)	0.58 ± 0.05—b	0.40 ± 0.02—c	0.73 ± 0.07—a	0.52 ± 0.13—b,c
Insulin (pmoles/L)	101 ± 28—b	37 ± 7—b	288 ± 42—a	48 ± 10—b
Glucose (mmol/L)	7.8 ± 0.65	7.3 ± 0.43	7.8 ± 0.20	6.3 ± 0.30
Body lipid (%w/w)	26 ± 1.6—a	15 ± 0.7—b	18 ± 1.4—b	15 ± 1.4—b
Bone Zn (mmol Zn/kg)	4.1 ± .28—a	1.4 ± 0.04—b	3.3 ± 0.09—a	3.6 ± 0.8—a
Weight gain (21 days)	129 ± 3—a	20 ± 1—c	41 ± 4—b	43 ± 3—b
Food intake (% of +Zn)	100 ± 1—a	52 ± 3—b	54 ± 1—b	54 ± 1—b

Values are expressed as means ± SEM.
Diets were AIN-93 formulations modified with spray dried eggwhite as the protein source.
Means with different letters in a horizontal row are significantly different, $P < 0.05$.

mally high leptin levels in −Zn rats could have reduced synthesis and release of NPY in the hypothalamus, and decreased appetite, thus providing an explanation for reduced appetite during zinc deficiency. However, our observation of lower blood leptin concentrations in −Zn rats suggests that leptin is down-regulated during zinc deficiency, providing a regulatory signal for increased food intake.

In Study 2, leptin levels were also lowest in −Zn rats. Body lipid generally correlated with leptin levels. In parallel with the results from Study 1, −Zn and PF-R rats had similar body fat, yet −Zn rats had lower leptin levels than PF-R rats. Highest leptin levels were found in PF-F rats, emphasizing the important role of insulin on postprandial circulating leptin levels.[26] The trend toward higher carcass lipid observed in PF-F group may reflect circulating lipids in the post prandial state.

Plasma leptin was positively correlated with plasma insulin ($R = 0.78$, $P < 0.0001$). The close association between the increase in plasma insulin and leptin in the PF-F control group supports evidence that plasma insulin stimulated leptin release.[26] Insulin has been shown to increase both *ob* mRNA and circulating leptin in rodents. In rats, the level of *ob* mRNA in adipose increased with food intake and correlated with plasma insulin concentrations.[27,28] Administration of insulin in vivo and in vitro increased ob mRNA levels.[28,29] The release of leptin by rat white adipocytes in response to insulin was blocked by a β-3 adrenergic receptor antagonist[30,31] in cell culture and in mice.[32]

Importantly, the present data may be related to the observation that zinc deficiency may be associated with hypogonadism.[33] With the discovery of the leptin receptor and its splice variants in tissues other than the brain, including the testis, prostate, and ovary, among others,[34,35] it may be possible that circulating leptin is a necessary signal for reproductive tissues to develop and function normally, providing a hypothesis linking zinc deficiency and reproductive dysfunction.[36]

Our observations show that plasma leptin concentrations are down regulated during zinc deficiency. During zinc deficiency-induced anorexia, circulating leptin concentrations are low, which should stimulate an increase in hypothalamic NPY if the "leptin-NPY" portion of the "leptin-NPY-appetite" axis is functional. Increases in hypothalamic NPY peptide and mRNA content during zinc deficiency have been reported,[17,18] which are consistent with reduced leptin levels, but inconsistent with the reduced intake associated with zinc deficiency. Reduced galanin peptide levels have been measured during zinc deficiency,[18,37] which may have a role in decreased appetite. Little is known regarding the regulation of other appetite-regulating factors such as corticotropin releasing hormone or melanocyte stimulating hormone during zinc deficiency. These peptides may mediate reduced appetite during zinc deficiency. Alternatively, it is possible that zinc deficiency changes normal processing of the pro-NPY peptide. Processing of thyrotropin releasing hormone is reduced by zinc deficiency.[38] Incompletely processed NPY would not mediate its appetite-stimulating effect. These possibilities need further investigation to resolve the paradox between low leptin, high NPY, and reduced appetite during zinc deficiency.

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