Zinc Status and Interleukin-1β-Induced Alterations in Mineral Metabolism in Rats

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Abstract. **Changes in zinc (Zn) metabolism and interleukin-1β (IL-1β) release occur as part of the physiological response to tissue injury and trauma. In the present study, the influence of Zn status on the response to continuous low-dose IL-1β administration was evaluated. Rats were fed 50 μg Zn/g (adequate zinc; AZn) or 5 μg Zn/g (marginal zinc; MZn) diets for 14 days. On day 15, rats were infused via osmotic minipumps, with IL-1β (2.3 ng/hr) or saline (control, C) and euthanized 1, 3, or 7 days later. In the AZn rats, IL-1β infusion resulted in increased plasma copper (Cu) concentrations and ceruloplasmin (Cp) activity, and decreased iron (Fe) concentrations throughout the 7d period. These effects were most pronounced on d1 and d3. A similar trend was observed in the MZn rats, but IL-1β-induced increases in plasma Cu and Cp activity were less than in the AZn fed rats. In MZn and AZn IL-1β infused rats, plasma Zn was decreased on Day 1, and Day 3, respectively, compared with their respective controls. AZn IL-1β-infused rats were characterized by high liver Fe, Zn, and metallothionein (MT) concentrations on Day 1; by Day 7, only MT concentrations remained elevated. Liver MnSOD activity was 13%–29% higher in both the AZn- and MZn-IL-1β-infused rats than their respective controls on Day 3 and Day 7, with most significant increase observed on Day 7. These data show that Zn status can influence the response to low-dose IL-1β; this influence of Zn should be considered when IL-1β is given therapeutically.** [P.S.E.B.M. 1994, Vol 206]

As one consequence of tissue injury or infection, the cytokine, interleukin-1β (IL-1β) is released (1, 2). This polypeptide acts in part to induce the acute-phase response, which involves a complex cascade of biological events, including hormone production (i.e., cortisol, glucagon, and insulin), changes in the distribution of minerals between storage and transport pools, T-cell and B-cell activation, production and release of neutrophils, and the synthesis of several hepatic proteins (3, 4).

It has been noted that many of the circumstances now known to stimulate IL-1β release, such as tissue injury or burns, wound healing, endotoxemia, infection, or general inflammation, also induce alterations in zinc (Zn) and iron (Fe) metabolism (5, 6). A single bolus injection, ip, of a crude IL-1 fraction lowered plasma Zn and Fe concentrations and raised liver Zn concentrations (7). Since Zn plays an important role in wound healing and in mediating immune function (6, 8), it has been postulated that Zn could regulate the expression of IL-1 activity (8). This concept is supported by the observation that *in vitro,* Zn enhances the proliferative response of murine thymocytes to IL-1 (9). In addition, IL-1 production is reduced in Zn-deficient lymphocyte cultures (10) and in Zn-deficient rats, the response to a single injection of IL-1α is impaired compared with control rats (11).

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Although a few studies employing low-dose continuous IL-1β administration have been reported, none, to our knowledge, have examined Zn-IL-1β interactions. In addition, studies concerning nutrient-cytokine interactions have typically not employed low-dose, chronic cytokine administration, such as might occur with chronic inflammation (12). The present study examined the influence of continuous low-dose IL-1β infusion on cytokine-induced changes in mineral metabolism in healthy adult rats over a 7-day period. Considering that marginal Zn status may occur with high frequency in some human populations (6, 13), the effects of marginal Zn deficiency on the effects of IL-1β were also examined.

Materials and Methods

Animals and Diets. Ninety virgin female Sprague–Dawley rats weighing 180–200 g were purchased from Charles River Breeding Laboratories (Wilmington, MA) and individually housed in suspended stainless steel cages in a controlled environment (12:12-hr light:dark cycle; 22°–23°C). Historic and biochemical data on female rats from our laboratory have been extensive, and previous studies by others (11, 12) have suggested no differences in response to IL-1β in male or female rats. Rat chow (Ralston Purina Co., St. Louis, MO) and double deionized water were fed *ad libitum* for a 7-day acclimation period. The rats were then fed a purified egg-white protein based diet, containing either 50 μg Zn/g diet (adequate Zn; AZn), or 5 μg Zn/g diet (marginal Zn; MZn), for 14 days (14). Animals were weighed and examined daily.

Experimental Procedure. On Day 15, rats were randomly assigned to one of the following treatment groups for periods of 1 day, 3 days, or 7 days: (i) IL-1β infusion; (ii) saline infusion (Sal); or (iii) untreated controls (NT). For infusion of IL-1β or saline, the animals were anaesthetized with methoxyflurane (Pitman-Moore, Inc., Washington Crossing, NJ) for 60–90 sec before implantation of the infusion pump. Beginning at the level of the scapulae, a subcutaneous tunnel was made posteriorly on the animal's back for implantation of an osmotic minipump (Model 2001; Alza Corp., Palo Alto, CA). The pumps were filled with either >95% pure human IL-1β (R & D Systems, Inc., Minneapolis, MN) reconstituted in 0.9% sterile saline solution or 0.9% sterile saline solution only. The wound site was closed with 9-mm sterile autoclips (Becton Dickinson and Co., Parsippany, NJ). IL-1β was infused at a rate of 2.3 ng IL-1β/hr. Thus, rats received 55.2 ng IL-1β after 1 day, 165.6 ng after 3 days, and 386.4 ng after 7 days. Previous studies have shown the osmotic minipump to be an effective means for continuous infusion of IL-1α or β (12, 15) and the dose infused in the current study was selected to be approximately 1/10 the dose reported to affect food intake and body weight (12), yet similar to doses used therapeutically (16). In addition, human IL-1β has been shown to be active in the rat (11, 15). On Day 1, Day 3, and Day 7 after implantation, rats from each group were humanely euthanized by overexposure to carbon dioxide, and blood was taken by cardiac puncture. The liver was perfused immediately, *in situ,* with ice-cold 0.9% saline, then removed and frozen in liquid nitrogen. Blood was centrifuged for 20 min at 1800*g* and 4°C. Plasma was removed from the cell layer and divided for quantitation of ceruloplasmin (Cp) oxidase activity and determination of mineral concentrations. Thymus was removed, weighed, frozen in liquid nitrogen, and stored at −70°C.

Plasma and Tissue Analysis. For mineral analysis, tissues and plasma were wet-ashed with 16 *N* HNO_3, evaporated by heat and diluted with 0.1% LaO_3/1.0 *N* HNO_3 as described by Clegg *et al.* (17). Diluted samples were analyzed by flame atomic absorption spectrophotometry (Model 55; Thermo-Jarrel Ash, Wilmington, MA) for Zn, copper (Cu), Fe, and manganese (Mn) concentrations. Certified reference solutions (1000 μg metal/ml) were used to generate standard curves for each element. A sample of the National Bureau Standard (NBS) bovine liver 1577b, (U.S. Dept. of Commerce National Inst. of Standards and Technology, Gaithersburg, MD) was included with the samples to ensure accuracy and reproducibility. Results are expressed as nmol/g wet-tissue or nmol/ml plasma.

Plasma Cp activity was measured following the oxidation of o-dianisidine dihydrochloride as described by Schosinsky *et al.* (18). Activity is expressed as U/l plasma where 1 U is defined as 1 mmole substrate oxidized/min.

Plasma concentrations of IL-1β were assayed using a commercial immunoassay kit (Quantikine; R & D Systems, Minneapolis, MN) and read on a Molecular Devices Kinetic Microplate reader (Molecular Devices Corp., Menlo Park, CA).

Plasma titers of fibrinogen, an acute-phase reactive protein in the rat, were determined by Ouchterlony double immunodiffusion (19). Briefly, neat and serially diluted serum and goat-anti-rat fibrinogen (Organon Teknika Corp., West Chester, PA) were added to prepunched wells in pH 7.2 buffered 1% agarose double diffusion plates (Kallestad, Chaska, MN). In accordance with standard protocol in clinical laboratories, a two-dilution factor increase in titer at 48 hr was considered a significant change in antigen/antibody response.

Fractionation of plasma proteins as an additional assessment of acute phase proteins, was performed by agarose zone electrophoresis as described in the Bio-Phoresis Horizontal Electrophoresis Cell Instruction Manual (BioRad Laboratories, Richmond, CA).

Briefly, plasma samples were electrophoresed on 1% agarose (standard low m_r agarose, BioRad Laboratories, Richmond, CA) gel at constant voltage (500 V) for about 2 hr or until the albumin front migrated at least 3.0 cm. Plasma proteins were identified by their mobility relative to albumin using a bromophenol blue dye marker. Protein fractions were scanned and quantitated using the Beckman DU-64 spectrophotometer modified for densitometry with the instrument's Gel Scan module (Beckman Instruments, Fullerton, CA).

Aliquots of liver (0.5 g) were homogenized in 0.25 *M* sucrose, 10 m*M* Tris-HCl buffer (25% w/v, pH 7.4) and centrifuged at 15,000*g* for 30 min at 4°C. Metallothionein (MT) levels were determined in the supernate fraction using the cadmium-heme saturation method described by Onosaka and Cherian (20). Data are expressed as nmol MT/g tissue.

For superoxide dismutase (SOD) activity liver was homogenized in 0.25 *M* sucrose, 10 m*M* Tris-HCl, pH 7.4 (10% w/v). Following sonication and centrifugation at 10,000*g* for 30 min at 4°C, total SOD activity was measured in the supernate fraction by its ability to inhibit the auto-oxidation of pyrogallol as described by Marklund and Marklund (21). MnSOD activity was measured under the same conditions with the addition of 1 m*M* KCN to the assay buffer to inhibit the activity of CuZnSOD. Activity of CuZnSOD was calculated by subtracting MnSOD activity from total activity. One unit of SOD activity is defined as the amount of enzyme needed for 50% inhibition of pyrogallol oxidation. MnSOD and CuZnSOD activities are expressed as units of activity per mg of protein. Protein was determined by the BioRad dye-binding assay (Richmond, CA).

Statistical Analysis. Fisher's PLSD test was used as a posthoc method to identify significant differences between the means. Statistical significance was established at $P < 0.05$. Results are presented as mean ± SE.

Results

Within both the AZn and MZn diet groups, results for the NT and Sal treatment groups were similar for each of the variables examined. Thus, to facilitate data presentation and interpretation, the NT and Sal groups were combined to represent the control (Con) response.

Dietary Zn did not significantly affect the baseline body weights of the rats prior to treatment. IL-1β infusion did not influence the weight gained over the experimental period in either AZn or MZn rats (Fig. 1).

Assay of plasma IL-1β levels did not detect measurable concentrations above the 20 pg/ml limit of the assay kit. Integrity of the kit was confirmed with IL-1β standards provided with the kit and the IL-1β inserted into the osmotic minipumps. Titers of plasma fibrinogen were significantly higher, as denoted by a two dilution change in titer, on Day 1 in IL-1β-infused rats fed the AZn diet than controls (data not shown). In the AZn rats, fibrinogen titers were not significantly affected by IL-1β on Day 3 and Day 7. In the MZn rats, plasma fibrinogen titers were not affected by IL-1β infusion at any of the time points assayed. Further evaluation of IL-1β-induced changes in acute phase proteins revealed a decrease in plasma albumin on Day 1 and a trend toward an increase in the α-$_1$ globulin fractions in IL-1β-infused rats on Day 3 compared with their controls (Table I). Significant dietary and IL-1β induced changes in α-$_2$ globulin fraction were also observed on Day 3. No significant changes in serum proteins were observed on Day 7 (data not shown).

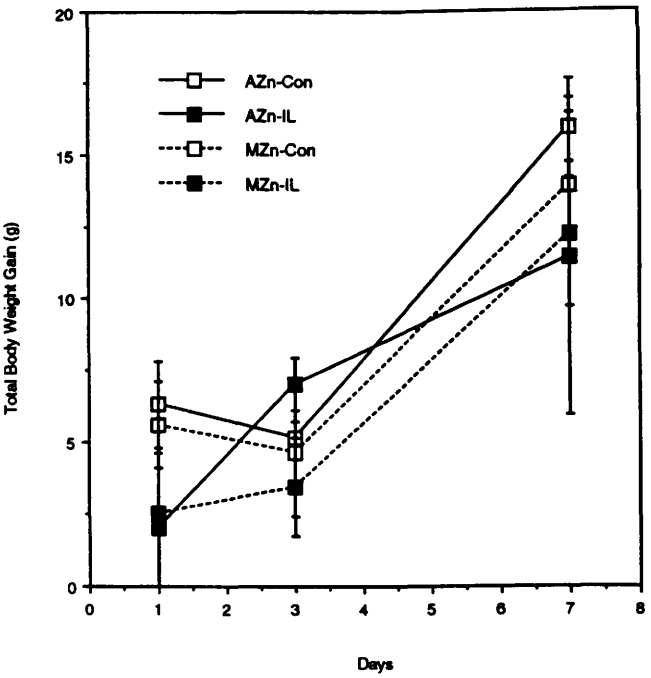

Figure 1. Total body weight gain after 1, 3, and 7 days of IL-1β infusion in the Sprague–Dawley rats. Data are expressed as mean ± SE of 5–10 animals/group. AZn, adequate Zn; MZn, marginal Zn; Con, control; IL, IL-1β.

Plasma Zn concentrations on Day 1, Day 3, and Day 7 were significantly lower in the MZn groups than in the AZn groups, demonstrating that marginal Zn deficiency was induced by the dietary treatment throughout the study period (Fig. 2). After 1 day of IL-1β treatment, plasma Zn concentrations were similar in both the AZn IL-1β and AZn control rats; however, after 3 days of infusion, plasma Zn concentrations were significantly lower in AZn IL-1β-infused rats than in AZn controls. After 7 days of infusion, plasma Zn values were again similar in the two groups. However, in the MZn rats, after 1 day of infusion, plasma Zn concentrations in the IL-1β group were sig-

Table I. Plasma Protein Fractionation in IL-1β Infused Sprague–Dawley Rats

	Albumin		α_1-Globulin		α_2-Globulin		β-Globulin		γ-Globulin		Albumin—globulin ratio	
	1	3	1	3	1	3	1	3	1	3	1	3
AZn-Con	54.5 ± 1.4[a]	50.6 ± 4.0	9.1 ± 1.5	6.7 ± 1.7	4.2 ± 1.7	3.9 ± 0.7	29.6 ± 1.8[a]	36.9 ± 1.7	2.3 ± 0.7	4.4 ± 1.5	1.20 ± 0.06[a]	1.02 ± 0.14
AZn-IL	49.5 ± 0.9[b]	44.3 ± 3.6	9.7 ± 1.0	9.2 ± 1.5	5.1 ± 0.9	7.6 ± 1.1	28.9 ± 1.9[a]	32.5 ± 3.9	5.3 ± 1.4	3.9 ± 1.2	1.02 ± 0.07[a]	0.87 ± 0.13
MZn-Con	48.4 ± 1.4[b]	44.9 ± 1.9	7.8 ± 2.7	9.0 ± 1.3	3.2 ± 0.7	6.4 ± 1.6	33.8 ± 1.8[a]	34.0 ± 1.4	2.3 ± 0.8	5.8 ± 0.7	1.07 ± 0.13[a]	0.82 ± 0.07
MZn-IL	37.6 ± 2.3[c]	42.7 ± 0.9	14.3 ± 1.3	13.5 ± 0.9	3.1 ± 0.1	10.6 ± 0.5	40.6 ± 2.5[b]	35.4 ± 3.8	5.4 ± 1.2	3.6 ± 0.4	0.60 ± 0.05[b]	0.67 ± 0.03
P values												
Treatment (T)	0.0002	NS	NS	0.0364	NS	0.0048	NS	NS	0.0158	NS	0.0034	NS
Diet (D)	0.0001	NS	NS	0.0437	NS	0.0349	0.0021	NS	NS	NS	0.0082	NS
T × D	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[a] Data expressed as mean ± SE of 3–6 animals/group.
[b] Data represent percentages of each fraction.
[c] Those values within columns containing different superscripts represent significant differences ($P \leq 0.05$); NS, not significant; AZn, adequate Zn; MZn, marginal Zn; Con, control; IL, IL-1β.

nificantly lower than in their respective controls, but on Day 3 and Day 7, plasma Zn concentrations were similar in both groups.

Liver Zn concentrations were significantly higher in the AZn IL-1β-infused groups after 1 day and 3 days than in the AZn control rats (Fig. 2). In MZn rats, however, IL-1β infusion increased liver Zn concentrations only toward the observed AZn control levels.

Thymus Zn concentrations were significantly higher in the MZn IL-1β-infused rats than in their respective controls after 1 day of treatment, but concentrations were similar after 3 days and 7 days of treatment. IL-1β infusion did not significantly affect thymus Zn concentration in the AZn rats at any of the time points tested (Fig. 2). Thymus Cu and Fe concentrations were similar among the groups on Day 1, Day 3, and Day 7 (Table II).

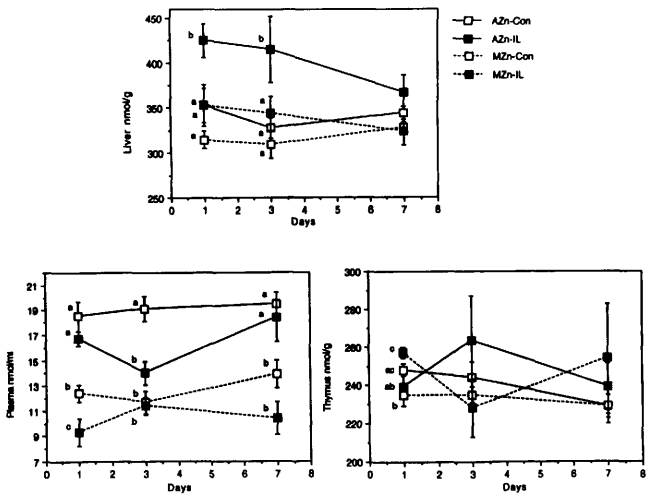

Figure 2. The influence of IL-1β on tissue mineral Zn concentrations in the Sprague–Dawley rats at 1, 3, and 7 days. Data are expressed as mean ± SE of 5–10 rats/group. At each time point, symbols denoted with different superscripts represent significant differences ($P \leq 0.05$). AZn, adequate Zn; MZn, marginal Zn; Con, control; IL, IL-1β.

Marginal Zn status alone had no significant effect on plasma Cu concentrations (Table II) or Cp activity (Table III). Plasma Cu concentrations and Cp activity were higher in both of the IL-1β-infused groups than in their respective controls throughout the experimental period.

Dietary Zn status did not significantly affect plasma Fe concentrations. For both AZn and MZn rats, plasma Fe concentrations in the IL-1β-infused groups were significantly lower than in their respective controls at Day 1 and Day 3, although Day 7 values were similar among the groups (Table II).

Liver Cu concentrations were not affected by diet or IL-1β infusion; liver Fe concentrations were affected by diet on Day 3 only (Table II).

Dietary Zn status did not influence liver Mn concentrations. After 3 days of IL-1β infusion, both AZn and MZn IL-1β-infused groups had significantly lower liver Mn concentrations than their respective controls (Table II). Liver Mn concentrations also tended to be lower in the IL-1β groups on Day 1 and Day 7.

Liver CuZnSOD and MnSOD activities were not affected by diet at the time points measured, however, liver CuZnSOD on Day 7 and liver MnSOD on Day 3 and Day 7 were affected by IL-1β infusion. IL-1β infusion resulted in higher liver MnSOD activity on Day 7 in both the AZn and MZn rats than in their respective controls (Table III).

Liver MT concentrations were significantly higher in the AZn rats than in the MZn rats (Table III). After 1 day of IL-1β infusion, liver MT levels in the AZn IL-1β-infused rats were markedly higher than in the AZn controls. Liver MT values were also significantly elevated on Day 3 and Day 7 in the AZn IL-1β rats, but these elevations were less than those observed on Day

Table II. Effects of IL-1β on Plasma, Liver, and Thymus Mineral Concentrations in Sprague–Dawley Rats

	Cu			Fe			Mn		
	1	3	7	1	3	7	1	3	7
Liver (nmol/g)									
AZn-Con	60 ± 2	58 ± 2	57 ± 2	2557 ± 123	2530 ± 154[a]	3118 ± 142	32 ± 1[a]	30 ± 1[ac]	35 ± 1
AZn-IL	65 ± 3	63 ± 4	66 ± 5	3248 ± 148	2559 ± 258[a]	2981 ± 85	28 ± 2[a]	24 ± 1[b]	32 ± 2
MZn-Con	59 ± 4	57 ± 2	60 ± 1	2944 ± 139	3676 ± 347[b]	3016 ± 170	34 ± 1[b]	33 ± 2[a]	38 ± 2
MZn-IL	59 ± 2	64 ± 4	61 ± 3	3471 ± 304	2989 ± 286[ab]	3042 ± 209	30 ± 1[ab]	27 ± 1[bc]	34 ± 1
P values									
Treatment (T)	NS	0.0392	NS	0.0024	NS	NS	0.0124	0.0020	0.0389
Diet (D)	NS	NS	NS	NS	0.0152	NS	NS	NS	NS
T × D	NS	NS	NS	NS	NS	NS	NS	NS	NS
Plasma (nmol/ml)									
AZn-Con	24.6 ± 1.3[a]	22.9 ± 1.0[a]	24.9 ± 1.5[a]	57.8 ± 5.0[a]	67.9 ± 4.4[a]	80.3 ± 4.6	ND	ND	ND
AZn-IL	37.4 ± 2.5[b]	51.1 ± 2.7[b]	33.2 ± 2.5[b]	31.2 ± 4.8[b]	47.7 ± 4.2[b]	68.6 ± 5.3	ND	ND	ND
MZn-Con	24.4 ± 1.4[a]	24.5 ± 1.6[a]	29.2 ± 1.7[ab]	62.3 ± 3.8[a]	82.6 ± 4.6[c]	67.5 ± 3.7	ND	ND	ND
MZn-IL	38.7 ± 2.8[b]	46.2 ± 4.9[b]	41.8 ± 3.8[c]	30.2 ± 4.1[b]	50.0 ± 3.6[b]	63.4 ± 6.6	ND	ND	ND
P values									
Treatment (T)	0.0001	0.0001	0.0001	0.0001	0.0001	NS	ND	ND	ND
Diet (D)	NS	NS	0.0079	NS	NS	NS	ND	ND	ND
T × D	NS	NS	NS	NS	NS	NS	ND	ND	ND
Thymus (nmol/g)									
AZn-Con	22.1 ± 1.8	23.2 ± 1.7	23.5 ± 1.4	501 ± 64	580 ± 74	629 ± 56	ND	ND	ND
AZn-IL	23.9 ± 1.8	22.5 ± 2.1	21.4 ± 2.0	554 ± 103	470 ± 78	606 ± 66	ND	ND	ND
MZn-Con	21.2 ± 1.2	22.8 ± 1.4	27.3 ± 1.5	473 ± 34	604 ± 59	631 ± 48	ND	ND	ND
MZn-IL	22.0 ± 1.8	27.3 ± 1.3	30.7 ± 4.6	490 ± 102	564 ± 91	664 ± 21	ND	ND	ND
P values									
Treatment (T)	NS	NS	NS	NS	NS	NS	ND	ND	ND
Diet (D)	NS	NS	0.0074	NS	NS	NS	ND	ND	ND
T × D	NS	NS	NS	NS	NS	NS	ND	ND	ND

[a] Data are expressed as mean ± SE of 5–10 rats/group.
[b] Those values within columns containing different superscripts represent significant differences ($P \leq 0.05$).
[c] NS, not significant; ND, not determined; AZn, adequate Zn; MZn, marginal Zn; Con, control; IL, IL-1β.

1. In contrast, liver MT levels in the MZn rats were only affected by IL-1β infusion after 1 day of treatment. The rise in liver MT levels following IL-1β treatment was less pronounced in the MZn rats.

Discussion

Neither dietary Zn status nor infusion of IL-1β had a significant effect on body weight during the study period. Previous studies observed a reduction in body weight when rats were injected twice daily with IL-1 or when infusions of IL-1α exceeded 0.48 mg/d (12, 22). However, our data show that even after 7 days of low dose IL-1β infusion, at a level which stimulated the acute phase response, as determined by elevated plasma fibrinogen titers and changes in the albumin and globulin fractions, anorexia was not observed.

Table III. Effects of IL-1β on Antioxidant Status and Stress-Induced Proteins in Sprague–Dawley Rats

	Liver CuZnSOD (U/mg)			Liver MnSOD (U/mg)			Liver MT (nmol/g)			Plasma Cp activity (U/liter)		
Days	1	3	7	1	3	7	1	3	7	1	3	7
AZn-Con	14.6 ± 0.7	14.3 ± 0.7	14.7 ± 0.5	3.1 ± 0.2	3.3 ± 0.1	3.1 ± 0.2[a]	6.2 ± 1.5[ac]	5.6 ± 0.7[a]	4.7 ± 0.4[a]	184 ± 10[a]	177 ± 11[a]	197 ± 10[a]
AZn-IL	14.3 ± 0.9	15.4 ± 0.8	16.1 ± 0.6	3.5 ± 0.2	3.5 ± 0.2	4.0 ± 0.1[b]	17.7 ± 1.2[b]	14.5 ± 2.4[b]	8.2 ± 1.9[b]	294 ± 29[b]	380 ± 48[b]	285 ± 28[b]
MZn-Con	14.3 ± 0.5	14.0 ± 0.5	15.0 ± 0.5	3.0 ± 0.2	3.1 ± 0.1	3.1 ± 0.1[a]	4.1 ± 1.1[a]	2.1 ± 0.6[c]	2.4 ± 0.4[c]	169 ± 10[a]	198 ± 22[a]	228 ± 14[a]
MZn-IL	13.0 ± 1.3	14.4 ± 1.0	16.3 ± 0.4	3.4 ± 0.2	3.7 ± 0.3	3.8 ± 0.3[b]	9.2 ± 1.9[c]	4.5 ± 0.7[ac]	1.8 ± 0.3[c]	269 ± 23[b]	365 ± 28[b]	344 ± 26[b]
P values												
Treatment (T)	NS	NS	0.0275	NS	0.0351	0.0006	0.0001	0.0001	NS	0.0001	0.0001	0.0001
Diet (D)	NS	NS	NS	NS	NS	NS	0.0020	0.0001	0.0001	NS	NS	0.0195
T × D	NS	NS	NS	NS	NS	NS	0.0460	0.0040	0.0145	NS	NS	NS

[a] Data are expressed as mean ± SE of 5–10 rats/group.
[b] Those values within columns containing different superscripts represent significant differences, $P \leq 0.05$.
[c] NS, not significant; ND, not determined; AZn, adequate Zn; MZn, marginal Zn; Con, control; IL, IL-1β.

Thus, with respect to its potential for clinical use, anorexia may not be a problem as long as the IL-1β dose is modest. To date, in the few studies that have examined potential clinical uses of IL-1β, the doses and duration given were similar to the concentrations used in the present study (16).

Reduction of plasma Zn concentrations in the MZn IL-1β-infused rats were observed earlier than in the AZn IL-1β-infused rats. In general, these data agree with previous observations following a single injection of a crude IL-1 fraction or recombinant human IL-1α (7, 11). However, the significance of these results suggests a potential nutrient-cytokine interaction, as observed on Day 3, indicating a difference in IL-1β-induced alterations in plasma Zn metabolism associated with marginal Zn status.

The mechanisms underlying the hypozincemia induced by IL-1β have not been completely established; however, it may in part be associated with the induction of liver MT synthesis (11, 23). Injection of crude IL-1 fractions or IL-1α increased concentrations of hepatic MT (7, 11). Data from the current study of the AZn rats are consistent with previous work, however, MZn rats showed suppressed IL-1β induced induction of liver MT. Thus, marginal Zn deficiency can alter the normal metabolic pathway of Zn during an acute-phase-like response. In addition, it has been suggested that MT plays a key role in overall Zn homeostasis (8). Therefore, in this study, Zn homeostasis may have been compromised in MZn rats infused with IL-1β.

Elevation of plasma Cp activity and plasma Cu concentrations throughout the 7-day time period indicates the effectiveness of IL-1β infusion inducing an acute-phase-like response. These data are consistent with previous studies following injection of crude IL-1 or IL-1α in rats (5, 24, 25) and suggest that the effects of IL-1β on Cu metabolism are not affected by marginal Zn status.

The mechanism(s) by which IL-1β infusion induces hypoferremia in rodents is not fully understood, but studies have shown that lactoferrin and transferrin can play key roles in sequestering Fe during the acute-phase response (25–27). We observed hypoferremia up to the 3 day time point in the IL-1β infused rats which agrees with previous studies with crude IL-1 fractions (7, 25).

As suggested by an earlier report, accumulation of Zn into the thymus during inflammation and tissue injury can potentially enhance immune system function (9). The observation that Zn concentrations were higher in the MZn IL-1β-infused rats than in the AZn IL-1β-infused rats on Day 1, is surprising, and may have been spurious. Further studies need to be directed toward the influence of IL-1β on the relationship between thymic function and mineral metabolism.

Several studies have observed increased MnSOD mRNA levels (28) and MnSOD protein (29) in hepatocytes over a period of 12 hr following a single high dose of IL-1 or following a 1- to 3-day period using serial doses of IL-1. It has been suggested that the increase in MnSOD activity is part of the response to oxygen radicals that are generated during the inflammatory response (28, 30). Consistent with the above, liver MnSOD activity was slightly higher on Day 1 and Day 3 in the AZn and MZn IL-1β-infused rats than in controls. Following 7 days of IL-1β infusion, both AZn and MZn rats had significantly higher levels of liver MnSOD activity than their respective controls. This increase in activity occurred in the groups despite slightly lower concentrations of liver Mn in these animals at all time points tested, suggesting that IL-1β altered the normal trafficking of Mn in the liver. Nevertheless, the data suggest a continuous low dose of IL-1β may potentially enhance the antioxidant system by stimulating MnSOD synthesis (31).

The data from the present study indicate that some of the effects of IL-1β infusion were persistent while others were transient. It has been suggested that continuous infusion of IL-1β can lead to tolerance, possibly through the production of IL-1 receptor antagonists (IL-1ra) which compete with IL-1β for its receptor, thereby reducing its activity (28, 32). Further studies investigating tolerance or adaptation to effects of IL-1β should investigate IL-1ra production as a possible mechanism.

In conclusion, our data suggest that marginal Zn deficiency alters the shift in mineral and MT metabolism which occurs in response to a continuous infusion of a low-dose of IL-1β; such a shift could possibly diminish the normal physiological response to IL-1β following injury or infection. In addition, considering that cytokines, such as IL-1β are being explored for therapeutic use, the present data underscore the need to monitor the nutritional status of the patient prior to, as well as during, therapy.

The opinions and assertions contained herein are the private views of the authors and are not to be construed as official nor do they reflect the views of the Department of the Army or the Department of Defense (AR 360-5).

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1. Abe H, Rodgers KE, Ellefson D, DiZerega GS. Kinetics of interleukin-1 secretion by murine macrophages recovered from the peritoneal cavity after surgery. J Surg Res **47**:178–182, 1989.
2. Wahl SM, Wong H, McCartney-Francis N. Role of growth factors in inflammation and repair. J Cell Biochem **40**:193–199, 1989.
3. Klasing KC. Nutritional aspects of leukocytic cytokines. J Nutr **118**:1436–1446, 1988.
4. Mizel SB. The interleukins. FASEB J **3**:2379–2388, 1989.
5. Grimble RF. Nutrition and cytokine action. Nutr Res Rev **3**:193–210, 1990.
6. Prasad AS. Clinical, biochemical, and pharmacological role of zinc. Annu Rev Pharmacol Toxicol **20**:393–426, 1979.
7. Klasing KC. Effect of inflammatory agents and interleukin-1 on iron and zinc metabolism. Am J Physiol **247**:R901–R904, 1984.
8. Cossack ZT. The role of zinc in endotoxin fever. Int J Vitam Nutr Res **61**:57–60, 1991.
9. Winchurch RA. Activation of thymocyte responses to interleukin-1 by zinc. Clin Immunol Immunopathol **47**:174–180, 1988.
10. Flynn A, Loftus MA, Finke JH. Production of interleukin-1 and interleukin-2 in allogeneic mixed lymphocyte cultures under copper, magnesium and zinc deficient conditions. Nutr Res **4**:673–679, 1984.
11. Huber KL, Cousins RJ. Maternal zinc deprivation and interleukin-1 influence metallothionein gene expression and zinc metabolism of rats. J Nutr **118**:1570–1576, 1988.
12. Otterness IG, Seymour PA, Golden HW, Reynolds JA, Daumy GO. The effects of continuous administration of murine interleukin-1α in the rat. Physiol Behav **43**:797–804, 1988.
13. Keen CL, Gershwin ME. Zinc deficiency and function. Annu Rev Nutr **10**:415–431, 1990.
14. Keen CL, Peters JM, Hurley LS. The effects of valproic acid on ^{65}Zn distribution in the pregnant rat. J Nutr **119**:607–611, 1989.
15. Hermus ARMM, Sweep CGJ, Demacker PNM, van der Meer MJ, Kloppenburg PWC, van der Meer JWM. Continuous infusion of interleukin-1β in rats induces a profound fall in plasma levels of cholesterol and triglycerides. Arterioscler Thromb **12**:1036–1043, 1992.
16. Aggarwal BB, Pocsik E. Cytokines: From clone to clinic. Arch Biochem Biophys **292**:335–359, 1992.
17. Clegg MS, Keen CL, Lonnerdal B, Hurley LS. Influence of ashing techniques on the analysis of trace elements in animal tissue. I. Wet ashing. Biol Trace Elem Res **3**:107–115, 1981.
18. Schosinsky KH, Lehmann HP, Beeler MF. Measurement of ceruloplasmin from its oxidase activity in serum by use of o-dianisidine dihydrochloride. Clin Chem **20**:1556–1563, 1974.
19. Kabat EA, Mayer MM. Experimental Immunochemistry. Springfield, IL: Charles C. Thomas, 1961.
20. Onosaka S, Cherian MG. Comparison of metallothionein determination by polarographic and cadmium saturation methods. Toxicol Appl Pharmacol **63**:270–274, 1982.
21. Marklund S, Marklund G. Involvement of the superoxide anion radical in the auto-oxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem **74**:469–474, 1974.
22. Mengozzi M, Ghezzi P. Defective tolerance to the toxic and metabolic effects of interleukin 1. Endocrinology **128**:1668–1672, 1991.
23. Cousins RJ, Leinart AS. Tissue-specific regulation of zinc metabolism and metallothionein genes by interleukin 1. FASEB J **2**:2884–2890, 1988.
24. Barber EF, Cousins RJ. Interleukin-1-stimulated induction of ceruloplasmin synthesis in normal and copper-deficient rats. J Nutr **118**:375–381, 1988.
25. Gordeuk VR, Prithviraj P, Dolinar T, Brittenham GM. Interleukin 1 administration in mice produces hypoferremia despite neutropenia. J Clin Invest **82**:1934–1938, 1988.
26. Uchida T, Yamagiwa A, Nakamura K. The effect of interleukin-1 on iron metabolism in rats. Eur J Haematol **46**:1–5, 1991.
27. Van Snick JL, Masson RL, Heremans JF. The involvement of lactoferrin in the hyposideremia of acute inflammation. J Exp Med **140**:1068–1084, 1974.
28. Dougall WC, Nick HS. Manganese superoxide dismutase: A hepatic acute phase protein regulated by interleukin-6 and glucocorticoids. Endocrinology **129**:2376–2384, 1991.
29. Ono M, Kohda H, Kawaguchi T, Ohhira M, Sekiya C, Namiki M, Takeyasu A, Taniguchi N. Induction of Mn-superoxide dismutase by tumor necrosis factor, interleukin-1 and interleukin-6 in human hepatoma cells. Biochem Biophys Res Commun **182**:1100–1107, 1992.
30. Sacks T, Moldow CF, Craddock PR, Bowers TK, Jacob HS. Endothelial damage provoked by toxic oxygen radicals released from complement-triggered granulocytes. Prog Clin Biol Res **21**:719–726, 1978.
31. Masuda A, Longo DL, Kobayashi Y, Appella E, Oppenheim JJ, Matsushima K. Induction of mitochondrial manganese superoxide dismutase by interleukin 1. FASEB J **2**:3087–3091, 1988.
32. Granowits EV, Santos AA, Poutsiaka DD, Cannon JG, Wilmore DW, Wolff SM, Dinarello CA. Production of interleukin-1 receptor antagonist during experimental endotoxaemia. Lancet **338**:1423–1424, 1991.