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ZINC STATUS AND INTERLEUKIN-1ß INDUCED MINERAL METABOLISM ALTERATIONS IN RATS§

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ABSTRACT

Interleukin-1ß (IL-1ß) can alter Zn metabolism. Changes in Zn metabolism and IL-1ß release occur in response to tissue injury and trauma. In this study, we examined whether Zn status modifies the effects of low dose IL-1ß administration on mineral metabolism. Rats were fed 50 µg Zn/g (AZn) or 5µg Zn/g (MZn) diets for 14d. On d 15, rats were infused via osmotic minipumps, with IL-1ß (2.3 ng/h) or saline (control, C) and euthanitized 1, 3 or 7d later. In the AZn rats, IL-1ß infusion resulted in increased plasma Cu concentrations and ceruloplasmin (Cp) activity, and decreased Fe concentrations throughout the 7d period, that were most pronounced on d1 and d3. A similar trend was observed in the MZn rats, but IL-1ß-induced increases in plasma Cu and Cp were less than in the AZn fed rats. In MZn and AZn IL-1ß infused rats, plasma Zn was decreased at d1 and d3, respectively, compared with their respective controls. AZn IL-1ß-infused rats were characterized by high liver Fe, Zn and metallothionein (MT) concentrations at d1; by 7d, only MT concentrations remained elevated. These data show that Zn status can influence the response to low dose IL-1ß. This influence of Zn should be considered when IL-1ß is given to stimulate wound healing.

INDEX TERMS: Continuous infusion, Copper, Ceruloplasmin, Metallothionein, Zinc, IL-1ß, Rats

INTRODUCTION

As one consequence of tissue injury, the cytokine, interleukin-1ß (IL-1ß) is released (1,37). 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 (22, 26). IL-1ß can also trigger a redistribution of zinc (Zn) from the plasma pool into the liver, thymus, and bone marrow (4,7). This redistribution of Zn is thought to play an important role in immune system function and initiation of tissue repair (12,15,20,34), although the mechanism(s) underlying these cytokine-nutrient interactions is not well characterized.

Recent investigations suggest that both Zn and IL-1ß enhance the response to tissue injury and trauma by stimulating wound healing (3,29,30,32). Because IL-1ß can stimulate the rate of wound healing, it has been suggested that it may have clinical value in some circumstances (2). Since IL-1ß induces marked changes in Zn metabolism, it is reasonable to assume that the effect of IL-1ß on Zn distribution among Zn pools may be altered by dietary Zn status. Considering that previous investigators had examined the effect of a single high dose of IL-1ß on mineral metabolism (18,21), we studied the influence of continuous low-dose IL-1ß infusion on cytokine-induced changes in mineral metabolism in healthy adult rats over a period of seven days. Furthermore, we examined the

influence of marginal Zn deficiency on IL-1ß-induced changes in mineral metabolism.

MATERIALS AND METHODS

<u>Animal and Diets</u>: 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 h light/dark cycle; 22-23° C). Rat chow (Ralston Purina Co., St. Louis, MO; 55 µg Zn/g) and double deionized water were fed ad libitum for a 7-d acclimation period. The rats were then assigned to a semi-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 d. Animals were weighed and examined daily.

<u>Experimental Procedure</u>: On d 15, rats were randomly assigned to one of the following treatment groups for periods of 1d, 3d, or 7d: 1) IL-1ß infusion; 2) saline infusion (Sal); or 3) no treatment (NT). For infusion of IL-1ß or saline, the animals were anesthetized 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-1ß (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-1ß was infused at a rate of 2.3 ng IL-1ß/h. Previous studies have shown the osmotic minipump to be an effective means for continuous infusion of IL-1α or ß (16,29) and the dose infused in the current study was approximately 1/10 the dose reported to affect food intake and body weight (29). At 1d, 3d, and 7d after implantation, rats from each group were humanely euthanitized by over-exposure to carbon dioxide and tissues were collected. Blood was taken by cardiac puncture and centrifuged for 20 min at 1800 x g and 4 °C. Plasma was removed from the cell layer and divided for quantitation of ceruloplasmin oxidase (Cp) activity and determination of mineral concentrations. The liver was perfused immediately, in situ, with ice cold 0.9% saline, then removed and frozen in liquid nitrogen. Thymus was removed, weighed, frozen in liquid nitrogen, and stored at -70 °C.

Plasma and Tissue Analysis: Zn, Cu, Fe, Mn, Ca, and Mg concentrations in plasma and tissues were analyzed after wet ashing with nitric acid, as described by Clegg et al. (6); values are expressed as nmol/g wet-tissue or nmol/ml plasma. Plasma Cp activity was determined spectrophotometrically at 540 nm using the o-dianisidine dihydrochloride method of Schosinsky et al. (33). Plasma concentrations of Il-1ß were assayed using a commercial immunoassay (Quantikine; R & D Systems, Minneapolis, MN). Plasma concentrations of fibrinogen, as an acute-phase reactive protein in the rat, were determined by Ouchterlony double immunodiffusion. The titers were determined at 24 and 48 hr, but only the 48 hr data were compared in accordance with standard protocol in clinical

laboratories. A significant difference was defined by at least a two dilution change in titer. Fractionation of plasma proteins as an additional assessment of acute phase proteins was performed by agarose zone electrophoresis as described in the BioPhoresis Horizontal Electrophoresis Cell Instruction Manual (BioRad Laboratories, Richmond, CA). Protein fractions were scanned and quantitated using the Beckman DU-64 spectrophotometer (Beckman Instruments, Fullerton, CA)

Aliquots of liver (0.5 g) were homogenized in 0.25 M sucrose buffer (25% w/v) (pH 7.4) and centrifuged at 15,000 x g for 30 min at 4 °C. Metallothionein (MT) was determined in the supernate fraction using the cadmium-heme saturation method, as described by Onosaka and Cherian (28). Liver superoxide dismutase (SOD) activity was determined in homogenates prepared in 0.25 M sucrose (10% w/v) as described by Marklund and Marklund (23). Following sonication and centrifugation at 10,000 x g for 30 min at 4 °C, the pellet was discarded and total SOD activity was measured in the supernate fraction by its ability to inhibit the auto-oxidation of pyrogallol. Manganese SOD (MnSOD) activity was measured under the same conditions with the addition of 1 mM KCN to the assay buffer. Cyanide was used to inhibit the activity of copper-zinc SOD (Cu-ZnSOD). Activity of Cu-ZnSOD was calculated by subtracting MnSOD activity from total activity. MnSOD and Cu-ZnSOD values are expressed as units of activity per mg of protein. Protein was determined by the Bio-Rad dye-binding assay (Bio-Rad Laboratories, Richmond, CA).

Statistical Analysis: Differences among treatment groups were determined by analysis of variance. Fisher's LSD test was used as a posthoc method to identify significant differences between the means. Significance was established at $p \leq 0.05$.

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 response.

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

Determination of plasma IL-1ß levels did not detect measurable concentrations above the 20 pg/ml limit of the assay kit. Integrity of the kit was confirmed with IL-1ß standards with the kit and the IL-1ß inserted into the osmotic minipumps. Plasma concentrations of fibrinogen were significantly higher, as denoted by a two dilution change in titer, at 1d in IL-1ß-infused rats fed the AZn diet than controls (data not shown). In the AZn rats, fibrinogen concentrations were not significantly affected by IL-1ß at 3d and 7d. In the MZn rats, plasma fibrinogen concentrations were not affected by IL-1ß infusion at any of the time points assayed. Further evaluation of IL-1ß-induced changes in acute phase proteins observed a decrease in plasma albumin and a trend toward an

increase in the globulin fractions in IL-1ß-infused rats compared with their controls (Table 2). The data, however, only achieved statistical significance in the MZn+IL group at 1d. These changes were less observable at 3d (Table 2) and not seen at 7d (data not shown).

Plasma Zn concentrations at 1d, 3d, and 7d were significantly lower in the MZn groups than in the AZn groups, demonstrating that marginal zinc deficiency was induced by the dietary treatment throughout the study period (Fig. 1A). After 1d of IL-1ß treatment, plasma Zn concentrations were similar in both the AZn IL-1ß and AZn control rats; however, after 3d of infusion, plasma Zn concentrations were lower (27%; $p \leq 0.05$) in AZn IL-1ß-infused rats than in AZn controls. After 7d of infusion, plasma Zn values were again similar in the two groups. After 1d of infusion, plasma Zn concentrations in the MZn IL-1ß group were significantly lower than in the MZn controls; at 3d and 7d, plasma Zn concentrations were similar in both groups.

Dietary Zn treatment alone had no significant effect on plasma Cu concentrations. Plasma Cu concentrations were higher in both of the IL-1ß-infused groups compared to their respective controls throughout the experimental period (Fig. 1B). Dietary Zn had no significant effect on plasma Cp activity in either group. IL-1ß infusion resulted in significant increases in plasma Cp activity at 1d, 3d, and 7d (Fig. 1D).

Dietary Zn treatment did not significantly affect plasma Fe concentrations. For both AZn and MZn rats, plasma Fe concentrations in the IL-1ß-infused groups were significantly lower compared with

their respective controls at 1d and 3d, although 7d values were similar among the groups (Fig. 1C).

Plasma Ca and Mg concentrations were not significantly affected by either diet or IL-1ß infusion at any of the time points assayed (Table 3).

Liver Zn concentrations tended to be significantly higher in the AZn groups than in the MZn groups (Fig. 2A). Liver Zn concentrations were significantly higher in the AZn IL-1ß-infused groups after 1d and 3d compared with the AZn control rats. In MZn rats, IL-1ß infusion increased liver Zn concentrations toward the observed AZn levels (Fig. 2A)

Liver MT concentrations were significantly higher in AZn rats than in MZn rats (Fig. 2B). After 1d of IL-1ß infusion, liver MT levels in the AZn IL-1ß-infused rats were markedly higher compared with the AZn controls (Fig. 2B). Liver MT values were also elevated at 3d and 7d in the AZn IL-1ß rats, but these elevations were less than that observed at 1d. In contrast, liver MT levels in the MZn rats were only affected by IL-1ß infusion after 1d of treatment.

Liver Cu, Fe (Fig. 2) and Mg (Table 3) concentrations were not affected by either diet or IL-1ß infusion at any of the time points.

Liver Ca concentrations in the AZn IL-1ß-infused and control rats were similar at 1d, 3d, and 7d. In contrast, after 1d, MZn IL-1ß infused rats had higher liver Ca concentrations compared with the MZn controls; this difference was not observed at 3d or 7d (Table 3).

Dietary Zn did not influence liver Mn concentrations. After 3d of IL-1ß infusion, both AZn and MZn IL-1ß-infused groups had

significantly lower liver Mn concentrations than their respective controls (Table 3).

The distri[illegible]ion of Zn, Cu, Fe, Mn, Ca, and Mg concentrations, as a percentage of the mineral concentrations in liver 15,000 x g sup[illegible]rnate and the resulting pellet, was not influenced by either diet or IL-1ß treatment at any time point (data not shown).

Thymus Zn concentrations were significantly higher in the MZn IL-1ß-infused rats than in their respective controls after 1d of treatment, but concentrations were similar after 3d and 7d of treatment. IL-1ß infusion did not significantly affect thymus Zn concentration in the AZn rats at any of the time points tested (Table 4). Thymus Cu and Fe (Table 4), and Ca and Mg (Table 3) concentrations were similar among the groups at 1d, 3d, and 7d.

Liver MnSOD and Cu-ZnSOD activities were not significantly different between the AZn and MZn groups. IL-1ß infusion resulted in higher liver MnSOD activity at 7d compared with controls. IL-1ß had no significant effect on liver Cu-ZnSOD activity (Table 5).

DISCUSSION

Zn is essential for normal cell proliferation and differentiation, immune function, and tissue repair (12,15,34). At tissue injury sites, macrophages and monocytes produce cytokines such as IL-1ß (1,5,8). The injection of high concentrations of IL-1ß is associated with marked changes in Zn metabolism (7,17). However, given the observation that marginal Zn status may occur with a high frequency in some populations (12,20), it seems prudent to ascertain if Zn status alters the response to IL-1ß infusion.

Neither dietary Zn status nor infusion of IL-1ß had a significant effect on body weight throughout the present study period. Others observed a reduction in body weight when rats were injected twice daily with IL-1 or when infusions exceeded 0.48μg/d (25,29). However, our data show that even after 7 days of low dose IL-1ß infusion, at a level which stimulated the acute phase response, as determined by elevated plasma fibrinogen concentration and changes in the albumin and globulin fractions, anorexia was not observed. Thus, with respect to its potential for clinical use, anorexia may not be a problem as long as the IL-1ß dose is modest. To date in the few studies that have examined potential clinical uses of IL-1ß, the doses and duration given were similar to the concentrations used in the present study (2).

Plasma Zn concentrations were low after 3d of IL-1ß infusion in the AZn rats, and after 1d in the MZn rats, compared with their respective controls. This result suggests that IL-1ß reduces plasma Zn concentration more rapidly in marginal Zn status. Thus, a patient who is marginally Zn deficient may be at risk if IL-1ß therapy triggers a Zn deficiency that retards the healing process.

The mechanisms underlying the hypozincemia induced by IL-1ß have not been completely established; however, it may in part be associated with the induction of liver MT synthesis (7,17). In support of this concept, liver Zn and MT concentrations were significantly higher in the AZn IL-1ß-infused rats compared with their respective controls after 1d and 3d of IL-1ß infusion. However, there was little influence of IL-1ß on liver Zn or MT concentrations in the MZn rats. The biological significance of elevated liver MT

levels in an acute phase response is not well understood, but our data suggest that marginal Zn deficiency altered the normal metabolic pathway of Zn during an acute-phase-like response.

After 7d of IL-1ß infusion, plasma Cp activity and Cu concentrations remained significantly elevated in both the AZn and MZn rats. However, the concentrations of liver MT and Zn in the AZn rats had markedly decreased after 7d of IL-1ß infusion, indicating that some effects of IL-1ß infusion are continuous while others are more transient. Our data suggest that the Zn, which may have been sequestered initially by the liver, was released slowly from the liver pool into the plasma, resulting in plasma Zn levels that were not affected by IL-1ß infusion after 7d of treatment. Alternatively, plasma Zn concentrations may have slowly increased in this group if a smaller fraction of newly absorbed zinc was being sequestered by MT in the liver. Our data suggest that by 7d, either the animals had become adapted to the continuous IL-1ß infusion, or that IL-1ß receptor antagonists which have no IL-1ß activity, were being produced, competing with IL-1ß for its receptors, and thereby reducing IL-1ß activity (11,14). In addition, prostaglandin E2 may inhibit IL-1ß translation via cAMP (9). IL-1ß can stimulate the production of arachidonic acid, the prostaglandin precursor. Therefore, the effects of IL-1ß may have been attenuated after 7d of infusion. Further studies are required to elucidate the mechanisms underlying our observations.

Similar to its effects on plasma Zn, IL-1ß infusion had a marked effect on plasma Fe concentrations initially, but by 7d, this cytokine did not affect plasma Fe concentrations. The mechanism(s) by which

IL-1ß 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 (13,35,36). Our data show that by 7 d of IL-1ß infusion, plasma Fe concentration had returned to normal, indicating adaptation (19).

As suggested by an earlier report, accumulation of Zn into the thymus during inflammation and tissue injury can potentially enhance immune system function (38). Our data show that thymus Zn concentration in the MZn IL-1ß-infused rats was significantly higher than their respective controls after 1d but not 3 or 7d. Further studies could be directed toward the influence of IL-1ß on the relationship between thymic function and mineral metabolism.

Several studies have observed increased MnSOD mRNA levels (11) and MnSOD protein (27) in hepatocytes over a period of 12h using a single high dose or over 1-3 days using serial doses of IL-1. These observations suggest that MnSOD may be part of the response to protect hepatocytes from oxgen radicals that may be generated during the inflammatory response (10,11,31). Our study shows that liver MnSOD activity was slightly higher at 1d and 3d in the AZn and MZn IL-1ß-infused rats. After 7d of IL-1ß infusion, both AZn and MZn had significantly higher levels of liver MnSOD activity compared with their respective controls. Liver MnSOD activity was increased in the IL-1ß-infused rats despite lower liver Mn concentration at all time points. These findings indicate that a continuous low dose of IL-1ß can potentially enhance the anti-oxidant system by stimulating MnSOD synthesis (24).

Others have infused or injected IL-1 (7,13,35), using doses ranging from 250 ng/h to 10 µg and have observed responses in the liver and plasma simlar to those we have observed by using continuous infusion of 2.3 ng IL-1ß/h. Using the continuous low dose of IL-1ß is more physiologically relevant, because, like hormones, only a trace quantity of IL-1ß is produced in the body (2). Therefore, larger doses may potentially initiate more rapid side effects.

In conclusion, our data suggest that marginal Zn deficiency alters the shift in mineral metabolism in response to continuous infusion of a low-dose of IL-1ß. In addition, the results suggest that Zn status may affect the acute phase response to trauma and tissue injury by possibly modifying the physiological response to cytokines, such as IL-1ß. Nutritional status of the patient may also be important in the therapeutic use of cytokines.

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§ TEXT FOOTNOTES

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)

The experimental studies of the authors described in this report were reviewed and approved by the Institutional Review Committee/Animal Care and Use Committee at Letterman Army Institute of Research and by the University of California, Davis Animal Health and Welfare Committee. The manuscript was peer reviewed for compliance prior to submission for publication. In conducting the research described here, the authors adhered to the "Guide for the Care and Use of Laboratory Animals," DHEW Publication (NIH) 85-23.

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Table 1: Total weight gained after IL-1ß infusion

Days	d1	d3	d7
AZn-IL	6.3±1.5	5.1±0.6	15.9±1.7
AZn+IL	2.0±2.6	9.2±0.7	11.4±5.5
MZn-IL	5.6±1.5	4.6±2.2	13.9±2.5
MZn+IL	2.5±3.3	3.4±1.7	12.2±2.5

Total body weight gained (g) in female S-D rats after continuous infusion of 2.3 ng/h IL-1ß for 1d, 3d, and 7d. Rats were fed adequate Zn (AZn) and marginal Zn (MZn) diets. Rats were infused with either IL-1ß (+IL) or saline (-IL). Values are means ± S.E.M. ; n=5-6 in the +IL treated groups in both dietary groups; n=10 in the -IL treated groups in both dietary groups.

Table 2 Plasma Protein Fractionation in IL-1ß Infused Rats

	Day 1				Day 3			
	AZn-IL	AZn+IL	MZn-IL	MZn+IL	AZn-IL	AZn+IL	MZn-IL	MZn+IL
Albumin	52.0±3.1	46.2±2.1	48.6±1.5	37.6±2.3*+	50.6±4.0	45.9±4.5	46.4±1.8	42.7±0.9
α_1-Globulin	9.0±1.5	7.1±1.7	7.8±2.7	14.3±1.3 +	6.7±1.7	8.2±1.5	9.0±1.3	13.5±0.9
α_2-Globulin	4.2±1.7	5.2±1.1	3.2±1.1	3.1±0.2	3.9±0.9	7.4±0.9	6.4±1.6	10.6±0.9
ß-Globulin	32.1±3.7	28.0±1.7	34.9±1.8	40.6±5.5	36.9±2.9	33.0±3.9	35.6±3.5	35.4±5.2
γGlobulin	2.3±0.7	4.6±1.2	2.3±0.8	5.4±1.2	4.4±1.5	4.2±1.1	5.8±0.7	3.6±0.4
Albumin: Globulin Ratio	1.10±0.13	1.05± 0.10	1.08±0.12	0.61±.06*+	1.07±0.19	0.91±0.18	0.87±0.06	0.75±0.03

Data expressed as mean ± S.E. of 3-6 animals/group. Data represent percentages of each fraction.
*Significantly different ($p<0.05$) from respective control.
+Significantly different from respective dietary Zn control.

Table 3. Effect of IL-1ß infusion on plasma, liver and thymus minerals.

Elements	Mn			Ca			Mg		
Days	1	3	7	1	3	7	1	3	7
Plasma									
AZn-IL	---	---	---	2544±107	2433±96	2350±60	945±19	887±20	984±40
AZn+IL	---	---	---	2652±130	2668±102	2783±217	927±24	877±34	1078±58
MZn-IL	---	---	---	2484±78	2546±86	2227±79	932±16	982±33	922±26
MZn+IL	---	---	---	2690±107	2726±69	2312±90	999±32	898±43	922±26
Liver									
AZn-IL	32±1	30±1	35±1	523±31	452±31	410±21	7365±164	7143±166	7264±113
AZn+IL	28±2	24±1*	32±2	595±35	522±44	419±30	7806±215	7306±171	7244±58
MZn-IL	34±1	33±2	38±2	465±40	488±18	521±42	7371±162	7175±184	7350±78
MZn+IL	30±1	27±1*	34±1	703±57*	538±17	454±15	7688±179	7624±196	7445±200
Thymus									
AZn-IL	---	---	---	200±16	211±17	188±18	7169±184	7379±257	6646±257
AZn+IL	---	---	---	224±41	402±189	168±27	6628±668	7294±224	7294±379
MZn-IL	---	---	---	192±18	212±43	186±8	6951±214	6958±141	6856±102
MZn+IL	---	---	---	218±45	215±69	225±47	7684±203	6638±241	6791±247

Adequate Zn (AZn) and marginal Zn(MZn) rats were infused with IL-1ß for 1d, 3d, and 7d. Values are means ± S.E.M. in nmol/ml plasma or nmol/g wet-tissue: n=5-6, +IL-1ß ; n=10, -IL-1ß in both dietary groups: * Significantly different from respective controls.

Table 4 Effect of IL-1ß on thymus minerals

Elements	Zn			Cu			Fe		
Days	1	3	7	1	3	7	1	3	7
AZn-IL	248±3	244±8	229±9	22±2	23±2	24±1	500±64	580±74	629±56
AZn+IL	239±6	263±24	239±12	24±2	23±2	21±2	554±103	470±78	606±66
MZn-IL	235±6	235±5	229±6	21±1	23±1	27±2	473±34	604±59	631±48
MZn+IL	257±3*	228±15	254±29	22±2	27±1	31±5	490±102	564±91	664±21

Adequate Zn (AZn) and marginal Zn (MZn) rats were infused with 2.3 ng/h IL-1ß for 1d, 3d, and 7d. Values are means ± S.E.M.: Values are expressed as nmol/g wet-tissue. n=5-6, IL-1ß infused rats, (+IL); n=10, controls, (-IL) in both dietary groups.

* Significantly different from respective controls.

Table 5 Effect of IL-1ß on liver MnSOD and Cu-ZnSOD activities

Days	1	3	7
MnSOD			
AZn-IL	3.0±0.2	3.2±0.1	3.0±0.2
AZn+IL	3.5±0.2	3.5±0.2	4.0±0.1*
MZn-IL	3.0±0.2	3.1±0.1	3.1±0.1
MZn+IL	3.4±0.2	3.7±0.3	3.8±0.3*
Cu-ZnSOD			
AZn-IL	15±0.7	14±0.7	15±0.5
AZn+IL	14±0.9	15±0.8	16±0.6
MZn-IL	14±0.5	14±0.5	15±0.5
MZn+IL	13±1.3	14±1.0	16±0.4

Liver MnSOD and Cu-ZnSOD activities in adequate Zn (AZn) and marginal Zn (MZn) rats infused with IL-1ß for 1d, 3d and 7d.

Values are means ± S.E.M. Values are expressed as Units/mg protein.

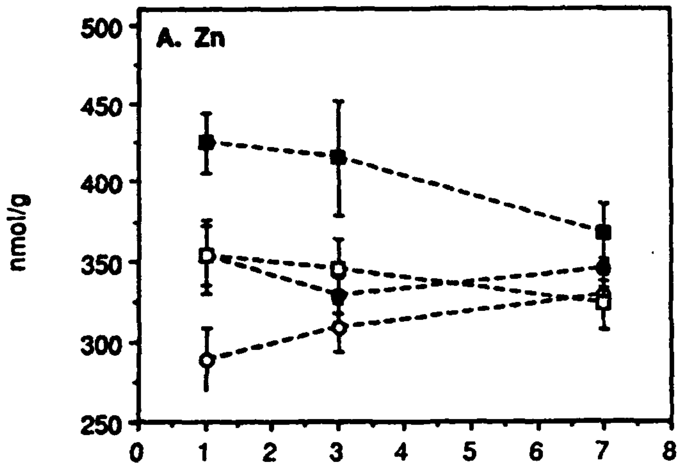
n=5-6, IL-1ß infused rats, (+IL) in both dietary groups.

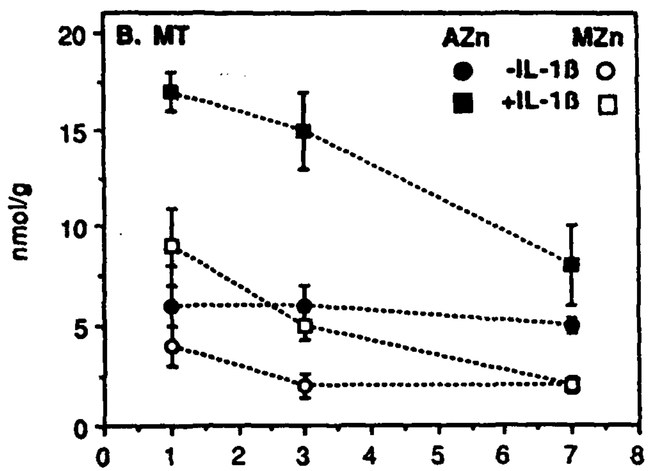
n=10, controls, (-IL) in both dietary groups.

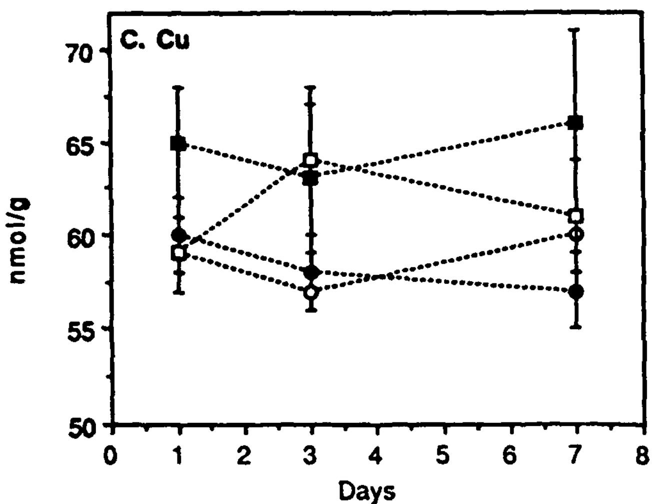
*Significantly different from respective controls.

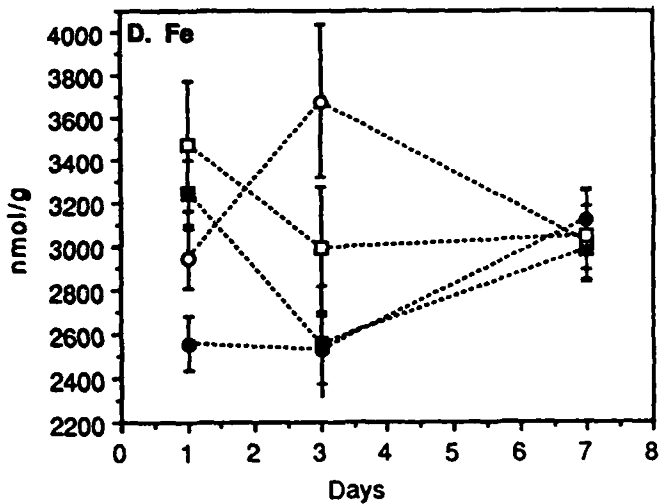
Figure 1. Plasma minerals and ceruloplasmin oxidase activity in adequate Zn (AZn) and marginal Zn (MZn) rats after 1d, 3d, and 7d of IL-1ß infusion. Values are mean ± S.E.M.; n=5-6, IL-1ß-infused rats (+IL-1ß); n=10, controls, (-IL-1ß) in both dietary groups.

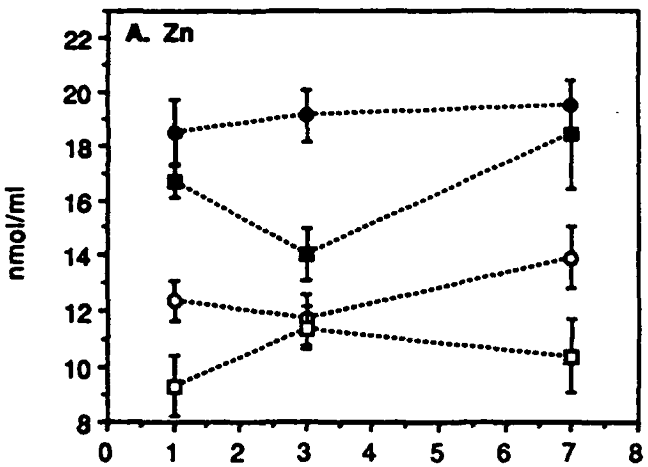
Figure 2. Liver minerals and liver metallothionein (MT) levels in adequate Zn (AZn) and r inal Zn (MZn) rats after IL-1ß infusion for 1d, 3d, and 7d; Values are means ± S.E.M.; n=5-6, IL-1ß-infused rats, (+IL-1ß); n=10, controls, (-IL-1ß) in both dietary groups.

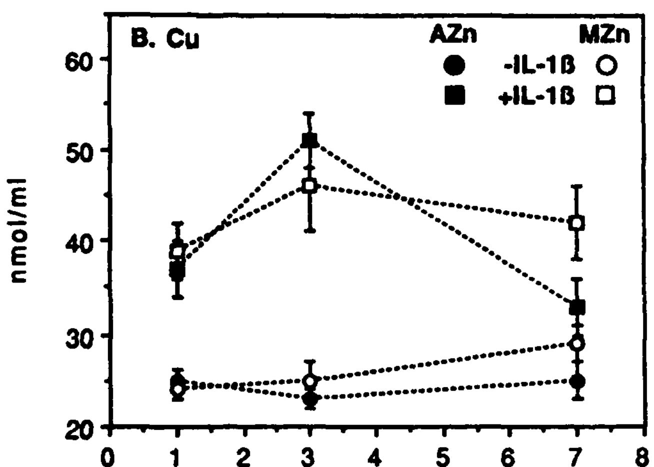

A. Zn
nmol/g
500
450
400
350
300
250
0
1
2
3
4
5
6
7
8

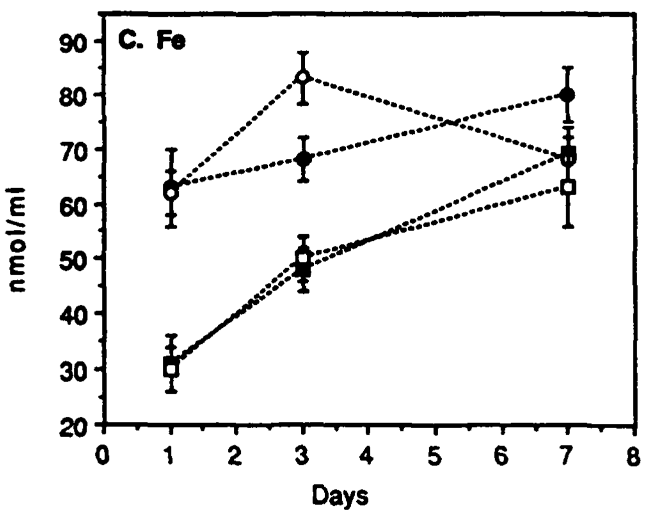

B. MT
AZn
MZn
-IL-1ß
+IL-1ß
nmol/g
20
15
10
5
0
0
1
2
3
4
5
6
7
8

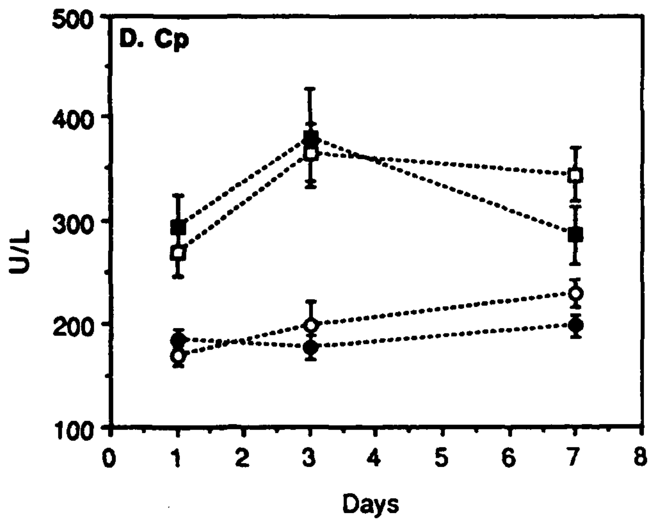

C. Cu
nmol/g
70
65
60
55
50
0
1
2
3
4
5
6
7
8
Days


D. Fe
nmol/g
4000
3800
3600
3400
3200
3000
2800
2600
2400
2200
0
1
2
3
4
5
6
7
8
Days


A. Zn
nmol/ml
22
20
18
16
14
12
10
8
0 1 2 3 4 5 6 7 8


B. Cu
AZn
MZn
-IL-1ß
+IL-1ß
nmol/ml
60
50
40
30
20
0 1 2 3 4 5 6 7 8


C. Fe
nmol/ml
90
80
70
60
50
40
30
20
0 1 2 3 4 5 6 7 8
Days


D. Cp
U/L
500
400
300
200
100
0 1 2 3 4 5 6 7 8
Days