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# Zinc Metabolism and Homeostasis in Rats Fed a Wide Range of High Dietary Zinc Levels (39358)

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The essentiality of zinc and its importance in animal and human nutrition are well known. However, many key aspects of zinc metabolism and homeostasis remain to be established (1). In studies with calves (2-5), adding 600 ppm supplemental zinc to a practical diet drastically increased zinc in certain tissues, especially liver, kidney, and pancreas, indicating a breakdown of zinc homeostasis. With rats the same diet did not materially affect the zinc content of liver and kidneys suggesting that homeostatic control mechanisms for zinc are much more effective in this species than in calves (6).

In this study effects of a wide range of dietary zinc levels (38 to 8438 ppm) on zinc and $^{65}$Zn metabolism and homeostasis in rats were investigated.

*Materials and methods.* Sixty-eight male Cherokee S-D Albino rats, initially weighing 100-120 g and being about 35 days of age, were fed a practical corn-soybean diet (2) containing 38 ppm zinc, or the same diet supplemented with 1200, 2400, 3600, 4800, 6000, 7200, or 8400 ppm zinc as ZnO for 21 days. The rats, maintained individually in stainless steel cages, were fed and given distilled water *ad libitum*. The rats were observed routinely throughout the experiment for clinical symptoms of toxicity such as skin lesions, diarrhea, muscular incoordination, and reduced feed intake.

Fourteen days after initiation of dietary treatments, each rat was anesthetized and given by gavage an oral tracer dose of 29.8 $\mu$Ci $^{65}ZnCl_2$ in acetate buffer (pH 4.7, sp act of 4.25 mCi/mg Zn). Total fecal collections were made for 7 days after dosing, at which time the rats were anesthetized with diethyl ether and sacrificed by exsanguination. Blood, heart, liver, kidneys, round muscle, and tibia samples were taken.

Zinc in feed, feces, and tissues was determined by atomic absorption spectroscopy, after nitric-perchloric-sulfuric acid wet ashing of samples (7). $^{65}$Zn activity was determined with an automated gamma ray test tube changer system with a NaI (T1) well crystal.[1] Because of nonhomogeneity of variances (due to large differences in mean values), the data were transformed to common logarithms before testing by analysis of variance and Duncan's Multiple Range Test procedures (8).

*Results.* The high zinc diets did not affect weight gains or feed consumption or cause clinical toxicity symptoms. Average daily feed consumption for controls and those receiving 8400 ppm added zinc were 18 and 19 g, respectively, while weight gains were 3.5 and 3.3 g per day, respectively.

Stable zinc excreted in the feces increased linearly with each additional increment of dietary zinc (Fig. 1A). In contrast, total fecal excretion of $^{65}$Zn increased sharply from 65% of the dose in controls to 86% of the dose in rats given 1200 ppm added zinc (Fig. 1B). Further additions of dietary zinc up to 8400 ppm had little effect of fecal $^{65}$Zn excretion (Fig. 1B).

Liver, kidney, and tibia stable zinc increased ($P < 0.05$) with added dietary zinc up to 2400 ppm (Table I). Although data were variable, a plateau in zinc concentration of these tissues was evident from the 2400 to about 7200 ppm added zinc. Further increases of zinc in liver, kidney, and tibia occurred when supplemental zinc was increased from 7200 to 8400 ppm. Muscle and heart zinc were not appreciably affected by any of the dietary treatments.

The high $^{65}$Zn content in tissues of control rats (38 ppm dietary Zn) (Table II) relative to those given supplemental zinc indicates much more labile zinc. Although only a small fraction of a kilogram of tissues is

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[1] Model 709, Baird Atomic, Inc., Cambridge, Mass.

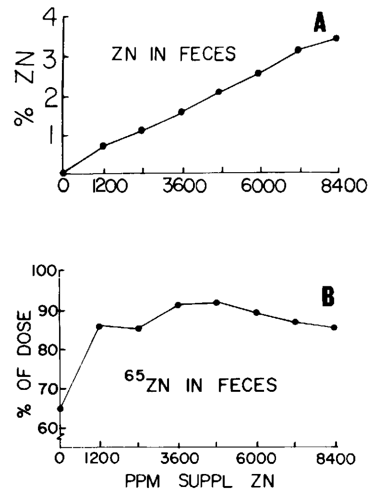

FIG. 1. Effect of feeding high levels of dietary zinc for 21 days on total fecal excretion of: (A) stable zinc (as percentage zinc in fecal dry matter); and (B) $^{65}$Zn from a single oral dose. All values are means of nine rats per treatment except for control and 1200 ppm supplemental zinc groups which had six and eight rats, respectively.

involved, the $^{65}$Zn data are presented per kilogram to obtain convenient sized numbers (Table II).

Feeding 1200 ppm supplemental zinc sharply reduced $^{65}$Zn retention in all tissues but further added zinc to 8400 had much less effect (Table II). Muscle and heart $^{65}$Zn declined significantly when added zinc was increased from 1200 to 8400 ppm. In contrast, bone $^{65}$Zn increased when added dietary zinc was advanced from 3600 to 6000 ppm and remained fairly constant with still higher zinc intakes (Table II).

*Discussion.* Homeostatic control mechanisms regulate tissue concentration of essential elements with different degrees of effectiveness in animals consuming varying intakes (1, 9, 10). In calves, these control mechanisms for zinc operate through changes in absorption, endogenous fecal excretion, and tissue turnover rates (1, 9). In this study, with young growing rats, linear

TABLE I. EFFECT OF DIETARY ZINC LEVEL ON STABLE ZINC IN SELECTED TISSUES.$^{a}$

| Dietary zinc added (ppm) | Tissues (ppm zinc, dry tissue) | | | | |
|---|---|---|---|---|---|
| | Liver | Kidneys | Heart | Tibia | Muscle (round) |
| Control$^{a}$ | 92 ± 3$^{b}$ | 96 ± 9$^{b}$ | 71 ± 5$^{b}$ | 246 ± 31$^{b}$ | 43 ± 2$^{b}$ |
| 1200 | 170 ± 30$^{bc}$ | 145 ± 21$^{bc}$ | 69 ± 2$^{b}$ | 456 ± 85$^{bc}$ | 46 ± 5$^{b}$ |
| 2400 | 242 ± 36$^{cd}$ | 237 ± 43$^{cd}$ | 71 ± 3$^{b}$ | 866 ± 161$^{cd}$ | 60 ± 9$^{b}$ |
| 3600 | 201 ± 42$^{c}$ | 220 ± 45$^{cd}$ | 73 ± 5$^{b}$ | 631 ± 160$^{bc}$ | 52 ± 6$^{b}$ |
| 4800 | 184 ± 40$^{bc}$ | 229 ± 52$^{cd}$ | 75 ± 3$^{b}$ | 748 ± 208$^{c}$ | 48 ± 5$^{b}$ |
| 6000 | 210 ± 50$^{c}$ | 209 ± 38$^{cd}$ | 78 ± 3$^{b}$ | 685 ± 165$^{c}$ | 43 ± 3$^{b}$ |
| 7200 | 243 ± 27$^{cd}$ | 238 ± 41$^{de}$ | 73 ± 3$^{b}$ | 904 ± 153$^{cd}$ | 48 ± 5$^{b}$ |
| 8400 | 379 ± 41$^{d}$ | 408 ± 48$^{e}$ | 94 ± 6$^{c}$ | 1407 ± 204$^{d}$ | 51 ± 7$^{b}$ |

[a] Values are mean ± SE for nine rats except control and 1200 ppm zinc supplemental groups which has six and eight rats, respectively. Control diet had 38 ppm zinc.

[bcde] Values in the same vertical column not followed by the same letter are significantly different ($P < 0.05$) as determined by analyses of variance of data transformed to common logarithms.

TABLE II. TISSUE $^{65}$ZN DISTRIBUTION AS AFFECTED BY DIETARY ZINC.$^{a}$

| Dietary zinc added (ppm) | Tissues (% of dose/kg fresh tissue) | | | | | |
|---|---|---|---|---|---|---|
| | Liver | Kidneys | Heart | Tibia | Muscle (Round) | Blood |
| Control$^{a}$ | 192.0 ± 6.4$^{b}$ | 145.9 ± 7.0$^{b}$ | 117.3 ± 4.1$^{b}$ | 525.4 ± 54.3$^{b}$ | 78.2 ± 4.7$^{b}$ | 38.6 ± 2.0$^{b}$ |
| 1200 | 7.7 ± 0.9$^{c}$ | 5.4 ± 0.6$^{cd}$ | 4.8 ± 0.6$^{c}$ | 25.9 ± 3.3$^{e}$ | 3.5 ± 0.6$^{c}$ | 1.6 ± 0.2$^{c}$ |
| 2400 | 6.2 ± 1.2$^{cd}$ | 4.4 ± 0.8$^{de}$ | 2.8 ± 0.4$^{cd}$ | 29.3 ± 4.2$^{e}$ | 2.8 ± 0.5$^{cd}$ | 1.4 ± 0.3$^{cd}$ |
| 3600 | 4.4 ± 0.6$^{d}$ | 3.3 ± 0.4$^{e}$ | 1.9 ± 0.3$^{def}$ | 37.0 ± 5.6$^{e}$ | 2.1 ± 0.2$^{cde}$ | 0.8 ± 0.1$^{e}$ |
| 4800 | 5.4 ± 0.5$^{cd}$ | 4.2 ± 0.4$^{de}$ | 1.6 ± 0.2$^{ef}$ | 57.3 ± 5.4$^{d}$ | 1.7 ± 0.1$^{def}$ | 0.8 ± 0.1$^{e}$ |
| 6000 | 8.1 ± 1.8$^{c}$ | 6.3 ± 1.3$^{cd}$ | 2.2 ± 0.5$^{de}$ | 97.2 ± 22.6$^{c}$ | 1.5 ± 0.5$^{ef}$ | 1.1 ± 0.3$^{de}$ |
| 7200 | 6.2 ± 0.5$^{cd}$ | 5.6 ± 0.6$^{cd}$ | 1.3 ± 0.2$^{f}$ | 91.2 ± 8.6$^{c}$ | 1.3 ± 0.1$^{f}$ | 0.9 ± 0.05$^{de}$ |
| 8400 | 6.3 ± 0.7$^{cd}$ | 6.3 ± 0.6$^{c}$ | 1.2 ± 0.2$^{f}$ | 92.8 ± 9.0$^{c}$ | 1.2 ± 0.1$^{f}$ | 1.0 ± 0.1$^{de}$ |

[a] Values are means ± SE for nine rats except control and 1200 ppm zinc supplemental groups which had six and eight rats, respectively. Control diet had 38 ppm zinc.

[bcdef] Values in the same vertical column not followed by the same letter are significantly different ($P < 0.05$) as determined by analyses of variance of data transformed to common logarithms.

increases in fecal excretion of zinc with each increased in dietary zinc contributed substantially to homeostatic control. The decreased retention of stable zinc and the contrasting plateau of fecal $^{65}$Zn retention indicates a more rapid zinc turnover rate and suggests higher endogenous losses when added dietary zinc was elevated above 1200 ppm (Fig. 1).

The sharp increases of stable zinc in the liver, kidney, and tibia with the highest zinc intake suggest some breakdown in zinc homeostatic control. The relatively constant heart and muscle zinc, regardless of dietary level, is consistent with previous rat (6) and calf (2–4) studies. Similarly, the plateau in zinc concentrations of liver, kidney, and tibia indicates good homeostatic control occurred in these tissues when supplemental dietary zinc was increased from 2400 to 7200 ppm. Failure of $^{65}$Zn retention to decline in liver, kidney, and tibia when added zinc was elevated above 2400 ppm suggests that an increasing zinc turnover rate in these tissues is a factor in the homeostatic control. The increase in zinc turnover rates was less evident in muscle and heart $^{65}$Zn data. Apparently, $^{65}$Zn metabolism in bone is quite different from that of more biologically active tissues (10, 11).

In contrast to the 8400 ppm zinc required for major elevations of zinc in liver and kidney of rats, only 600 ppm added dietary zinc in calf diets causes much larger elevations in tissue zinc (2, 3). Thus, when high dietary zinc is fed, rats have much more effective homeostatic control of zinc levels in body tissues than calves.

*Summary.* Zinc metabolism and homeostasis were studied in young growing rats fed a 38 ppm zinc diet with added zinc levels ranging from 0 to 8400 ppm for 21 days. High dietary zinc did not cause toxicity symptoms. Stable zinc in feces increased linearly with dietary zinc intake but fecal $^{65}$Zn, from a single oral dose, did not increase above the 1200 ppm dietary level. Stable zinc in liver, kidney, and tibia increased two to three times with 2400 ppm added zinc, but was not further elevated until 8400 ppm was fed. Stable zinc in muscle and heart was not affected appreciably by dietary zinc level. In all tissues, $^{65}$Zn retention was drastically reduced with 1200 ppm added dietary zinc. Additional dietary zinc reduced $^{65}$Zn in muscle and heart but had little effect on liver and kidney $^{65}$Zn. The data indicate that rats have fairly effective homeostatic control mechanisms for tissue zinc below about 7200 ppm dietary zinc. Whereas, with dietary zinc up to about 1200 ppm, decreasing absorption is the main route of homeostatic control, above this level, more rapid zinc turnover rates and increasing endogenous zinc excretion appear to have major importance.

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