The importance of the body's need for zinc in determining Zn availability in food: a principle demonstrated in the rat

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1. The hypothesis that the availability of zinc in a food is limited by factors in the food was tested against the hypothesis that Zn absorption is homeostatically regulated by the body according to its need for Zn.

2. The experimental model used was the short-term administration to rats of a parenteral nutrition solution with no added Zn in an attempt to increase their need for Zn in an anabolic phase.

3. The absorption and retention of ^{65}Zn from a piece of endosperm-wheat crisp-bread in rats injected intraperitoneally with the parenteral nutrition solution was more than 40% higher than that in a control group injected with physiological saline (9 g sodium chloride/l).

4. The results indicate that the availability of Zn in the bread is not fixed but variable and dependent on the body's need for Zn.

Many investigations have been made on the amount of zinc absorbed by humans and animals from different foods and meals. Several of these have indicated that the presence of factors such as dietary fibre or phytic acid in a food limits the intestinal absorption of Zn from the food (Prasad, 1979; Sandström *et al.* 1980; Sandberg *et al.* 1982). However, other studies have shown no significant differences in the availability of Zn in diets high and low in fibre and phytic acid when the availability was measured as Zn balance (Sandstead *et al.* 1978) or serum Zn concentration (Sandström *et al.* 1983).

Zinc homeostasis involves both Zn absorption, which is incompletely regulated, and the excretion of excess endogenous Zn (Weigand & Kirchgessner, 1978). When the Zn in the diet or test meal exceeds the body's need, factors in the food may affect Zn absorption to some extent but Zn homeostasis will be maintained and Zn balance and Zn concentrations in tissues and fluids will vary very little. This may explain the differences in the results of studies in which different measures of Zn availability have been used. It also indicates that differences in Zn absorption from diets with more than adequate Zn contents may be of little nutritional consequence as they only result in differences in Zn excretion. Moreover, it is possible that the body could increase its absorption of Zn should its need increase.

Thus, there appear to be two opposing hypotheses which seek to explain the limitation or the regulation of the absorption of Zn. According to the first of these, factors in the foods limit the availability of Zn for absorption and the Zn in each food has a characteristic availability. According to the second hypothesis, the body regulates its absorption and retention of Zn from foods according to its need. One way of testing these two hypotheses would be to increase the body's need for Zn and then measure the absorption of Zn from a test meal. If the absorption of Zn increases when the body's need for Zn is increased, the absorption measured under normal conditions could hardly be used to represent available Zn as more Zn would be available to an animal in need of Zn.

Kay *et al.* (1976) observed symptoms of Zn deficiency in humans who were given total parenteral nutrition for long periods of time. The solutions used in parenteral nutrition at that time were generally rich in energy and amino acids but low in Zn. Their study indicates that these solutions stimulate anabolism but do not satisfy the body's need for Zn. Latimer

et al. (1980) made similar observations in infants with short-bowel syndrome and suggested that the development of clinical Zn deficiency might have been accelerated by an increased need for Zn in an anabolic phase. The same suggestion is made in a clinical report by Gordon & White (1978).

Thus it seemed logical to attempt to test the hypotheses of Zn availability and regulation against each other using parenteral nutrition in rats to increase their need for Zn. The absorption of Zn (^{65}Zn) from endosperm-wheat crisp-bread was measured and compared in rats given intraperitoneal injections of a parenteral nutrition solution with no added Zn, and in control rats given physiological saline (9 g sodium chloride/l).

MATERIALS AND METHODS

The thirty-two male albino rats of the Sprague-Dawley strain (Anticimex, Stockholm, Sweden) used in the study were 8 weeks old (300 g) at the start of the experiment. They were divided into four groups of eight by formal randomization and housed in groups of four in plastic cages. The rats were fed on a diet of endosperm-wheat crisp-bread (Veteknäcke; AB Wasabröd, Filistad) and deionized water *ad lib.* The crisp-bread contained (/kg) 11 mg Zn, 2 mmol phytic acid, 30 g fibre, 110 g protein and 90 g fat. After 4 d on the bread and water diet, the rats were deprived of food but not water for 10 h during the night before being given ^{65}Zn on a piece of the crisp-bread or by intraperitoneal injection.

Two groups (bread groups) were given 25 μCi carrier-free ^{65}ZnCl$_2$ (142 Ci/g Zn) in 30 μl 0·1 M-hydrochloric acid (Amersham International plc, Amersham, Bucks) on a 2 g piece of the same endosperm-wheat crisp-bread which had been given to all the rats earlier. Each rat was observed until it had eaten the entire piece of bread, which they all did within 0·5 h. One of these groups was then injected intraperitoneally with 5 ml glucose–amino acid solution (Vamin-Glukos®, 9 g N/l, Vitrum AB, Stockholm) while the other, a control group, was injected with an amount of physiological saline (9 g sodium chloride/1) containing an equal amount of Zn. Injections were given three times at 2·5 h intervals. The glucose–amino acid solution contained (g/l): 4·1 L-aspartic acid, 9·0 L-glutamic acid, 3·0 L-alanine, 3·3 L-arginine, 1·2 L-cysteine/cystine, 2·1 glycine, 2·4 L-histidine, 3·9 L-isoleucine, 5·3 L-leucine, 2·6 L-lysine, 1·9 L-methionine, 5·5 L-phenylalanine, 8·1 L-proline, 7·5 L-serine, 3·0 L-threonine, 1·0 L-tryptophan, 0·5 L-tyrosine, 4·3 L-valine, 100 glucose, 0·6 KCl, 0·55 CaCl$_2$O . 2H$_2$O and 0·37 MgSO$_4$O . 7H$_2$O, and was buffered to pH 5·2 with sodium hydroxide and potassium hydroxide. The Zn concentration was 0·25 mg Zn/l.

Two groups (injection groups) received no ^{65}Zn on the bread but were instead injected intraperitoneally with the same dose of ^{65}Zn in 0·5 ml physiological saline solution 0·55 h after the first of three 5 ml injections of the glucose–amino acid solution or physiological saline. Immediately after the first injection of either the glucose–amino acid solution or saline, all thirty-two animals were given free access to the original bread and water diet.

At 6, 12, 24 and 96 h after the first injection, 0·5 ml blood from the tail of each rat was collected in acid-washed, borosilicate glass tubes, allowed to clot and centrifuged. Samples of serum were taken and diluted tenfold with 0·1 M-HCl. The Zn concentrations in the serum samples were determined using a Varian Techtron Model AA-6DB atomic absorption spectrophotometer operating at 213·9 nm (Hallmans,1978). Samples of Zn in 0·1 M-HCl were used as references.

The whole-body retention of ^{65}Zn was measured within 0·5–1 h after the rats had consumed the bread with ^{65}Zn or after they were given the ^{65}Zn injection and thereafter daily, except during the weekend, for 9 d. During these measurements the rats were immobilized in a plastic tube and placed in a whole-body scintillation counter with 76 × 76 mm sodium iodide (T1) scintillation detector and an Atomenergi Model 3207 single

channel analyser calibrated to count at the 1·116 MeV gamma peak of ^{65}Zn. A 25 μCi ^{65}Zn injection solution was diluted to 100 ml with 0·1 M-HCl and measured as a reference. The background counting rate was measured daily and subtracted from all activity measurements.

The ^{65}Zn retained and the ^{65}Zn absorbed were defined as follows (Heth & Hoekstra, 1965):

$$^{65}\text{Zn retained at time } T = \frac{\text{whole-body }^{65}\text{Zn activity at time } T}{\text{whole-body }^{65}\text{Zn at time } 0},$$

$$^{65}\text{Zn absorbed} = \frac{^{65}\text{Zn retained in a rat given }^{65}\text{Zn on bread}}{\text{mean }^{65}\text{Zn retained in group injected with}^{65}\text{Zn}}.$$

Thus the ^{65}Zn activity retained in the group injected with ^{65}Zn was used to represent complete absorption and to correct for excretion of absorbed ^{65}Zn. During the period following ^{65}Zn administration, as the unabsorbed ^{65}Zn was excreted from the rats given the activity on a piece of bread, the ratio of ingested:injected ^{65}Zn decreased asymptotically to a level which was used to represent the gross absorption of Zn from the bread at the time of ^{65}Zn administration.

The differences between group means for different variables were tested using Student's t test for unpaired observations. We chose to reject the null hypotheses at $P < 0.01$. The t test was modified if the variances were significantly different ($P < 0.01$; F test) (Snedecor & Cochran, 1967).

RESULTS

The rats did not gain weight on the bread and water diet during the experiment. No differences were seen among the groups.

At 6 h after the first intraperitoneal injections there was no significant difference in the serum Zn concentration between the groups given glucose–amino acid solution and the groups given saline (Fig. 1). At 12 and 24 h after the first injections, the mean serum Zn concentration in the two groups given glucose–amino acid solution was significantly lower than that in the groups given saline. By 96 h after the first administration of the respective solutions there was again no significant difference in the serum Zn concentration between the groups.

The whole body retention of ^{65}Zn did not differ between the two groups given ^{65}Zn intraperitoneally (injection groups). The ^{65}Zn retention in the bread group given glucose–amino acid solution was higher than that in the group given saline (Fig. 2). The mean gross absorption of ^{65}Zn was calculated to be 0·53 in the bread group given glucose–amino acid solution and 0·37 in the bread group given saline (Fig. 3).

DISCUSSION

It has been demonstrated in humans that chronic parenteral nutrition with glucose–amino acid solutions of the type used in the present study causes symptoms of Zn deficiency which have been alleviated by the administration of Zn (Kay *et al.* 1976). In the present study, rats were fasted overnight, given the test meal, given the parenteral nutrition solution intraperitoneally in three 5 ml doses over a period of 5 h, and thereafter allowed to eat at will. While this experimental situation is hardly equivalent to parenteral nutrition in humans, it is assumed that the administration of glucose and amino acids stimulates anabolism and that, as the body has no appreciable, readily available Zn reserves, this should in turn create a need for Zn. The three 5 ml injections of the glucose–amino acid solution used in the
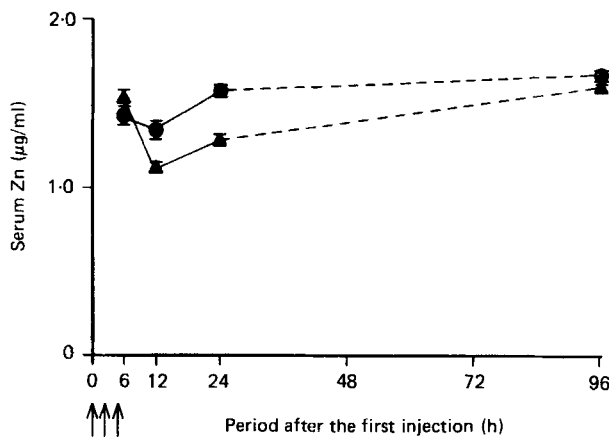
Fig. 1. Serum zinc concentrations at various time intervals after the first intraperitoneal injections of glucose–amino acid (▲) and physiological saline (9 g sodium chloride/l) (●). The results are means with their standard errors represented by vertical bars. ↑↑↑, Intraperitoneal injections. For details of procedures, see p. 60.
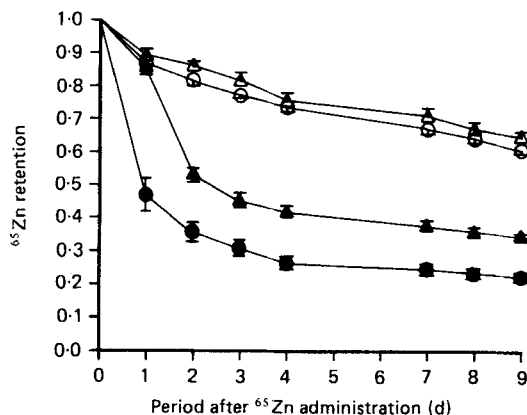

Fig. 2. The retention of ^{65}Zn activity at various times after the administration of ^{65}Zn on bread (▲, ●) or in an intraperitoneal injection (△, ○). The results are means with their standard errors represented by vertical bars. Glucose–amino acid injections (▲, △); physiological saline injections (●, ○).

present study provided 135 mg N and 42 kJ, or approximately 1 day's N intake and one-third of 1 day's energy intake in a 300 g male rat, and at the same time provided only 4 μg Zn, which is approximately 3% of its net requirement for normal daily growth (Rogers, 1979). The 2 g piece of bread given to the rats immediately before the first injection contained 22 μg Zn.

The effects of the intraperitoneal injections of glucose–amino acid solution were dramatic. They resulted in a more than 40% increase in the absorption of Zn from the ^{65}Zn-labelled crisp-bread (Fig. 3). Most of the unabsorbed ^{65}Zn was excreted during the 2nd day after the injections of glucose–amino acid solution rather than during the 1st day as in the group injected with saline (Fig. 2). This may have been due to a loss of appetite in the parenteral nutrition group prolonging the intestinal transit time. Finally, the serum Zn concentration was significantly reduced both at 12 and 24 h after the first injection (Fig. 1) which may indicate that the Zn was removed from serum to take part in protein synthesis.
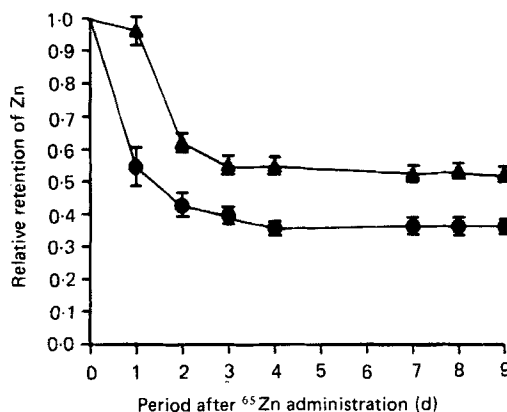
Fig. 3. The relative retention of zinc calculated as the ratio, retention of ^{65}Zn activity administered on bread: mean retention of injected ^{65}Zn activity at various times after administration of glucose–amino acid solution (▲) or physiological saline (●). The results are means with their standard errors represented by vertical bars. The average of the values for days 7–9 has been taken to represent the gross absorption.

The absorption of ^{65}Zn from the bread in the control group (0·37) can hardly represent the availability of Zn in the bread in the sense that it is an upper limit for absorption. On the contrary, it would appear that the average absorption of Zn from the bread varies considerably depending on the experimental conditions and that its availability is probably at least 40 % more than the measure obtained under ordinary circumstances. It is quite evident that values for Zn absorption from food obtained under conditions in which the body's need for Zn is not increased can hardly be used as measures of the availability of Zn in these foods because much more Zn is probably available to the body should it need it. It is unlikely that an absolute upper limit on Zn availability exists and more likely that the Zn remaining in the unabsorbed intestinal contents is less available the more Zn has already been removed.

Thus the answer to our question of what determines Zn absorption, the availability of Zn in the food or the body's homeostatic regulation according to its need for Zn, is most likely that both play important roles. However, more experiments must be made in which these two hypotheses are critically tested against one another in order to explain the differences in the results of earlier studies.

REFERENCES

Gordon, W. & White, P. J. (1978). *South African Medical Journal* **54**, 823–824.
Hallmans, G. (1978). *Acta Dermato-Venereological* **58**, Suppl., 80.
Heth, D. A. & Hoekstra, W. G. (1965). *Journal of Nutrition* **85**, 367–374.
Kay, R. G., Tasman-Jones, C., Pybus, J., Whiting, R. & Black, H. (1976). *Annals of Surgery* **183**, 331–340.
Latimer, J. S., McClain, C. J. & Sharp, H. L. (1980). *Journal of Pediatrics* **97**, 434–437.
Prasad, A. S. (1979). *Zinc in Human Nutrition*. Boca Raton, Florida: CRC Press.
Rogers, A. E. (1979). In *The Laboratory Rat*, vol. 1, *Biology and Diseases*, pp. 123–152 [H. J. Baker, J. R. Lindsey and S. H. Weisbroth, editors]. New York: Academic Press.
Sandberg, A.-S., Hasselblad, C. & Hasselblad, K. (1982). *British Journal of Nutrition* **48**, 185–191.

Sandstead, H. H., Munoz, J. M., Jacob, R. A., Klevay, L. M., Reck, S. J., Logan, G. M., Dintzes, F. R., Inglett, G. E. & Shney, W. C. (1978). *American Journal of Clinical Nutrition* **31**, 180–184.

Sandström, B., Andersson, H., Bosaeus, J., Flakheden, T., Göransson, H. & Melkersson, M. (1983). *Human Nutrition: Clinical Nutrition* **37**C, 295–300.

Sandström, B., Arvidsson, B., Cederblad, A. & Björn-Rasmussen, E. (1980). *American Journal of Clinical Nutrition* **33**, 739–745.

Snedecor, G. & Cochran, W. (1967). *Statistical Methods*, 6th ed. Ames, Iowa: Iowa State University Press.

Weigand, E. & Kirchgessner, M. (1978). *Nutrition and Metabolism* **22**, 101–112.