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ZINC BIOAVAILABILITY

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Zinc was not recognized as an essential nutrient in mammalian nutrition until 1934 (1). In 1956, the existence of conditioned zinc deficiencies in certain human diseases was suggested (2), and the occurrence of zinc deficiency on a dietary basis was documented in 1963 (3). In 1973, Sandstead (4) warned that zinc deficiency might be widespread even within the U.S. population. Three basic mechanisms could contribute to endemic zinc deficiency: 1) the dietary intake could be insufficient; 2) the absorption of zinc could be reduced; or 3) zinc could be lost in excessive amounts from the body's reserves.

The RDA for zinc for various age-groups is given in Table 1 (5). In order for adult females to consume their requisite zinc intake, the average mixed diet would have to contain 7.5 mg of zinc per 1000 kcal. Recent surveys (6) have shown that most groups spontaneously consume between 50-67 percent of the recommended levels. However, the amount of zinc that must be absorbed daily by an adult has been estimated to be from 2.2 (7) to 4.5 mg (8). This absorption requirement increases with excessive endogenous losses of zinc (8). However, sweat loss -- which can amount to from 1 to 4 mg per liter -- would only affect North Americans under extraordinary conditions of temperature (e.g. steelworkers) or exertion (i.e. athletes). Thus, as suggested by the Expert Committee on Trace Elements in Human Nutrition in 1973 (7), the biological availability of zinc from different diets would be a significant determinant of human nutrition with respect to zinc. If the intake of zinc is deficient or losses excessive, a sufficiently high efficiency of absorption of dietary zinc could still provide enough mineral to maintain balance; on the other hand, even if the dietary intake is equal to or greater than the RDA, if the fractional absorption is sufficiently poor, the zinc demands of the organism will not be met. In recent years, studies in our laboratories, and in other centers around the world, have scrutinized the factors affecting zinc bioavailability.

METHODS FOR STUDYING ZINC ABSORPTION IN HUMANS

One of the barriers that has limited our understanding of this subject has been the lack of a perfect methodological approach to the quantification of zinc absorption in human subjects. The various methods in use are outlined in Table 2. The various advantages and disadvantages are detailed. We have used the change-in-plasma-zinc technique most extensively as the index of zinc absorption.

ZINC ABSORPTION FROM VARIOUS FOODSTUFFS

Studies in laboratory animals showed that zinc in foods of animal origin appeared to be more bioavailable than zinc from vegetal sources (9,10). It was to the diet of high-extraction wheat flour, baked into an unleavened bread, that Reinhold (1) ascribed the genesis of the human zinc-deficiency syndrome in the Middle East. At first, phytic acid was thought to be the inhibitory constituent. Indeed, Pecoud et al (12) showed that a 2:1 phytate: zinc molar ratio reduced the uptake of zinc into serum after a 50 mg dose of stable zinc was ingested by healthy volunteers. More recently, a dominant role for dietary fiber has been suggested. Metabolic balance studies in human volunteers suggest that dietary fiber inhibits zinc absorption (13,14).

The traditional diet in southern Mexico and northern Central America consists of corn tortillas and whole black beans. These foods are rich in phytates. A day's ration of a typical rural Guatemalan diet contained 93 g of dietary fiber (15). At the Institute of Nutrition of Central America and Panama in

Guatemala City, we undertook to evaluate the effects of this diet on zinc absorption. When 25 mg of zinc as zinc sulfate was mixed into a meal of tortillas (120g), black beans (120g), sweet roles (40g) and coffee (250 ml), there was no net rise in plasma zinc (16) (Table 3). However, we were unable to discriminate between the effects of the various components of the meal. Coffee had a mild inhibitory effect (16). To examine and differentiate the impact of the corn and the beans, we sought a palatable source of larger quantities of zinc. We used whole, raw oysters. One-hundred twenty grams of these oysters provided 108 mg of zinc. When the 120 g portions of tortillas or black beans were consumed with an equivalent amount of oysters, we could clearly discern a more potent inhibitory effect from tortillas than from black beans (Fig. 1). The extrinsic tags -- zinc sulfate or whole oysters-- do not necessarily reflect the behavior of the intrinsic zinc in the foods, but they should define what happens to the zinc in foods consumed along with these two high-fiber, high-phytate foods.

ZINC ABSORPTION/IRON ABSORPTION

The isthmus-wide survey in Central America in 1967 revealed a substantial public health problem of iron deficiency anemia. The basis of this disorder was a combination of a low consumption of red meat and the poor bioavailability of iron in the tortilla and bean diet. During the last decade, various strategies were proposed: to improve iron nutriture of iron deficient populations one would involve supplementation of the diet with ascorbic acid to improve the absorption of the intrinsic, nonheme iron; another was the fortification of a common vehicle (salt or sugar) with the iron chelate, NaFeEDTA. We undertook to determine the effects of ascorbic acid and of NaFeEDTA on zinc absorption.

Ascorbic Acid: Using the increment in plasma zinc as the index, we determined that the absorption of zinc in the presence of 0.5, 1.0 and 2.0 g of vitamin C was equivalent to that of zinc in aqueous solution alone (Fig.2) (18). The addition of 2 g of ascorbic acid to a mixture of 108 mg of stable zinc and 120 g of black beans had no effect on the uptake of zinc.

NaFeEDTA: The effect of NaFeEDTA on zinc absorption was evaluated. Addition of the amounts of this chelate that would be in a cup of coffee (15 mg) or in a day's ration (40 mg) at the enrichment provided in a trial of sugar fortification (19), had no effect on plasma zinc increments (18). Higher doses of NaFeEDTA -- 115 and 308 mg, respectively, -- caused progressive reduction in zinc absorption (18). Studies in animals suggest that EDTA can increase zinc bioavailability (20,21). These animal experiments were conducted with solid diets, whereas our human studies involved aqueous solutions. It is not inconceivable that in the context of meals, NaFeEDTA would show an enhancing effect on zinc absorption. Preliminary studies in animals would support this possibility (Batres R, Viteri FE: unpublished observations).

Zinc: Iron Interaction: In 1970, Hill and Matrone (22) formulated a comprehensive thesis regarding the competitive interaction of chemically similar minerals of the transition series of the Periodic Table of Elements. Several animal experiments have demonstrated a competitive interaction between zinc and iron (23-27). In healthy human volunteers, we examined the increments in plasma zinc that followed oral doses of 25 mg of zinc in CocaCola, administered alone, with 25 mg (1:1), 50 mg (2:1) and 75 mg (3:1) of iron as ferrous sulfate. As shown in Figure 2, there was a progressive reduction in plasma uptake of zinc with graded increases in iron: zinc ratio. No inhibition of zinc absorption was seen when 75 mg of iron as heme chloride was ingested with the zinc solution. When the zinc was administered as raw oysters (60 g of oysters; 54 mg of zinc), a 2:1 iron: zinc ration constituted by adding 50 mg of ferrous iron to the meal failed to affect zinc uptake (28). There are practical consequences to these findings. For instance, a majority of the vitamin-mineral preparations that specified a content of iron and zinc had a ratio of iron to zinc of 3:1. It is likely that the iron in these compounds reduced the biological availability of their zinc (28). Moreover, the RDAs for infants less than 6 months of age for iron and zinc are 10 and 3 mg, respectively; this would represent a 3.3: 1 iron:zinc ratio. Care would have to be taken that a commercial infant food, formulated to meet the specifications of the RDA (5), not have a reduced zinc availability due to excessive supplementation with iron (28).

We have extended these observations on iron:zinc intestinal competition to determine the nature of the interaction (28). We have found that trivalent, oxidized ferric iron is a less potent inhibitor of zinc uptake than is ferrous iron; however, addition of ascorbic acid to a ferric iron and zinc mixture increases the inhibition of zinc uptake to that observed with an equivalent amount of ferrous iron. Moreover, the iron nutriture of an individual influences the interaction of iron and zinc in the intestine, suggesting that the competition occurs prior to or at the step by which the organism regulates the transfer of iron to the body.

ENHANCEMENT OF ZINC ABSORPTION

In addition to factors that reduce the efficiency of zinc absorption, researchers have been interested in host and dietary factors that might enhance the absorption of dietary zinc. As reported earlier, ascorbic acid failed to increase zinc bioavailability from aqueous solutions or foods administered to healthy human volunteers. In metabolic balance studies conducted at the University of California, McDonald and Margen (29) found, however, that a California red wine (Zinfandel) either with or without its natural complement of alcohol, increased the apparent absorption and retention of zinc from a formula diet. A third of a liter of wine was consumed during each meal of the balance periods. Using the change-in-plasma-zinc approach, we were able to show a small, but statistically significant, increase in the rise of plasma zinc in the third hour of an absorption test in which 25 mg of zinc mixed with cheese was fed with a 250 ml of Zinfandel wine (29). It is thought that the congeners of wines are responsible for this enhancing effect on zinc absorption.

The observation that breast-milk retards the development of the symptoms of acrodermatitis enteropathica* or induces remissions, led scientists to investigate the possibility that some quality of human milk improved the biological availability of the intrinsic zinc. Earlier, Evans et al (30) had reported that a low-molecular-weight binding-ligand of pancreatic origin was instrumental in the efficient absorption of zinc by experimental animals. It was postulated that the same or similar chemical species was responsible for the enhanced absorption of zinc from human milk. The chemical identity of this zinc-binding ligand remains in doubt. Evans and Johnson (31) have suggested that it is picolinic acid, a metabolite of tryptophan produced in the biosynthesis of niacin in mammals. Hurley et al (32) have advanced evidence that cirtic acid was the physiologically-important ligand. Recently, human experiments have addressed the question of the activity of these two

* Acrodermatitis enteropathica is an inborn error of metabolism, autosomal in nature, which is characterized by severe manifestations of zinc deficiency and in which a genetic defect in zinc absorption has been postulated as the pathogenesis of systemic zinc depletion.

chemicals on zinc absorption by the human intestine. Boosalis et al (33) have shown that the plasma zinc curve following the administration of zinc dipicolinate to patients with acquired pancreatic insufficiency was greater than that produced by zinc sulfate. Casey (34), however, found no effect of picolinic acid or citric acid, in 2:1 ligand;zinc ratios, on the absorption of 25 mg of zinc mixed into milk with a 2% protein content in patients with acrodermatitis enteropathica. We have recently observed that various picolinic acid to zinc ratios with free picolinic acid or preformed zinc dipicolinate did not enhance the uptake of zinc into the plasma of normal subjects when the doses were administered as aqueous solutions (35). Moreover, when the absorption of the zinc was partially inhibited by the presence of a standard breakfast meal, neither picolinic acid nor citric acid produced any major return of the plasma zinc curves toward the aqueous zinc situation. Curiously, however, the expected inhibition of zinc uptake in the presence of a 2:1 iron;zinc ratio was completely reversed when the ingested solution contained a 2:2;1 picolinic acid:iron:zinc composition (35).Whether some interference with the inhibition of zinc absorption by iron is a truly physiological role of picolinic acid under normal dietary circumstances awaits further investigation.

HOMEOSTATIC REGULATION OF ZINC ABSORPTION.

Just as the absorption of iron is regulated by the nutritional status of the host, so there seems to be a homeostatic control of zinc absorption as well. The principal evidence for this feed-back control of zinc absorption comes from the laboratory of Prof. Robert Cousins at Rutgers. He has presented evidence that high levels of dietary zinc or total-body zinc induce the synthesis of metallothione in which, in turn, traps newly absorbed zinc in the mucosal cells (36,37). In studies conducted in human volunteers in Texas, Freeland-Graves et al (38) showed an increase in the rise of serum zinc following an oral dose of 50 mg of zinc as zinc sulfate after three weeks on a high-fiber, high phytate diet. We have supplemented the daily rations of young institutionalized children in a public welfare shelter in Guatemala with either 22.4 or 44.8 mg of zinc daily for 28 days; no reduction in zinc absorption as measured by the uptake of zinc into the plasma after a fasting dose of 22.4 mg of zinc was observed comparing pre-supplementation responses to post-supplementation responses in the cohort receiving the lower zinc dosage, and there was no difference between the absorption curves of the low-and high-dose cohorts at 28 days (Solomons NW; unpublished observations) Thus, with the duration, dose, and diet used in these pediatric experiments, no down-regulation of zinc absorption by zinc supplementation has been demonstrated.

CONCLUSION

Zinc is an essential nutrient. Many populations throughout the world may be at risk of zinc deficiency. Although insufficient intake and excessive loss can produce zinc deficiency, it is likely that the contribution of dietary and host factors to the biological availability of the zinc in diet is a crucial determinant of zinc nutriture in a large number of situations. Despite the technical difficulties and limitations in the experimental determination of zinc absorption in man, recent years have seen an expansion of the experimental data regarding the factors influencing zinc bioavailability in humans. Various plant constituents clearly reduce zinc absorption with dietary fiber and phytic acid being the primary offenders. A competition between iron and zinc may decrease zinc absorption under certain circumstances. Wine appears to enhance the absorption of zinc as does human breast milk. The precise mechanism by which the intrinsic zinc in maternal milk becomes better absorbed is not full delineated, but the presence of picolinic acid and citric acid have been postulated as playing important roles. Conclusive evidence for the participation of either or both of these chemicals in zinc absorption remains to be developed. Zinc absorption appears to be homeostatically regulated in animals, and mounting evidence for a feed-back control of zinc absorption in humans has been forthcoming. To insure that human diets will provide sufficient zinc for normal nutriture and to prevent and treat zinc deficiency in susceptible hosts will require an exnension of our present knowledge of the factors governing zinc bioavailability in man.

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Table 1. Recommended Dietary Allowances for Zinc

Population	Age(yr)	Allowance(mg)
infants	0.0-0.5	3
	0.5-1.0	5
children	1-10	10
males	11+	15
females	11+	15
pregnant women	---	20
lactating women	---	25

From Recommended Dietary Allowances, 9th Edition, 1980.

Table 2. Experimental Methods Used to Study Zinc Absorption in Human Subjects

I. Metabolic Balance

II. Intestinal Perfusion

III. Radioisotopic Tracers (^{65}Zn, ^{69}Zn)

IV. Stable Isotope Tracers (^{67}Zn, ^{68}Zn, ^{70}Zn)

V. Change in Circulating Zinc Concentration

Table 3. The Change in Plasma Zinc Concentration Following Aqueous Zinc, Zinc Mixed into a Guatemalan Meal, and the Same Meal Without Added Zinc

	(intervals post-ingestion)					
	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
110 mg $ZnSO_4 \cdot 6H_2O$ in water (N = 5)	61±12[a,b]	68±10	52±6	38±9	18±8	2±7
110 mg $ZnSO_4 \cdot 6H_2O$ plus a Guatemalan meal[c] (N = 4)	- 2±2	- 6±2	- 4±5	-11 ±2	- 7±4	- 4±6
Guatemalan meal[c] alone (N = 5)	- 5±5	- 10±5	- 12±5	- 18±4	- 8±7	- 16±6

a) change in plasma zinc concentration, ug/dl
b) mean ± SEM
c) 250 ml coffee, 120 g tortillas, 120 g beans, 40 g sweet rolls

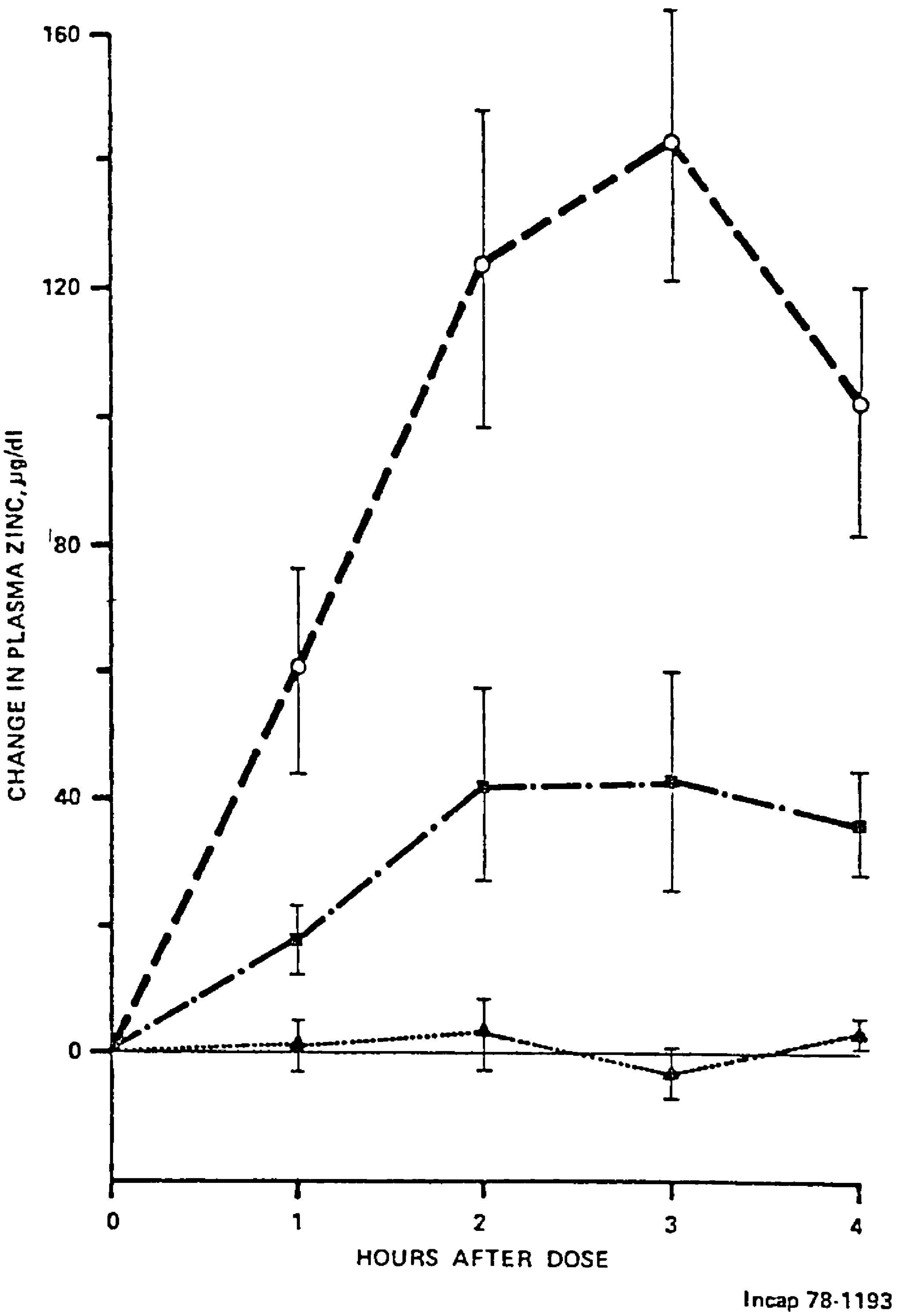

Figure 1. The change in plasma zinc concentration after ingestion of 120 g of raw Atlantic oysters containing 108 mg of zinc when consumed alone (dashed line, N = 6), or with 120 g of black bean gruel (dashed-dotted line, N = 5), or with 120 g of corn tortillas (dotted line, N = 4).