EFFECT OF EXOGENOUS IRON AND CALCIUM ON THE BIOACCESSIBILITY OF ZINC FROM FOOD GRAINS

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INTRODUCTION

Marginal zinc deficiency and suboptimal zinc status have been recognized in many population groups in both less developed and industrialized countries. Although the cause of suboptimal zinc status in some cases may be inadequate dietary intake of zinc, inhibitors of zinc absorption are likely the most common causative factors (Prasad, 2003). We have recently reported the zinc bioaccessibility values in cereals and pulses consumed in India, and observed differences in the extent of bioaccessibility of zinc and iron from these grains (Hemalatha et al. 2007a). We have also reported that germination and fermentation of food grains generally improves bioaccessibility of zinc and iron (Hemalatha et al. 2007b). Apart from inherent inhibitors of mineral absorption such as phytate, tannin and insoluble dietary fibre, minerals that are similar in chemical configuration are likely to compete with each other at the site of absorption, thus coming in the way of their bioavailability (Gibson, 1994).

Although it is inferred that iron probably does not affect zinc absorption at the molar ratios inherent in the food grains, pharmacological supplements of the same may have a bearing on the bioavailability of zinc. Supplements containing iron and multiple trace elements and minerals are used by millions of people world-wide. It is also a common practice to take iron supplements during pregnancy in the developing countries. Iron was shown to decrease zinc absorption in human in a dose dependent manner when given in a water solution but not when given with the meal (Valberg et al. 1985; Sandstrom et al. 1985). Iron supplements have been reported to decrease zinc absorption in pregnant women (Simmer et al. 1987), and lower serum zinc concentrations were observed in teenage pregnant women taking daily multi-vitamin supplements containing 18 mg iron than in those taking multivitamin supplements without any iron (Dawson et al. 1989). Calcium inherent in the grain had a negative influence on zinc bioaccessibility in cereals (Hemalatha et al. 2007a). While calcium has made a difference in zinc bioaccessibility at the levels normally inherent in the grain especially in cereals, higher doses of the same as encountered in therapeutic supplementation of calcium, is expected to further modulate zinc bioaccessibility from food grains. The present investigation was made with the following objectives: a) To examine the influence of exogenously added iron and calcium at therapeutic levels on the bioaccessibility of zinc from selected food grains.

MATERIALS AND METHODS

Materials

Cereals - rice (*Oryza sativa*), wheat (*Triticum aestivum*) and sorghum (*Sorghum vulgare)*, and legumes - chickpea (*Cicer arietinum*), decorticated green gram (*Phaeolus aureus*) and decorticated red gram (*Cajanus cajan*) were procured locally, cleaned and used for the studies. Pepsin, pancreatin (porcine) and bile extract (porcine) were obtained from Sigma Chemical Co., St. Louis, USA. All chemicals used here were of analytical grade. Triple distilled water was employed during the entire study. Acid-washed (2% nitric acid in water) glassware was used throughout the study.

ZINC, IRON AND CALCIUM

Grain and legume samples were ground finely and ashed in a muffle furnace at 550°C for 5 h, the ash was dissolved in conc. HCl and made up to a known volume with triple distilled water. Zinc, iron and calcium content were determined by atomic absorption spectrometry (Shimadzu AAF-6701). Calibration of measurements was performed using commercial standards. All measurements were carried out with standard flame operating conditions as recommended by the manufacturer. The reproducibility values were within 2.0% for both zinc and iron. In the case of calcium, lanthanum chloride was added to the mineral solution at a final concentration of 0.2%, to avoid interference from phosphate.

BIOACCESSIBILITY OF ZINC AND IRON

Bioaccessibility of zinc and iron in the samples was determined by the *in vitro* method of Luten et al. (1996) involving simulated gastrointestinal digestion with suitable modifications, as described by us earlier (Hemalatha et al. 2005). Briefly, the food grain samples were subjected to simulated gastric digestion by incubating with pepsin at pH 2.0 for 2 h. Segments of dialysis tubing containing a solution of sodium bicarbonate in concentrations equimolar to sodium hydroxide as determined by titratable acidity, were inserted into the gastric digesta and incubation was continued further in the presence of pancreatin, to simulate intestinal digestion. The dialysable fraction of the mineral which represents the bioaccessible portion was quantitated by Atomic Absorption Spectrophotometry.

HEAT PROCESSING OF FOOD GRAINS

Ten grams of the food grains were pressure-cooked in 30 ml of triple distilled water for 10 min (15 p.s.i.). The cooked samples were homogenized and used for the determination of mineral bioaccessibility as described above.

STUDIES WITH EXOGENOUS IRON AND CALCIUM

Rice and decorticated green gram were independently examined for the effect of exogenous iron, while rice and wheat were employed for the study on the influence of exogenous calcium. Appropriate additions of exogenous iron (as ferrous sulphate) and calcium (as calcium carbonate) was made to these grains so as to provide 1, 2, 3 and 4 times the intrinsic concentration of these minerals. A combination of rice and decorticated red gram (5:1), which represents the major constituent of a meal as recommended by the Indian Council of Medical Research for Indians was used for the studies on the effect of therapeutic levels of iron and calcium on the bioaccessibility of zinc, both in raw and cooked forms. Exogenous iron (as ferrous sulphate) at a level that provides 5 mg of iron and calcium (as calcium carbonate) to provide 83 mg calcium, were added individually to 10 g portions of the cereal-legume combination. In the case of cooked grains, the exogenous minerals were added to the grains after cooking. Bioaccessibility of zinc and iron from these samples was determined as described above.

STATISTICAL ANALYSIS

Determination of zinc and iron bioaccessibility in these variations of food samples, as well as all other chemical analyses was carried out in five replicates. Statistical analysis of analytical data was done according to Snedecor and Cochran (1976), using Student's t-test.

RESULTS AND DISCUSSION

Influence of exogenous iron on zinc bioaccessibility

Fig.1 presents the zinc bioaccessibility from two representative grains - green gram and rice as influenced by moderate amounts of exogenously added iron. Exogenous iron had a negative influence on zinc bioaccessibility from green gram only when the ratio of extrinsic to intrinsic iron was not less than 4. The zinc: iron molar ratio varied from 0.48 to 0.10 in the green gram samples examined in this study for zinc bioaccessibility. Thus, the effect of exogenous iron on zinc bioaccessibility from green gram was apparent only when the zinc: iron molar ratio was equalized to 0.12 or lower. Effect of exogenous iron on zinc bioaccessibility was not seen in the case of rice because even at the highest concentration of exogenous iron examined (which corresponded to a ratio of extrinsic to intrinsic iron of 4), the zinc: iron molar ratio was 0.14. The zinc: iron molar ratio varied from 0.70 to 0.14 in the rice samples examined here for zinc bioaccessibility. Consequently, the critical zinc: iron molar ratio is probably around 0.12, (as observed in the case of decorticated green gram) to exert an inhibitory influence on zinc bioaccessibility. This was substantiated in a subsequent study wherein zinc bioaccessibility was examined in a grain combination of rice and decorticated red gram (5:1) and employing therapeutic level (60-times intrinsic level) of exogenous iron. Iron added to a rice - red gram mix at therapeutic levels (which corresponded to a 60-fold excess of that natively present in the grain combination) had a significant reducing effect on the bioaccessibility of zinc, both in the raw as well as cooked grain samples, the negative effect being slightly more pronounced in the case of cooked grains (Fig.2).

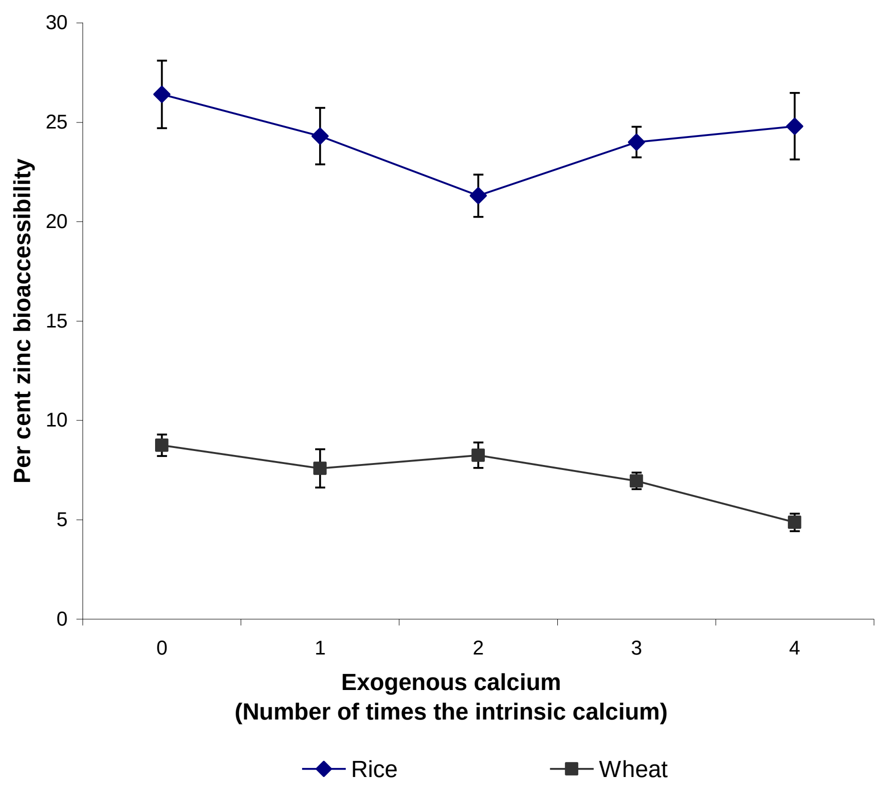

Fig.3 Zinc bioaccessibility from rice and wheat in presence of moderate amounts of exogenous calcium

Values are mean ± SEM of 5 independent determinations.

* Significant decrease (P < 0.05)

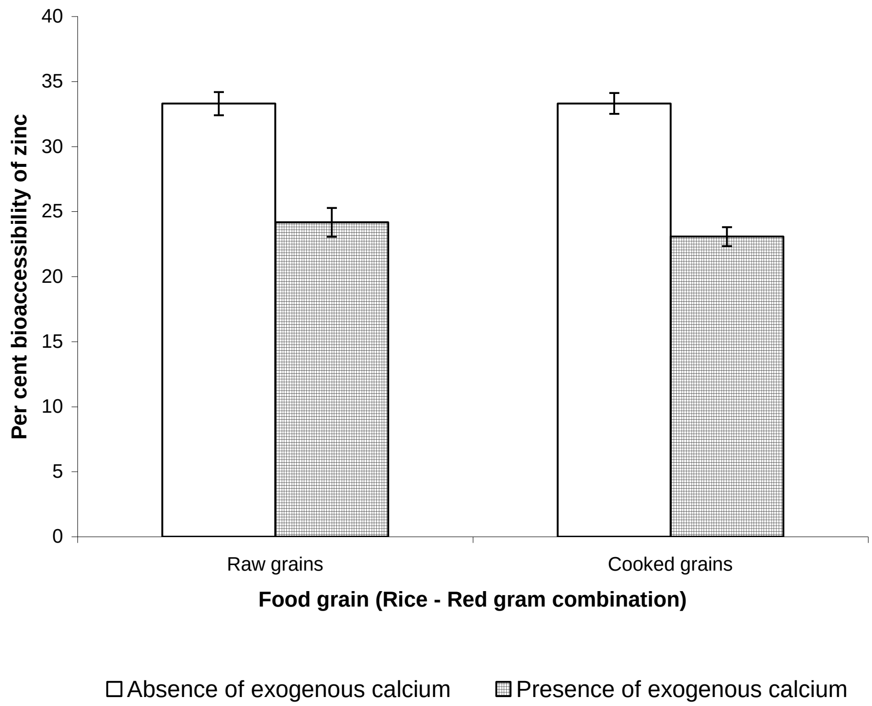

Fig.4 Zinc bioaccessibility from the combination of rice and decorticated red gram in presence of exogenous therapeutic levels of calcium

Values are mean ± SEM of 5 independent determinations.
* Significant decrease (P < 0.05)

In brief, presence of moderate amounts of exogenous iron did not severely inhibit zinc bioaccessibility from the grains. However, when the levels of exogenous iron corresponded to supplemental levels, there was a significant reduction in zinc bioaccessibility from the grains. The results suggested that the negative effect of exogenous iron is probably discernible only when zinc: to iron molar ratio exceeds 1:8, as encountered during supplemental iron regimen. Moderate amounts of exogenously added calcium negatively influenced the bioaccessibility of zinc from wheat. As in the case of iron, supplemental levels of calcium present along with the grain exerted a significant negative influence on zinc bioaccessibility from the tested food grains. The negative influence of such high exogenous levels of iron and calcium on zinc bioaccessibility was seen in both raw and cooked grains. Thus, our study suggests that in the Indian context, zinc supplementation may be necessary whenever iron and calcium supplements are recommended to compensate for this reduction in zinc bioaccessibility.

SUMMARY

In the present study, we have assessed the influence of exogenous iron and calcium equivalent to their supplemental levels on the bioaccessibility of zinc from food grains that generally are the major components of meal in India. Bioaccessibility measurement was made by a procedure involving equilibrium dialysis during simulated gastrointestinal digestion. Exogenous iron equivalent to therapeutic

levels (5 mg per 10 g of cereal-legume combination) significantly reduced the bioaccessibility of zinc from the food grains tested, the percent reduction being 32.4. Exogenous calcium equivalent to therapeutic levels (83 mg per 10 g of the cereal-legume combination) also significantly reduced (by 27.4%) the bioaccessibility of zinc from the tested food grains. The negative influence of exogenous iron and calcium was similar in both raw and cooked grains. Such negative influences on the bioaccessibility of zinc were however not seen when exogenous iron and calcium were only moderate (up to four times the intrinsic level). The observed negative influence of supplemental iron and calcium on zinc bioaccessibility suggests that zinc supplementation may be necessary in the Indian context, whenever iron and calcium supplements are taken, to compensate for the reduction in zinc bioaccessibility.

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