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Zinc Inhibits Nonheme Iron Bioavailability in Humans

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ABSTRACT

There is increasing concern about potential negative interactions in combined iron and zinc supplementation. The aim of the present study was to determine the dose-response effect of zinc, given as a solution, on iron bioavailability. Twenty-two healthy adult women were selected to participate in the study. Iron, with or without zinc was given as an aqueous solution on d 1, 2, 14, and 15 of the study. Iron bioavailability was measured on the basis of erythrocyte incorporation of ^{55}Fe or ^{59}Fe 14 d after administration. Subjects received 0.5 mg of iron together with graded zinc concentrations (0–11.71 mg). No significant effect of zinc on iron absorption was found at Zn : Fe molar ratios up to 2 : 1. At 5 : 1, 10 : 1, and 20 : 1 molar ratios, a dose-dependent inhibitory effect on iron absorption was observed (28–40% of iron absorption inhibition; one-way repeated-measures ANOVA, $F = 4.48$, $p = 0.02$). In conclusion, zinc administration combined with iron in an aqueous solution leads to the inhibition of iron bioavailability, which occurs in a dose-dependent way. This negative interaction should be considered for supplementation programs with both microminerals.

Index Entries: Zinc; iron; bioavailability; interaction; humans.

INTRODUCTION

Iron deficiency is the single most common nutritional disorder worldwide and the main cause of anemia in infancy, childhood, and pregnancy *(1)*. It is prevalent in most of the developing world, where it coexists with other conditions such as zinc, vitamin A, and folate deficiencies *(2)*.

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Combined supplementation with iron and zinc is one of the strategies that can be used to improve the iron and zinc status of a population. However, there is concern about potential negative interactions between these two micronutrients.

Most of the information on the interaction between nonheme iron and zinc has been obtained from studies that have provided these two minerals simultaneously either as a supplement or fortified food.

Studies performed in humans have shown an inhibitory effect of zinc on iron absorption; however, conflicting results have been obtained when this interaction was studied in different food matrixes *(3–7).* A decrease in iron absorption is observed when iron and zinc are given together in a solution or supplied in wheat flour *(3–5,7).* However, this negative effect was not observed when zinc and iron were provided in a composite hamburger meal, premature formula, or human milk *(4,5).*

The few studies that have assessed whether simultaneous administration of iron and zinc affects iron bioavailability have not established the dose-response curve and the threshold at which zinc could impair iron absorption *(3–7).*

The aim of our study was to determine, using a double radioisotopic technique in humans, the dose-response curve of the inhibitory effect of zinc on nonheme iron bioavailability. Iron and zinc were administered in an aqueous vehicle to better understand interactions when medicinal supplements are given together.

MATERIALS AND METHODS

Iron (Fe) absorption studies were performed in 2 groups of 11 women between the ages of 30 and 46 yr (40 ± 4 yr). None were pregnant, as confirmed by a negative test for human chorionic gonadotropin in urine; all were using intrauterine devices at the time of the study and were in apparent good health. A written, informed consent was obtained from all the volunteers before the studies began. The protocol was approved by the Ethics Committee of the Institute of Nutrition and Food Technology.

Iron isotopes (^{59}Fe and ^{55}Fe) of high specific activity were used as tracers for the Fe bioavailability studies (Du Pont de Nemours, Wilmington, DE). Aqueous solutions containing either Fe alone, as ferrous sulfate, or Fe and zinc (Zn), as zinc sulfate, were mixed with isotopes immediately before administration to the subjects. The doses of radioisotopes used were approved by the Chilean Commission on Nuclear Energy.

Because it is critical for appropriate interpretation of results that each subject acts as his/her own control, constraints regarding the administration of Fe isotopes for more than four times to each subject forced us to approach this objective by carrying out two separate studies. Study I examined Fe absorption from one dose of 0.5 mg Fe given either alone or with one dose of Zn (at 0.5 : 1, 1 : 1, and 2 : 1 Zn : Fe molar ratios), whereas Study II assessed

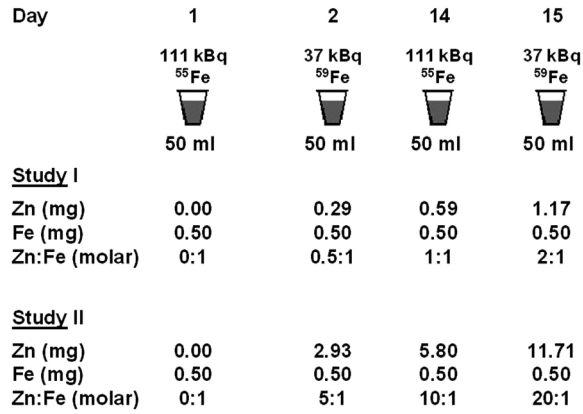

Fig. 1. Experimental design.

Fe absorption from one dose of 0.5 mg Fe given either alone or with one dose of Zn (at 5 : 1, 10 : 1, and 20 : 1 Zn : Fe molar ratios). In both studies, 50 mL of the labeled solutions was administered on d 1, 2, 14, and 15. Fe solutions were labeled with 111 kbq ^{55}Fe on d 1 and 14 and with 37 kbq ^{59}Fe on d 2 and 15 (Fig. 1). The preparations were consumed after an overnight fast, and no food or beverages other than water were allowed for the following 4 h.

Venous blood samples were obtained on study day 14 to measure the circulating radioactivity and thus calculate absorption of Fe provided on d 1 and 2. A second venous sample was obtained on d 28 to determine the increase in red blood cell radioactivity and thus calculate absorption of Fe provided on d 14 and 15.

Hemoglobin (Hb) and mean cell volume (MCV) (CELL-DYN 1700; Abbott Diagnostics, Abbott Park, IL), serum iron, total Fe-binding capacity and transferrin saturation (Sat) *(8),* Zn-protoporphyrin (ZPP) (ZP Hemato-fluorometer Model 206D; AVIV Biomedical Inc., Lakewood, NJ), and serum ferritin (SF) *(9)* were assessed to evaluate the Fe status of the subjects. All women with Hb concentrations <120 g/L were classified as anemic. Those who had normal Hb levels with two or more abnormal biochemical measurements of Fe status were classified as Fe deficient (MCV<80 fL and/or ZPP >1.24 μmol/L red blood cells (RBC) and/or Sat <15 and/or SF <12 μg/L). Fe-deficiency anemia was defined as Hb concentration <120 g/L plus two or more abnormal biochemical measurements of Fe status.

For the calculation of total radioactivity ingested, radioactivity was counted in sextuplicate from labeled solution aliquots. Measurement of blood radioactivity was performed from duplicate venous samples according to the technique of Eakins and Brown *(10).* The samples were counted, allowing sufficient time to obtain a counting error of <3% using a liquid scintillation counter (LS 5000 TD; Beckman Instruments, Fullerton, CA). Radioactivity from labeled solution aliquots and venous samples were counted simultaneously at the end of the study, to avoid an error in the calculation of Fe absorption resulting from the decay of isotopes between

Table 1
Fe Nutrition Status of the Studied Subjects

Study	Hb (g/L)	MCV (fL)	ZPP (umol/L RBC)	Sat (%)	SF* (ug/L)
I (n=11)	128 ± 22	85 ± 11	1.93 ± 1.42	18.3 ± 9.8	20 (7-54)
II (n=11)	137 ± 10	88 ± 7	1.24 ± 0.48	25.1 ± 14.5	23 (12-43)
P**	NS	NS	NS	NS	NS

Note: Mean ± SD, except where noted.
* Geometric mean and range ±1 SD.
** Mann-Whitney *U*-test.

administration and the absorption measurement 14 d later. In addition, absorption on d 28 of labeled Fe administered on d 14 and 15 was corrected for the isotopes provided on d 1 and 2 by subtracting the radioactivity of the blood sample of d 14 from RBC radioactivity of d 28. The percentages of Fe absorption were calculated on the basis of blood volumes estimated for height and weight *(11)* and assuming an 80% incorporation of the radioisotope into the erythrocyte *(12).* This method is reproducible in our laboratory with a coefficient of variation of 5%.

Because the percentages of Fe absorption and serum ferritin have skewed distributions, these values were first converted to their logarithms before calculating means and standard deviations. The results were retransformed to antilogarithms to recover the original units and then expressed as geometric means and ± 1 SD ranges. Statistical analyses included Mann–Whitney *U*-test and one-way repeated-measures analysis of variance (ANOVA) with Zn dose as the within-subject independent variable (Statistica for Windows, release 4.5; StatSoft Inc., Tulsa, OK). Post hoc comparisons were performed using the Newman–Keuls test. All comparisons were done at the 5% level of significance.

RESULTS

The Fe nutritional status of most of the subjects who participated in these studies was normal. Only 4 out of 22 women presented Fe-deficiency anemia and 2 had Fe deficiency without anemia. No significant differences in the Fe nutrition status values were found between the subjects who participated in Study I and those who participated in Study II (Table 1).

The absorption of 0.50 mg of Fe in the presence of graded concentrations of Zn is shown in Tables 2 and 3. The fitted dose-response curve shows that Fe absorption decreases progressively as the Zn : Fe ratio increases (Fig. 2). However, a significant negative effect of Zn on Fe absorp-

Table 2
Effect of Graded Doses of Zn (0–1.17 mg) on Fe Absorption of 0.5 mg Fe (Study I; *n*=11 Subjects)

Zn dose (mg)	0	0.29	0.59	1.17
Fe dose (mg)	0.50	0.50	0.50	0.50
Zn:Fe (molar ratio)	0:1	0.5:1	1:1	2:1
Iron absorption (%) [1,2]	50.8	40.6	38.8	39.5
	(31.0-83.1)	(23.9-68.9)	(19.9-75.8)	(26.5-59.0)

[1] Geometric mean (range±1SD).
[2] One-way repeated-measures ANOVA, p = NS.

Table 3
Effect of Graded Doses of Zn (0–11.71 mg) on Fe Absorption of 0.5 mg Fe (Study II; *n*=11 Subjects)

Zn dose (mg)	0	2.93	5.80	11.71
Fe dose (mg)	0.50	0.50	0.50	0.50
Zn:Fe (molar ratio)	0:1	5:1	10:1	20:1
Iron absorption (%) [1,2]	45.5^a	32.9^b	31.7^b	27.4^b
	(28.0-74.0)	(16.7-64.8)	(14.1-71.1)	(14.8-50.9)

[1] Geometric mean (range±1SD).
[2] One-way repeated-measures ANOVA, F = 4.48, p <0.02. Neuman–Keuls post hoc test; a different superscript letter indicates a statistically significant difference (p<0.05).

tion only occurred at molar ratios Zn : Fe ≥ 5 : 1 (one-way repeated-measures ANOVA, F = 4.48, p = 0.02; Newman–Keuls post hoc test, p <0.05). A 28% and 40% Fe absorption inhibition was observed at Zn : Fe molar ratios 5 : 1 and 20:1, respectively.

DISCUSSION

The mean Fe absorption of Fe given alone in an aqueous solution observed in the present study was comparable with previously published values *(13)*. We provide new information by demonstrating that the impairment of Fe bioavailability occurs in a dose-dependent way when Fe and Zn are administrated together in a water solution and in fasting conditions. The pattern of the dose-response curve suggests that both minerals compete for a saturable mechanism. A threshold for the inhibi-

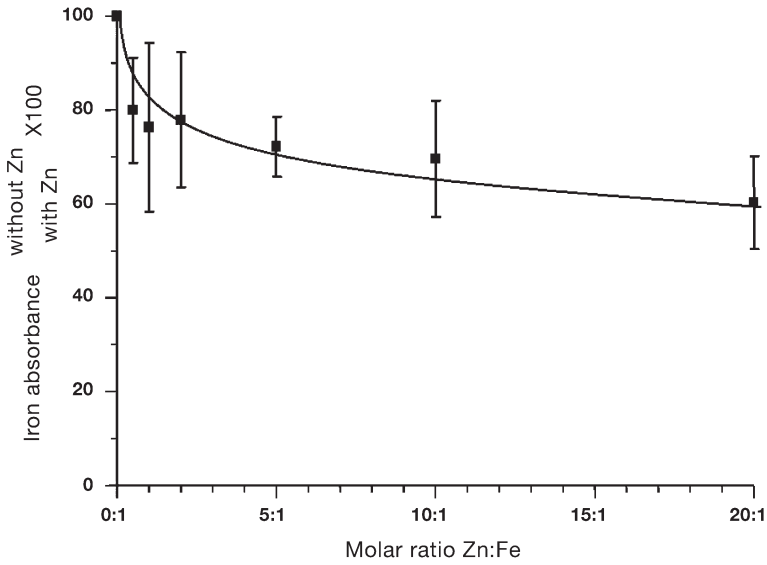

Fig. 2. Fitted dose-response curves of corrected geometric mean (±SEM) percentages of absorption of Fe in competition with Zn. Absorption of the 0.5-mg dose of Fe given without Zn (i.e., the reference dose) is defined as 100% absorption.

tion of Fe bioavailability was observed at a Zn : Fe molar ratio of 5 : 1. Although the majority of the decline in Fe absorption seen across the range 1 : 0 to 20 : 1 Zn : Fe molar ratio already occurred at a molar ratio of 1 : 1, it was not statistically significant, probably because of a lack of statistical power. To ascertain this possibility we combined data of Fe absorption, at a Zn : Fe molar ratio of 1 : 1, obtained in the current study with that from another unpublished study also performed by us. This new analysis obtained from 25 subjects discarded this possibility. In fact, geometric mean absorption of 0.5 mg Fe given alone or with Zn at a molar ratio 1 : 1 was 42.1% (range ±1 SD: 22.9–77.5%) and 34.1% (range±1 SD: 18.3–66.8%), respectively (p = NS).

Previous studies have not established the dose-response curve of the inhibitory effect of Zn on Fe absorption because they usually examined the effect of a reduced number of Zn to Fe molar ratios *(3–7).* Two radioisotopic studies have described a decrease in Fe absorption when Zn and Fe were given together at 4.2 : 1 and 0.9 : 1 Zn : Fe molar ratios in aqueous and saline solution, respectively *(4,5).* No inhibitory effect on Fe absorption was observed in women receiving a prenatal supplement containing Fe and Zn in a molar ratio of 0.2 : 1 or when a very small concentration of Fe (0.01 mg) was provided at a 255 : 1 Zn : Fe molar ratio in an aqueous solution *(4,6).* On the other hand, Crofton et al. *(3),* using the Fe postabsorptive plasma curve as a surrogate of Fe absorption, did observe a reduction of Fe absorption from a water solution at a Zn : Fe molar ratio of 1 : 1.

Studies in vitro have also confirmed the negative effect of Zn on Fe uptake. Wien et al. *(14)* demonstrated, in intestinal brush-border mem-

brane vesicles prepared from rats fed an Fe-deficient diet, that equimolar concentrations of Zn and Fe reduced Fe uptake. Recently, we described in Caco-2 cells, a human epithelial intestinal cell line, that Zn inhibits Fe uptake in a dose-related way. A 50% inhibition of Fe uptake was found at a Zn : Fe molar ratio of 1.7 : 1 *(15).*

The mechanisms involved in the interaction between Zn and Fe are not fully understood. It has been proposed that Zn and Fe compete for a shared absorptive pathway. This negative interaction could be explained by a competitive binding to the divalent metal transporter 1 (DMT1), a proton-coupled transporter of a variety of divalent metals *(16).* However, some recent studies performed in Caco-2 cells have questioned the role of DMT1 on Zn uptake *(17–20).* Recently it has been postulated that there is a common pathway of Fe and Zn uptake, different from the DMT1, located in the apical membrane of the intestinal cell *(19).* Nevertheless, the possibility that Zn can compete with Fe for transporters in plasma or in their utilization by different tissues should be considered. It has been shown that transferrin, the main Fe plasma transporter, can also bind Zn *(21).* On the other hand, Zn can block the Fe storage capacity of ferritin *(22,23).* Given that in the present study we measured Fe bioavailability by looking at Hb incorporation of Fe, we cannot establish if the negative interaction between Fe and Zn occurred at the absorptive level and/or during tissue utilization. Further research is required to fully elucidate the mechanisms of the negative interaction that occurs between Zn and Fe.

CONCLUSION

Combined Zn and Fe administration in aqueous solution leads to the inhibition of Fe bioavailability, which occurs in a dose-dependent way. This negative interaction should be considered for supplementation programs with both microminerals.

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