New insights about iron bioavailability inhibition by zinc

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Abstract

Objective: We measured the effects of lower and higher doses of zinc (Zn) given as an aqueous solution on the bioavailability of iron (Fe).

Methods: Fourteen healthy subjects received a solution with 0.5 mg of elemental Fe as ferrous sulfate given alone or with 0.59 mg of Zn as zinc sulfate (molar ratio Zn:Fe 1:1). Fourteen days after they received a second solution with 10 mg of Fe given alone or with 11.71 mg of Zn (molar ratio Zn:Fe 1:1). Iron bioavailability was assessed by erythrocyte incorporation of iron radioisotopes ^{55}Fe and ^{59}Fe.

Results: No significant effect of Zn on Fe bioavailability was observed at lower doses; however, at higher doses Fe bioavailability was inhibited by 56% ($P < 0.001$, repeated measures analysis of variance).

Conclusion: The inhibitory effect of Zn on Fe bioavailability depends on the total amount of both minerals present in the intestinal lumen. This fact should be considered when designing a supplementation program if Fe and Zn are to be provided together.

Keywords: Iron absorption; Zinc; Iron; Interaction; Human

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 micronutrient deficiencies such as zinc, vitamin A, and folate [2,3]. The exact prevalence of zinc deficiency is not known, but it is estimated that the magnitude might not be too different from that for iron [4]. This is probably because the diet of populations in the developing world is based mainly on foodstuffs that not only have low iron and zinc concentrations, but also the bioavailability of these minerals is poor.

Combined supplementation with both micronutrients is one strategy that can be used to improve the iron and zinc status of a population. However, there is concern about the negative interactions between these two minerals. Studies performed in humans have shown an inhibitory effect of zinc on iron absorption, but it is has not been well established whether this interaction depends on the absolute amount of iron and zinc in the supplement and/or on the molar ratio between these two minerals. This information could help design rational guidelines for iron and zinc supplementation programs.

Therefore, the aim of the present study was to measure the effects of lower and higher doses of zinc on iron bioavailability when both are provided as an aqueous solution in a 1:1 molar ratio.

Materials and methods

Subjects

Fourteen healthy women 35 to 44 y of age were selected to participate in the study. None of the women were pregnant before the study, as confirmed by a negative test result for human chorionic gonadotropin in urine, and all were using an intrauterine device as a method of contraception at the time of the study. A written, informed consent was

This research was supported by grant 1040874 from FONDECYT-Chile.
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obtained from all the volunteers before the isotopic studies. The protocol was approved by the ethics committee of the Institute of Nutrition and Food Technology.

Isotopic studies

Iron isotopes (^{59}Fe and ^{55}Fe) of high specific activity were used as tracers (Du Pont de Nemours, Wilmington, DE, USA). Aqueous solutions containing iron alone, as ferrous sulfate, or iron and zinc, as zinc sulfate, were mixed with the isotopes immediately before administration to the subjects. A total amount of 50 mL of the labeled solutions was administered to each subject. The radioisotope dose and protocols had been previously approved by the Chilean Commission on Nuclear Energy.

On day 1 of the study subjects received 0.5 mg of iron labeled with 111 kBq of ^{55}Fe and on day 2 they received 0.59 mg of zinc and 0.5 mg of iron labeled with 37 kBq of ^{59}Fe (molar ratio 1:1 zinc to iron). A venous blood sample was obtained 12 d later (day 14 of the study) to measure the circulating radioactivity and to determine the iron status of the subjects. These samples also provided baseline values of ^{55}Fe and ^{59}Fe radioactivity in red blood cells for the next set of absorption studies. On day 14, subjects were given 10 mg of iron labeled with 111 kBq of ^{55}Fe and on day 15 of the study they received 11.71 mg of zinc and 10 mg of iron labeled with 37 kBq of ^{59}Fe (molar ratio 1:1 zinc to iron). A final venous sample was obtained on day 31 to determine the increase in red blood cell radioactivity.

Blood analyses

Hemoglobin (CELL-DYN 1700, Abbott Diagnostics, Abbott Park, IL, USA), transferrin saturation [5], Zn-protoporphyrin (ZP Hematofluorometer Model 206D, AVIV Biomedical Inc., Lakewood, NJ, USA), and serum ferritin [6] were assessed to evaluate the iron status of the subjects. Iron deficiency was defined if a subject had a normal hemoglobin and at least two positive indicators of deficiency (transferrin saturation $<$15%, Zn-protoporphyrin $>$1.24 μmol/L red blood cells, and serum ferritin $<$12 μg/dL) and iron deficiency anemia if a subject had two positive indicators of deficiency and a hemoglobin level $<$120 g/L.

For the calculation of total radioactivity ingested, radioactivity was counted in sextuplicate from labeled solution aliquots. The measurement of blood radioactivity was performed from duplicate venous samples according to the technique of Eakins and Brown [7]. All samples were analyzed using a liquid scintillation counter (Tri-Carb 1500TR, Packard Instruments Co., Downers Grove, IL, USA) allowing sufficient time to obtain a counting error $<$3%. 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 iron absorption due to decay that had occurred between administration of the isotopes and the absorption measurement 14 d later. In addition, the absorption of iron administered on days 14 and 15 was corrected for the isotope that had been administered on days 1 and 2 by subtracting the radioactivity of the blood sample of day 14 from red blood cell radioactivity of day 28. The percentages of iron absorption were calculated on the basis of blood volumes estimated for height and weight [8] and assuming an 80% incorporation of the radioisotope into the erythrocyte [9]. This method is reproducible in our laboratory with a coefficient of variation of 5%.

Statistical methods

Because the percentages of iron bioavailability and serum ferritin have skewed distributions, these values were first converted to their logarithms before performing any statistical analyses. The results were retransformed to the antilogarithms to recover the original units and then expressed as geometric means $\pm$ 1 SD ranges. Statistical analyses performed include repeated measures analysis of variance and Scheffé's post hoc tests to establish significant differences in iron absorption (Statistica 4.5 for Windows, StatSoft Inc., Tulsa, OK, USA). All comparisons were done at the 5% level of significance.

Results

The general characteristics and iron statuses of subjects are presented in Table 1. The means $\pm$ SDs for age, height, and weight of the subjects were 39.4 $\pm$ 3.4 y, 156 $\pm$ 8 cm, and 63.3 $\pm$ 10.1 kg, respectively. The means $\pm$ SDs for hemoglobin and zinc protoporphyrin concentrations and transferrin saturation were 130 $\pm$ 11 g/L, 1.27 $\pm$ 0.44 μmol/L of red blood cells, and 22.7 $\pm$ 13.8%, respectively. Furthermore, the geometric mean (1 SD range) for serum ferritin was 22.1 (8.8–55.4). Two of the 14 subjects had iron-deficiency anemia and two had iron deficiency without anemia.

When equimolar concentrations of zinc to iron (1:1) were administered to the subjects, no significant effect of zinc on iron bioavailability was observed at lower doses (0.59 mg of zinc and 0.50 mg of iron; Table 2). However, at higher doses (11.71 mg of zinc and 10 mg of iron) zinc had a 56% inhibitory effect on iron bioavailability (repeated measures analysis of variance, $F = 23.92$, $P < 0.001$; Scheffé's post hoc test, $P < 0.002$).

Discussion

Most of the information on the interaction between nonheme iron and zinc has been obtained from studies that provided these two minerals simultaneously as a supplement. We previously demonstrated that zinc impairs iron bioavailability in a dose-dependent way when both microminerals are administered together as a water solution in

Table 1
General characteristics and iron status of subjects

Subject no.	Age (y)	Height (cm)	Weight (kg)	Hb (g/L)	Transferrin saturation (%)	ZPP (μmol/L RBC)	Serum ferritin (μg/L)
1	37	149	71.6	125	24.0	1.06	37.1
2	41	161	71.1	140	44.3	0.91	47.8
3	42	144	53.8	137	41.8	1.01	48.4
4	36	173	81.1	124	8.6	1.06	3.9
5	39	164	70.1	140	19.7	1.21	42.0
6	35	156	52.1	137	12.8	0.86	75.9
7	41	152	56.8	130	22.1	1.11	24.6
8	35	146	45.8	128	47.2	1.52	36.9
9	44	159	73.2	146	24.6	1.21	31.5
10	35	156	64.4	134	15.2	1.36	6.5
11	38	163	60.8	124	21.6	1.01	12.8
12	44	150	57.0	105	4.1	2.53	5.2
13	43	152	72.2	115	5.1	1.77	15.2
14	41	155	56.4	140	26.9	1.16	32.4
Mean	39.4	156	63.3	130	22.7	1.27	22.1*
SD	3.4	8	10.1	11	13.8	0.44	8.8–55.4

Hb, hemoglobin; RBC, red blood cells; ZPP, Zn-protoporphyrin
* Geometric mean and range of ±1 SD.

fasting conditions [10]. We found that at low doses of iron (0.5 mg) the threshold for the inhibition of iron bioavailability was at a zinc-to-iron molar ratio ≥5:1 [10], whereas in the present study we observed that zinc does inhibit iron bioavailability at a 1:1 zinc-to-iron molar ratio, when higher doses of iron (10 mg) and zinc (11.71 mg) are provided. At higher doses zinc reduced iron absorption by approximately 50%. This finding could explain some limitations in the efficacy of combined supplementation with both nutrients. Furthermore, because the solution with zinc was provided the day after the solution without zinc, iron absorption could have been reduced by the prior administration of a large dose of iron due to a mucosal blockage. This might explain why zinc appeared to reduce iron absorption from the higher doses but not the lower doses. Nevertheless, this explanation is unlikely, because we previously demonstrated that daily administration of iron doses higher than those provided in the present study does not produce a mucosal blockage [11].

Previous studies have shown an inhibition of iron bioavailability produced by combined administration of zinc with 0.3 or 3.0 mg of iron, provided as aqueous solutions, at zinc-to-iron molar ratios of 3.4:1 and 4.3:1, respectively [12,13]. In contrast, combined administration of 2.99 mg of zinc and 0.01 mg of iron (zinc-to-iron molar ratio 255:1) did not inhibit iron absorption [13]. Two other studies using higher doses of iron have analyzed the effect of zinc on iron absorption. Crofton et al. [14] using the iron postabsorptive plasma curve as a surrogate of iron absorption found a reduction of iron absorption from a water solution containing 27.5 mg of zinc and 23.5 mg of iron (zinc-to-iron molar ratio 1:1), whereas no effect on iron bioavailability was found in pregnant women receiving a prenatal supplement containing 60 mg of iron and 15 mg of zinc (zinc-to-iron molar ratio 0.2:1) [15].

The mechanisms involved in the interaction between zinc and iron are not fully understood. This negative interaction could be explained by a competitive binding to the transporter protein DMT1, which participates in divalent metal transport [16]. However, some studies performed in Caco-2 cells have questioned the role of DMT1 on zinc uptake [17–20]. Furthermore, it has recently been postulated

Table 2
Effect of 0.59 and 11.71 mg of Zn on the bioavailability of 0.50 and 10 mg of Fe, respectively (Zn:Fe molar ratio 1:1)

	Iron absorption (%)			
	A	B	C	D
Zn dose (mg)	0	0.59	0	11.71
Fe dose (mg)	0.50	0.50	10.0	10.0
Subject				
1	14.4	11.2	18.6	10.9
2	26.7	22.8	20.4	2.8
3	22.8	15.6	6.6	6.5
4	100.6	63.4	59.5	45.3
5	22.3	25.5	14.2	2.2
6	34.6	26.5	11.1	1.8
7	36.9	38.4	23.0	12.0
8	18.9	12.3	3.4	1.9
9	49.5	38.7	21.0	4.8
10	103.2	79.5	28.1	13.5
11	16.5	47.2	23.8	19.2
12	93.3	48.1	49.3	32.9
13	45.5	37.2	48.2	39.0
14	62.1	50.4	17.2	9.8
Mean*	37.4	31.8	19.4	8.6
SD	19.1–73.4	17.5–57.7	8.9–42.1	2.8–26.3

Fe, iron; Zn, zinc
* Geometric mean ± 1 SD range. Repeated measures analysis of variance, $F = 23.92$, $P < 0.001$. Scheffé's post hoc test: A versus B, NS; A versus C, $P < 0.002$; A versus D, $P < 0.001$; B versus C, NS; C versus D, $P < 0.002$.

that there is a common pathway of iron and zinc uptake, different from the DMT1, located in the apical membrane of the intestinal cell [21].

The different pattern of response of the inhibitory effect of zinc on iron absorption observed when lower or higher doses of zinc and iron are provided could be explained by the difference in the abundance of both cations as they compete for a limited number of shared transporters at the gut. At higher doses of both minerals all shared transporter molecules are occupied and the inhibitory effect of zinc on iron absorption can be observed even at a zinc-to-iron molar ratio of ~1:1. Conversely, at low doses of iron, shared transporter molecules remain available until the zinc-to-iron molar ratio reaches a value >3:1. An alternative explanation for these findings is that at low iron concentrations, iron and zinc might be competing for a transporter with a high affinity for iron, whereas at higher iron concentrations both minerals would compete for a low-affinity transporter. Further research is needed to elucidate the exact mechanism for this negative competition and the transporter or binding molecules involved in this interaction.

Conclusions

The inhibitory effect of zinc on iron bioavailability depends on the molar ratio of zinc to iron and on the total amount of both minerals presents in the intestinal lumen. These aspects should be considered for combined supplementation programs with zinc and iron.

Acknowledgments

The authors thank Dr. Daniel López de Romaña for his critical review of the manuscript, and Ms. Angélica Letelier for her technical assistance.

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