

# Acute inhibition of iron absorption by zinc

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## Abstract

Iron and zinc deficiencies are the most common nutritional deficiencies worldwide. Combined zinc and iron supplementation is one of the strategies used to prevent these deficiencies. Zinc has an inhibitory effect on iron absorption. The objective of the study was to determine the duration of the inhibitory effect of zinc on iron absorption. Fifteen healthy subjects were selected to participate in the study. Subjects received a water solution with 0.5 mg of elemental iron, as ferrous sulfate, given alone or with zinc (11.71 mg), as zinc sulfate, in a molar ratio 20:1 zinc to iron, provided simultaneously with iron or 30 and 60 minutes before iron administration. The double radioisotopic technique was used to measure iron absorption. An inhibitory effect of zinc on iron absorption was observed when both mineral were given simultaneously; however, this inhibitory effect was not observed when zinc was administered 30 or 60 minutes before (analysis of variance for repeated measures,  $F = 5.96$ ,  $P < .002$ ; Scheffé post hoc test,  $P < .006$ ). In conclusion, zinc administration with iron in aqueous solution leads to the inhibition of iron bioavailability. However, this inhibitory effect lasts less than 30 minutes. The timing of this negative interaction should be considered for supplementation programs with both minerals.

*Keywords:* Zinc; Iron; Iron bioavailability; Interaction; Human

## 1. 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 [2,3].

Combined supplementation with iron and zinc is one of the strategies, which can be used to improve the iron and zinc status of a population. However, studies performed in humans have shown an inhibitory effect of zinc on iron absorption and that combined supplementation with both minerals could be less efficacious than single supplementation with iron in reducing the prevalence of anemia and

improving iron status [4-10]. An unsolved issue is how separated should be given iron and zinc to avoid the negative effects of zinc on iron absorption.

The aim of the study was to determine, using a double radioisotopic technique in humans, the timing of the inhibitory effect of zinc on iron bioavailability.

## 2. Methods and materials

### 2.1. Subjects

Fourteen healthy women between 32 and 45 years old ( $36.6 \pm 4.4$  years old) were selected to participate in the study. None of the women were pregnant, as confirmed by a negative test for human chorionic gonadotropin in urine; all were using an intrauterine device as a method of contraception at the time of the study and were in apparent good health, and none had consumed any vitamin or mineral

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supplements in the previous 6 months. 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, Santiago, Chile.

## 2.2. Isotope studies

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

Iron and zinc were administered in 50 mL of an aqueous solution. The preparations were consumed after an overnight fast, and no food or beverages other than water were allowed for the following 4 hours. No additional dietary restrictions were provided. On day 1, the subjects received 0.5 mg of iron labeled with 111 kBq of  $^{55}\text{Fe}$ ; on day 2, they received 11.71 mg of zinc and 0.5 mg of iron labeled with 37 kBq of  $^{59}\text{Fe}$  (molar ratio, 20:1 zinc to iron). A venous blood sample was obtained 2 weeks later (day 14) to measure the circulating radioactivity and to determine the iron status of the subjects. This same sample also provided baseline values of  $^{55}\text{Fe}$  and  $^{59}\text{Fe}$  radioactivity in red blood cells for the next set of absorption studies. On day 14, subjects were given 11.71 mg of zinc, and 30 minutes later, a dose of 0.5 mg of iron labeled with 111 kBq of  $^{55}\text{Fe}$  was provided; on day 15, they received 11.71 mg of zinc, and 60 minutes later, a dose of 0.5 mg of iron labeled with 37 kBq of  $^{59}\text{Fe}$  was provided. A final venous sample was obtained on day 31 to determine the increase in red blood cell radioactivity.

## 2.3. Blood analyses

Hemoglobin and mean cell volume (CELL-DYN 1700; ABBOTT Diagnostics, Abbott Park, Ill), serum iron, total iron binding capacity and transferrin saturation [11], zinc protoporphyrin (ZP Hematofluorometer Model 206D; AVIV Biomedical Inc, Lakewood, NJ), and serum ferritin [12] were assessed to evaluate the iron status of the subjects.

For the calculation of total radioactivity ingested, radioactivity was counted in sextuplicate from labeled solution aliquots. Measurement of blood radioactivity was

performed in duplicate venous samples according to the technique of Eakins and Brown [13]. The samples were counted allowing sufficient time to obtain a counting error of less than 3% using a liquid scintillation counter (LS 5000 TD; Beckman Instruments, Fullerton, Calif). 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 the decay of isotopes between administration and the absorption measurement 14 days later. In addition, absorptions of labeled iron administered on days 14 and 15 were corrected for the isotope given 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 [14], and assuming an 80% incorporation of the radioisotope into the erythrocyte [15]. This method is reproducible in our laboratory with a coefficient of variation of 5%.

## 2.4. Statistical methods

Because the percentages of iron absorption and serum ferritin have skewed distributions, these values were first converted to their logarithm before calculating means and SDs, and performing statistical analyses. The results were retransformed to antilogarithm to recover the original units and then expressed as geometric means and  $\pm 1$  SD ranges. Statistical analyses included analysis of variance for repeated measures and Scheffé post hoc test order to establish significant differences in iron absorption and Pearson correlation (Statistica for Windows, release 4.5; StatSoft Inc, Tulsa, Okla). All comparisons were done at the 5% level of significance.

## 3. Results

Mean  $\pm$  SD of laboratory measures of iron nutrition status were the following: hemoglobin,  $137 \pm 10$  g/L; mean cell volume,  $89 \pm 8$  fL; zinc protoporphyrin,  $1.17 \pm 0.30$   $\mu\text{mol/L}$  red blood cell; transferrin saturation,  $23.2 \pm 8.4\%$ . The geometric mean and range of 1 SD of serum ferritin were  $23 \mu\text{g/L}$  ( $15\text{--}37 \mu\text{g/L}$ ).

The inhibitory effect of zinc on iron absorption was observed when both microminerals were administered simultaneously (analysis of variance for repeated measures,

Table 1

Effect of 11.71 mg of zinc given simultaneously with iron, 30 minutes and 60 minutes before the administration of iron on the absorption of 0.50-mg iron (n = 14)

Zinc administration (min)		Simultaneous	-30	-60
Zinc dose (mg)	0	11.71	11.71	11.71
Iron dose (mg)	0.50	0.50	0.50	0.50
Iron absorption (%) <sup>a</sup>	41.9 (25.6-68.7)	25.0 <sup>b</sup> (13.3-47.1)	35.0 (22.9-53.4)	40.0 (23.4-68.4)

<sup>a</sup> Geometric mean ( $\pm 1$  SD range). ANOVA for repeated measures,  $F = 5.96$ ,  $P < .002$ .

<sup>b</sup> Scheffé post hoc test,  $P < .006$  compared to control (iron absorption without zinc).

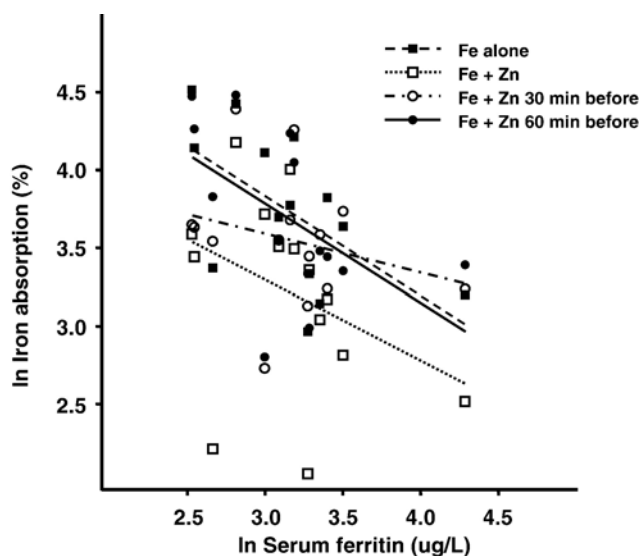


Fig. 1. Correlation between iron absorption and serum ferritin. Iron was given either alone or with zinc, which was provided either simultaneously or 30 and 60 minutes before the iron dose. Values are expressed as natural logarithm.

$P < .002$ ; Scheffé post hoc test,  $P < .006$ ). This inhibition was not observed when zinc was given 30 or 60 minutes before administering iron (Table 1).

A negative significant correlation was observed between serum ferritin and iron absorption of iron given alone ( $r = -0.59$ ,  $P < .03$ ) or when zinc was provided 60 minutes before iron ( $r = -0.54$ ,  $P < .05$ ). However, no statistically significant association was observed when zinc was provided simultaneously with iron ( $r = -0.37$ ) or 30 minutes before ( $r = -0.27$ ). Fig. 1 shows the regression lines among serum ferritin and iron absorption with and without zinc.

#### 4. Discussion

Studies performed in humans have shown an inhibitory effect of zinc on iron absorption when both minerals are administered together in fasting conditions [4-7]. We had previously found that zinc administration combined with iron in an aqueous solution leads to the inhibition of iron absorption, which occurs in a dose-dependent way [16]. The threshold for this inhibitory effect was at a Zn/Fe molar ratio of 5:1, and a 28% and 40% of iron absorption inhibition was observed at a Zn/Fe molar ratios of 5:1 and 20:1, respectively. On the other hand, there are some reports demonstrating that the combined supplementation with iron and zinc was less efficacious than single supplementation with Fe in reducing the prevalence of anemia and in improving iron status [8-10].

Because it is recognized that iron and zinc deficiencies are present concomitantly in vulnerable populations, the study of the timing of the inhibitory effect of zinc on iron absorption is key for the improvement of supplementation

strategies. Our results confirmed the inhibitory effect of zinc on iron absorption and provided new information by demonstrating that the impairment of iron bioavailability is of short duration. In fact, the inhibition of iron absorption was observed when zinc and iron were administered simultaneously, and it was not appreciated when zinc was given 30 or 60 minutes before iron administration. In the current study, we selected a zinc-to-iron molar ratio of 20:1 to assure a maximum acute iron absorption inhibition, despite that this ratio is much higher than what would be found in foods or supplements.

The mechanisms involved in the interaction between zinc and iron are not fully understood. It has been proposed that zinc and iron 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 [17]. However, some recent studies performed in Caco-2 cells have questioned the physiologic role of DMT1 on zinc uptake [18-22]. Recently, it has been postulated 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]. Nevertheless, the possibility that zinc can compete with iron for transporters in plasma or in their use by different tissues should be considered. It has been shown that transferrin, the main iron plasma transporter, can also bind zinc [23]. On the other hand, zinc can block the iron storage capacity of ferritin [24,25]. Given that in the present study we measured iron bioavailability by looking at hemoglobin incorporation of iron, we cannot establish if the negative interaction between iron and zinc occurred at the absorptive level and/or during tissue utilization.

In conclusion, zinc administration with iron in aqueous solution leads to the inhibition of iron bioavailability. However, this acute inhibitory effect lasts less than 30 minutes. The timing of this negative interaction should be considered for supplementation programs with both microminerals.

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