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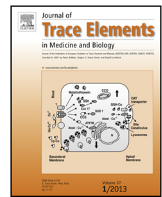

BIOAVAILABILITY

Effect of phytic acid, tannic acid and pectin on fasting iron bioavailability both in the presence and absence of calcium

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ABSTRACT

Objective: To determine the effect of phytic acid, tannic acid and pectin on fasting non-heme iron bioavailability in both the presence and absence of calcium.

Research methods: Twenty-eight apparently healthy adult females participated in two iron absorption studies using radioactive iron isotopes (^{59}Fe and ^{55}Fe). One group received 5 mg of iron (as $FeSO_4$) alone (control), together with 10 mg of phytic acid, 100 mg of tannic acid and 250 mg of pectin (study A), on different days. The second group received the same iron doses and compounds as the other group, plus 800 mg of calcium ($CaCl_2$) (study B). The compounds were administered after an overnight fast, and no food or beverages were consumed for the following 3 h. Iron status and circulating radioactivity were measured in venous blood samples.

Results: The geometric means of iron bioavailability (range ± 1SD) for iron alone, iron with phytic acid, iron with tannic acid, and iron with citrus pectin were 25.0% (11.9–52.0); 18.9% (9.9–35.8); 16.8% (8.7–32.3); and 21.1% (10.2–43.9), respectively (repeated-measures ANOVA, $p < 0.02$ (Dunnett's post hoc: control vs tannic acid $p < 0.05$). When 800 mg of calcium was added (study B), iron bioavailability was 16.7% (10.1–27.5); 13.2% (7.1–24.6); 14.8% (8.8–25.1); and 12.6% (5.5–28.8), respectively (repeated-measures ANOVA, NS).

Conclusions: Tannic acid decreases the fasting bioavailability of non-heme iron, however this effect did not exist in the presence of calcium. No effect was observed by phytic acid or citrus pectin on fasting non-heme iron bioavailability in both the presence and absence of calcium.

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Introduction

Iron deficiency anemia remains the most prevalent nutritional deficiency in developed and developing countries [1]. In addition to this, it has been estimated that a large proportion of adolescents and adults do not reach the current dietary recommendations for calcium [2]. The interaction between calcium supplementation and compounds present in foods and beverages that contain iron may be an important factor in the deficiency of both of these nutrients. Both micronutrient deficiencies may produce negative consequences on health. Adequate dietary calcium throughout the life cycle is important for ensuring bone mineralization, for rickets prevention in children, and for long-term bone loss prevention [3]. Iron deficiency adversely affects the cognitive development of children [4], increases maternal and infant mortality, and reduces physical work capacity in adulthood [5,6].

Diets characterized by low iron bioavailability [7], are one of the main causes of iron deficiency anemia. The following compounds have been recognized as inhibitors of non-heme iron absorption: phytate, some proteins (soy, milk and egg yolk), calcium [8], zinc [9], manganese [10], and tannic acid [11]. It has been postulated that pectin may also inhibit the absorption of non-heme iron [12,13]. Regarding the effect of calcium, it has been suggested that this mineral interacts with components in food matrices, inhibiting the absorption of non-heme iron. One study showed an inhibitory effect of phytic acid on iron absorption in the presence of calcium [14]. Although it has been thought that calcium may have a direct inhibitory effect on iron absorption, a recent article [15] showed that doses ≤800 mg of calcium (Ca molar ratio: Fe ≤ 225:1), do not diminish the bioavailability of 5 mg of non-heme iron. Evidence on the interaction between calcium and phytic acid and their effect

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on the absorption of non-heme iron is scarce. The effect of the interaction between calcium and tannic acid, and the interaction between calcium and pectin, on the bioavailability of non-heme iron, is unknown. Thus, the present study investigates the effect of one dose of phytic acid, tannic acid and pectin on the fasting bioavailability of 5 mg of non-heme iron, in both the presence and absence of calcium.

Subjects and methods

Subjects

Twenty-eight apparently healthy multiparous adult women voluntarily participated in two iron absorption studies. All women were using contraception methods. Exclusion criteria were pregnancy (confirmed by a negative human gonadotropin chorionic urine test), lactation and the use of micronutrient supplements within 6 months prior to the start of the study.

Ethics

A written informed consent was obtained from 6677809‴. The Ethics Committee of the Institute of Nutrition and Food Technology at the University of Chile approved the protocol, and the Chilean Commission of Nuclear Energy approved the radioactive isotope doses.

Experimental design

We conducted two experimental studies designed as block controlled studies of iron absorption. In each study, iron absorption was compared within the same subject. Study A ($n=15$) was developed to determine the effect of doses of phytic acid (10 mg), tannic acid (100 mg) and citrus pectin (250 mg) on the bioavailability of 5 mg of Fe ($FeSO_4$). Study B ($n=13$) was designed to determine the effect of doses of phytic acid (10 mg), tannic acid (100 mg) and citrus pectin (250 mg) on the bioavailability of 5 mg of Fe ($FeSO_4$) in the presence of 800 mg of $CaCl_2$. In both studies, the same subject underwent a control dose (day one) and three treatments (day two, 14 and 15). Table 1 shows which compounds were administered by day of study. For both studies A and B, iron radioactive isotopes of high specificity ^{59}Fe or ^{55}Fe (PerkinElmer, Inc., Boston, MA, USA), were given in a carrier of 5 mg of Fe as ferrous sulfate; 37 kBq of ^{59}Fe (days one and 14) and 111 kBq of ^{55}Fe (days two and 15), to provide each dose of radioactivity. Two isotopes (different emission of radioactivity) were used in order to test simultaneous treatments in the same individuals.

The delivered iron dose was mixed with distilled and deionized water to provide 50 mL of solution. The amount of solution ingested by each subject was calculated from the difference between the weight of the glass before (glass filled) and after intake (vacuum vessel). Eight hundred mg of calcium were given as $CaCl_2$ (Calcium Chloride anhydrous granular Merck- F1562091 905) packaged in gelatin capsules. Ten mg of phytic acid (phytic acid sodium salt hydrate, Sigma – P0109), 100 mg of tannic acid (Merck 100773 tannic acid) and 250 mg of citrus pectin (pectin from citrus fruit, 55–70% esterified Sigma – P9436), were also given in gelatin capsules. The subjects were required to attend the indicated days having fasted for at least 8 h. The ingestion of these compounds was supervised by the researchers in order to verify that they ingested these compounds immediately after ingesting the iron dose. They were instructed to not eat or drink in the 3 h following the ingestion of the compounds.

In each study, venous blood samples were collected 14 days after the administration of the compounds. Iron status was determined in 10 mL of blood. Twenty mL of blood drawn from each subject were processed according to the Eakins and Brown technique [16]. A 14 day follow-up was chosen since it has been demonstrated that 80% of radioactively labeled absorbed iron is incorporated into hemoglobin after this amount of time [17]. The scintillation liquid was added according to the standards and the samples were processed and brought to the liquid scintillation counter (Packard TriCarb 1600TR system Scintillation Counter, Meriden CT). The liquid scintillation counter gave the measure of radioactivity incorporated to hemoglobin (Hb) in the circulating erythrocytes as counts per minute (cpm). Samples were counted a sufficient number of times to ensure less than 3% error. With the weight and height of each subject, taken at the beginning of the studies, body blood volume was estimated [18]. For the calculations of iron bioavailability, it was assumed that 80% of the absorbed radioactivity was incorporated into Hb of circulating erythrocytes, independently of body iron status [17]. The following formula was used to calculate the bioavailability of iron:

$$\%\text{Fe Bioavaialability} = \frac{(\text{cpm/mL}) \times \text{volemia}}{(\text{cpm/mL}) \times \text{intake weight}} \times \frac{100}{0.8}$$

Biochemical and hematological determinations

Hemoglobin and mean corpuscular volume (MCV) were determined by electronic cell counter (CELL – DYN 3200, Abbott Diagnostics, Abbott Park, IL, USA). Transferrin saturation (Sat%) was calculated [19]. Zinc protoporphyrin (ZPP) was determined by hematofluorimetry (ZP – M206D, AVIV Biomedical Inc., Lakewood, NJ, USA). Serum ferritin (SF) was determined by enzyme immunoassay (ELISA) [20], as well as serum transferrin receptor (sTfR) (ELISA – Ramco Laboratories Inc., Houston, TX, USA). Body iron content was calculated by the following formula: (Body iron (mg/kg) = $-(\log (R/F_{\text{ratio}}) - 2.829)/0.1207$). Body iron values (mg/kg) were considered either positive (surplus iron stores) or negative (iron deficiency in tissues) [21]. Iron deficiency anemia was classified as Hb < 120 g/L [22,23], with two altered iron status parameters. Iron deficiency without anemia was labeled to the women with two or more altered parameters and Hb > 120 g/L. Iron depleted stores were defined as serum ferritin values <12 μg/L, VCM < 80 fL [20], Zpp > 70 g dL RBC [23], Sat < 15% [20] and SF < 12 μg/L [20] were defined as altered parameters.

Sample size calculation

A sample size of nine subjects was calculated for each study (studies A and B), using the software PRIMER, version 3.02, option "power and simple size ANOVA". The sample size was calculated with an alpha of 0.05, a power of 80%, an expected residual standard deviation of three, a number of treatment groups of four and a minimum detectable difference of 5%. For each study, 15 volunteers were considered in order to account for possible participants lost due to the rejection of intake, and/or the presence of diarrhea or vomiting, producing significant losses of the administered compounds.

Statistics

Statistical analyses were performed using the statistical software GraphPad PRISM version 6.01 (GraphPad Software, Inc, La Jolla, CA, USA). Non-normally distributed variables were converted to their natural logarithms to perform statistical tests and then reconverted to their original units to be reported as geometric means with ranges (−1SD, +1SD). We used the Student t-test for unpaired samples to identify differences between age, body mass index (BMI), parameters of iron status and control bioavailability of iron (day one) between studies A and B. For each study, repeated

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