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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.

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_{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

Table 1
Compounds used by each day of the study design.

Study	Day			
	1 (^{59}Fe)	2 (^{55}Fe)	14 (^{59}Fe)	15 (^{55}Fe)
A	5 mg Fe ($FeSO_4$)	5 mg Fe ($FeSO_4$) + 10 mg phytic acid	5 mg Fe ($FeSO_4$) + 100 mg tannic acid	5 mg Fe ($FeSO_4$) + 250 mg citrus pectin
B	5 mg Fe ($FeSO_4$) + 800 mg Ca ($CaCl_2$)	5 mg Fe ($FeSO_4$) + 800 mg Ca ($CaCl_2$) + 10 mg phytic acid	5 mg Fe ($FeSO_4$) + 800 mg Ca ($CaCl_2$) + 100 mg tannic acid	5 mg Fe ($FeSO_4$) + 800 mg Ca ($CaCl_2$) + 250 mg citrus pectin

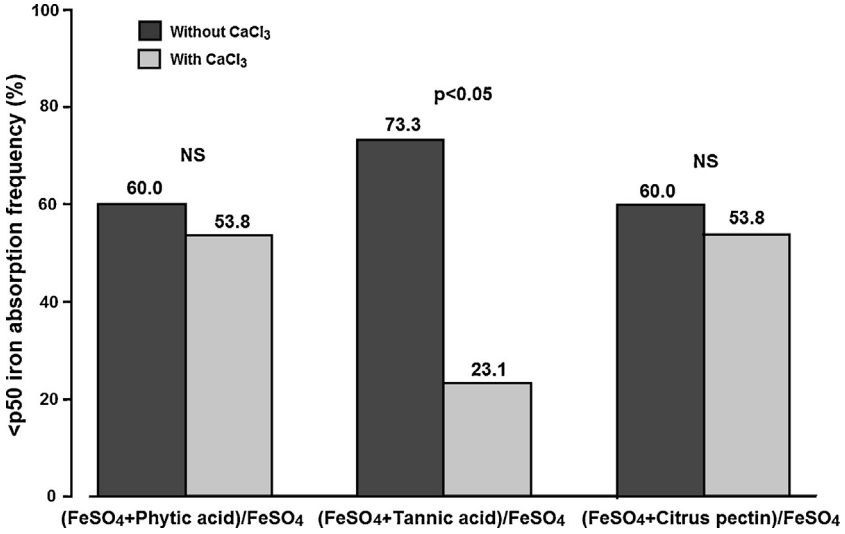

Fig. 1. Relationship between the effect of phytic acid, tannic acid, and pectin, on fasting bio-availability of non-heme iron in both the presence and absence of calcium. (Expressed as a ratio of the non-heme iron bioavailability with phytic acid (A), tannic acid (B) and citrus pectin (C), on presence and absence of calcium). The frequency of iron absorption below the 50th percentile was calculated for study A and study B. $p < 0.05$ obtained after comparing the ratios medians of bioavailability of non-heme iron by treatment in both the presence and absence of calcium, using the Fisher's exact test.

measures ANOVA with Dunnett's post hoc were applied in order to assess the effect of treatments. The ratio of bioavailability of non heme iron (bioavailability of iron by treatment/bioavailability of control iron) was used to compare the bioavailability of Fe by treatment in absence (study A) and presence (study B) of calcium. Because the ratios did not have normal distribution even after transforming the values, we applied the non-parametric Fisher's exact test to compare the medians. Fig. 1 shows this information and was plotted using the software R. A p-value <0.05 was considered as significant.

Results

Baseline characteristics

All women who voluntarily participated in these two studies were adults aged 30–47 years. None of the volunteers presented iron deficiency anemia. In study A, two women had iron deficiency without anemia and one iron depleted stores. According to the calculation of body iron, a volunteer in study A and one in study B presented iron deficiency in tissues. In study B, three women had iron deficiency without anemia (data not shown). Table 2 summarizes the general characteristics such as age, BMI and iron status biomarkers. Only Hb differed between groups, being higher ($p < 0.05$) in participants from study A (154 ± 9 g/L) vs study B (144 ± 11 g/L).

Iron bioavailability on day one (control) in study A averaged 25.0% (11.9–52.2), a figure that did not differ compared with 16.7% (10.1–27.5) in day one (control) ($t = 1.66$, N.S.) for study B.

Effect of phytic acid, tannic acid and pectin on fasting non heme iron bioavailability

In study A, there was a significant difference in the bioavailability of non-heme iron after treatment ($F = 4.29$, $p < 0.02$). The Dunnett's post hoc test showed a significant difference between control (day one) and the bioavailability of non-heme iron in the presence of 100 mg of tannic acid ($p < 0.05$). No difference was observed in iron bioavailability between the control and the presence of phytic acid or citrus pectin with 55–70% esterification (Table 3).

Table 2
Baseline characteristics of the participants by group.

	Studies of non-heme iron bioavailability	
	A ($n = 15$)	B ($n = 13$)
Age (years)[a]	39 ± 4	38 ± 5
BMI (kg/m^2)[a]	26.3 ± 2.7	26.8 ± 3.7
Hb (g/L)[a,*]	154 ± 9	144 ± 11
MCV (fL)[a]	86 ± 3	84 ± 5
Zpp (μg/dL RBC)[a]	74 ± 13	75 ± 26
Sat (%)[b]	23.1 (16.3–32.7)	23.1 (14.3–37.6)
FS (μg/L)[b]	24 (13–46)	30 (12–72)
sTfR (mg/L)[b]	4.4 (3.3–5.8)	6.4 (3.6–9.0)
Body iron (mg/kg)[a]	4.7 ± 2.7	4.5 ± 3.9

BMI, body mass index; MCV, mean corpuscular volume; Zpp, zinc protoporphyrin; Sat, transferrin saturation; FS, serum ferritin; sTfR, soluble transferrin receptor.
[a] Values are mean ± 1 standard deviation (SD).
[b] Values are geometric mean (range ± 1SD).
[*] Significant differences between groups $p < 0.05$.

Table 3
Effect of phytic acid, tannic acid and pectin, on the bio-availability of fasting non-heme iron, study A.

Subject	Non-heme iron bioavailability (%)				Absorption ratio[b]		
	Day 1 (a) $^{59}FeSO_4$	Day 2 (b) $^{55}FeSO_4$ + phytic acid	Day 14 (c) $^{59}FeSO_4$ + tannic acid	Day 15 (d) $^{55}FeSO_4$ + citrus pectin	b/a	c/a	d/a
1A	16.4	11.2	10.1	18.8	0.69	0.62	1.15
2A	26.6	12.3	14.9	14.7	0.46	0.56	0.55
3A	21.7	17.6	25.5	32.2	0.81	1.18	1.48
4A	19.9	22.7	9.4	14.5	1.14	0.47	0.73
5A	49.3	30.9	52.1	46.4	0.63	1.06	0.94
6A	50.0	36.8	35.3	38.0	0.74	0.71	0.76
7A	6.0	4.3	4.2	5.8	0.72	0.71	0.96
8A	20.2	21.3	14.3	28.0	1.06	0.71	1.38
9A	20.8	12.9	13.1	23.5	0.62	0.63	1.13
10A	5.3	16.0	7.5	6.1	2.99	1.41	1.14
11A	45.0	18.2	19.8	18.8	0.40	0.44	0.42
12A	36.2	38.6	32.3	36.4	1.07	0.89	1.01
13A	74.3	58.9	27.9	92.5	0.79	0.38	1.25
14A	39.6	10.8	18.5	14.8	0.27	0.47	0.37
15A	30.5	25.5	18.2	14.9	0.84	0.60	0.49
Mean[a]	25.0	18.9	16.8*	21.1	0.76	0.67	0.85
−1SD	11.9	9.9	8.7	10.2	0.44	0.46	0.54
+1SD	52.2	35.8	32.3	43.9	1.30	0.98	1.31

[a] Geometric mean (range of ±1SD).
[b] Absorption ratio ((bioavailability of iron by treatment/bioavailability of control iron).
* Difference compared with bioavailability of the non-heme iron dose (day 1) $p < 0.05$, repeated measures ANOVA (Dunnett's post hoc).

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

In study B, no differences were observed in the bioavailability of non-heme iron after treatment in the presence of 800 mg Ca ($CaCl_2$) ($F = 1.00$, $p = 0.37$) (Table 4).

Relationship between phytic acid, tannic acid and pectin on fasting non heme iron bioavailability in both the presence and absence of calcium

There was a significantly higher frequency of subjects below the 50th percentile of iron absorption in subjects (73.3%) who received tannic acid without calcium compared with those who received tannic acid with calcium (23.1%) (Fisher test $p = 0.0107$) (Fig. 1B). No significant differences were found between phytic acid ($X^2(1) = 0.1436$; $p = 0.7074$), and citrus pectin with 55–70% esterification, ($X^2(1) = 0.1436$; $p = 0.3524$) when fasting non-heme iron bioavailability ratios were compared in the presence and absence of calcium; after categorizing by iron absorption below the 50th percentile per each study (Fig. 1A and C).

Discussion

The present study investigates the effect of one dose of phytic acid, tannic acid and pectin on fasting bioavailability of 5 mg of non-heme iron in both the presence and absence of calcium. In the presence of 100 mg of tannic acid, we observed a significant decrease in the bioavailability of 5 mg of iron ($FeSO_4$). It has been estimated that 100 mg of tannic acid is the approximate amount found in one cup of tea (250 mL), prepared with 5 g of tea leaves/L of water [24]. In fasting conditions, without the presence of other compounds in the food matrix, Disler et al. [25] reported a significant decrease in the absorption of a dose of ferric chloride or ferrous ascorbate, when consumed with 250 mL of tea. Disler et al. [11] also postulated that tannins might form non absorbable complexes with iron, in the intestinal lumen, decreasing its absorption. The present article indicates that 100 mg of tannic acid significantly decreases the bioavailability of 5 mg of non-heme Fe. This finding is consistent with the available evidence reported by Disler et al.

In our study, there was no effect of administering 10 mg of phytic acid on fasting bioavailability of 5 mg of iron ($FeSO_4$). A study conducted with wheat rolls fortified with 10 mg of phytic acid indicates a 55.7% reduction in the bioavailability of one dose of 4.1 mg of non-heme iron [26]. It is important to mention that the effect of phytic acid on the bioavailability of non-heme iron in the presence of foods has been studied. The present article is the first study showing the effect of phytic acid on iron bioavailability when ingested alone. We speculated that under the experimental conditions of our study, the low pH reached in the adult stomach due to the absence of food, could favor the presence of larger amounts of iron in the ferrous state (Fe^{2+}) [27], making it readily available to be absorbed in the proximal intestine. It is possible that higher amounts (>10 mg) of phytic acid are required to observe significant reductions in the bioavailability of 5 mg of iron ($FeSO_4$), in the absence of other compounds in the food matrices.

Using 250 mg of citrus pectin with 55–70% esterification, we observed no effect on the fasting bioavailability of 5 mg of iron ($FeSO_4$). The available evidence shows that most of the studies focused on the effect of pectin and the way in which it influences the absorption of iron. These studies have mainly been carried out in vitro [28,29] or in vivo, using animal models [12,30–34]. There are few human studies that have studied the effect of pectin on iron bioavailability using isotope labeling methodologies. Moreover, the available studies have contradictory results [13,35]. Monnier et al. [13] in an essay focused on the kinetics of iron with radioactive isotopes in patients with idiopathic hemochromatosis, reported a 46.4% reduction in the fractional absorption of a dose of non-heme iron (when pectin was offered in quantities equivalent to 9 g/m^2 body surface). In the study published by Monnier et al., pectin was offered with cold water. This aspect by itself may affect the digestive process. The authors did not clarify the source of pectin used, the degree of methylation, nor the molecular weight. Moreover, they studied this problem in subjects under clinical conditions. All these conditions do not allow us to accurately extrapolate these results to the general population, especially to community-dwelling subjects. In another study, conducted in humans, Cook et al. [35] found no significant differences in the bioavailability of 4.4 mg of non-heme iron when provided with 5 g of citrus pectin in muffins. Similarly to the study by Monnier et al., the authors did not specify the

Table 4
Effect of phytic acid, tannic acid, and pectin, on the bio-availability of fasting non-heme iron in the presence of calcium, study B.

Subject	Non-heme iron bioavailability (%)				Absorption ratio[b]		
	Day 1(a) $^{59}FeSO_4 + CaCl_2$	Day 2 (b) $^{55}FeSO_4 + CaCl_2$ + phytic acid	Day 14 (c) $^{59}FeSO_4 + CaCl_2$ + tannic acid	Day 15 (d) $^{55}FeSO_4 + CaCl_2$ +citrus pectin	b/a	c/a	d/a
1B	21.4	15.1	13.1	13.4	0.70	0.61	0.63
2B	22.9	11.5	9.4	27.8	0.50	0.41	1.21
3B	24.8	28.2	27.3	20.0	1.14	1.10	0.81
4B	18.4	27.7	14.8	13.2	1.50	0.80	0.71
5B	13.3	8.0	23.3	19.2	0.60	1.75	1.44
6B	6.2	5.1	9.6	9.7	0.82	1.56	1.58
7B	17.0	15.4	13.7	5.9	0.91	0.80	0.35
8B	35.6	36.4	32.7	59.8	1.02	0.92	1.68
9B	21.9	16.7	21.9	11.4	0.76	1.00	0.52
10B	7.0	5.1	6.7	7.0	0.72	0.95	1.00
11B	13.3	7.6	11.7	13.2	0.57	0.88	1.00
12B	24.9	15.4	7.4	1.8	0.62	0.30	0.07
13B	13.5	11.8	27.1	15.6	0.87	2.00	1.15
Mean[a]	16.7	13.2	14.8	12.6	0.79	0.89	0.75
−1SD	10.1	7.1	8.8	5.5	0.58	0.52	0.33
+1SD	27.5	24.6	25.1	28.8	1.07	1.52	1.74

[a] Geometric mean (range of ±1SD).
[b] Absorption ratio ((bioavailability of iron by treatment/bioavailability of control iron) There were no significant differences in iron bioavailability across groups compared with the bioavailability of the non-heme iron dose (day 1), repeated measures ANOVA (Dunnett's post hoc).

molecular weight, nor the degree of methylation of the pectin used. Moreover, Cook and colleagues did not include a dose of ascorbic acid as an enhancer to the absorption of non-heme iron.

In our study, we believe that another factor involved with the absence of an effect on iron bioavailability by pectin was the high degree of digestion and metabolism of these compounds in the colon. This aspect has been reported in studies performed in humans [36,37]. In vitro, it has been also observed that the ability of pectin to bind iron dependent upon pH [29]. At lower pH there is less binding capacity [29]. It is possible that iron released by pectin in the colon could be absorbed [38]. These results do not rule out that higher amount of pectin to have an inhibitory effect on the bioavailability of non-heme iron. It should be also taken into account that this study used a citrus pectin with a high degree of esterification, so these results should not be extrapolated to pectins from other sources or low methoxyl pectins.

In this study we did not observe a relationship between the bioavailability of 5 mg of non-heme iron ($FeSO_4$) and the absence or presence of 800 mg of Ca ($CaCl_2$), when delivered in a fasting state to non-pregnant, premenopausal, adult women. In agreement with these results, a previous study performed by us, and recently published by Gaitan et al. [15] found that doses ≤800 mg of Ca ($CaCl_2$) did not decrease the bioavailability of 5 mg of Fe ($FeSO_4$) in subjects with similar characteristics. Also, a study focused on the effect of calcium on uptake, efflux, and net absorption of non-heme iron using the intestinal-like epithelial Caco-2 cell found that calcium did not have an effect on the net absorption of non-heme iron [39].

Administration of 100 mg of tannic acid in the presence of 800 mg of calcium, displayed no significant effect on the bioavailability of 5 mg of Fe ($FeSO_4$) in a fasted state. The effect of tannic acid on the bioavailability of non-heme iron indicates that the availability was higher in the presence of calcium vs in its absence. This suggests that under these experimental conditions, calcium could protect non-heme iron from the inhibitory effect of tannic acid on its bioavailability. In this regard, we speculated that calcium ions at high concentrations (molar ratio Ca: Fe 225:1) could be bound to the hydroxyl groups of the tannic acid molecule, preventing iron ions from interacting and allowing for greater amounts of iron available for absorption. It is necessary to design experimental studies to further explore this effect.

In this study, 10 mg of phytic acid in the presence of 800 mg of calcium ($CaCl_2$) did not significantly affect the fasting bioavailability of 5 mg of non-heme iron ($FeSO_4$). In food matrices, it has been reported that higher amounts of available phytic acid capture non-heme iron in the presence of calcium [14]. It has been speculated that phytic acid and calcium may form complexes that decrease the action of phytases, leaving higher amounts of phytic acid available to interact with iron. High amounts of phytases have been observed in cereals [40] and baker's yeast [41]. Under fasting conditions, without the presence of other components from the food matrices, the effect of phytic acid on the bioavailability of non-heme iron in the presence of calcium has not been previously characterized. It should also be emphasized that no relevant phytase activity has been observed in the secretions of the human gastrointestinal tract [42]. The results observed in this study may be due to the fact that under these experimental conditions, greater amounts of phytic acid are required to observe a significant effect on the absorption of non-heme iron, even in the presence of high doses of calcium. We speculated that the absence of other components in the food matrices, including phytases, could diminish the inhibitory effect of phytic acid on the bioavailability of non-heme iron in the presence of calcium. Under the experimental conditions of our study, 10 mg of phytic acid did not significantly alter the bioavailability of 5 mg of non-heme iron in the presence of calcium ($CaCl_2$).

Citrus pectin with 55–70% esterification, in doses of 250 mg, in the presence of 800 mg of calcium ($CaCl_2$), did not significantly affect the fasting bioavailability of 5 mg of iron ($FeSO_4$). Although pectins may bind iron [28] and calcium [43], the effect of the pH in its interaction with calcium a iron [29,43], and the high degree of digestion and metabolism in the human may explain these results. This study does not exclude the possibility that a significant effect on the bioavailability of non-heme iron in the presence of calcium may be observed with higher amounts of pectin, or pectin with different molecular weights or degrees of methylation.

Conclusions

One-hundred mg of tannic acid significantly decreases the fasting bioavailability of 5 mg of non-heme iron ($FeSO_4$), while 10 mg of phytic acid and 250 mg of high methoxyl citrus pectin had no effect. The same compounds in the presence of 800 mg of calcium ($CaCl_2$), did not affect the bioavailability of 5 mg $FeSO_4$.

Conflict of interest

The authors declare no conflicts of interest.

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