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One-month of calcium supplementation does not affect iron bioavailability: A randomized controlled trial

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ABSTRACT

Objectives: Calcium (Ca) and iron (Fe) are essential minerals for normal growth and development. Although previous studies have shown that Ca inhibits acute Fe absorption, there is no evidence of the possible long- or medium-term effects of Ca supplementation on Fe bioavailability. The aim of this study was to determine the effect of 34 d of Ca supplementation on heme Fe and non-heme Fe bioavailability in non-pregnant women of ages 33 to 47 y.

Methods: This was a prospective, randomized, double-blind, placebo-controlled trial. Twenty-six healthy women (40 ± 5 y) were randomly assigned to receive either 600 mg of elemental Ca/d as $CaCO_3$ (Ca group, n = 13) or a placebo (P group, n = 13) for 34 d. Heme Fe and non-heme Fe bioavailability were determined before and after treatment using ^{55}Fe and ^{59}Fe radioisotopes. A two-factor, repeated-measures analysis of variance was used to assess differences by treatment and timing.

Results: The geometric mean (range ± 1 SD) of heme Fe bioavailability before and after treatment was 16.5% (8.3–32.8) and 26% (15.5–43.6) for the Ca group and 21.8% (13.0–36.6) and 25.1% (16.5–38.3) for the P group. Non-heme Fe bioavailability before and after treatment was 39.5% (19.9–78.7) and 34.1% (19.1–60.6) for the Ca group, and 44.6% (24.9–79.7) and 39.3% (24.3–63.4) for the P group. There were no differences in either heme Fe or non-heme Fe bioavailability either at baseline or after treatment.

Conclusion: The administration of calcium supplements for 34 d does not affect iron bioavailability. This trial is registered with Controlled-trials.gov, number ISRCTN 89888123.

Introduction

Calcium (Ca) and iron (Fe) are essential micronutrients for normal growth and development [1,2]. Iron deficiency (ID) is the most prevalent nutritional disorder and the most common cause of anemia worldwide [3]. A decreased dietary intake of Fe is considered the main cause of ID, but it can also be due to other causes, such as increased menstruation in women [3]. ID is commonly the consequence of both low intake and low bioavailability of Fe from the diet [4]. On the other hand, Ca has a recognized role in the prevention of osteoporosis in non-pregnant women [5,6], especially among those with low dietary intake of this mineral [7]. According to current recommendations, Ca intake should be 1000 mg/d in women ages 19 to 24 y [8]. The administration of Ca supplements with meals has been recommended to improve Ca absorption [6,9]. Available evidence on the effect of Ca supplementation on Fe absorption is contradictory and heterogeneous. Some studies have shown an acute inhibitory effect of Ca on both heme and non-heme Fe absorption when provided in food [10–17]. Moreover, this inhibitory effect occurred regardless of the kind of Ca salt used [10–17]. It has been reported that the duration of this inhibitory effect is less than 2 h [18]. On the other hand, Fe status seems to be unaffected by the long-term intake of Ca supplements [19–21]. To date, the effect of Ca supplementation on Fe absorption or Fe bioavailability is unknown [22]. Calcium supplementation could affect Fe bioavailability, especially among

FP and MO designed the research; IRC and FP analyzed the data; AB and DLdR assisted with data interpretation; IRC and AB wrote the paper; FP had primary responsibility for the final content. All authors read and approved the final manuscript. None of the authors had a conflict of interest.

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Table 1
Baseline characteristics of participants

Variables	Ca group (n = 13)	P group (n = 13)	*P*-value*
Age (y)	39 ± 5	39 ± 4	0.70
BMI (kg/m^2)	26 ± 3	27 ± 3	0.27
Energy intake (kcal)	2040 ± 482	2257 ± 464	0.25
Protein intake (g)	68 ± 25	70 ± 21	0.78
Fat intake (g)	101 ± 27	108 ± 32	0.55
Carbohydrate intake (g)	218 ± 50	252 ± 54	0.11
Dietary calcium intake (mg)†	410 (277–606)	342 (209–561)	0.31
Iron intake (mg)	12.8 ± 3.7	13.3 ± 3.3	0.76

BMI, body mass index
Data presented as mean ± SD
* Student's *t* test.
† Geometric mean (range of ± SD).

vulnerable groups, increasing the risk for developing ID anemia (IDA) [3]. Therefore, the objective of this study is to determine if 34 d of supplementation of 600 mg of elemental Ca will have an effect on both heme and non-heme Fe bioavailability in non-pregnant women ages 33 to 47 y.

Participants and methods

Participants

Community-dwelling, apparently healthy non-pregnant women (ages 33–47 y) were recruited. All women were using intrauterine devices as a contraception method. Exclusion criteria were pregnancy (confirmed by a negative human gonadotropin chorionic urine test), lactation at the beginning of the study, and use of micronutrient supplements within 12 mo before the start of the study.

This experiment was conducted in the Micronutrient Laboratory at the Institute of Nutrition and Food Technology (INTA), University of Chile, between March and August 2011. The participants were recruited from South East Santiago, a low-income area in Chile's capital.

Ethics

A written informed consent was obtained from each volunteer before the study. 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.

Study design

The study was designed as a prospective, double-blind, placebo-controlled trial, with balanced randomization (1:1). Participants were randomly assigned by lottery to receive either 600 mg of Ca as calcium carbonate ($CaCO_3$) (Ca group) or a placebo (P group) for a period of 34 d, defining this period as a medium-term Ca supplementation. We decided to use $CaCO_3$ because it is the most common form used and has low cost. The lottery process was done by the project manager, assigning two different colors until the identification of two groups with the same number of participants. To ensure compliance and blinding of both participants and investigators, both supplements were provided as a chewable tablet of similar appearance, the study staff supervised their intake and neither the participants nor staff knew the type of supplement administered. Supplements were consumed with meals. Iron radioisotopes ^{55}Fe and ^{59}Fe (NEN Life Science Products, Inc., Boston, MA) were used as tracers of Fe bioavailability. Radiolabeled heme and non-heme Fe doses were ingested on an empty stomach on days 1 and 2 and on days 48 and 49. Non-heme Fe radioisotope (0.7 μCi ^{59}Fe, as $FeCl_3$) was administered in 50 mL of an aqueous solution with 3 mg Fe as ferrous sulfate ($FeSO_4$). Heme Fe radioisotope (3.0 μCi of ^{55}Fe as heme) was administered as four capsules with extract of sheep red blood cells (RBCs) dried with 3 mg of heme Fe. On days 14 and 62, 20 mL of venous blood were drawn after an overnight fast to measure circulating radioactivity (cpm/mL). Radiolabeled heme [23] was obtained by intravenous injection of 10 mCi of ^{55}Fe citrate (NEN, Life Science Products, Inc., Boston, MA) into a 6-mo-old sheep, sacrificing the animal 2 wk later, and removing the stroma by lysing and centrifugation.

Fe bioavailability

Radiolabeled compounds of ^{55}Fe and ^{59}Fe were counted by quadruplicate for the calculation of amount of radioactivity ingested (NEN Life Science Products, Inc., Boston, MA). Iron bioavailability was assessed by the double isotopic method as described in an earlier study [24]. Iron bioavailability was calculated assuming that 80% of the Fe absorbed was incorporated into hemoglobin (Hb) of circulating erythrocytes 14 d after ingestion [25]. No food or drink (except water) was allowed 3 h after compound administration. The circulating radioactivity was counted as many times as necessary to obtain less than 3% error in a liquid scintillation counter (Packard Tri-Carb 1600TR Scintillation Counter System, Meriden CT). To calculate Fe bioavailability at days 48 and 49, counted radioactivity was subtracted from that of days 1 and 2 to prevent overexposure.

Biochemical and hematologic determinations

A venous blood sample (10 mL) was obtained by venipuncture from the ulnar artery before an 8-h fast on day 1 to determine Fe status: Hb and mean corpuscular volume (MCV) (Electronic counter cell CELL-DYN 1700, Abbott Diagnostics, Abbott Park, IL), serum Fe, percent of transferrin saturation (TS) [26,27], zinc protoporphyrin (ZP) (ZP-M206D Hematofluorimeter, AVIV Biomedical Inc., Lakewood, NJ), and serum ferritin (SF) by enzyme-linked immunosorbent assay [28].

Anemia was defined as Hb below 120 g/L and IDA as Hb below normal plus ≥ 2 abnormal laboratory measurements (MCV < 80 fL, ZP > 70 μg/dL RBCs, SF < 15 μg/L or TS < 15%) [28]. Iron deficiency without anemia (IDWA) was defined as normal Hb plus ≥ 2 abnormal laboratory results; depleted Fe stores were defined as SF < 15 μg/L. Fe status was considered to be normal when all of these laboratory indexes were within the reference range [28].

Anthropometry and dietary intake estimation

Weight and height were measured on day 1 using a SECA Hispanic precision electronic balance (0.1 kg sensitivity) with stadiometer (0.1 cm sensitivity) (Model 700, SECA Mechanical column scales, SECA Corporation, Columbia. MD). Body mass index was calculated as weight divided by height in squared meters (kg/m^2). The anthropometric variables were also used to estimate blood volume in accordance with Tulane et al [29]. A Semi-Quantitative Food Frequency Questionnaire was used to quantify the daily intake of macronutrients, Fe and Ca, based on the assessment of dietary intake in the last 30 d [30].

Table 2
Iron status biomarkers

Variables	Treatment groups						P^1	P^2	P^3
	Calcium (n = 13)			Placebo (n = 13)					
	Before	After	Delta	Before	After	Delta			
Hb (g/L)*	141 ± 11	141 ± 11	0.6 ± 8.6	142 ± 8	145 ± 11	2.8 ± 6.2	0.41	0.57	0.71
MCV (fL)*	86 ± 5	86 ± 5	0.2 ± 0.8	87 ± 4	86 ± 4	−0.3 ± 0.5	0.70	0.95	0.85
TS (%)*	22.5 ± 11.3	24.5 ± 11.3	2.0 ± 11.2	19.1 ± 6.2	19.7 ± 8.9	0.7 ± 9.1	0.13	0.61	0.80
ZP (μg/dL GR)†,‡	68 (55–83)	65 (52–80)	0.9 (0.8–1.1)	70 (52–95)	60 (47–78)	0.9 (0.7–1.1)	0.58	0.04	0.53
SF (μg/L)†,‡	14.2 (4.5–44.5)	18.5 (7.3–46.8)	1.3 (0.9–1.8)	17.8 (7.8–40.6)	24.9 (14–44.2)	1.4 (1–2)	0.08	0.01	0.15

ANOVA, analysis of variance; Hb, hemoglobin; MCV, mean corpuscular volume; SF, serum ferritin; TS, transferrin saturation; ZP, free erythrocyte protoporphyrin
Data presented as mean ± SD
* *P*-values of two-factor repeated-measures ANOVA: 1. Treatment group; 2. Timing (before vs. after); and 3. Interaction (treatment group # timing).
† Geometric mean (range of ± 1 SD).
‡ *P*-values of ANCOVA for delta of iron status biomarkers in: 1. Treatment group; 2. Baseline values (as covariates); and 3. Interaction (treatment group # baseline values).

Table 3
Heme Fe bioavailability*

Ca group† participants	Before	After	Delta‡	Ratio	**P group**† participants	Before	After	Delta‡	Ratio
1	12.8	13.0	1.2	1.02	14	18.3	35	16.7	1.91
2	3.6	18.2	14.6	5.06	15	17.4	19.5	2.1	1.12
3	19.2	31.0	11.8	1.61	16	20.2	38.6	18.4	1.91
4	12.7	15.8	3.1	1.24	17	16.6	33.9	17.3	2.04
5	31.1	43.5	12.4	1.4	18	48.5	47.1	−1.4	0.97
6	44.7	54.6	9.9	1.22	19	26.7	38.0	11.3	1.42
7	19.6	55.2	35.6	2.82	20	20.4	23.1	2.7	1.13
8	24.1	28.2	4.1	1.17	21	31.3	26.6	−4.7	0.85
9	7.3	14.6	7.3	2.00	22	33.2	15.7	−17.5	0.47
10	10.1	15.4	5.3	1.52	23	5.5	10.4	4.9	1.89
11	16.6	27.2	10.6	1.64	24	31.9	24.2	−7.7	0.76
12	18.4	21.5	3.1	1.17	25	20.2	20.1	−0.1	1.00
13	38.9	44.3	5.4	1.14	26	24.6	20.5	−4.1	0.83
Geometric mean	**16.5**	**26.0**	**9.5**	**1.58**		**21.8**	**25.1**	**2.9**	**1.15**
− 1 SD	**8.3**	**15.5**	**2.5**	**1.01**		**13.0**	**16.5**	**3.0**	**0.74**
+ 1 SD	**32.8**	**43.6**		**2.47**		**36.6**	**38.3**		**1.79**

Bold values highlight geometric mean and SD.
* Data expressed as percentage of absorption.
† Participant name initials.
‡ Delta expressed as standard mean error.

Sample size calculation

A sample size of 28 participants (14 per group) was estimated to detect a 5% difference in either non-heme or heme Fe bioavailability ($\alpha = 0.05$, $\beta = 0.20$) between groups [31]. There were no changes in the outcomes after finishing the trial.

Statistics

Because Fe bioavailability, SF and ZP concentrations and dietary Ca intake had a skewed distribution, values were log-transformed before calculating means and SD or performing statistical analyses. The results were then retransformed into their antilogarithms to recover the original units and were expressed as geometric means ± 1 SD range. Given that the values for SF and ZP were significantly different at baseline, we compared the changes in SF and ZP using analysis of covariance with treatment groups and the corresponding baseline value as covariates; a two-factor repeated-measures analysis of variance was used to determine differences in Fe bioavailability by treatment group, timing, and interaction; and to compare the other Fe status biomarkers between groups.

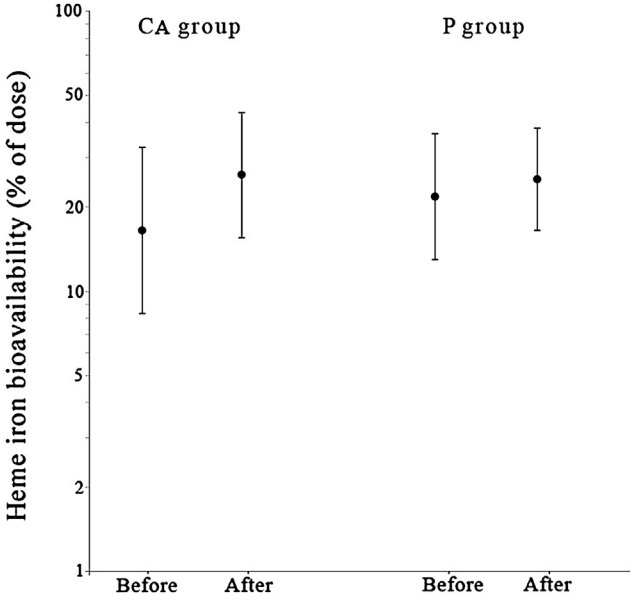

Fig. 1. Effect of calcium supplementation on heme Fe bioavailability. Two-way ANOVA for heme Fe bioavailability: Treatment: F = 0.68 ($P = 0.4145$); timing: F = 3.94 ($P = 0.0530$); interaction: F = 1.10 ($P = 0.2991$). Y axis: logarithmic scale.

All analyses were performed using the statistical software STATA 11.0 (Stata Corp LP, College Station, Texas). A P-value < 0.05 was considered significant.

Results

Twenty-six participants completed the research protocol because two women withdrew voluntarily. The analysis was done according to the original assigned groups. There were no significant differences in age or dietary intake estimations (Table 1) or in Hb, MCV, or TS between groups at baseline or at the end of the trial. SF and ZP were significantly ($P < 0.05$) higher in the placebo group at baseline (Table 2). The change in SF and ZP was not significantly different between treatment groups. No reported harmful or unintended effects were reported in any participant in either group. At baseline, only 1 participant in each group had IDA; 7 in the Ca group and 2 in the P group had IDWA; and 1 was classified as having IDS in each group (data not shown).

There were no differences in heme Fe bioavailability by treatment over time, with no interaction between these two variables (Table 3). The geometric mean (range ± 1 SD) of heme Fe bioavailability before and after supplementation for the Ca group was 16.5% (8.3–32.8) and 26% (15.5–43.6), respectively and for the P group, 21.8% (13.0–36.6) and 25.1% (16.5–38.3), respectively (two-way analysis of variance [ANOVA]: treatment: F = 0.68 [P = NS]; timing: F = 3.94 [P = NS]; interaction: F = 1.10 [P = NS]) (Fig. 1).

Similarly, there were no differences in non-heme Fe bioavailability by treatment over time, with no interaction between these two variables (Table 4). The geometric mean (range ± 1 SD) of non-heme Fe bioavailability before and after supplementation for the Ca group was 39.5% (19.9–78.7) and 34.1% (19.1–60.6), respectively, and for the P group 44.6% (24.9–79.7) and 39.3% (24.3–63.4), respectively (two-way ANOVA: treatment: F = 0.65 [P = NS]; time: F = 0.72 [P = NS]; interaction: F = 0.00 [P = NS]) (Fig. 2).

Discussion

In the present study, Ca supplementation for 34 d did not reduce either heme Fe or non-heme Fe bioavailability in non-pregnant women ages 33 to 47 y.

Table 4
Non-heme Fe bioavailability*

Ca group† participants	Before	After	Delta‡	Ratio	**P group**† participants	Before	After	Delta‡	Ratio
1	37.3	17.5	−19.8	0.47	14	42.2	25.7	−16.5	0.61
2	15.6	14.7	−0.9	0.94	15	26.8	35.9	9.1	1.34
3	58.9	22.7	−36.2	0.39	16	84.1	62.1	−22.0	0.74
4	13.6	17.2	3.6	1.26	17	57.6	67.2	9.6	1.17
5	45.1	24.2	−20.9	0.54	18	33.1	29.0	−4.1	0.88
6	96.1	81.5	−14.6	0.85	19	59.9	61.3	1.4	1.02
7	34.8	60.4	25.6	1.74	20	32.0	35.0	3.0	1.09
8	35.3	36.3	1.0	1.03	21	91.5	85.0	−6.5	0.93
9	20.5	34.4	13.9	1.68	22	60.9	39.6	−21.3	0.65
10	19.7	26.6	6.9	1.35	23	17.0	18.3	1.3	1.08
11	98.3	68.8	−29.5	0.70	24	59.6	52.5	−7.1	0.88
12	65.3	61.6	−3.7	0.94	25	16.5	19.5	3.0	1.18
13	90.0	49.5	−40.5	0.55	26	85.1	34.9	−50.2	0.41
Geometric mean	**39.5**	**34.1**	**−8.9**	**0.86**		**44.6**	**39.3**	**−7.7**	**0.88**
− 1DE	**19.9**	**19.1**	**5.5**	**0.53**		**24.9**	**24.3**	**4.6**	**0.63**
+ 1DE	**78.7**	**60.6**		**1.40**		**79.7**	**63.4**		**1.22**

* Data expressed as percentage of absorption.
† Participant name initials.
‡ Delta expressed as standard mean error.

Several studies have reported an acute inhibitory effect of Ca supplementation on the absorption of both heme and non-heme Fe in test meals [10–17,32,33]. Most of the available evidence is contradictory. One study reported that 600 mg of elemental Ca, as either citrate or phosphate, decreased the absorption of non-heme Fe in a test meal by approximately 50% on an empty stomach, but this effect was not observed when the dose was provided as calcium carbonate [32]. Additionally, another study reported that Ca supplementation had no effect on dietary non-heme Fe absorption when both compounds were administered with at least a 2-h difference [18]. Some studies suggest an inhibitory effect of Ca supplementation on heme Fe absorption when both compounds are administered in test meals [14,15].

Our results suggest that providing Ca supplements as $CaCO_3$ with meals for a period of 34 d does not have an effect on non-heme or heme Fe bioavailability. We decided to use Ca as $CaCO_3$ because it is low cost and the most widespread salt used in supplementation programs. However, there are promoting and inhibitory factors in meals that could affect our findings. In this sense, one group of researchers [14,33] has suggested that part of the inhibitory effect of Ca on Fe absorption in the short-term is caused by the enzymatic degradation of phytate, a potent non-heme Fe inhibitor [34]. On the other hand, we had previously shown that Ca does not inhibit non-heme or heme Fe absorption in the absence of a meal, supporting no influence of Ca supplementation on heme and non-heme Fe without dietary influence [35]. However, it is important to mention that this study was done with only 15 d of follow-up. Therefore, the strengths of the present study is in showing no effect of 34 d of Ca supplementation in the presence of meals.

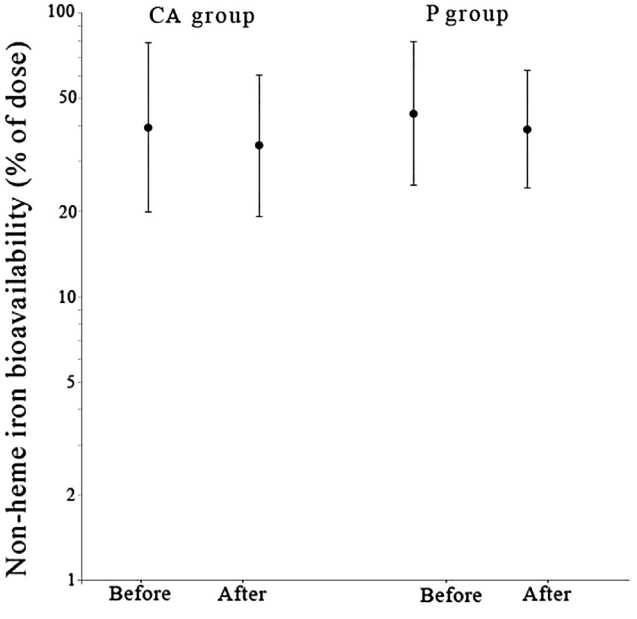

Fig. 2. Effect of calcium supplementation on non-heme Fe bioavailability. Two-way ANOVA for non-heme iron bioavailability: treatment: F = 0.65 (P = 0.4250); timing: F = 0.72 (P = 0.4001); interaction: F = 0.00 (P = 0.9467).

As far as we know, the effects of Ca supplementation on Fe absorption per se had not been previously investigated [22]. These effects had only been studied indirectly looking at the changes of Fe status biomarkers, mainly through the determination of SF [19–21,36,37]. None of these studies reported a negative effect of Ca supplementation on Fe status [19–21].

One of the limitations of the present study was a lack of assessment into the molecular mechanisms involved in the effect of Ca supplementation on Fe bioavailability. Nevertheless, our results suggest that the body is able to regulate the response of Fe absorption despite a prolonged exposure to Ca; probably through an adaptive response of the enterocyte [20,38]. We suggest that the acute effect of Ca supplementation on Fe bioavailability can be an important modulator of a subsequent response in the development of the intestinal crypt-villus unit, thus promoting Fe absorption efficiency; however this process is poorly understood. The effect of Ca on these mechanisms needs to be further studied.

A recent study reported a decrease in the expression of the specific non-heme Fe transporter DMT1 in the apical membrane of Caco-2 cells treated for 4 h with either 2.5 mM Ca, 30 mM non-heme Fe (as ferric ammonium citrate) or both. The authors also showed a decrease in the concentration of cytoplasmic ferritin levels with levels of Ca at 1.25 and 2.5 mM. Nevertheless, there were no differences in the expression of ferroportin in the basolateral membrane. These findings suggest that the effect of Ca on the absorption of non-heme Fe seems to be related with the expression and translocation of Fe transport proteins [39].

Thirty-four days of supplementation should allow the assessment of the effect on more than three replacement cycles

of the intestinal epithelium surface [40]. Our data indicates that it is possible to provide Ca supplementation to non-pregnant women without having negative effects on Fe bioavailability. Although we did not evaluate Ca body status, it is recognized that non-pregnant women are at risk for low Ca status due to low dietary intake [7,41]. Our results have potential implications for public health. However, additional studies are needed to confirm or refute this proposition.

Conclusions

A period of 34 d of daily supplementation with 600 mg of elemental Ca, as $CaCO_3$, did not decrease heme Fe nor non-heme Fe bioavailability in non-pregnant women.

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