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Applied nutritional investigation

Reducing iron deficiency anemia in Bolivian school children: Calcium and iron combined versus iron supplementation alone

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ABSTRACT

Objective: The aim of this study was to determine the effect of combined calcium and iron versus single iron supplementation on iron status in Bolivian schoolchildren.
Methods: Children ages 6 to 10 y old (N = 195), were randomly assigned to receive either 700 mg Ca (as calcium carbonate) plus 30 mg Fe (as ferrous sulfate) (Ca + Fe group) or 30 mg Fe (as ferrous sulfate) (Fe group). The doses were administered daily, from Monday to Friday, between meals at school over 3 mo. Iron status was assessed at baseline and after intervention. Additionally, overall nutritional status was assessed by anthropometry and an estimation of dietary intake.
Results: At baseline, the prevalence of anemia in the Ca + Fe group and the Fe group were 15% and 21.5%, respectively. After 3 mo follow-up, the prevalence of iron deficiency anemia dropped significantly ($P < 0.001$) to 3% in both groups (χ^2 = NS). Iron dietary intake was within recommended levels, but calcium intake only covered 39% of the Recommended Daily Intake.
Conclusion: Combined calcium and iron supplementation is equally as effective as single iron supplementation in reducing the prevalence of iron deficiency anemia in Bolivian school children.

Introduction

Iron (Fe) is an essential mineral for humans, involved in many biological processes. Fe is needed for oxygen transport, storage, and erythropoiesis; being involved in cell division and the synthesis of hemoglobin (Hb) [1]. This micronutrient is also essential for the proper functioning of many enzymes. Iron deficiency is probably the most common nutritional deficiency disorder in the world especially in developing countries and is the most common cause of anemia worldwide [2].

The World Health Organization (WHO) estimates that about 39% of children age <5 y, 48% of children ages 5 to 14 y, 42% of all women, and 52% of pregnant women in developing countries have iron deficiency anemia (IDA) [3]. The etiology of IDA is multifaceted and often several factors are at play in the individual with anemia. Additionally, the condition is inherently associated with poverty and is therefore particularly prevalent in the developing world where the problem is often exacerbated by limited access to appropriate health care and treatment options [3].

It has been reported that other nutrients can interact with Fe thus altering Fe absorption and their nutritional status. Calcium (Ca) is the only micronutrient in the diet that may inhibit both heme and non-heme Fe absorption. Some studies have shown that the acute intake of Ca interferes with the absorption of Fe and that this effect is dose-dependent. These studies, however, do not isolate the effect of Ca from other dietary components [4–7]. A recent study demonstrated that doses up to 800 mg of calcium as Ca chloride do not affect the absorption of a dose of 5 mg Fe as ferrous sulfate on an empty stomach [8]. This latter finding has prompted us to test the effect of Ca and Fe on the iron status of Bolivian schoolchildren, allowing us to assess the feasibility of using this mixture of minerals in supplementation programs. We hypothesized that the combined supplementation of Ca and Fe has the same effect as Fe supplementation alone on the Fe status of Bolivian schoolchildren.

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The authors have no conflicts of interest.
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Materials and methods

Study design

An experimental, double-blind, randomized controlled trial was performed. Participants were randomized into two groups: The first group was given 700 mg Ca as calcium carbonate and 30 mg Fe as ferrous sulfate (Ca + Fe group), and the second group received 30 mg single Fe as ferrous sulfate (Fe group). Medical personnel administered the supplements as a chewable tablet of similar appearance at the time of school entry. Supplementation lasted 3 mo, with children receiving the compounds daily from Monday to Friday. The Fe dose of 30 mg/d was chosen as recommended by the WHO/Food and Agriculture Organization (FAO) for supplementation programs in children ages 6 to 10 y [9]. The dose of 700 mg Ca/d was also chosen as recommended by the WHO/FAO for supplementation in schoolchildren [10].

Sample size calculation

A sample size of 88 children per group was calculated assuming an alpha error of 5%, a power of 80%, and a difference in the prevalence of anemia of 15% at the end of the study. The sample size was increased by 10% to allow for losses, calculating 194 children in all.

Participants

Between August and December 2010, 195 schoolchildren (6–10 y) of both sexes were selected. They were apparently healthy and were students from a public school (social class was measured with a standardized and validated survey focused on acquisitions and salary) in the city of Sucre, Bolivia. Exclusion criteria were the presence of severe IDA (hemoglobin [Hb] <83 g/L corrected by altitude at 2790 m.a.s.l.), intake of vitamin or mineral supplements in the past 6 mo and any gastrointestinal disorders that could interfere with Fe absorption.

Ethics

Written, informed consent was obtained from all the volunteers before the studies began. Participation was voluntary, no remuneration was provided, and all children were free to withdraw at any stage of the study. Parents or guardians received detailed information about the study and those who agreed to participate, also signed an informed consent. The protocol was approved by the Ethics Committee of the Institute of Nutrition and Food Technology, University of Chile, before its execution, which was conducted in accordance with the Helsinki Declaration. Moreover, the study was approved by health and education authorities from Sucre, Bolivia.

Biochemical and hematologic determinations

A fasting venous blood sample was obtained at baseline and at the end of the study between 0700 and 0800 after an overnight of 8 to 10 h to measure Hb, serum ferritin (SF), and C-reactive protein (CRP). In the laboratory of Biochemistry at the University of San Francisco Xavier de Chuquisaca, Sucre-Bolivia, Hb was determined by electronic cell counter (Horiba ABX Micros 60) and serum was separated and frozen at −22 °C. In the laboratory of micronutrients at INTA, University of Chile, Santiago, SF was measured by enzyme-linked immunosorbent assay (ELISA) [11]. CRP was determined by turbidimetry (Orion Diagnostica, Espoo, Finland). The upper normal cutoff value of 5 mg/L was used to indicate the presence of inflammation or infection [12,13]. IDA was defined by Hb <134 g/L using a cutoff adjusted for age and corrected for altitude [14]. Iron depleted stores (IDS) were defined as normal with normal Hb and SF <30 μg/L [15].

Anthropometric measurements and nutritional status assessment

Weight and height were measured in accordance with the anthropometric indicators measurement guide, food and nutrition technical assistance project, and the U.S. Academy for Educational Development [16]. Weight was measured using a Camry digital scale with a maximum capacity of 120 kg and 10 g accuracy. Height was measured with a portable stadiometer fixed to the wall (Seca-206), with a capacity of 220 cm and 0.1 cm accuracy. The assessment of nutritional status *Z* scores was calculated according to the 2007 WHO growth standards for children ages 5 to 19 y [17]. Stunting and underweight were defined as a *Z* score < −2 SD for height/age and weight/age, respectively [18]. The body mass index (BMI) for age was also calculated and ranked to determine nutritional status in four intervals: Severe thinness, <−3 SD; thinness, <−2 SD; overweight, >+1 SD (equivalent to BMI 25 kg/m^2 at 19 y); obesity: >+2 SD (equivalent to BMI 30 kg/m^2 at 19 y).

Dietary intake estimation

Two 24-h dietary recalls at baseline (one for weekdays and another for weekends) were obtained from the parents or guardians of each child using visual models of foods [19]. Calorie and nutrient intake were calculated using the Excel program and according to the database of the Chilean food composition database; a dietary database that includes food composition information from national, regional, and international databases, such as those developed by the U.S. Department of Agriculture [20].

Statistical analysis

Because SF concentrations had a skewed distribution, these values were log-transformed before calculating means, SD, or performing statistical analyses [21]. SF is an acute-phase protein that increases with inflammation and infection. In this sense, one approach widely used to reduce the effect of inflammation on SF is to exclude individuals with inflammation on the statistical analysis. However, this approach can significantly reduce the sample size, especially in developing countries where many asymptomatic individuals present with chronic inflammation leading to biased results [22,23]. For this reason, and in an attempt to adjust the high prevalence of inflammation observed before (96.6% Fe group and 94.4% Ca + Fe group) and after supplementation (100% Fe group and 96.5% Ca + Fe group) we did not exclude children with elevated CRP (>5 mg/L) [12,13], increasing the cut point for SF to 30 mg/L [2,15].

Sixteen children who had no data in the second measurement because of parental refusal to obtain a second blood sample, were dropped from the analysis. No children were removed due to severe IDA. Continuous variables that showed Gaussian distribution were presented as means and SD and were compared through Student's *t* tests. In addition, the χ^2 test was used to evaluate differences between proportions (comparison of prevalence of anemia and Fe status). The effect of Ca supplementation on Fe status biomarkers before and after supplementation was determined by two-way repeated-measures analysis of variance, considering Ca (700 mg) and time as variables. *P*-value < 0.05 was considered significant.

The software package Statistica for Windows 6.0 (StatSoft Inc., Tulsa, OK, USA) was used for statistical analysis and the program R Studio (R-Tools Technology Inc. Richmond Hill, Ontario, Canada) for plotting figures.

Results

The protocol was completed for 179 schoolchildren; 89 in the Fe group and 90 in the Ca + Fe group. The mean age was 8.3 ± 1.3 y (range 6–10 y). There were no significant differences on demographic and socioeconomic indicators between the two groups (Table 1). It was also noted that the study population had a high level of poverty, with most of them receiving the minimum salary and insufficient resources to cover their basic needs (data not shown).

Before supplementation, it was observed that most children had weights and heights below the normal growth curve. There was no significant difference in the percentage of malnourished children in accordance with *Z* score for weight/age (<2 *z*) neither in the indicator of height/age before supplementation (χ^2 = NS) (data not shown).

Table 1
Anthropometric and dietary characteristics of school children at baseline

	Fe group	Ca + Fe group	*P*-value*
N	89	90	
Age (y)†	8.2 ± 1.4	8.4 ± 1.3	
Girls (%)	42.7	45.6	
z Weight/age	−0.8 ± 0.9	−0.9 ± 0.9	0.386
z Height/age	−1.3 ± 0.9	−1.3 ± 0.8	0.528
z BMI/age	0.0 ± 1.0	−0.1 ± 0.9	0.570
Dietary Fe (mg)‡	11.8 ± 3.5	12.2 ± 4.1	0.534
Dietary Ca (mg)	366.0 ± 130.5	369.1 ± 118.7	0.871

BMI, body mass index; Ca, calcium, Fe, iron
* Significant *P* value for Student's *t* test <0.05.
† Mean and SD.
‡ Reference Nutrient Intake 5% bioavailability,15.2 mg.

Table 2
Effect of calcium supplementation on iron status in school children supplemented with iron

Fe status*	Fe group (n = 89)		Ca + Fe group (n = 90)		Two-way repeated-measures ANOVA†		
	Before	After	Before	After	Treatment effect	Time effect	Interaction
Hb(g/L)‡	140 ± 8	147 ± 8	141 ± 8	148 ± 7	0.398	0.000	0.725
SF (μg/L)§	27 ± (17–44)	38 ± (25–57)	28 ± (17–46)	35 ± (24–52)	0.368	0.000	0.052
CRP (mg/L)	7.9 ± 2.5	7.7 ± 2.0	8.2 ± 3.1	7.7 ± 2.8	0.814	0.270	0.723

ANOVA, analysis of variance; Ca, calcium; CRP, C-reactive protein; Fe, iron; Hb, hemoglobin; SF, serum ferritin
* Mean and SD.
‡ Significant value for two-way ANOVA < 0.05.
§ Geometric mean (−1 SD +1 SD).

The energy and nutrient intake was similar in both groups (Student's *t* test, NS). Regarding mineral intake, 79% of children ingested Fe as recommended by the WHO for a diet with low Fe bioavailability (5%) [9], Ca intake was very low in both groups (Table 1). Only 32% had covered their requirements for Ca, and 99% of children in both groups had Ca intakes below the estimated average requirement [24].

Table 2 shows the mean and SD for Fe status biomarkers and CRP. There were no significant differences on Hb or SF concentrations before or after supplementation in either group. After 3 mo of supplementation, a significant increase ($P < 0.001$) was noted in the levels of both indicators (two-way repeated-measures analysis of variance).

At baseline, 21.5% of the Fe group and 15% of the Ca + Fe group had anemia (χ^2 = NS). Both treatments were able to significantly ($P < 0.001$) reduce IDA to 3%. The prevalence of IDS was 56% and 51% for Fe and Ca + Fe groups, respectively, showing a significant ($P < 0.001$) reduction to 26% and 30%, respectively, after supplementation without differences by study group (χ^2 = NS) (Fig. 1).

In a cumulative plot of Hb concentrations of all children, before and after supplementation, the whole curves for Fe and Ca + Fe groups are shifted to the right, displaying that as a population, the children consuming the Fe or the Ca + Fe supplements had improved Hb concentrations (Fig. 2).

Discussion

The effect of 3 mo of combined supplementation of Fe and Ca and Fe status was investigated in a group of Bolivian schoolchildren considered at risk for IDA. Supplementation of 30 mg of Fe and 700 mg of Ca daily for 3 mo on an empty stomach was equally as effective as the supplementation of single 30 mg Fe in reducing IDA. The mean (±SD) Hb concentration at enrollment was 140 g/L Fe group and 141 g/L Ca + Fe group. After intervention, both groups increased levels by 7 g/L. Similarly, there was a significant reduction in the prevalence of IDS. The geometric mean (interquartile range) SF concentration was 27 (17–44) μg/L and 28 (17–46) μg/L for the Fe group and the Ca + Fe group, respectively. At the end of the study, the geometric mean SF concentrations averaged between groups were 38 (25–57) μg/L in the Fe group and 35 (24–52) μg/L in the Ca + Fe group.

To our knowledge, this is the first study to measure the combined administration of Ca and Fe as supplements. Most of the other studies have been aimed at measuring the acute effect of Ca supplementation on dietary Fe absorption. In this context, one study [4] provided some of the most relevant evidence regarding an inhibitory effect of Ca on Fe absorption. However, in this study the potential inhibitory effect of other compounds present in the food matrix were not isolated. Other studies also showed an inhibitory effect of Ca on Fe absorption, but they used the same basic methodology (single meals, dual-radioisotope labeling, and red blood cell iron incorporation) [5–7]. A recently published study [8] demonstrated that Ca in doses <800 mg (as chloride), on an empty stomach, caused no decrease in the absorption of 5 mg of Fe (as sulfate) (molar ratio Ca:Fe; 223:1). However, doses ≥1000 mg of Ca were shown to decrease non-heme Fe absorption by ~50% [8].

Based on short-term experiments (effect of Ca on Fe absorption), it has been postulated that chronic Ca supplementation could affect Fe status. One group studied the effect of 1200 mg of calcium carbonate supplementation daily for 6 mo in 11 adults with adequate Fe deposits, reporting no effect of Ca supplementation on Fe status biomarkers (plasma ferritin, Hb, hematocrit, and zinc protoporphyrin concentrations) [25]. Another study evaluated whether 1200 mg of Ca (calcium glycerophosphate) supplementation of infant formula affects the Fe

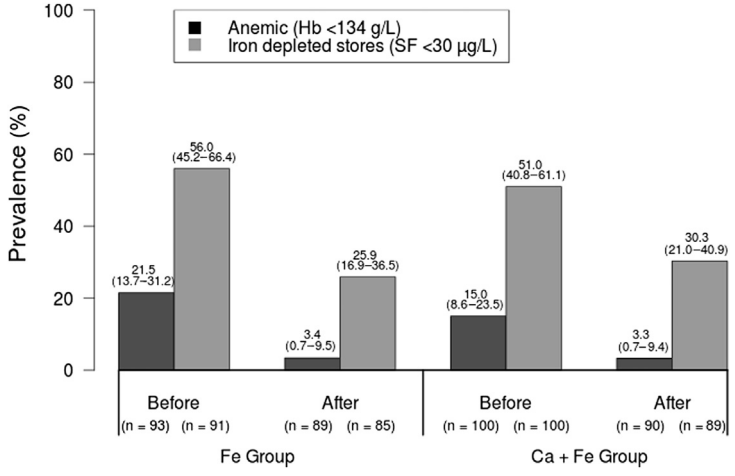

Fig. 1. Prevalence of anemia and iron status. Ca, calcium; Fe, iron; Hb, hemoglobin; SF, serum ferritin.

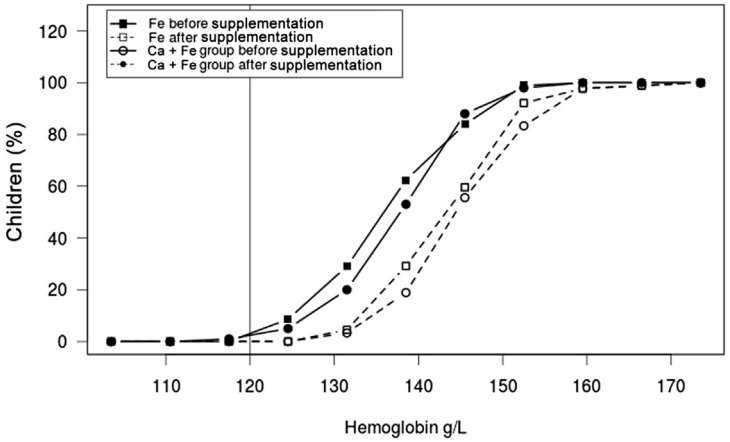

Fig. 2. Cumulative frequency of hemoglobin before and after supplementation. Ca, calcium; Fe, iron.

status of healthy full-term infants. After 4 to 9 mo of supplementation, no differences were observed between the experimental and control groups on hematocrit, ferritin, erythrocyte protoporphyrin, and total iron-binding capacity [26]. Furthermore, lactating women were given 1000 mg of Ca (as carbonate) for 6 mo, and no significant effects on Hb or Fe were found compared with controls [27]. Similar results were found in a study that identified adolescents with high (1000–1300 mg/d), and low intake (<713 mg/d) of Ca from more than 1000 participants. The participants were randomly assigned to receive Ca 500 mg/d as calcium carbonate or placebo. After 1 y, no differences in mean Hb, SF, or transferrin receptors (TfR) were observed [28].

It should be noted that most of the studies discussed here measured the effect of Ca supplementation on dietary Fe in normal and adult populations. To our knowledge, this is the first study evaluating the effect of combined Ca and Fe supplementation in children and in a country with a high risk of IDA [29,30]. However, like the studies just discussed, we did not observe an effect on Fe status with the long-term Ca + Fe supplementation. These differences in the effects of Ca in the short- or long-term are likely to be due to adaptation to a high intake of Ca as regulatory mechanism of Fe absorption to maintain Fe homeostasis [31].

Short-term regulation of Fe absorption in the small intestine is given, especially for two proteins, the divalent metal transporter 1 and ferroportin [32], but little is known about the response of these proteins to factors other than Fe status. Nevertheless, it is possible to expect an adaptation to changes in the Ca cell content [33]. Meanwhile, in the long-term regulation of Fe, there are Fe regulatory elements and Fe regulatory proteins which upon detecting cellular concentrations of Fe can increase or decrease the expression of certain important proteins in cellular homeostasis of Fe and thus counteract the effects of Ca [34]. However, more research is still needed on this point.

Finally, a limitation of this study is that Hb and SF were the only iron status markers used. The combination of Hb, SF, TfR, and parameters of infection (CRP, α1 acid glycoprotein) represent the best approach to measure Fe status and infection, and TfR appears to be largely unaffected by inflammatory disorders. However, it is accepted as a criterion for the diagnosis of IDA a reduction of Hb (or hematocrit) together with a positive therapeutic trial, or a reduction of Hb plus one or more of the other laboratory altered tests, such as SF [3]. More studies should be conducted using other markers of iron nutritional status specifically those not affected by infectious-inflammatory disorders.

Conclusion

Combined CA and Fe supplementation is equally as effective as single Fe supplementation in reducing the prevalence of IDA in Bolivian school children.

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