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Effect of Daily Supplementation with Iron and Zinc on Iron Status of Childbearing Age Women

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Abstract The objective was to determine the effect of daily supplementation with 30 mg of iron (Fe) plus 30 mg of zinc (Zn) for 3 months on Fe status of women of childbearing age. This was a randomized double-blind, placebo-controlled trial. Eighty-one women (18–45 years) were randomly assigned to receive either a daily single dose of 30 mg of Fe (group 1; n=28) and 30 mg of Fe plus 30 mg of Zn (group 2; n=26) or placebo (n=27) for 3 months. Hemoglobin (Hb), mean corpuscular volume, serum Fe, total iron-binding capacity, transferrin saturation, erythrocyte Zn protoporphyrin, serum ferritin (SF), serum transferrin receptor (TfR), total body Fe, serum Zn, and high-sensitivity C-reactive protein were measured at baseline and at the end of the study. At baseline, 3.7, 28.4, and 3.7 % of women had iron-deficiency anemia (IDA), Fe deficiency without anemia, and depleted Fe stores, respectively. No significant differences on Fe status were found between groups before supplementation. After supplementation, group 2 showed a significant increase of Hb and total body Fe and a significant decrease of TfR compared with placebo (p<0.05). Moreover, serum Zn increased significantly in group 2 compared with group 1 (p<0.01) and placebo (p<0.01). In conclusion, daily supplementation with 30 mg of Fe plus 30 mg of Zn for 3 months improved significantly the Fe and Zn status of women, compared with those who received placebo. The positive effect of Fe supplementation on Fe status is enhanced by combined Zn supplementation.

Keywords Anemia · Iron status · Zinc status · Supplementation · Micronutrients

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Introduction

Iron (Fe) deficiency is the single most common nutritional disorder worldwide. World Health Organization (WHO) has estimated that 40 % of the population (2 billion people) have anemia, of which half is due to iron deficiency, mainly affecting infants, children, and women of fertile age [1, 2]. It has been suggested that this situation coexists with zinc (Zn) deficiency in developing countries [3, 4]. The International Zinc Nutrition Consultative Group (IZINCG) has reported that 1.2 billion people worldwide are at risk of inadequate intake of Zn [3].

The supplementation has been developed as a strategy to prevent these deficiencies and to improve Fe and Zn status. However, some studies conducted in women of fertile age have reported a negative interaction between both minerals when given together in aqueous solution, with an inhibitory effect of Zn on Fe absorption [5–8]. Nevertheless, there are contradictory results on the effects of combined Fe and Zn supplementation on Fe status [9–11, 22–27]. Therefore, the aim of our study was to determine the effect of daily supplementation with 30 mg of Fe plus 30 mg of Zn on the Fe status of women of childbearing age.

Materials and Methods

Subjects

Eighty-seven apparently healthy women between 18 and 45 years of age were selected to participate in the study. None of the volunteers had consumed vitamin or mineral supplements in the previous 6 months, and none were pregnant or currently breastfeeding at the time of the study.

Study Protocol

This study was a randomized, double-blind, placebo-controlled clinical trial. Participants were randomized to receive a daily dose of either 30 mg of elemental Fe (group 1), 30 mg of elemental Fe plus 30 mg of Zn (group 2), or a placebo for 3 months. Fe and Zn were given as ferrous sulfate and zinc sulfate, respectively.

To monitor compliance, the supplements were administered on an empty stomach between meals by study personnel, who visited the participants' homes on a daily basis to deliver the supplement and verify its consumption during the 3-month period. Supplements and placebos, identical in appearance, were prepared by a local pharmacy (Farmacias Cruz Verde, Santiago, Chile).

Anthropometric Measurements

Weight and height were measured at baseline and at the end of the study using a SECA Hispanic precision electronic balance (0.1-kg sensitivity) and stadiometer (0.1-cm sensitivity) (Model 700, SECA Mechanical column scales, SECA Corporation, Columbia, MD), respectively.

Hematological and Biochemical Parameters

A fasting blood sample (15 mL) was obtained at baseline and end of the study to measure hemoglobin (Hb), mean corpuscular volume (MCV) (CELL-DYN 3200, Abbott Diagnostics, Abbott), serum Fe, total iron-binding capacity (TIBC), transferrin saturation (Sat) [12], Zn protoporphyrin (Zpp) (ZN Hematofluorometer Model 206D, AVIV Biomedical Inc., Lakewood, NJ, USA), serum ferritin (SF), soluble serum transferrin receptor (TfR) (enzyme-linked immunosorbent assay, Ramco Laboratories Inc., Houston, TX, USA), serum Zn (atomic absorption spectrometer, model 2280, PerkinElmer Life and Analytical Sciences), and high-sensitivity C-reactive protein (CRP) (Turbidimetry, Kit QCA). Total body Fe was estimated from the ratio of TfR to SF using the equation developed by Cook et al. [13].

Anemia was defined as Hb below 12 g/dL [1] and iron-deficiency anemia (IDA) as anemia plus ≥2 other abnormal indices for Fe status (MCV<80 fL, Zpp>70 μg/dL red blood cell (RBC), Sat<15 %, TfR>8.6 mg/L, and SF<15 μg/L. Iron deficiency without anemia (ID) was defined as normal Hb with ≥2 abnormal indices for Fe status. Depleted Fe stores were defined as only SF <15 μg/L [14]. Fe status was considered to be normal when all of these laboratory indexes were within the reference range. Zn deficiency was defined as serum Zn <70 μg/dL [3].

Dietary Intake

A Food Frequency Questionnaire (FFQ) was implemented by a nutritionist to quantify intake of macronutrients, Fe, and Zn of all participants at the beginning and end of the study. Dietary intakes were converted to nutrient intakes using data from the Chilean Food Composition Table and [15], whenever needed, complemented by data from 108 commonly consumed Chilean foods [16] and by the US Department of Agriculture National Nutrient Database for Standard Reference [17].

Ethical Approval

A written informed consent was obtained from all participants before the study. The protocol was approved by the Ethics Committee of the Institute of Nutrition and Food Technology (INTA), Santiago, Chile. This study was performed in accordance with the Helsinki Declaration (1964) and was approved by the Ethics Committee at INTA, University of Chile, before their execution.

Sample Size

A sample of 28 participants per group (84 total) was estimated. Calculation was based on multiple-arm comparisons, with ~10 % withdrawals of follow-up and 0.05 α error and 80 % power to detect a difference of 2.5 mg/kg on total body Fe levels among the three groups, using ANOVA for repeated measures.

Statistical Analyses

Normally distributed variables are presented as means and standard deviations (SD). Because distributions of SF, CRP, and dietary intake measurements were skewed, the values were transformed to their natural logarithms. The results of SF, CRP, and dietary intake measurements were then retransformed into antilogarithms to recover the original units and are shown as geometric means and range±1 SD.

A one-way ANOVA with a Scheffé post hoc test was used to compare the age, and anthropometric and hematological measurements between treatment groups at baseline. A two-way ANOVA for repeated measures test was used to determine the differences of hematological and dietary measurements within and among the groups (effect of treatment, time of the study, and interaction of treatment and time). In cases where a significant interaction was observed between treatment and time, a one-way ANOVA with post hoc Scheffé test was used to compare the change between baseline and final measurements (delta) among the three treatment groups.

All statistical analyses were performed with STATA, version 10.0 (Stata Corp, College Station, TX, USA). The statistical significance level was set at $p<0.05$.

Results

General and Biochemical Characteristics of Participants at Baseline

A total of 87 women were enrolled in the study. Six women dropped out before completing the supplementation period. One woman was receiving an Fe supplement for anemia treatment. Three declared digestive intolerance and two participants did not comply with the study protocol (Fig. 1). Women who dropped out of the study were not significantly different in baseline characteristics compared with the group of women that completed the protocol.

All participants consumed supplements for an average of 88 days. During the study, there were no differences in the compliance among the three treatment groups.

General characteristics of women are shown in Table 1. The mean age was 33.5±8.8 years. There were no statistically significant differences in age or in anthropometric indices among treatment groups.

Table 1 General characteristics of participants at baseline (mean ± SD)

Variables	Placebo (n=27)	Group 1 30 mg Fe (n=28)	Group 2 30 mg Fe+ 30 mg Zn (n=26)	p value[a]
Age (year)	32.0±8.0	33.5±9.0	34.9±9.5	N.S.
Weight (kg)	67.3±11.2	68.5±9.7	66.6±8.8	N.S.
Height (m)	1.57±0.1	1.56±0.1	1.57±0.1	N.S.
BMI (kg/m^2)	27.2±4.1	28.3±5.0	27.0±3.4	N.S.

N.S. not significant

[a] One-way ANOVA

Effect of Supplementation on Laboratory Parameters

There were no statistically significant differences in indicators of iron status at baseline. None of the three treatments had an effect on all biochemical parameters studied. Nevertheless, there was a significant interaction (time×treatment) for Hb, TfRs, and total body Fe levels ($p<0.05$) (Table 2). In this sense, group 2 had a significant increase in Hb levels compared with placebo (Δ0.4 vs Δ−0.3 g/dL) ($p<0.05$) (Fig. 2a). The positive change in total body Fe concentration was also significantly higher for group 2 compared with placebo (Δ1.5 vs Δ−0.1 mg/kg) ($p<0.05$) (Fig. 2b). In addition, group 2 had a significant decrease in TfRs compared with placebo (Δ−1.7 vs 0.2 mg/L) ($p<0.05$) (Fig. 2c).

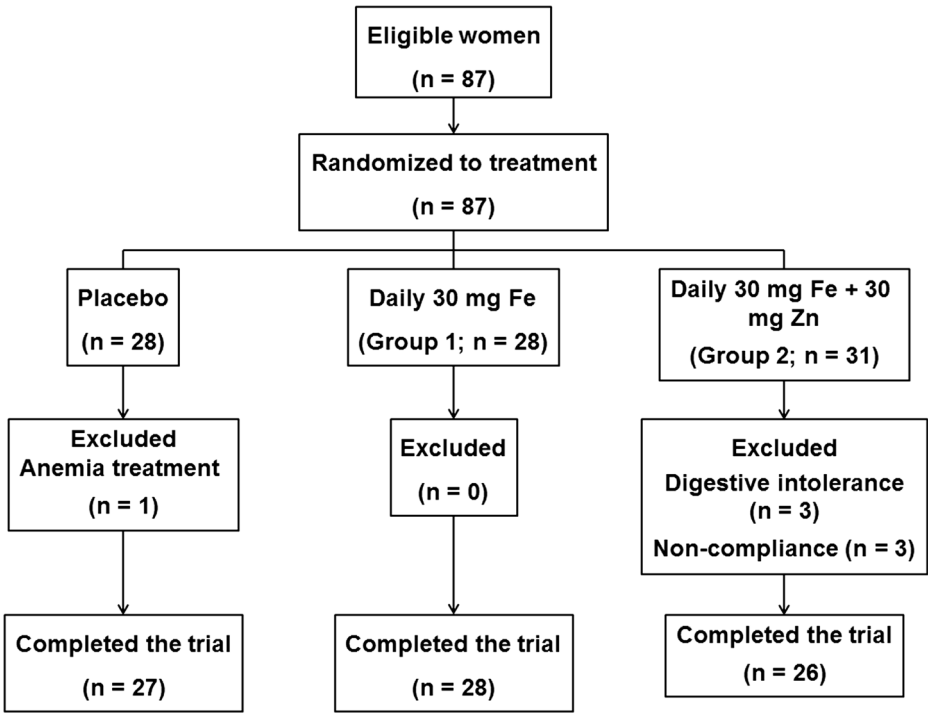

Fig. 1 Flow chart of participants in the study

Table 2 Baseline and final biochemical variables of participants (mean ± SD)

Variables	Placebo (n=27)	Baseline		Final		p value[a]			
		Group 1 (n=28)	Group 2 (n=26)	Placebo (n=27)	Group 1 (n=28)	Group 2 (n=26)	Treatment	Time	Treatment×time
Hb (g/dL)	15.2±0.7	15.2±1.4	14.8±1.6	14.9±0.7	15.2±0.9	15.2±1.0	N.S.	N.S.	0.03
MCV (fL)	84.7±3.0	84.2±4.1	82.4±7.6	84.7±2.9	84.9±2.7	83.7±5.9	N.S.	0.01	N.S.
Serum iron (μg/dL)	77.1±29.9	76.3±27.2	65.7±26.9	89.2±38.8	92.0±38.1	97.9±42.33	N.S.	<0.01	N.S.
TIBC (μg/dL)	356.3±54.1	332.9±34.0	348.9±72.9	362.6±47.1	326.7±33.5	333.1±67.1	N.S.	N.S.	N.S.
Sat (%)	22.1±9.2	23.2±8.4	20.3±9.7	24.9±10.8	28.6±12.0	29.4±11.2	N.S.	<0.01	N.S.
Zpp (μg/dL RBC)	74.2±18.7	80.8±26.8	86.1±32.6	73.8±15.6	71.0±16.3	72.4±17.6	N.S.	<0.01	N.S.
TfR (mg/L)	6.3±1.9	6.6±3.9	8.5±3.8	6.5±1.9	5.8±1.8	6.8±2.2	N.S.	0.01	0.03
Serum ferritin (μg/L)[b]	22.5 (12.5–40.5)	24.3 (11.3–52.4)	20.3 (8.0–51.6)	23.1 (13.5–40.5)	30.2 (11.1–53.4)	25.9 (12.6–53.4)	N.S.	<0.01	N.S.
Total body iron (mg/kg)	3.4±2.9	3.6±3.5	2.0±4.6	3.3±2.6	4.6±2.4	3.5±3.2	N.S.	<0.01	0.03
Serum zinc (μg/dL)	94.5±42.7	86.6±13.4	81.1±9.0	86.7±11.9	92.6±21.8	111.4±21.1	N.S.	<0.01	<0.01
CRP (mg/L)[b]	2.5(0.6–10.7)	3.2 (0.8–13.9)	2.4 (0.6–10.2)	3.0 (0.9–10.1)	4.1 (1.0–16.5)	3.2 (0.7–13.6)	N.S.	N.S.	N.S.

Hb hemoglobin, *MCV* mean corpuscular volume, *TIBC* total iron binding capacity, *Sat* transferrin saturation, *Zpp* zinc protoporphyrin, *TfR* soluble transferrin receptor, *CRP* C-reactive protein, *N.S.* not significant

[a] Two-way ANOVA for repeated measures: time (baseline − final), treatment and interaction (time × treatment)

[b] Geometric mean ± range 1 SD

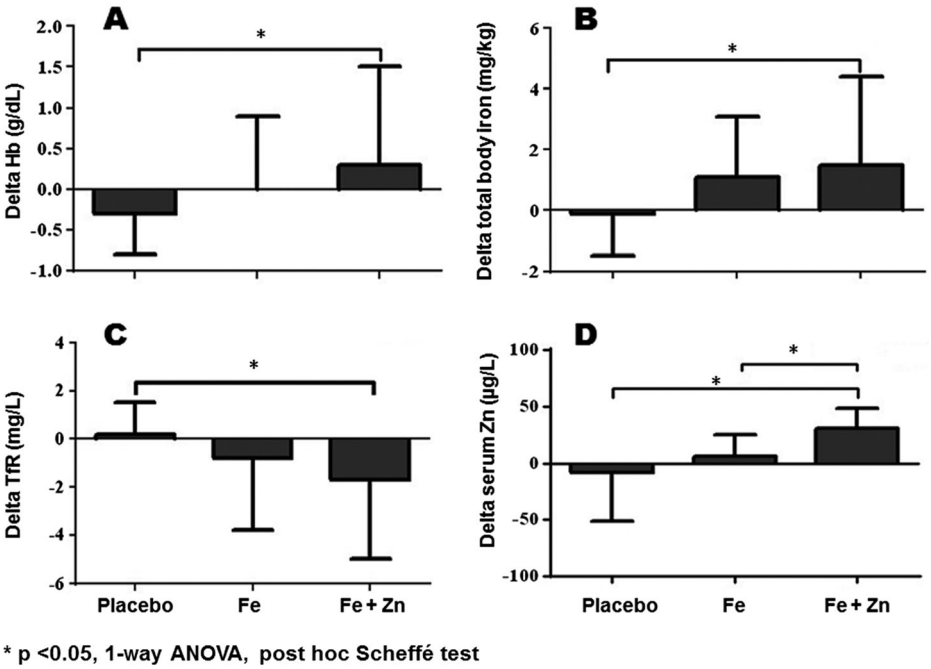

Fig. 2 Changes after supplementation (delta after–before) in hemoglobin (**a**), total body iron (**b**), serum transferrin receptor (**c**), and serum zinc (**d**)

Overall, group 1 did not show significant changes of Fe status indices when compared to group 2 and placebo (Table 2 and Fig. 2). MCV, serum Fe, total body Fe, Sat, Zpp, and SF levels only showed a significant time-related changes ($p<0.01$) (Table 2).

Regarding Zn status, there was a significant interaction in Zn levels ($p<0.01$). Group 2 showed a significant increase in serum Zn concentration compared to both group 1 (Δ30.3 vs 6.0 μg/dL) and placebo (Δ30.3 vs −7.8 μg/dL) ($p<0.01$) (Table 2 and Fig. 2d), with no significant differences between group 1 and placebo. Also, there was a significant difference in Zn levels comparing the times of the study ($p<0.01$) (Table 2).

Moreover, no significant changes were found in CRP levels among and within the treatment groups (Table 2).

Prevalence of Anemia, Iron Deficiency, and Zinc Deficiency

The baseline prevalence of IDA, ID without anemia, Fe-depleted stores, and normal Fe status was 0, 29.6, 7.4, and 63.0 %, respectively (placebo); 3.6, 25.0, 0, and 71.4 % (group 1); and 7.7, 30.8, 3.8, and 57.7 % (group 2). At the end of the study, these figures were 0, 29.6, 3.7, and 66.7 % (placebo); 0, 17.9, 0, and 82.1 % (group 1); and 3.8, 11.6, 3.8, and 80.8 % (group 2). No significant differences were found comparing by treatment groups at baseline and end of the study.

The baseline prevalence of Zn deficiency was 0 % (placebo), 7.1 % (group 1), and 0 % (group 2). At the end of the study, the prevalence of zinc deficiency was 3.7 % (placebo), 3.6 % (group 1), and 0 % (group 2). There were no significant differences among groups at baseline and end of the study.

Dietary Intake

At baseline of the study, the prevalence of adequate intake of Fe and Zn was 95 and 94 %, respectively. At the end of the study, this prevalence was 97 and 95 %, respectively, without considering the supplements. In both times of the study, there were no significant differences on nutrient intake when it was compared between the treatment groups (Table 3). Energy (kcal) and protein intake were significantly different comparing by time of the study ($p<0.05$). There were no significant differences on nutrient intake comparing by treatment groups (Table 3). Energy (kcal) and protein intake were significantly different comparing by time of the study ($p<0.05$).

Discussion

Combined supplementation with Fe and Zn is a strategy that has been used to improve the status of both micronutrients in the population [1, 3]. However, acute studies have shown that Zn can have a negative effect on the absorption of a single dose of Fe [5, 18]. We have previously reported that a low dose of Zn has a significant inhibitory effect on the absorption of 0.5 mg of Fe at Zn/Fe molar ratios ≥5:1 [7] and that if both minerals are provided at higher doses (11.7 mg of Zn and 10 mg of Fe), the negative effect is observed at a Zn/Fe molar ratio of 1:1 [6]. Similar inhibitory effects have been observed

Table 3 Nutrient intake of participants (geometric mean ± range 1 SD)

Variables	Placebo (n=27)	Baseline		Final		p value[a]			
		Group 1 (n=28)	Group 2 (n=26)	Placebo (n=27)	Group 1 (n=28)	Group 2 (n=26)	Treatment	Time	Treatment × time
Energy (Kcal)	2474.1 (1806.1–3389.1)	2370.5 (1833.9–3064.2)	2347.1 (1794.2–3070.4)	2243.0 (1655.7–3038.7)	2232.4 (1823.6–2732.7)	2213.7 (1686.62905.6)	N.S.	0.02	N.S.
Protein (g)	97.8 (70.7–135.4)	95.8 (67.6–135.7)	98.5 (73.5–131.8)	88.1 (66.6–116.5)	87.4 (69.6–110.0)	87.0 (67.0–113.1)	N.S.	0.002	N.S.
Carbohydrates (g)	409.2 (297.5–562.8)	392.7 (298.3–517.1)	390.9 (293.2–521.1)	369.7 (273.7–499.2)	378.9 (303.0–474.0)	373.9 (270.5–516.9)	N.S.	N.S.	N.S.
Fat (g)	46.6 (30.5–71.1)	41.6 (27.3–63.3)	40.8 (27.5–60.6)	43.5 (28.5–66.4)	39.6 (28.5–54.9)	39.4 (28.2–54.9)	N.S.	N.S.	N.S.
Iron (mg)	17.3 (13.6–22.0)	16.5 (10.9–24.9)	16.4 (11.9–22.8)	16.8 (12.1–23.4)	16.1 (12.0–21.4)	15.8 (9.2–27.0)	N.S.	N.S.	N.S.
Zinc (mg)	11.6 (8.4–15.9)	10.8 (7.3–16.1)	11.1 (8.1–15.2)	10.8 (7.7–15.1)	10.6 (8.4–13.4)	10.0 (7.5–13.4)	N.S.	N.S.	N.S.

N.S. not significant

[a] Two-way ANOVA for repeated measures: time (baseline − final), treatment and interaction (time × treatment)

by Rossander-Hultén et al. in women who received 3 mg of Fe plus 15 mg of Zn in aqueous solution compared with those who received Fe alone [19]. Moreover, a study in male and female adults reported that 51 mg of Fe showed an inhibitory effect on the absorption of 6 mg of Zn [20].

In the present study, we showed that women who receive 30 mg of Fe plus 30 mg of Zn (Zn/Fe molar ratio of 0.9:1) had a significant improvement in Hb, total body Fe, and TfRs levels compared to those who received placebo. Two previous studies, one with anemic women of childbearing age and the other with Fe-deficient women, also showed that women who received Fe and Zn supplements had a significant higher increase in Hb levels compared with those who received Fe alone [9, 21]. Similar results have been reported in children who received combined supplementation, with a significant improvement in SF, Hb [22, 23], and TfR levels compared to Zn alone and placebo [24–26]. Moreover, Lind et al. showed that children supplemented with Fe and the combined supplemented group increased their Hb and SF levels. However, contrary to our results, the improvement of these parameters was significantly higher in the Fe-supplemented group [10]. On the other hand, studies in pregnant women supplemented with 60 mg of Fe plus 15 mg of Zn showed no differences in Fe status compared with those who received Fe alone [11, 27].

The difference in results among these studies might not be a consequence of the negative interaction of these minerals during absorption but rather could be explained by differences in either baseline Fe status of participants, doses of Fe and Zn, and/or their molar ratio, supplements administered separately or together, duration of supplementation trial and sensitivity and specificity of iron status parameters utilized to measure the effect.

It has been speculated that Fe and Zn could compete for Divalent Metal Transporter-1 (DMT-1), the most important Fe transporter, which participates in the transport of a variety of divalent metals [28, 29]. However, there are doubts over the physiological role of this transporter on cellular Zn uptake because Zn has a low affinity for DMT-1 [28–30]. Furthermore, studies in Caco-2 cells have reported that Zn uptake is not altered by the presence of an antibody against DMT-1, which supports the notion that the interaction does not occur during transport into the cell [31]. On the contrary, it has been showed that intracellular Zn is decreased in Caco-2 cells transfected with sh-ribonucleic acid (RNA) plasmids to selectively inhibit expression of DMT-1[32].

Possible explanations for our results are related with cellular homeostatic changes that modify the localization and expression of Fe transporters after chronic supplementation of both micronutrients. Studies in Caco-2 cell have shown that the exposure to Zn (100 μM) improves the uptake of Fe through significant increases in the expression of DMT-1 and the pH-dependent Fe uptake [33]. Similar results were reported by Iyengar et al. [34] who showed that supplementation of Fe

and Zn increases ferroportin expression and the apical localization of DMT-1 through an increase in RNA-bound form of Fe-responsive proteins (IRP-1 and IRP-2) and in the expression of IRP-2. In contrast, Fe supplementation decreases the RNA-bound form of IRP proteins and does not have a significant effect either on the expression of IRP-2 or on increasing the localization of DMT-1 in the apical duodenum [34].

On the other hand, as we expected, we found that the Fe- and Zn-supplemented group showed higher levels of serum Zn compared to the Fe alone and placebo groups. Similar results were reported in Brazilian women who received a daily supplement with 25 mg of Zn plus 25 mg of Fe during 8 weeks [35].

Our study showed that the Fe supplementation had no significant effect on Fe status. Moreover, in this group, the prevalence of women with normal Fe status increased from 71.4 to 82.1 % at the end of the study; nevertheless, these changes were not significant. These results could be due the low IDA prevalence in the participants. In Chile, the last National Health Survey showed a prevalence of anemia of 5 % in women of childbearing age [36], which is possibly due to the country's mandatory fortification of flour with Fe, as ferrous sulfate, which has an absorption of ~10 % [2, 37, 38]. An average daily intake of 239 g of fortified bread has been reported for Chilean women of childbearing age [39]. A second possible contributor to the observed low levels of anemia and to negative results of Fe supplementation in this study could be the increase in meat consumption over the last decade (2001–2011), considered a good source of both minerals (Fe and Zn), at a rate of 1.9 % per year, reaching a consumption of 84.7 kg per person per year [40].

The strengths of our study include the randomized controlled trial (RCT) design and the inclusion of personnel who visited the participants' homes on a daily basis to deliver the supplement and verify its consumption.

Also, the estimation of total body Fe provides us information about early changes of Fe status.

Unlike several studies that have reported a negative interaction in the absorption of Fe and Zn, we observed that the supplementation of 30 mg of Fe plus 30 mg of Zn improved Hb and total body Fe levels in childbearing age women. In this sense, more research is needed to corroborate our results in Fe- and Zn-deficiency populations.

Conclusion

Daily supplementation with 30 mg of Fe plus 30 mg of Zn for 3 months significantly improved Fe and Zn status of women, compared with those receiving placebo. The positive effect of Fe supplementation on Fe status is enhanced by combined Zn supplementation.

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