

## Iron and Vitamin C Co-Supplementation Increased Serum Vitamin C Without Adverse Effect on Zinc Level in Iron Deficient Female Youth

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### ABSTRACT

**Background:** Iron supplementation can decrease the absorption of zinc and influence other antioxidants levels such as vitamin C. This study aimed to investigate the effect of iron supplements alone and in combination with vitamin C on zinc and vitamin C status in iron deficient female students.

**Methods:** In a double-blind randomized clinical trial, 60 iron deficient students were selected from 289 volunteers residing in dormitory. After matching, subjects were randomly assigned into two groups: Group I (50 mg elemental iron supplements) and Group II (50 mg elemental iron + 500 mg ascorbic acid). Serum ferritin, iron, serum zinc, and plasma vitamin C concentrations were measured by using enzyme-linked immunosorbent assay, spectrophotometer, atomic absorption spectrometer, and colorimeter, respectively after 6 and 12 weeks supplementation. Student's *t*-test and repeated measures analysis of variance were applied to analyze the data using SPSS software.

**Results:** Serum zinc levels had no significant differences between 2 groups at the baseline; however, its concentration decreased from  $80.9 \pm 4.2$ - $68.9 \pm 2.7$   $\mu\text{g}/\text{dl}$  to  $81.2 \pm 4.5$ - $66.1 \pm 2.9$   $\mu\text{g}/\text{dl}$  ( $P < 0.001$ ) in Groups I and II, respectively after 6 weeks of supplementation. Continuous supplementation increased serum zinc concentration to baseline levels ( $79.0 \pm 2.9$   $\mu\text{g}/\text{dl}$ ;  $P < 0.01$ ) in Group I and  $70.5 \pm 3.1$   $\mu\text{g}/\text{dl}$  in Group II following 12 weeks of supplementation. Plasma vitamin C increased from  $3 \pm 0.1$ - $3.3 \pm 0.2$   $\text{mg}/\text{dl}$  to  $2.7 \pm 0.1$ - $4.2 \pm 0.2$   $\text{mg}/\text{dl}$  ( $P < 0.01$ ) in Groups I and II, respectively. At the end of study, plasma vitamin C significantly increased from  $3.3 \pm 0.3$ - $4.7 \pm 0.3$  ( $P < 0.01$ ) to  $4.2 \pm 0.2$ - $7.1 \pm 0.2$  ( $P < 0.001$ ) in Groups I and II, respectively.

**Conclusions:** Iron supplementation with and without vitamin C led to reduction in serum Zn in iron-deficient female students after 6 weeks. However, the decreasing trend stops after repletion of iron stores and Zn levels returned to the approximately baseline values after 12 weeks.

**Keywords:** Female, iron deficiency, iron supplementation, serum zinc, vitamin C

## INTRODUCTION

Iron deficiency anemia (IDA) and zinc deficiency are the widespread nutritional problems with high prevalence in women of reproductive age living in developing countries such as Iran.<sup>[1-3]</sup> The prevalence of IDA was found to be 16.6% in Iranian women of childbearing age and was not significantly different between rural and urban settings.<sup>[4,5]</sup> In humans, iron is an essential component of proteins involved in oxygen transport. It is also essential for the regulation of cell growth and differentiation.<sup>[6,7]</sup> Zinc presents in the zinc fingers of DNA, RNA, and enzymes structures and is required for the essential body's biochemical reactions.<sup>[8-11]</sup> Iron supplementation is suggested by health organizations as an effective strategy to prevent iron deficiency and anemia specially in developing world, whereas there is no regulated policy to prevent other elements' deficiency such as zinc.<sup>[12,13]</sup> On the other hand, interventions to combat mild Fe deficiency in women of childbearing age may affect Zn nutriture.<sup>[14]</sup> Interaction between iron and zinc has been shown during intestinal absorption in animal models.<sup>[15-17]</sup> Therefore, excess iron supplementation interfere intestinal absorption or plasma diffusion of bivalent elements presenting in foods (such as zinc) and results in deficit of other trace elements. It could cause impaired zinc absorption, too.<sup>[18-21]</sup>

Currently, it has been suggested that in addition to possible interaction between iron and other elements like zinc, accumulation of iron in tissues could increase oxidative stress. Moreover, higher level of iron has been known as a major source of free radicals production<sup>[22,23]</sup> and the measurement of plasma antioxidants like vitamin C determines the effects of iron on free radicals production.<sup>[24,25]</sup> Until now, some studies have been shown that ascorbic acid acts as an antioxidant in the presence of high level of iron and traps free radicals, and prevents the diffusion of these components to membrane of red blood cells and low density lipoprotein particles.<sup>[26,27]</sup>

Regarding the importance of zinc and its interaction with iron, the effect of excess iron on elevation of oxidative stress, and production of free radicals, we aimed to evaluate the effect of iron supplementation alone and iron with vitamin C on zinc and vitamin C status in iron deficient female students.

## METHODS

This study was a double-blinded clinical trial. The required sample size was determined using serum levels of ferritin as a key dependent variable.<sup>[10]</sup> The study power was considered 80%. Because of dropping samples during the study, 30 subjects were considered for each group at the start of the study. Two hundreds eighty-nine students enrolled from the Al-Zahra Dormitory Complex affiliated to Shahid Beheshti University of Medical Sciences. All participants agreed to attend in the screening phase of the study in written consent form. Detailed information about age, height, weight, body mass index (BMI), history of disease, menstrual status, medication, and supplement use were collected by completing questionnaire. Venous blood in fasting state (5 ml) was drawn and divided into parts: 1 ml transferred into tubes (containing 0.2 cc ethylenediaminetetraacetic acid 5%) and 4 ml was collected in hemolysis tubes and was transferred to the Nutritional Research Lab of the National Nutrition and Food Technology Research Institute. Hemoglobin, hematocrit, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and ferritin were measured in this phase. Sixty nonanemic iron deficient volunteers with hemoglobin levels higher than 12.5 mg/dl and serum ferritin levels < 23 ng/ml<sup>[28]</sup> were selected in our study. Subjects were attended in information session and signed written consent form. Participants with history of thalassemia, diabetes, gastrointestinal, liver, kidney, inflammatory, and infectious diseases were excluded from the study. Furthermore, participants who were not interested to continue consuming less 75 out of 90 capsules during the study period, were withdrawn from the study. After matching, 60 subjects based on their age, BMI, serum ferritin were allocated in 2 groups: Receiving iron (50 mg/d elemental iron; Group I) and iron + vitamin C (50 mg/d elemental iron + 500 mg/d vitamin C; Group II) for 12 weeks. Supplements were ferrous fumarate with or without vitamin C made in Iran Daru Company and similar in shape, color, and taste.

To determine serum levels of iron, zinc and plasma levels of vitamin C, 10 ml venous blood samples were drawn at the beginning, week 6 and 12 of intervention. Hemoglobin, ferritin, iron, vitamin C, and zinc were quantified using cell counter, enzyme-linked immunosorbent assay (commercial kits, RADIM, Italy), spectrophotometry (commercial kit, Zeist

Chemistry), colorimeter (using DNPH 4, 2),<sup>[29]</sup> and atomic absorption spectrometer, respectively.

Energy, iron, zinc, vitamin C, and other macro and micronutrients intake were estimated using three 24-h dietary recalls (at baseline, week 6 and 12) throughout the study. Dietary data was analyzed using a widely used nutritional software package (Food Processor II Windows v. 7.6; ESHA Research, Salem, OR). Physical activity of participants was assessed using validated questionnaire about time spent on physical activities and leisure time activities at baseline and at the end of the study.<sup>[30]</sup>

### Statistical analysis

Statistical analysis was performed using SPSS for Windows (version 17; SPSS Inc., Chicago, IL, USA). Kolmogorov-Smirnov test was applied to ensure normal distribution of variables. Quantitative variables (type of supplements and period of the study) were compared using two-way repeated measures analysis of variance. In addition, quantitative variables of each group were compared using paired *t*-test at the beginning, week 6 and 12 of the study. To manage any interfering variables, BMI, physical activity, iron, and vitamin C intake were considered as a covariance in the model.  $P < 0.05$  was considered as significant.

## RESULTS

From 30 subjects allocated to each group, 3 subjects from Group I (iron) and 5 subjects from Group II (iron + vitamin C) were excluded due to the irregular use of supplements or health complications. Therefore, final analyses were done on 27 and 25 subjects in intervention groups, respectively [Figure 1].

Mean of age, height, weight, BMI, and selected nutrient intakes of the subjects at the baseline are presented in Table 1. There were no significant differences for these variables between Groups I and II.

Mean of physical activity were not different between Groups I and II at the beginning of the study, too ( $10.16 \pm 1.9$  and  $14.6 \pm 2.8$  h/week, respectively). Baseline relative frequencies of subjects with serum zinc levels lower than  $70 \mu\text{g}/\text{dl}$  are shown in Figure 2. Serum levels of zinc lower than normal status were found in 14.3% and 16.7% of subjects, respectively.

The mean and standard error of mean for hemoglobin, serum iron, ferritin, zinc and vitamin C

**Table 1:** Baseline characteristics and selected nutrient intakes of participants in each intervention group

	Groups (n=30)	
	I (iron)	II (iron+vitamin C)
Age (year)	20.5±1.5	21.1±2.2
Height (cm)	160.8±5.9	159.5±6.8
Weight (kg)	55.4±6.8	55.3±6.5
BMI (kg/m <sup>2</sup> )	21.4±2.5	21.8±2.5
Energy (kcal/day)	1941.6±495.1	2110.6±788.5
Protein (g/day)	65.9±21.4	61.6±28.2
Carbohydrate (g/day)	261.7±94.5	295.8±123.0
Fat (g/day)	71.8±22.1	79.3±36.4
Vitamin C (mg/day)	77.4±42.6	90.8±57.6
Iron (mg/day)	13.2±3.9	18.6±13.5
Zinc (mg/day)	10.3±3.6	9.87±4.5

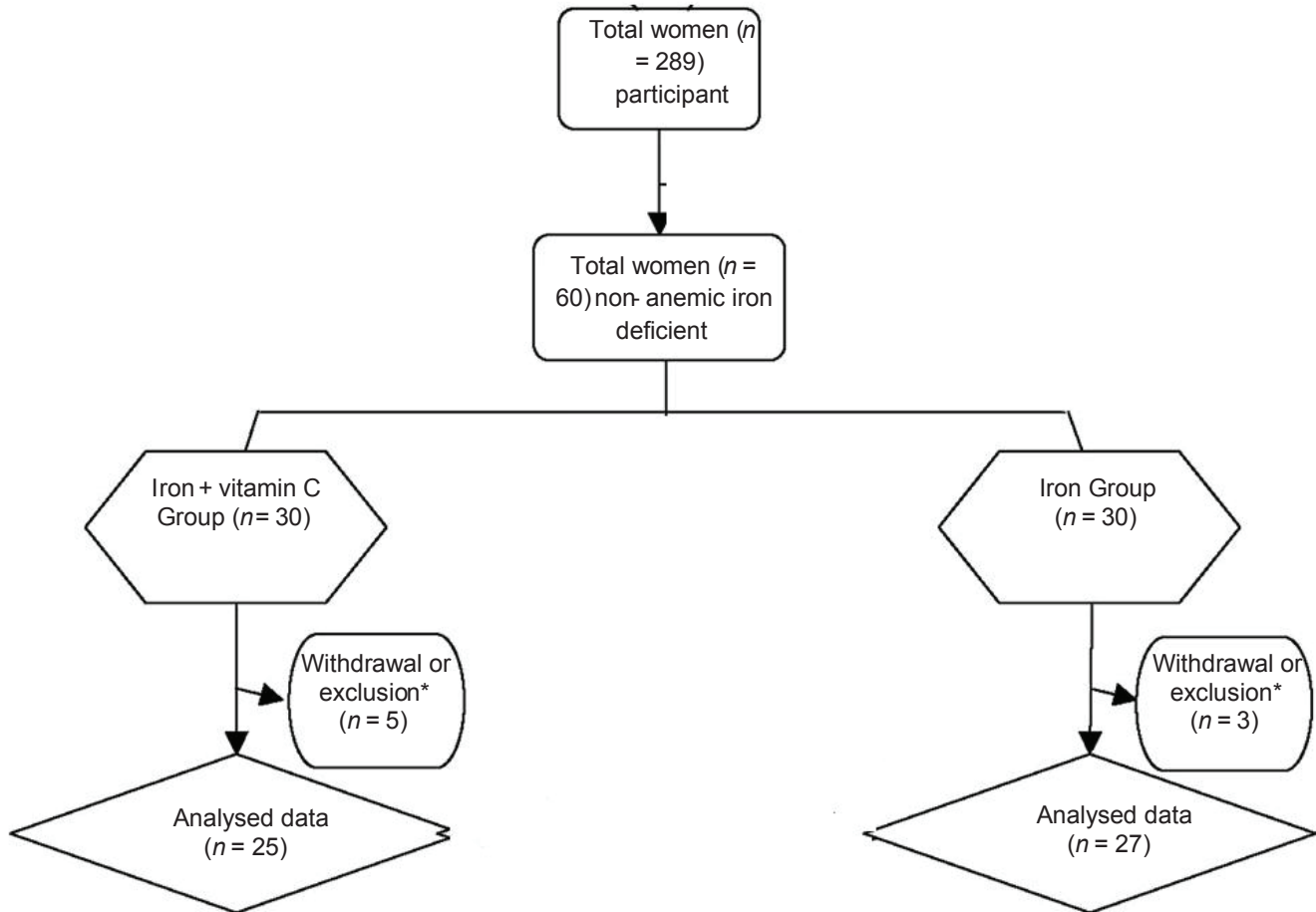
No significant differences between two groups. BMI=Body mass index

before and after intervention were shown in Table 2. Mean levels of hemoglobin in week 6 significantly increased compared with the beginning of the study in both groups; however, its level decreased at the end study compared with mid-term of the study. Hemoglobin level at the end of the study was significantly higher than baseline levels ( $P < 0.001$ ) and was not significantly different between two groups.

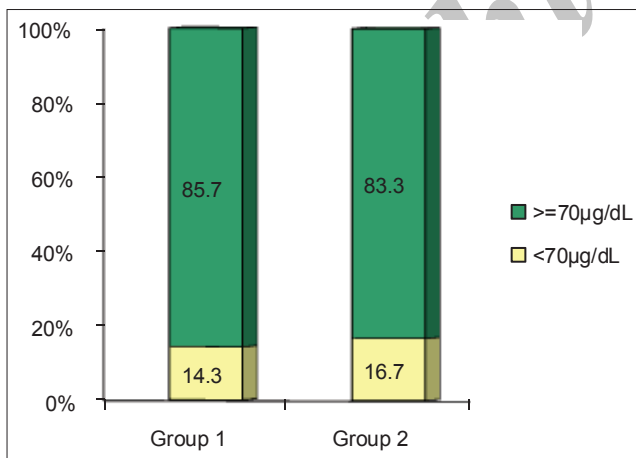
Serum levels of iron after 6 weeks supplementation was significantly higher than baseline levels in both groups. Serum levels of ferritin significantly increased after 6 weeks supplementation compared to baseline ( $P < 0.001$ ). Continuous supplementation for 12 weeks significantly increased serum ferritin level of Group II, but not in Group I.

Mean plasma vitamin C values increased significantly in Group I after 6 weeks supplementation ( $P < 0.01$ ), but this increase was not significant in Group II. At the end of the study, plasma vitamin C was significantly increased compared to week 6 in both groups ( $P < 0.001$ ). There was no significant difference at the beginning of study in terms of vitamin C.

Serum zinc levels had no significant differences at baseline in both groups. Its level decreased significantly after 6 weeks supplementation ( $P < 0.001$ ), however, after 12 weeks supplementation significantly increased to baseline levels in Group I, but not in Group II [Table 2].



**Figure 1:** Recruitment, withdrawals, exclusions, and intervention group numbers



**Figure 2:** Relative frequency of subjects with serum zinc levels lower than normal at the beginning of the study

## DISCUSSION

Baseline anthropometric profile and age of subjects had no significant differences in both groups. In addition, mean of energy, protein, iron, and vitamin C intake were not different between

groups. Therefore, we were able to compare interested variables in our study.

We found a significant reduction of serum zinc levels after 6 weeks of supplementation. Haidar *et al.* showed that serum zinc of pregnant women significantly decreased when they received iron supplements for 3 months.<sup>[31]</sup> In agreement with our findings Seyyed Shariat- Doust *et al.* have reported that iron supplementation of healthy women (hemoglobin 13.2 g/dl) caused significant decrease of serum zinc levels.<sup>[32]</sup> In addition, in a study by Ziaei *et al.*, iron supplementation in pregnant women with hemoglobin > 13.2 g/dl reduces serum levels of copper and zinc.<sup>[33]</sup> O'Brien *et al.* observed similar findings, too.<sup>[18]</sup> In contrast, Harvey and his colleagues' findings showed no reduction in serum zinc of pregnant women after iron supplementation.<sup>[34]</sup> In addition, Falahi *et al.* have reported iron supplementation for 4 months increased serum levels of zinc.<sup>[35]</sup> However, in most of the studies, iron supplementation did not affect the biochemical status of zinc, but the data are not clear regarding morbidity outcomes.<sup>[36-38]</sup> On the

**Table 2:** Mean and standard error of hemoglobin, serum iron, ferritin, zinc and vitamin C of subjects during different periods between groups

Indices	Group	No.	Time (week)*			P value		
			0	6	12	Time	Group	Time x group
Hemoglobin (g/dl)	I (Fe)	27	13.5±0.2 <sup>a*</sup>	14.3±0.2 <sup>b</sup>	13.9±0.1 <sup>c</sup>	0.001		0.64
	II (Fe+vitamin C)	25	13.2±0.2 <sup>a</sup>	13.8±0.2 <sup>b</sup>	13.6±0.1 <sup>c</sup>	0.001		
	Total	52	13.3±0.1 <sup>a</sup>	14±0.1 <sup>b</sup>	13.7±0.1 <sup>c</sup>	0.001	0.12	
Serum iron (µg/dl)	I (Fe)	27	69.3±5.5 <sup>a*</sup>	95.8±7.6 <sup>b</sup>	86.6±7.5 <sup>b</sup>	0.001		0.95
	II (Fe+vitamin C)	25	68.8±5.5 <sup>a</sup>	92.9±8 <sup>b</sup>	88.3±8 <sup>b</sup>	0.001		
	Total	52	69.0±3.8 <sup>a</sup>	94.4±5.5 <sup>b</sup>	87.4±5.5 <sup>b</sup>	0.01	0.93	
Ferritin (ng/dl)	I (Fe)	27	16.0±1.5 <sup>a*</sup>	37.9±2.6 <sup>b</sup>	40.8±3.6 <sup>b</sup>	0.001		0.12
	II (Fe+vitamin C)	25	14.9±1.6 <sup>a</sup>	38.8±2.7 <sup>b</sup>	47.2±3.7 <sup>c</sup>	0.001		
	Total	52	15.5±1.1 <sup>a</sup>	38.4±1.93 <sup>b</sup>	44.1±2.6 <sup>c</sup>	0.01	0.53	
Vitamin C (mg/l)	I (Fe)	27	3.0±0.1 <sup>a</sup>	3.3±0.2 <sup>b</sup>	4.7±0.3 <sup>c</sup>	0.001		0.01
	II (Fe+vitamin C)	25	2.7±0.1 <sup>a</sup>	4.2±0.2 <sup>a</sup>	7.1±0.3 <sup>b</sup>	0.001		
	Total	52	2.9±0.1	3.8±0.1	5.9±0.2	0.01	0.01	
Zn (µg/dl)	I (Fe)	27	80.9±4.2 <sup>a</sup>	68.9±2.7 <sup>b</sup>	79.0±2.9 <sup>a</sup>	0.345		0.619
	II (Fe+vitamin C)	25	81.2±4.5 <sup>a</sup>	66.1±2.9 <sup>b</sup>	70.5±3.1 <sup>c</sup>	0.066		
	Total	52	81.1±4.3	67.1±2.8	75.0±3.0	0.016	0.365	

\*Mean value with different letters have different significance

other hand, the addition of zinc to iron folic acid supplements did not modify efficacy on iron status or improve zinc status.<sup>[39]</sup>

It has been suggested that decreased absorption of zinc after iron supplementation is the outcome of higher affinity of bivalent ions transporters (divalent metal ion transporter) to iron ion. Furthermore, it has been shown that iron could prevent diffusion and uptake of zinc from intestinal cells. Studies using animal models have shown that the higher ratio of iron to zinc has negative impact on absorbance of zinc from jejunum cells of intestine. Furthermore, there is no adverse interaction between iron and zinc within normal range of them. But at higher ratios, such as the present study, it could cause inhibitory effect on the serum zinc levels.<sup>[18]</sup>

In our study, zinc absorption improved in the presence of relatively higher levels of iron after 6 weeks of supplementation. The level of iron in Group I recovered to the baseline and in Group II increased compared to the beginning of the study. This elevation was higher in Group II rather than Group I. It has been suggested that iron uptake increases across intestinal cells at the lower iron levels and its absorption decreases when its level is normal. Consequently, less binding of iron to its receptor on intestinal cell membranes and less competition between zinc and iron in binding to their shared receptors results in higher zinc uptake.

Other studies have reported that iron supplementation to iron deficient individuals caused lower uptake of zinc.<sup>[40]</sup> Iron deficiency increased the inhibitory effect of iron on zinc absorption from 26 to 39% to 59-82%. It occurs after adaptation of intestinal cells in response to lower body stores of iron. Obviously, the effect of iron on uptake of zinc mostly has been reported in pregnant, breast feeding women, and athletes. As expected when iron deficiency status improves, this inhibitory effect is reduced and the serum level of zinc yields to its baseline level.<sup>[41]</sup> In addition, it has been suggested that significant increase of serum vitamin C levels after 6 weeks of iron + vitamin C supplementation could improve iron uptake and yield to lower uptake of zinc. In addition, lack of significant increase in zinc level could be the attributed to direct interaction of vitamin C and zinc. In this regard, Oladip *et al.* showed that supplementation of 200 mg vitamin C caused significant reduction of zinc uptake.<sup>[42]</sup>

The significant changes in both hemoglobin and serum iron concentrations in both groups are within normal limits, but do not seem clinically meaningful. These changes might be due to homeostatic mechanisms of iron absorption, including dietary regulator, which limits iron absorption and stores excess iron as ferritin.<sup>[43,44]</sup> Iron supplementation with iron alone

did not increase ferritin levels after 6 weeks, which can be attributed to decrease in iron absorption with duration of supplementation; However, it seems that co supplementation with iron and vitamin C had further effects on repletion of iron stores between 6 and 12 weeks supplementation.

The results of this study revealed that plasma vitamin C levels of both groups (Group I after 12 weeks and Group II after 6 and 12 weeks supplementation) increased. To the best of our knowledge, oxidative stress is associated with both iron deficiency and accumulation of iron.<sup>[22,23]</sup> Then, we quantified vitamin C level to consider the possibility of peroxidase activity of iron and accordingly reduction of vitamin C as an antioxidant. First, our findings indicated that iron supplementation improves iron deficiency and secondary did not cause excess iron accumulation. As a result, antioxidant activity of vitamin C was increased.<sup>[45]</sup> Therefore, elevation of vitamin C concentration of Group II has no conflict with our findings and it seems higher level of vitamin C of Group I relates to seasonal eating habits. First sampling was done on March, the period that vitamin C intake from foods are in its lowest level, while second sampling was done on August that consumption of vegetables and fruit (as main sources of vitamin C) is high. However, as a limitation of this study, our 24-h dietary recalls did not confirm it.

## CONCLUSIONS

Overall finding of this study confirmed that iron supplementation alone and combined with vitamin C causes reduction of serum zinc levels after 6 weeks; however, by repairing the iron stores, its absorption improves. In addition, iron supplementation for 12 weeks has no adverse effect on plasma vitamin C.

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