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## Iron Absorption From Elemental Iron-Fortified Corn Flakes In Humans. Role of Vitamins A and C<sup>1–3</sup>

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### Abstract

The objective of this study was to determine relative iron absorption from reduced iron-fortified Corn Flakes and the role of vitamins A and C improving absorption. The protocol included 87 subjects who received 4 meals composed of cereal, milk and sugar. Iron and different concentrations and combinations of vitamins A and C were added. Iron absorption was measured calculating radioactive iron incorporation into the blood of subjects. There was a significant 3.6-times increase in iron absorption when both vitamins were administered together. *In vitro* solubility tests with ferric chloride, electrolytic and reduced irons showed an important role of vitamins A and C enhancing iron solubility at pH 6 to the values found at pH 2. Addition of vitamins A and C to a ready-to-eat cereal significantly improves iron absorption by a process at least partially due, to the solubilizing effect of these vitamins on reduced iron. Addition of both vitamins in the same meal produced an increment in absorption corresponding to the additive factor of each vitamin. © 2003 Elsevier Inc. All rights reserved.

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### 1. Introduction

Iron deficiency and anemia are important problems affecting billions of people through the world. These are more prevalent in underdeveloped countries, during rapid growth periods

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and when requirements and/or losses are higher. For these reasons, vulnerable groups to develop iron deficiency and anemia are infants, young children, adolescents, women of reproductive age and pregnant women [1]. Strategies for combating iron deficiency include control of parasitic infections, improvement of sanitation, iron supplementation and iron fortification [2]. Of these strategies, iron fortification of basic foods is the most economical and more convenient approach and has the advantage that it does not require food habit modifications.

There are some important steps for a fortification program to be successful. Two of the key issues are the food vehicle and the iron compound. The food vehicle selected should reach the entire population and deliver most of the calories of the diet. It has to be consumed daily but at the same time with no risk of excessive consumption. In underdeveloped countries cereal flours, especially wheat and corn, are frequently used as fortification vehicles, because grain products are the staple foods for that populations [3]. Other common fortified foods are ready-to-eat and infant cereals, which in industrialized countries could provide a significant amount of iron [4] and its bioavailability could be significantly increased with the inclusion of vitamin C and, as reported recently, of vitamin A in the fortification formula [5,6].

The selection of the iron compound for fortification is important in order to avoid interactions of iron with the food vehicle or the total meal, because a minor change in organoleptic characteristics of the food will result in consumers' rejection. When the iron compound is added, it is necessary to evaluate possible changes in food color, taste or appearance with time and storage on adverse temperature and humidity conditions. Solubility, chemical reactivity, bioavailability and cost are other important issues when selecting an iron compound. For instance, ferrous sulfate is a highly bioavailable and relatively inexpensive compound, but because of its reactivity produces undesirable changes in some fortified foods. On the other hand, elemental iron (reduced, electrolytic or carbonyl) is also inexpensive but it has been reported to have a low bioavailability depending on particle size and the food vehicle to be fortified [7–9].

Iron compounds have been classified as water soluble (ferrous sulfate, ferrous gluconate, ferrous lactate, ferric ammonium citrate), poorly water soluble but soluble in diluted acids (ferrous fumarate, ferrous succinate, ferric sacharate), poorly soluble in water or acid solutions (ferric pyrophosphate, ferric orthophosphate, elemental iron) and protected compounds (hemoglobin, NaFeEDTA, iron-bisglycine chelate). To establish relative iron bioavailability of these compounds, absorption values are compared to a standard compound, which is ferrous sulfate, with a relative bioavailability of 100. The average relative bioavailability of reduced iron in humans shows a high variability ranging from 13 to 148 [4].

The objective of this study was to determine relative iron absorption from a ready-to-eat cereal (Corn Flakes) fortified or not with reduced iron, vitamin A and vitamin C. Iron absorption was measured without iron or vitamins addition, and with different concentrations and combinations of these nutrients (including concentrations found in commercially available Corn Flakes) to study the effect of each vitamin on reduced iron absorption and to evaluate if there is an additional enhancing effect when both vitamins are administered together. *In vitro* experiments to evaluate solubility of reduced iron, electrolytic iron and ferric chloride with pH changes in presence of vitamins A and C, were also performed.

## 2. Materials and methods

### 2.1. Human absorption studies

Iron absorption studies were performed in adult volunteers apparently in good health, although some of them presented mild to moderate iron deficiency and anemia. The subjects to study included males over 15 years of age, menopausal and child bearing age women. To the last group, a pregnancy test was performed. Each subject received 4 meals in each study and was allowed to participate in only one study. The first day of the experiment, pregnancy tests were performed and selected individuals were informed about the objectives and procedures of the study. Each volunteer signed a written consent form. The protocol was approved by the Committee for the protection of human subjects from the Venezuelan Institute for Scientific Research.

**Studies and ready-to-eat cereal:** Six studies were performed each including between 11 and 20 volunteers. Each person received 4 meals (denoted as 1-4) except for study 2, which included only 3 meals. The ready-to-eat cereal administered was Corn Flakes (W.K. Kellogg Institute, Battle Creek, Michigan) prepared according to the standard product fortification profile for Venezuela, which includes 10 micronutrients. Fortification profile (except for iron, vitamin A and vitamin C) per 100 g Corn Flakes was: vitamin B1 as thiamin mononitrate 0.93 mg, vitamin B2 as riboflavin 1.43 mg, vitamin B6 as pyridoxine hydrochloride 1.7 mg, vitamin B12 as cyanocobalamin 1.7 mg, niacin as niacinamide 15.8 mg, folic acid 667  $\mu\text{g}$  and zinc oxide 5 mg. Two lots of cereals were prepared: lot one contained Corn Flakes fortified with all vitamins and minerals except for iron, vitamin A and vitamin C and lot 2 was prepared without vitamins A and C.

Each meal contained 30 g of cereal, 120 mL of skim milk and 5 g of sugar. Studies 1 and 2 were performed with cereal from lot I, which contains neither added iron, nor vitamins A and C, while meals for studies 3-6 were prepared with Corn Flakes from lot II, with reduced iron but without vitamins A or C. The missing nutrients were added, at different combinations and concentrations, at our laboratory. Table 1 shows iron, vitamin A and vitamin C additions for each study. Study 3 meal 4 contained the concentration of iron, vitamin A and vitamin C usually added to the commercial product in Venezuela. Iron content of Corn Flakes from lot I was 0.15 mg/30g product, and from lot II (studies 3-6) was 3.5 mg/30 g, as reported by manufacturer. This value was confirmed at the laboratory by the digestion method [10].

It is important to highlight that the technical difficulties to obtain intrinsically labeled reduced iron and the relative insolubility of this iron source, does not allow making conclusions about absolute absorption values. The extrinsic radio isotopic labeling using  $^{59}\text{Fe}$  or  $^{55}\text{Fe}$  as ferric chloride, will uniformly label the non-heme iron pool, which includes the native iron and the reduced iron that has been solubilized. The remaining fraction can not be measured. The administration of a basal food without vitamin additions (as meal 1 for each of the 6 studies) allows to use each individual as its own control and to draw conclusions about the effect of vitamins A and C on the absorption of non-heme iron pool.

**Protocol:** MEAL 1, tagged with  $^{59}\text{Fe}$  (0.9  $\mu\text{Ci}/\text{person}$ ), was administered to the subjects after an overnight fast. MEAL 2, also extrinsically labeled, but with  $^{55}\text{Fe}$  (1.3  $\mu\text{Ci}/\text{person}$ ),

Table 1  
Iron, vitamin A and vitamin C added to a cereal portion (30 g)

	Vitamin A ( $\mu\text{g}$ RE/serving)	Vitamin C (mg/serving)	Iron (mg/serving)
STUDY 1			
Meal 1	0	0	0
Meal 2	0	0	3.5
Meal 3	150	0	0
Meal 4	0	15	0
STUDY 2			
Meal 1	0	0	0
Meal 2	0	0	3.5
Meal 3	150	15	0
STUDY 3			
Meal 1	0	0	3.5
Meal 2	150	0	3.5
Meal 3	0	15	3.5
Meal 4	150	15	3.5
STUDY 4			
Meal 1	0	0	3.5
Meal 2	300	0	3.5
Meal 3	0	15	3.5
Meal 4	300	15	3.5
STUDY 5			
Meal 1	0	0	3.5
Meal 2	150	0	3.5
Meal 3	0	30	3.5
Meal 4	150	30	3.5
STUDY 6			
Meal 1	0	0	3.5
Meal 2	300	0	3.5
Meal 3	0	30	3.5
Meal 4	300	30	3.5

was fed 4 hours later. No food or drink (except for water) was allowed between MEALS 1 and 2, and 4 hours after administration of MEAL 2. The protocol for the administration of radioactive food in the morning after an overnight fast and the afternoon of the same day was based on experiments previously published [11].

On day 15, blood (30 mL) was drawn to determine the hematological profile (hemoglobin concentration [12], serum iron [13], unsaturated binding capacity [14] and serum ferritin concentration [15]) and to measure radioactivity incorporation into red cells. Duplicate blood (10 ml) and triplicate samples of radioactive food were prepared for radioactive counting using the technique of Dern and Hart [16,17]. Iron absorption from each meal was calculated from the radioactivity in the subject's blood using an estimation of blood volume based on sex, weight and height [18].

On the same day (day 15) MEALS 3 and 4 were administered following the same protocol as for MEALS 1 and 2. On day 30, a blood sample was taken to measure radioactivity incorporation and serum ferritin concentration.

## 2.2. Solubility of iron compounds with pH changes

The effect of vitamins A and C on iron solubility at pH 2 and 6 was measured. Three iron compounds were tested: reduced iron (Mallinckrodt Inc, St. Louis, MO. Food grade, extra fine powder US STD #325), electrolytic iron (Fortitech Inc, New York NY, Lot N° 5-99EI) and ferric chloride (Sigma Chemicals, St. Louis, MO). Reduced iron was studied because it is the compound used to fortify Corn Flakes. Electrolytic iron is a comparable form of elemental iron and ferric chloride is the chemical form of the radioactive iron used to perform absorption studies. Iron solutions of each of the compounds mentioned containing 5 mg iron, were prepared in 0.1 mol/L HCl adding 2.2  $\mu$ mol (600  $\mu$ gRE) vitamin A and/or 284  $\mu$ mol (50 mg) vitamin C. (Commercially available Corn Flakes contains per 100g: 11.7 mg iron, 500  $\mu$ gRE and 50 mg Vitamin C).

Duplicate 1-mL aliquots were taken after 30 min at room temperature to measure soluble iron at pH2, and to the remaining solution, the pH was adjusted to 6 with careful addition of NaOH. After standing 10 min at room temperature, duplicate 1-mL aliquots from the top of the solution were taken, and iron was measured for all iron compounds with different concentrations and combinations of vitamins A and C by the digestion method [10].

## 2.3. Statistical analysis

Data analysis was based in comparisons (repeated measures-ANOVA with Bonferroni as a post-test) between absorption values for the 4 meals in each study. Geometric values and standard error were calculated for all absorption data and ferritin concentrations. Absorption data was analyzed in normal and iron deficient subjects and each subject was their own control. Paired t-test was used to compare solubility of iron compounds with pH changes.

# 3. Results

## 3.1. Subjects

Anthropometric and hematological parameters were determined for each of the 87 subjects that participated in the studies (Table 2) Most of the volunteers were women with a mean age of 28 y. They were in apparent good health, but some of them presented anemia or iron deficiency. From 17 subjects in study 1, 8 were anemic (7F, 1M) and 10 were iron deficient (9F, 1M). In study 2, out of 20 volunteers, 4 presented anemia (3F, 1M) and 3 iron deficiency (2F, 1M). From 12 subjects in study 3, 1 woman was anemic and 4 were iron deficient. Study 4 included 14 subjects, 2 women and 1 man were anemic and 4 women presented iron deficiency. In study 5, out of 11 subjects, 2 presented anemia and 5 iron deficiency (all women). From 13 volunteers in study 6, 1 male and 1 female were anemic and none presented iron deficiency, indicating that anemia was probably due to infection.

Table 2

Anthropometrical and hematological characteristics of the groups studied<sup>1</sup>

	STUDY 1	STUDY 2	STUDY 3	STUDY 4	STUDY 5	STUDY 6
n	17	20	12	14	11	13
SEX	15 F, 2 M	17 F, 3 M	10 F, 2 M	8 F, 6 M	10 F, 1 M	10 F, 3 M
AGE (YEARS)	29.71 ± 12.92	29.20 ± 11.02	39.29 ± 18.37	29.79 ± 18.86	29.64 ± 6.76	32.85 ± 11.51
WEIGHT (Kg)	59.06 ± 13.15	65.50 ± 10.41	59.50 ± 9.46	58.36 ± 16.95	63.18 ± 12.88	61.54 ± 15.78
HEIGHT (cm)	156.7 ± 6.21	156.20 ± 6.72	165.4 ± 5.96	160.0 ± 10.22	166.1 ± 4.37	161.8 ± 8.58
HEMOGLOBIN (g/L)	11.85 ± 0.77	12.89 ± 1.42	13.46 ± 1.30	13.21 ± 1.37	13.09 ± 1.71	12.86 ± 0.81
HEMATOCRIT (%)	38.29 ± 1.61	42.45 ± 4.64	41.86 ± 3.42	40.57 ± 3.32	41.09 ± 4.83	40.23 ± 2.35
SERUM IRON (µg/L)	77.00 ± 33.94	110.9 ± 48.09	73.43 ± 29.21	89.71 ± 28.85	77.18 ± 30.2	95.15 ± 30.07
UIBC (µg/L)	332.10 ± 89.19	252.05 ± 52.04	293.7 ± 38.23	254.3 ± 43.55	252.3 ± 45.92	205.5 ± 41.52
TIBC (µg/L)	409.11 ± 66.17	363.45 ± 46.29	367.1 ± 36.60	343.9 ± 39.60	329.5 ± 35.24	300.7 ± 48.15
TRANSFERRIN (µg/L)	20.29 ± 11.09	30.95 ± 12.25	19.71 ± 7.73	26.14 ± 8.04	23.55 ± 9.81	31.62 ± 8.40
FERRITIN µg/L	8 (6.9–9.3)	25 (23.7–26.1)	29 (27.4–30.3)	21 (19.7–22.1)	9 (7.4–10.3)	26 (24.7–27.1)

<sup>1</sup> Values are means ± SD. For ferritin concentration values are geometric means (±1 SEM).

### 3.2. Iron absorption studies

Results from the 6 studies performed are presented in tables 3 and 4. The data presented correspond to all the subjects included in each study, regardless of their iron status.

Studies 1 and 2 included experiments with Corn Flakes without added iron, vitamin A and vitamin C. Absorption from cereal without iron was significantly lower than absorption from reduced iron fortified cereal (mean 3.38 and 9.27%, respectively). Addition of vitamin A to Corn Flakes without added iron did not improve absorption while vitamin C significantly increased absorption from native (food) iron. Study 2 (meal 3) showed that simultaneous addition of vitamins A and C to cereal without extrinsic iron, significantly increased absorption compared to meal 1 (Table 3).

Studies 3 to 6 were performed with Corn Flakes containing iron added at the factory, but no vitamins A and C, so they were added at the laboratory in different concentrations and combinations.

Table 3

Iron absorption from ready-to-eat cereal Corn Flakes (CF) prepared at factory without iron, vitamin A and vitamin C addition<sup>1</sup>

	Iron absorption (%)				
	CF	CF + 3.5 mg reduced iron	CF + 150 µgRE	CF + 15 mg Vit. C	CF + 150 µgRE + 15 mg Vit. C
Study 1	2.96 ± 1.2 <sup>c</sup>	9.74 ± 1.2 <sup>a</sup>	2.53 ± 1.2 <sup>c</sup>	6.29 ± 1.2 <sup>b</sup>	
Study 2	3.79 ± 1.1 <sup>c</sup>	8.79 ± 1.1 <sup>a</sup>			5.82 ± 1.1 <sup>b</sup>

<sup>1</sup> Values are means ± SE. (n = 17 and 20 for studies 1 and 2, respectively). Means with dissimilar letters are significantly different p < 0.05.

Table 4

Iron absorption from ready-to-eat cereal Corn Flakes (CF) prepared at factory without vitamin A and vitamin C addition<sup>1</sup>

	Proportion of vitamins A and C	Iron absorption (%)			
		CF	CF + 150 $\mu$ gRE	CF + 15 mg Vit. C	CF + 150 $\mu$ gRE + 15 mg Vit. C
Study 3	Similar to commercial CF	6.4 $\pm$ 1.1 <sup>c</sup>	11.4 $\pm$ 1.2 <sup>b</sup>	11.9 $\pm$ 1.1 <sup>b</sup>	22.8 $\pm$ 1.1 <sup>a</sup>
Study 4	Double amount of vitamin A	7.5 $\pm$ 1.2 <sup>b</sup>	18.6 $\pm$ 1.1 <sup>a</sup>	15.7 $\pm$ 1.2 <sup>a</sup>	25.2 $\pm$ 1.2 <sup>a</sup>
Study 5	Double amount of vitamin C	7.5 $\pm$ 1.2 <sup>b</sup>	17.9 $\pm$ 1.2 <sup>a</sup>	25.4 $\pm$ 1.2 <sup>a</sup>	32.1 $\pm$ 1.2 <sup>a</sup>
Study 6	Double amount of vitamins A and C	6.1 $\pm$ 1.2 <sup>b</sup>	19.8 $\pm$ 1.1 <sup>a</sup>	20.5 $\pm$ 1.1 <sup>a</sup>	23.2 $\pm$ 1.1 <sup>a</sup>

<sup>1</sup> Values are means  $\pm$  SE. (n = 12, 14, 11 and 13 for studies 3, 4, 5 and 6, respectively). Means with dissimilar letters are significantly different  $p < 0.05$  for the same study.

In study 3 the breakfast administered contained the same concentration of vitamins A and C as commercially available Corn Flakes (meal 4). With this formulation there was a 3.6-fold increase in iron absorption compared to no vitamins addition (meal 1). When vitamin A or vitamin C were added separately, absorption was similar for both vitamins but significantly higher than absorption from meal 1, without vitamins addition (Table 4).

In study 4, meals 2 and 4, vitamin A concentration was doubled, compared to the concentration present in commercial Corn Flakes. Iron absorption increased significantly when vitamin A was present compared to the breakfast without added vitamins. However, as presented in Table 4, the inclusion of double amount of vitamin A produced a slight increase in absorption compared to the absorption from single doses of vitamin A (study 3, meal 2 in both studies). Absorption from meal 4 with vitamin C and double amount of vitamin A, was not different than absorption with a single doses of vitamin A.

When vitamin C concentration was increased from 15 to 30 mg/serving, (study 5, meals 3 and 4) iron absorption increased significantly compared to the basal breakfast with no vitamin addition (meal 1). Likewise, absorption from meal 3 in study 5 was substantially higher than the same meal in study 3 (single doses of vitamin C). Also, absorption was higher from meal 4 study 5, than from the same meal in study 3.

In study 6, meals 2 to 4, vitamin A and vitamin C concentrations were doubled (300  $\mu$ g RE/30 g cereal for vitamin A and 30 mg/30 g cereal for vitamin C) compared to the concentrations present in commercial Corn Flakes. As in the other studies included in Table 4, inclusion of either vitamin A or C, significantly increased iron absorption compared to meal 1, where no vitamins were added. However, differences when both vitamins were administered at double concentration in the same meal, were no different from absorption values obtained from Corn Flakes enriched with both vitamins in single doses.

Table 5

Effect of vitamin A (VA) and vitamin C (VC) on *in vitro* solubility of reduced iron, electrolytic iron and ferric chloride with pH changes from 2 to 6<sup>1,2</sup>

Iron compound <sup>2</sup>	Soluble Fe (mg) pH 2	Solubility (%) Compared to 5 mg Fe	Soluble Fe (mg) pH 6	Solubility (%) Compared to 5 mg Fe	Solubility (%) Compared to pH2
Reduced iron	1.32 ± 0.18 <sup>c</sup>	26.35	0.64 ± 0.21 <sup>b</sup>	12.88	48.86
Reduced iron + VA	1.35 ± 0.29 <sup>c</sup>	26.91	1.22 ± 0.24 <sup>c</sup>	24.47	90.93
Reduced iron + VC	1.34 ± 0.26 <sup>c</sup>	26.89	1.57 ± 0.55 <sup>c</sup>	31.43	116.90
Reduced iron + VA + VC	1.39 ± 0.29 <sup>c</sup>	27.86	1.40 ± 0.20 <sup>c</sup>	28.00	100.51
Electrolytic iron	2.76 ± 0.26 <sup>b</sup>	55.22	0.79 ± 0.20 <sup>b</sup>	15.87	28.73
Electrolytic iron + VA	2.86 ± 0.29 <sup>b</sup>	57.20	1.16 ± 0.51 <sup>e</sup>	23.13	40.44
Electrolytic iron + VC	2.94 ± 0.32 <sup>b</sup>	58.72	3.37 ± 0.65 <sup>d</sup>	67.32	114.65
Electrolytic iron + VA + VC	2.93 ± 0.34 <sup>b</sup>	58.60	2.98 ± 0.38 <sup>d</sup>	59.56	101.64
Ferric chloride	5.14 ± 0.20 <sup>a</sup>	102.84	0.32 ± 0.08 <sup>c</sup>	6.36	6.18
Ferric chloride + VA	5.15 ± 0.21 <sup>a</sup>	102.95	0.64 ± 0.30 <sup>b</sup>	12.88	12.51
Ferric chloride + VC	5.23 ± 0.25 <sup>a</sup>	104.56	4.86 ± 0.20 <sup>a</sup>	97.12	92.88
Ferric chloride + VA + VC	5.24 ± 0.06 <sup>a</sup>	104.75	4.93 ± 0.23 <sup>a</sup>	98.52	94.05

<sup>1</sup> Values are means ± SD, n = 6. Means in a column with dissimilar letters are significantly different p < 0.05.

<sup>2</sup> Initial iron concentration tested for all iron compounds: 5 mg.

### 3.3. Solubility tests

As shown in Table 5, solubility of reduced iron, electrolytic iron and ferric chloride at pH 2, is significantly different. For reduced iron only 27% of the iron was soluble after 30 min in 0.1 mol/L HCl, while 55% of the iron from electrolytic iron and 100% from ferric chloride were soluble under the same conditions. For the 3 compounds tested, raising the pH to 6 produced a further decrease on the percentage of soluble iron respect to the amount of iron initially added. The reduction on the percentage of soluble iron at pH 6 compared to the percentage of soluble iron at pH 2, was only 13.5% for reduced iron, 39.4% for electrolytic iron and 96.5% for ferric chloride.

The inclusion of vitamin A, vitamin C or mixtures of both vitamins in concentrations similar to those used in commercial Corn Flakes, produced significant increments in iron solubility. Vitamin A produces a 1.9-fold increase in solubility from reduced iron, while addition of vitamin C alone or in combination with vitamin A maintained 100% of the iron in a soluble form.

For electrolytic iron the addition of vitamin A before raising the pH to 6, produced a 1.4-fold increment in solubility compared to no vitamin A addition. As with reduced iron, solubility of electrolytic iron in the presence of vitamin C or mixtures of vitamins A and C, did not change with pH changes, remaining completely soluble.

The loss of iron solubility with pH changes was higher from ferric chloride, in spite of being the most soluble of the compounds tested at pH 2. However the inclusion of vitamin A was able to avoid precipitation of half of the iron, and vitamin C or mixtures of vitamin A and vitamin C maintained 100% of the iron in solution.

#### 4. Discussion

The most cost-effective intervention to increase iron intake is fortification. As iron deficiency and anemia are more prevalent in children, among other susceptible groups, targeted fortification of certain food items consumed by this age group, could help to reduce iron deficiency. Consumption of fortified ready-to-eat cereals would increase the intake of iron and vitamins and do not represent a risk of over consumption in adult men whose requirements are lower [19].

The balance between an iron compound with high bioavailability but also highly reactive, and compounds like elemental iron almost inert but poorly available, is an important issue to consider in food fortification, depending on the food vehicle selected. Ferrous sulfate is considered the gold standard regarding bioavailability, but it produces unacceptable changes in food color and taste.

Elemental irons as well as water insoluble sources such as ferric pyrophosphate and orthophosphate, have been used for many years to fortify foods, mainly because they do not change physical properties of foods. They are the iron compounds used in most of the countries to fortify wheat flour. In Mexico, a program to add nutrients to corn and wheat flours, promoted by the Ministry of Health, recommended extra fine reduced iron (30 mg/Kg), based on “absorption level, potential interactions among nutrients and a wide range of security to eliminate the risk of an adverse effect on health” [20].

There is a considerable body of evidence suggesting that bioavailability of elemental iron powders is low [21]. As has been demonstrated, bioavailability of elemental iron powders depends in part on the particle size and manufacturing processes. Cook et al. in 1973, demonstrated that reduced iron had a bioavailability comparable to that of ferrous sulfate (8.6 vs. 9.1%), when used to fortify wheat rolls, provided that iron of small particle size, prepared at their laboratory, was used [22].

Storage of elemental iron powders up to 9 months had no effect on organoleptic characteristics of wheat flour or relative biological values in rats. As particle size increases relative bioavailability decreases for reduced iron but not for electrolytic or carbonyl iron [23]. There are other evidences showing that decreasing particle size of an iron source, improves its bioavailability and maintains organoleptic properties in accelerated stability tests [24].

In other studies, the bioavailability of hydrogen-reduced iron was comparable to ferrous sulfate, when using a stable isotope-enriched form of hydrogen-reduced iron powder added to bread and fed to non-anemic female volunteers. Percentage absorption from 3 mg dose of iron as ferrous ascorbate was 64.8% and from 1 mg dose of reduced iron baked in a bread roll was 49.7% [25]. However, it has been reported that bioavailability of hydrogen-reduced iron powders in humans relative to ferrous sulfate, ranges from 13 to 148% [4].

There is still controversy about bioavailability of elemental iron due to the disparity of the results reported in the literature and the lack of information about physical and chemical characteristics of the iron powders used. Pla et al [26] found good correlation between solubility and availability for hydrogen-reduced, electrolytic but to some extent carbonyl iron. Crosby [27] defined carbonyl iron as metallic, reduced iron of small particle size ( $5\mu\text{m}$ ), non toxic and as effective as ferrous sulfate. In contrast, Hallberg et al 1986 [9], reported a low and variable bioavailability of carbonyl iron, prepared by neutron irradiation, in man.

Iron absorption studies are based on the “non-heme iron pool” concept [28–31]. Labeled soluble iron sources completely exchanges and labels this pool. The difference with partially soluble iron sources, such as elemental iron, is that theoretically the dissolved part of the reduced iron enters the pool, but it is difficult to predict how much iron is soluble and remains available under certain conditions. It is also possible that the radio iron used to label the pool, will exchange with some of the insoluble reduced iron and produce falsely low absorption values [32]. As reported by Hurrell, iron sources that fail to enter the common pool because they are relatively insoluble, have an uniformly low absorption [33].

To avoid the interference of confounding factors to interpret absorption studies, such as subject to subject variations as well as meal composition, the present studies were conceived using a single meal prepared exactly under the same conditions and also repeating one of the meals without any vitamin addition (meal 1) in each of six studies, in order to use each individual as their own control.

It is important to highlight that due to the low solubility of reduced iron and because of the difficulties to obtain this compound intrinsically labeled, these studies will only provide relative values which will allow us to evidence or not an effect of vitamins A and C on reduced iron absorption from Corn Flakes, compared to a “basal” breakfast without vitamins addition.

Studies 1 and 2 were performed to evaluate absorption from reduced iron compared to native iron and the effect of vitamins A and C. Vitamin A did not show an effect increasing iron absorption when added to cereal without iron fortification. In contrast, vitamin C was able to increase iron absorption from both, food and fortification irons. Simultaneous administration of vitamins A and C to Corn Flakes without added iron (study 1), showed a significant increment in iron absorption, although it was similar to the one obtained with only vitamin C, as shown in the previous study.

Studies 3 to 6 evaluated the effect of vitamins A and C from reduced iron fortified cereal. For all the studies, mean iron absorption from meal 1 was approximately 7%. Addition of vitamins A and C, at the concentrations used in commercially available Corn Flakes, produced a 1.8 and 1.9-times increment in iron absorption, respectively. Using stable isotope techniques Fairweather-Tate et al. [34] reported that iron absorption from reduced iron fortified Corn Flakes was 14.1%, but failed to find an effect of vitamin C enhancing absorption.

The simultaneous addition of vitamin A and vitamin C to Corn Flakes, produced a 3.6-times increase on iron absorption compared to no vitamin addition and a 2-times increase compared to the absorption from the cereal fortified with only one of the vitamins. This implies that both vitamins, at the concentrations used in commercially available Corn Flakes, were capable of enhance iron absorption at the same level, thus increasing iron absorption approximately 2 times when administered separately and almost 4 times when administered together.

An increase in vitamin A concentration did not produce further significant increments in iron absorption. However, addition of double amount of vitamin C, produced a significant 3.4 and 2.1-fold increment on iron absorption, compared to no vitamin C addition and a single doses, respectively. At the concentrations studied, vitamin C showed a stronger enhancing effect than vitamin A.

It is interesting to highlight that in studies 4,5 and 6, differences were no statistically significant between addition of vitamin A, C or both, when double amount of vitamins were used. This is different from the results obtained in study 3, since absorption in presence of vitamin A + vitamin C was significantly higher than absorption when only one of the vitamins was present.

It can be noticed that except for the inclusion of double amount of vitamin C, increases in vitamin concentration did not significantly increase iron absorption, neither the doubled vitamin alone nor administered simultaneously with the other vitamin in a single doses. To evaluate the values reported in study 5, higher than in the other studies, it is important to keep in mind that almost 50% of the subjects presented iron deficiency. The mean ferritin concentration for this group was 9  $\mu\text{g/L}$ , while it was 29, 21 and 26  $\mu\text{g/l}$  for studies 3, 4 and 6, respectively.

Solubility of iron compounds in acidic solutions, even among elemental irons, is highly variable. Ferric chloride, the iron salt used for radio isotopic labeling, was very susceptible to pH changes, resulting in a decrease in solubility of almost 100% when the pH was increased to 6. This diminution in solubility was higher than the reduction reported for electrolytic or reduced irons. It is interesting to point out that reduced iron was less soluble than electrolytic iron at pH 2, but it was also more resistant to precipitate when pH was increased. Results show that the total amount of iron at pH 6 (without vitamin additions) was similar for both compounds even though the amount soluble at pH 2 was different.

In presence of vitamin C, and in a lesser extent of vitamin A, solubility of ferric chloride, reduced and electrolytic irons at pH 6, increased to 100%, that is in comparison to the values found at pH 2.

In summary, addition of vitamin A or vitamin C, in the concentrations used to fortify commercial Corn Flakes, to a ready-to-eat cereal significantly improves iron absorption by a process at least partially due to the solubilizing effect of these vitamins on reduced iron. Addition of both vitamins in the same meal produced an increment corresponding to the additive factor of each vitamin. A further increase in vitamin A concentration (to double the amount of the original formula) does not produce further significant increases on iron absorption. Increments in vitamin C, showed a significant improvement in absorption, but results from both vitamins in the same meal, were no different from the values obtained with the original concentrations of vitamins.

Our results show that the addition of vitamins A and C at certain concentrations can act synergistically as enhancers of reduced iron absorption in fortified corn flakes. A window of opportunity is open to improve fortification programs with the addition of these two vitamins in the fortification profile of staple foods in needed populations. Further studies are required to assess the impact and cost effectiveness of including them in the fortification profile of common vehicles used in iron fortification programs.

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