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Full Length Research Paper

Effect of NaFe-EDTA and vita-min A on *in vitro* iron solubility from electrolytic and reduced iron

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The objective was to evaluate *in vitro* and *in vivo* of the effect of NaFe-EDTA and vitamin A on iron solubility and absorption from corn and wheat breads fortified with electrolytic or reduced iron. Subjects (178) were randomly selected to receive breads containing electrolytic or reduced iron, NaFe-EDTA and a combination of one elemental iron and NaFe-EDTA. One meal also included vitamin A (300.3 μgRE: 1000 IU). Meals contained radioactive and 5 mg cold iron/bread. Radioactivity in blood reflected iron absorption. Serum retinol was measured by HPLC. Solubility tests were performed increasing the pH of iron solutions from 2 to 6 and measuring iron in the supernatant. NaFe-EDTA significantly ($p < 0.05$) increased absorption from electrolytic and reduced iron above the calculated expected values of the compounds administered separately. The increase ranged from 12 to 49% depending on the elemental iron tested. Addition of vitamin A further and significantly ($p < 0.05$) increased iron absorption and there was a 55.7% prevalence of vitamin A deficiency. NaFe-EDTA also increased in vitro solubility of iron and vitamin A produced further and significant ($p < 0.05$) increments. Iron absorption and solubility from electrolytic and reduced iron was significantly enhanced by the inclusion of NaFe-EDTA and vitamin A in corn or wheat breads or in vitro. Serum retinol status was low in subjects studied and iron absorption was higher in retinol deficient subjects.

Key words: Electrolytic iron, NaFe-EDTA, vitamin A, iron bioavailability, corn, wheat.

INTRODUCTION

Iron deficiency and anemia are the major nutritional deficiencies throughout the world (WHO-UNICEF 1993; Viteri, 1994) and the strategies for combating them include control of parasitic infections, especially hookworm infection, improvement of sanitation, iron supplementation and food fortification (WHO-UNICEF, 1993; INACG, 1977). Of these strategies, iron fortification of basic foods is the most economical and most convenient approach and has the advantage that it does not require changes in food habits. In underdeveloped countries cereal flours, especially wheat and corn, are frequently used as fortification vehicles, because cereals are the staple food for those populations (INACG, 1982).

There are some important steps for a fortification program to be successful. 2 of the key issues are the food vehicle and the iron compound (Longfils et al., 2008). 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.

The selection of the iron compound for fortification is important not only in terms of amount, bioavailability and cost, but also regarding its reactivity with the food matrix. It is desirable to avoid interactions with the food vehicle or the total meal, because a minor change in organoleptic characteristics of the food will result in consumers' rejection. Bioavailability is a major concern about iron compounds, since the chemical form of the compound, its particle size, as well as the food matrix or the diet composition, greatly influence its absorption.

It has been reported that the inclusion of certain iron compounds could increase iron absorption from other iron sources. That is the case of NaFe-EDTA increasing iron

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Table 1. Basic protocols for the studies of iron absorption from corn or wheat breads fortified with electrolytic iron, reduced iron, NaFe-EDTA or mixtures of one elemental iron with NaFe-EDTA. Addition of vitamin A to elemental irons and NaFe-EDTA mixtures.

	Flour	Meal 1	Meal 2	Meal 3	Meal 4
Protocol 1 Electrolytic iron NaFe-EDTA Vitamin A	Corn	5 mg	5 mg	2.5 mg 2.5 mg	2.5 mg 2.5 mg 1000 IU
Protocol 2 Electrolytic iron NaFe-EDTA Vitamin A	Wheat	5 mg	5 mg	2.5 mg 2.5 mg	2.5 mg 2.5 mg 1000 IU
Protocol 3 Reduced iron NaFe-EDTA Vitamin A	Corn	5 mg	5 mg	2.5 mg 2.5 mg	2.5 mg 2.5 mg 1000 IU
Protocol 4 Reduced iron NaFe-EDTA Vitamin A	Wheat	5 mg	5 mg	2.5 mg 2.5 mg	2.5 mg 2.5 mg 1000 IU

iron absorption from ferrous sulfate (Martínez- Torres et al., 1979; INACG, 1998). Other substances are also able to increase iron absorption such as vitamin C, a meat factor and vitamin A (García-Casal, 1998).

Since cereals constitute the main source of calories and nutrients in many countries where iron deficiency problems are prevalent and due to the low iron absorption from cereal based foods and the extended use of elemental irons as fortificants, the objective of this study was to evaluate the effect of NaFe-EDTA and vitamin A on iron absorption from corn and wheat breads fortified with electrolytic iron or reduced iron. Serum retinol was measured in all subjects, in order to evaluate the vitamin A status of the population and correlate iron absorption to serum retinol levels. The effect of NaFe-EDTA and vitamin A on in vitro iron solubility from electrolytic and reduced iron was also evaluated.

SUBJECTS AND METHODS

Iron absorption studies were performed in 178 adult volunteers (41 men, 137 women) apparently in good health, although some of them presented mild to moderate iron deficiency and/or anemia. The subjects studied 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. A written consent form was signed by each volunteer. The study was approved by the Ethical Committee of the Venezuelan Institute for Scientific Research.

Meal 1, tagged with ^{59}Fe (0.9 μCi/person), was administered to the subjects after an overnight fast. MEAL 2, also extrinsically labeled, but with ^{55}Fe (1.3 μCi/person), was fed 4 h later. No food or drink (except for water) was allowed between meals 1 and 2, and 4 h 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 (Taylor et al., 1995).

On day 15, blood (30 ml) was drawn to determine the hematological profile (hemoglobin concentration (Crosby et al., 1954), serum iron (International committee for standardization in hematology 1978a), unsaturated binding capacity (international committee for standardization in hematology 1978b) and serum ferritin concentration (Flowers et al., 1986)) and to measure radioactivity incorporation into red cells. Duplicate (10 ml) and triplicate samples of radioactive food were prepared for radioactive counting using the technique of Dern and Hart (1961a,b). Iron absorption from each meal was calculated from the radioactivity in the subject's blood using an estimated of blood volume based on sex, weight and hei-ght (Nadler et al., 1962).

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.

Studies performed and meals administered

Volunteers received 4 meals according to the 4 basic protocols presented in Table 1. The 2 main differences between protocols were the non-enriched cereal flours used to prepare the bread, corn flour (a donation from Alimentos Polar Commercial. PROMESA Venezuela) or wheat flour (donated by Cargill de Venezuela) and the elemental iron tested, electrolytic iron (Fortitech Inc. Grade A- 131, -325 MESH Lot 5-99EI) or reduced iron (Mallinckrodt Inc. St Louis MO. Food grade extra fine powder US STD # 325).

Each volunteer received a 100 g bread, 50 g of white cheese and 10 g of margarine per meal. In meal 1bread was fortified with 5 mg

Table 2. Anthropometrical and hematological characteristics of the individuals participating in iron absorption studies[1]

	Protocol 1	Protocol 2	Protocol 3	Protocol 4
Cereal	Corn	Wheat	Corn	Wheat
Iron	Electrolytic	Electrolytic	Reduced	Reduced
n	44	68	29	37
Sex	36F,8M	49F,19M	24F,5M	28F,9M
Age (YEARS)	35.62± 12.58	34.19± 14.41	34.02± 12.59	36.0± 11.94
Weight	64.04± 11.09	58.05± 9.82	63.24± 11.55	64.78± 14.72
Height (cm)	160.25± 6.82	161.16± 8.41	162.17± 6.5	164.33± 7.54
Hemoglobing (/L)	13.13± 1.35	13.27± 1.30	13.07± 1.19	13.14± 1.21
Hematocrit (%)	38.84± 3.76	41.12± 3.55	39.83± 3.11	40.15± 3.49
Serum IRON (μg/L)	90.47± 30.03	102.13± 32.33	102.78± 34.81	94.36± 30.28
UIBC (μg/L)	250.16± 62.54	239.68± 68.49	222.33± 69.93	246.2± 47.87
TIBC (μg/L)	342.48± 45.08	339.87± 59.57	315.76± 73.3	328.0± 48.57
Transferrin (μg/L)	27.13± 10.23	30.54± 11.64	32.14± 11.63	26.86± 7.22
Ferritin (μg/L)	17.70± 3.19	19.31± 2.46	19.91± 2.94	20.79± 3.41
Serum RETINOL(μg/mL)	18.80± 6.09	18.42± 3.76	23.52± 9.08	17.83± 4.93

[1]Values are means ± SD.

of one elemental iron (as electrolytic iron or reduced iron, depending on the study) and meal 2 contained the same amount of iron but as NaFe EDTA (Sodium iron ethylene diamine tetraacetic acid from Sigma chemicals St Louis MO). For meal 3 bread was fortified with a mixture of one elemental iron (as electrolytic iron or reduced iron, depending on the study) + NaFe EDTA (2.5 mg of iron from each compound to a total of 5 mg) and the same iron mixture with 300.3 μg RE (1000IU) of vitamin A (water soluble retinol palmitate donated by DSM laboratories of Venezuela) was administered in meal 4.

It is important to highlight that the technical difficulties to obtain intrinsically labeled electrolytic or 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} Fe or ^{55} Fe as ferric chloride, will uniformly label the non-heme iron pool, which includes the native iron and the electrolytic iron that has been solubilyzed. The remaining fraction can not be measured. The administration of a meal enriched with electrolytic or reduced iron (as meal 1 for each of the studies) allows to use each individual as its own control and to draw conclusions about the effect of NaFe-EDTA and vitamin A on the absorption from electrolytic or reduced iron.

Serum retinol determinations

Blood samples obtained on days 15 and 30 of each study, were processed to obtain serum (centrifuged at 1060 x g, 10 min at 4°C), protected from light and frozen until used for retinol determinations by high performance liquid chromatography (HPLC) (Chow and Omaye, 1983).

Solubility of iron compounds with pH changes

Iron solubility was measured from solutions prepared at pH 2 (considered 100% solubility for that particular iron compound) and raised to pH 6. The iron compounds tested include electrolytic iron, reduced iron, NaFe-EDTA and combinations of either elemental iron with NaFe EDTA. To this mixture of irons, vitamin A was added to evaluate its effect on iron solubility.

Iron solutions of each of the compounds mentioned containing 5 mg iron, were prepared in 0.1 mol/l HCL. Vitamin A was prepared in water at 1.1 μM (300.3 μgER, 1000 IU).

Duplicate 1 ml aliquots were taken after 30 min at room temperature to measure soluble iron at pH 2 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 and combinations by the digestion method (Bothwell et al., 1979).

Statistical analysis

Data analysis was based in comparisons (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 will be its own control. Due to the low solubility of electrolytic iron and the difficulties to obtain this compound intrinsically labeled, these studies will only allow to evaluate the effect of NaFe-EDTA on iron bioavailability from electrolytic and reduced iron and a role of vitamin A increasing iron absorption.

Linear correlation analyses were performed to compare iron absorption and serum retinol levels in all subject recruited.

RESULTS

Subjects studied

Anthropometrical and hematological parameters were determined for each of the 178 subjects that participated

Table 3. Iron Absorption from wheat or corn breads fortified with electrolytic iron, NaFe-EDTA or mixtures of electrolytic iron with NaFe-EDTA. Effect of vitamin A on iron absorption from these iron mixtures.

		Meal 1	Meal 2	Meal 3	Meal 4	
			Iron	Absorption	(%)	
	n	Bread + electrolytic iron	Bread+NaFe EDTA	Bread + electrolytic iron + NaFe-EDTA	Bread + electrolytic iron + NaFe-EDTA + vitamin A	Expected calculated absorption from meal 3
Corn	44	4.15 ± 1.3^{a}	12.55 ± 1.1^{b}	10.22 ± 1.1^{b}	15.10 ± 1.2^{c}	8.36 ± 1.2
Wheat	68	5.00 ± 1.2^{a}	12.92 ± 1.2^{b}	9.99 ± 1.2^{b}	17.74 ± 1.1^{c}	8.96 ± 1.2

[1] Values are means ±SE. Means in the same row, with dissimilar letters are significantly different $p<0.05$.

Table 4. Iron Absorption from wheat or corn breads fortified with reduced iron, NaFe-EDTA or mixtures of reduced iron with NaFe-EDTA. Effect of vitamin A on iron absorption from these iron mixtures.

		Meal 1	Meal 2	Meal 3	Meal 4	
			Iron	Absorption	(%)	
	n	Bread + reduced iron	Bread+NaFe-EDTA	Bread + reduced iron +NaFe-EDTA	Bread +reduced iron +NaFe-EDTA + vitamin A	Expected calculated absorption from meal 3
Corn	29	3.17 ± 1.2^{a}	13.36 ± 1.1^{b}	11.66 ± 1.1^{b}	22.35 ± 1.2^{c}	8.26 ± 1.2
Wheat	37	7.55 ± 1.2^{a}	16.52 ± 1.1^{b}	17.99 ± 1.1^{b}	24.38 ± 1.2^{c}	12.06 ± 1.2

[1] Values are means ±SE. Means in the same row, with dissimilar letters are significantly different $p < 0.05$.

in the studies (Table 2). The subjects assigned to each protocol presented similar anthropometrical and hematological characteristics. Hemoglobin and ferritin concentrations were also similar for all groups. Most of the volunteers were women (137) with a mean age of 35 years. They were in apparent good health, but some of them presented anemia and/or iron deficiency. Prevalence of anemia and iron deficiency in the whole group studied was 20.8 and 29.2%, respectively. From all cases with anemia (37 out of 178), 21 cases were also iron deficient, indicating that for this volunteers iron deficiency is still the main cause of anemia, but also that approximately 43% of the anemia is not caused by iron deficiency. Anemia was defined as hemoglobin concentrations below 120 g/l for females and 130 g/l for males. The cutoff for iron deficiency was a serum ferritin concentration < 12 μg/l for females, and < 13 μg/l for males.

Iron absorption studies

Although all breads administered were enriched with the same amount of total iron (5 mg), absorption from corn or wheat breads fortified with NaFe- EDTA was 2.5 to 3 times higher than absorption from breads fortified with electrolytic iron (Table 3). When NaFe-EDTA and electrolytic iron were administered together, iron absorption increased above the expected additive values of each compound.

Based on absorption values obtained with 5 mg of iron from each compound, the expected absorption value from a mixture of NaFe-EDTA and electrolytic iron would be 8.36%. The experimental value obtained was 10.22%. The increase in absorption from the same iron mixture also occurred with wheat bread, obtaining an 11.5% increase above the expected value. Differences in absorption between the mixture of NaFe-EDTA and electrolytic iron were significantly different from electrolytic iron alone, but there was no difference between the iron mixture and NaFe-EDTA alone.

For both cereals, addition of vitamin A to the mixture of NaFe-EDTA and electrolytic iron produced a significant increase in absorption compared to the other conditions tested, except in the case of corn bread, where the increase was similar to NaFe-EDTA alone.

Experiments with reduced iron showed a similar pattern than electrolytic iron (Table 4) . Absorption from NaFe-EDTA was 2 to 4 times higher than absorption from the same amount of reduced iron and when both compounds were administered together, absorption increased 41% for corn bread and 49% for wheat bread, above the expected calculated value. Absorption from the mixture NaFe-EDTA and reduced iron was not different from the absorption obtained with NaFe-EDTA administered alone and the inclusion of vitamin A significantly increased iron absorption compared to either iron treatment tested, even NaFe-EDTA alone.

Serum retinol determinations

As shown in Table 2, all groups studied presented low serum retinol levels except for subjects in protocol 3, with a mean serum retinol concentration of 23.52 ± 9.08 μg/dl.

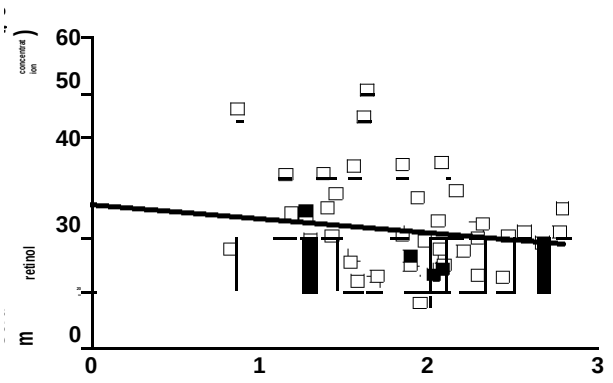

Figure 1. Linear regression analysis of iron absorption from corn or wheat breads fortified with reduced iron and serum vitamin A concentration. Corn or wheat breads extrinsically labelled with ^{59}Fe iron were administered to healthy subjects to measure iron absorption. Serum retinol concentration was measured by high performance liquid chromatogramphy and a regression analysis of both parameters was performed using Prism software. Values are means of 6 determinations for retinol concentrations and 2 determinations for iron absorption n = 66.

The cutoff point used in this study to define low levels of serum retinol was < 20 μg/dl. The prevalence of vitamin A deficiency in all subjects studied was 55.7%.

Linear correlation analysis to compare iron absorption and serum retinol levels showed a non significant correlation between these 2 parameters.

However, as shown in Figure 1, in all tests performed with reduced iron (either corn or wheat breads), the trend of the slope seems to indicate higher absorption values at lowers serum retinol levels.

Solubility of iron compounds with pH changes

Solubility of electrolytic iron was low even at pH 2. From 5 mg of iron initially added to acidic solutions, only 2.7 mg were soluble at this pH. Increasing the pH to 6 produced a further decrease in solubility rendering up to 70% of iron in the precipitate (Figure 2A). NaFe-EDTA was highly soluble at both, pH 2 and 6 and its presence in a solution containing electrolytic iron improves solubility for this source. Solubility of electrolytic iron at pH 6 increased 28.9% in presence of NaFe-EDTA. The expected values of soluble iron when mixtures of electrolytic iron and NaFe-EDTA at pH 2 were raised to 6, should be around 2.34 mg Fe (1.93 mg from NaFe-EDTA and 0.41 mg from electrolytic iron), and the value obtained experimentally was 3.01 mg. An additional increase in solubility was achieved with vitamin A. Iron in the supernatant increased from 3.01 mg in solutions containing NaFe-EDTA + electrolytic iron at pH 6, to 3.53 mg with vitamin A, a

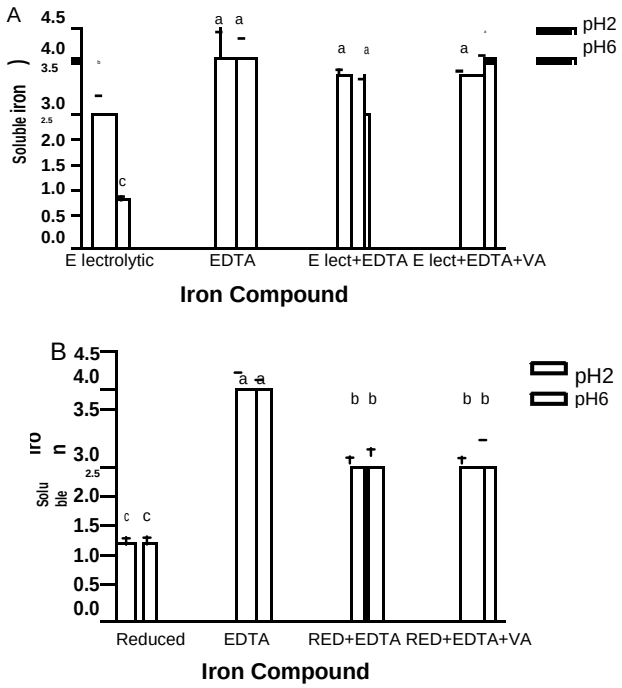

Figure 2. Effect of NaFe-EDTA (EDTA) and vitamin A (VA) on iron solubility from electrolytic (A) and reduced (B) iron. Iron solutions containing 5 mg of iron were prepared at pH 2, and the pH slowly raised to 6 with NaOH. After 10 min incubation, iron remaining in the supernatant was measured. Values are means±SD, n=6. Bars with dissimilar letters are significantly different $p < 0.05$.

further but no significant 17.3% increase. However the increment with vitamin A was significant (51.2%) when compared to the expected value for NaFe-EDTA + electrolytic iron. Differences were no statistically signifi-cant for soluble iron at pH 6 between NaFe-EDTA, NaFe-EDTA + electrolytic iron or NaFe-EDTA + electrolytic iron + vitamin A. Experiments with reduced iron (Figure 2B), showed that the initial solubility of this source at pH 2 was even lower than from electrolytic iron. From 5 mg initially added only 1.2 mg were soluble at pH 2, and exactly the same amount of iron remained soluble after rising the pH to 6.

Solubility of reduced iron was significantly increased (2.73 mg) in presence of NaFe-EDTA, compared to the calculated value of 2.53 mg and the addition of vitamin A produced a slight further increase of solubility (2.83 mg) from the mixture of NaFe-EDTA and reduced iron. Differences in solubility between NaFe-EDTA alone and mixtures of NaFe-EDTA + reduced iron or NaFe-EDTA + reduced iron + vitamin A, were statistically different.

DISCUSSION

For a fortification program to be successful one of the most important premises is to select the adequate iron

compound. Solubility, chemical reactivity, bioavailability and cost are 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 powders (reduced, electrolytic or carbonyl) are also inexpensive but they have been reported to show a low bioavailability depending on particle size and the food vehicle to be fortified (Cook and Reusser, 1983; Hallberg, 1982; Hurrell, 1997).

The use of radio isotopic techniques with electrolytic or reduced iron is of limited value, since it is not possible to predict its degree of solubilization and the fraction available for absorption. However, the use of these forms of iron repeatedly in the same subject, in a way that each subject can act as his/her own control, allows us to make some conclusions.

All absorption studies were performed in apparently healthy subjects and the inclusion of individuals in each protocol showed a similar distribution in terms of anthropometrical and hematological characteristics. It is interesting to point out that although the prevalence of anemia was 20.8% and in most of the cases it was produced by iron deficiency, there was a 43% of anemia cases that were produced by other causes. There is also noteworthy to point out the high prevalence of low levels of vitamin A in the subjects studied, although in most of the cases it was a mild deficiency (serum retinol concentration between 10 and 20 μg/dl).

Iron absorption from corn or wheat breads fortified with electrolytic or reduced iron was similar to previous reports with other iron salts, showing a slightly higher absorption from wheat than from corn breads. For both cereals, iron absorption was significantly higher from breads fortified with NaFe-EDTA compared to either elemental iron. When electrolytic or reduced iron powders were mixed with NaFe-EDTA to the same total iron concentration, absorption was increased to values not significantly different to NaFe-EDTA alone, indicating the possibility of mixing iron sources of different bioavailability (and cost) to obtain iron mixtures of high bioavailability for food fortification procedures. Increases in absorption were higher than the calculated expected values of the compounds administered separately. The increase above the expected values ranged from 12 to 49% depending on the elemental iron tested.

There are some interesting, although controversial evidences, about the role of Na_2-EDTA in iron absorption from ferrous fumarate and reduced iron using both radioactive and non-radioactive methods. In 2003, Walter et al. showed that Na_2-EDTA significantly increased iron absorption from corn tortillas enriched with ferrous fumarate, while Davisson et al. (2002) found no effect of Na_2-EDTA on absorption from ferrous fumarate on the same food vehicle, concluding that Na_2-EDTA is not an enhancer of iron absorption from compounds less soluble than ferrous sulfate. Later, Walter et al. (2004) showed no effect of Na_2-EDTA increasing iron absorption from tortillas fortified with reduced iron.

Other studies using Na_2-EDTA and different iron salts as ferrous sulfate, ferrous fumarate, ferric pyrophosphate and various food vehicles (wheat, corn, rice and milk) have been performed, with different outcomes. The addition of Na_2-EDTA to ferric pyrophosphate, produced a 1.7-fold increase in iron absorption from wheat rolls, although the increase was significantly higher with ferrous sulfate and Na_2-EDTA (Hurrell et al., 2000). Forti-fication of rice with ferrous sulfate and Na_2-EDTA showed a significant increase in absorption compared to ferrous sulfate alone (Hettiarachi et al., 2004). Addition of iron to dairy products showed a high dialyzability of iron from NaFeEDTA and an increase in Zn dialyzability from intrinsic Zn (Drago and Valencia, 2008). On the other hand, the evaluation of a milk- based weaning food program in Mexico, showed a very low absorption from a reduced iron and Na_2-EDTA mixture, compared to ferrous sulfate or ferrous fumarate (Perez-Expósito et al., 2005).

Although widely used in iron fortification programs with relative success (Wang et al., 2008), few studies have been performed to evaluate the use of NaFe-EDTA as an enhancer of availability of other iron compounds. Besides the initial studies with ferrous sulfate already mentioned, in 2001, Fairweather-Tate reported that there was significant higher iron absorption from a combination of reduced iron and NaFe-EDTA than from reduced iron alone, when administered in a cereal based breakfast (Fairweather-Tate et al., 2001).

Our results indicate that NaFe-EDTA had a significant effect on iron absorption from corn or wheat breads enriched with electrolytic or reduced iron, probably because NaFe-EDTA improved the solubility of these elemental iron powders. Vitamin A further increased electrolytic and reduced iron solubility, which is supported not only by the increase in absorption when vitamin A was included with the iron mixtures containing elemental iron + NaFe-EDTA to the breads, but also by in vitro tests, with the increase in soluble iron in presence of this vitamin.

Although it is important to increase the number of subjects evaluated, results from this study seem to indicate that, besides an effect on iron solubility, the vitamin A status of the individuals could influence iron absorption. Our data showed not only a high prevalence of vitamin A deficiency, but also a negative correlation between retinol levels and iron absorption, indicating that vitamin A could influence iron absorption depending on retinol status. This could partly explain, besides other considerations about food matrix or chemical form of compounds used the lack of effect of vitamin A on iron absorption in a previous report (Walczyk et al., 2003).

In vitro studies showed no change in solubility of NaFe-EDTA with increments in pH. NaFe-EDTA was capable of

increasing solubility of electrolytic and reduced iron when pH was raised to 6. *In vitro* experiments demonstrated that without inhibiting or competing factors present in foods, NaFe-EDTA was capable of keep a less soluble source of iron in solution. This finding could have some interesting applications, especially considering costs, for therapeutic or supplemental uses.

NaFe-EDTA and vitamin A affected solubility of electrolytic and reduced iron powders. Reduced iron resulted less soluble and less susceptible to the effect of NaFe-EDTA or vitamin A, than electrolytic iron in solubility experiments but more bioavailable in absorption studies. However, it is important to highlight that reduced iron although less soluble at pH 2, resulted in more iron soluble at pH 6 compared to electrolytic iron, since the increase in pH did not precipitate any additional iron from the amount that was soluble at pH 2.

In conclusion, as with previous studies showing an effect of NaFe-EDTA improving iron absorption from ferrous sulfate, our results with less soluble and less available forms of iron, also indicate an enhancing effect. Based in the use of cereal flours as fortification vehicles, these findings could be of use for food fortification programs worldwide. It is possible that the iron compound used, its solubility and its capability of enter the non-heme iron pool, as well as the food matrix, are important requirements in order to obtain an improvement in iron absorption, mediated by NaFe-EDTA. It would be interesting to know if the effect mediated by this compound and also by vitamin A, is only on the added iron powders, rendering them soluble to enter the non-heme iron pool, or if NaFe-EDTA affects the native food iron availability to the same extent.

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