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New property of vitamin A and ß-carotene on human iron absorption: effect on phytate and polyphenols as inhibitors of iron absorption

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RESUMEN. Nueva propiedad de la vitamina A y ß-caroteno sobre la absorción de hierro en humanos: efecto de los fitatos y polifenoles como inhibidores de la absorción de hierro. Ciento setenta y cuatro sujetos fueron estudiados con el fin de conocer la interacción de la vitamina A y el ß-caroteno con los inhibidores de la absorción de hierro, utilizando un desayuno basal conteniendo un pan preparado a partir de 100 g de harina precocida de maíz o de harina blanca de trigo obtenidas comercialmente y fortificadas con fumarato ferroso y vitaminas, mas 50 g de queso y 10 g de margarina. Este pan fue marcado con ^{59}Fe o ^{55}Fe. Se apreció que el porcentaje de absorción de hierro del desayuno preparado con harina de maíz administrado solo o con diferentes concentraciones de café fue prácticamente el mismo, mientras que la absorción de hierro del desayuno basal preparado con harina de trigo disminuyó de 6% cuando el desayuno es administrado solo, a menos de 2% cuando fue administrado con diferentes concentraciones de café. El único ingrediente presente en la harina de maíz y no en la de trigo, fue la vitamina A. Esta diferencia estimuló a los autores para practicar otros experimentos utilizando harina precocida de maíz o harina blanca de trigo fortificadas solamente con fumarato ferroso. Estos estudios demostraron que la vitamina A inhibe el efecto de los polifenoles y parcialmente el de los fitatos sobre la absorción de hierro. Estudios espectrofotométricos y de HPLC demostraron que ocurren interacciones entre la vitamina A y el hierro. Otros experimentos, que incluyeron 100 voluntarios, fueron practicados para evaluar el efecto de la vitamina A y el ß-caroteno sobre la absorción del hierro del maíz, trigo y arroz. La presencia de vitamina A aumentó la absorción de hierro hasta 3 veces en arroz, 2.4 veces en trigo y 1.8 veces en maíz. El ß-caroteno aumentó la absorción hasta 3 veces para los tres cereales estudiados. Esta información sugiere que la vitamina A y el ß-caroteno forman complejos con el hierro manteniéndolo soluble en el lumen intestinal y evitando el efecto inhibitorio de fitatos y polifenoles sobre la absorción de hierro.

Palabras clave: Hierro, vitamina A, ß-caroteno, humanos, fitatos, polifenoles.

SUMMARY. One hundred and seventy four human subjects were studied to find out the interaction of vitamin A or ß-carotene with the inhibitors of iron absorption, from a basal breakfast containing bread from either 100 g of precooked corn flour or 100 g of white wheat flour, 50 g of cheese and 10 g of margarine. Bread was labeled with either ^{55}Fe or ^{59}Fe. This bread was made from commercially flours fortified with iron as ferrous fumarate and vitamins. It was noticed that the percentage of iron absorption from the breakfast prepared with precooked corn flour given alone and with different concentrations of coffee was practically the same, while the iron absorption from the breakfast prepared from wheat flour decreased from 6% when the breakfast was given alone, to less than 2% when it was given with different concentrations of coffee. The only ingredient present in precooked corn flour and not in wheat flour was vitamin A This difference encouraged the authors to perform further experiments using precooked corn and wheat flours fortified only with ferrous fumarate. These studies demonstrated that vitamin A inhibits the effect of the polyphenol and partially inhibits the effect phytate on iron absorption. HPLC and spectrophotometric studies demonstrated an interaction between vitamin A and iron. Other experiments, which included 100 volunteers, were performed to test the effect of vitamin A and ß-carotene on iron absorption from corn, wheat and rice. The presence of vitamin A increased iron absorption up to 3 times for rice, 2.4 times for wheat and 1.8 times for corn. ß-carotene increased absorption almost 3 times for the three cereals tested, showing that both compounds were capable of preventing the inhibitory effect of phytates on iron absorption. This information suggest that vitamin A and ß-carotene form a complex with iron keeping it soluble in the intestinal lumen and preventing the inhibitory effect of phytates and polyphenols on iron absorption.

Key words: Iron, vitamin A, ß-carotene, humans, phytates, polyphenols.

INTRODUCTION

Vitamin A is vital nutrient for cellular differentiation, vision, bone growth, reproduction and integrity of the immune system (1). It is also essential for erythropoiesis. Deficiency of this vitamin results in anemia in humans and animals that is

reversed only by vitamin A supplementation (2-6).

ß-carotene is the most abundant provitamin A in foods. Approximately 10-50% of the total ß-carotene consumed is absorbed in the gastrointestinal tract and within the intestinal wall is partially converted into vitamin A. The efficiency of ß-carotene absorption decreases as intake increases and conversion to vitamin A is regulated by the vitamin A status of the individual. ß-carotene accumulation is not toxic, so it is considered as a safe source of vitamin A (7).

After our laboratory studied the iron absorption from diets consumed by different socioeconomic strata of the Venezuelan population (8), we continued the studies about the interaction of various micronutrient on iron absorption, especially since 1993 when was started a national program of fortification of precooked corn and wheat flours with iron and vitamins (Table 1).

TABLE 1
Enrichment of food vehicles in Venezuela

	Precooked maize flour /kg	White wheat flour/ kg
Vitaamin A, IU	9,500	-
Thiamin, mg	3.1	1.5
Riboflavin, mg	2.5	2.0
Niacin, mg	51.06	20.0
Iron* mg	50.0	20.0

Since 1994, 33.3 mg as ferrous fumarate + 16.7 mg as electrolytic iron

In 1994, one year after starting the fortification project in national scale, a survey was performed on 7,11 and 15 year old school children of both sexes. All subjects lived in Caracas and belonged to the low socioeconomic strata of the population. The comparison between the survey made in 1992, one year before the inception of the fortification program, and the 1994 survey for the same age, sex and socioeconomic strata of the population, made one year after the initiation of fortification, showed that the prevalence of iron deficiency and anemia had been significantly reduced from 37% to 15% and from 19% to 10%, respectively. This was confirmed by the iron reserves measured by the serum ferritin concentration of all subjects tested, which increased from the median of 15 g/L in 1992 to the median of 21 g/L in 1994 (9).

Such results were impressive and provocative since iron fortification usually takes a considerable longer time to produce favorable results. These results motivated the authors to start iron absorption studies to find out an explanation for such particular behavior originating from the fortified flours.

Iron Absorption Studies

The iron absorption studies were performed in one hundred and seventy four human subjects. The methods from iron absorption studies were already published (10,11,13).

Results of the experiments

This article condense the results of the experiments carried out on the effect of vitamin A and ß-carotene on iron absorption from a basal breakfast containing 100 g of either precooked corn flour or wheat flour enriched with iron and vitamins + 50 g of cheese and 10 g of margarine.

In the first experiment (Table 2), where the bread was prepared from enriched precooked corn flour, the percentage of iron absorption was practically the same from the breakfast given alone than from the one given with different concentrations of coffee. Iron absorption from the breakfast prepared from wheat flour decreased from 6.8%, when the breakfast was given alone, to 1% or less, when it was given with an infusion containing various concentrations of coffee (10).

Comparing the enrichment patterns of precooked corn flour and wheat flour, the only vitamin present in precooked corn flour and not in wheat flour is vitamin A. This difference encouraged the authors to perform further studies using precooked corn flour fortified only with 5 mg of iron as ferrous fumarate per 100 grams of flour.

Iron absorption from the bread prepared from non-enriched corn flour increased more than twice when 1000 IU of vitamin A were added during the preparation of the dough. This increase in absorption was not affected when coffee or tea infusions where given with the bread that contained vitamin A. These results indicate that the vitamin A prevents the effect of phytates and polyphenols on iron absorption. The effect of vitamin A on phytates was fully demonstrated in Table 3. Iron absorption from the bread prepared with precooked corn flour given alone, or with the addition of either 1000 IU of vitamin A or 304 U of phytase was studied. The iron absorption from precooked corn bread given alone was 3.6%, from the bread with vitamin A was 10.6% and from bread with phytase was 15,4% (11).

The incorporation of 1000 IU of vitamin A to the dough prepared with non-enriched wheat flour is ineffective because 50% of the vitamin A is denature by the effect of yeast in the dough and after baking was further reduced to about 100 IU. Dissolving 1000 IU of vitamin A in water and drinking it slowly while eating the bread prepared from non-enriched wheat flour, can compensate the losses. Also by incorporating 2000 IU of vitamin A in the dough instead of 1000 IU produced the same effect (10,13).

HPLC and spectrophotometric studies

In an attempt to find out if there is a direct interaction between iron compounds and vitamin A in food during the digestive process, spectrophotometric and HPLC studies were performed. Changes in absorbance or elution profiles provide evidence of the formation of vitamin A-iron complexes (Figure 1). When 0.01 M solutions of vitamin A and $FeCl_3$, are combined and passed through a C-18 column in the HPLC

TABLE 2

Iron absorption from a basal breakfast containing either precooked maize bread or wheat bread prepared from commmercially available fortified flour and several concentrations of coffee as a beverage

Subjects and sex	Hb g/dL	Serum transferrim saturation %	Serum ferritin concent. ug/L	Iron absorptopm (%) A Basal breakfast given alone	B Basal breakfast + American coffee (2g)	C Basal breakfast + espresso coffee (4g)	D Basal breakfast + Capuchino coffee (4g)
1) 4M 3F Mean	11.9	20	17	5.1	7.7	8.2	7.8
SEM	0.2	0.5	1	1.4	1.4	1.4	1.3
Statistics: A vs B- p>.05; A vs C- p>.0.5; A vs D- >.05							
2) 1M 9F Mean	14.3	27	26	4.4	5.3	4.6	
SEM	0.4	1	1	1.3	1.3	1.5	
Statistics: A vs B- p>.05; A vs C- p>.05; A vs E- p>.05							
Average: Mean 1 + 2	13.3	24	22	4.7	6.1	5.8	
SEM	0.4	2	1	1.3	1.5	1.5	
		BASAL BREAKFAST WITH WHITE WHEAT BREAD					
2M 8F Mean	12.9	29	28	6.8	1.2	0.4	
SEM	0.8	1	2	1.2	1.4	1.4	
Statistics: A vs B- p<0.05; A vs C-p<0.05							

TABLE 3

Effect of phytase and vitamin A on iron absorption from basal breakfast containing bread prepared from precooked corn flour enriched with 5 mg iron as ferrous fumaratet, 50 g cheese + 10 g margarine

Number and sex	Hb g/dL	Transferrim saturation %	Serum ferritin concent. µg/L	Iron absorption (%) A Basal breakfast alone	B Basal breakfast + 1000 IU vitamin A	C Basal breakfast + 304 U Phytase
4M 9F Mean	12.3	26	19	3.6	10.6	15.4
SE	0.4	2	1	1.1	1.1	1.1
Statistics: A vs B p< 0.05; A vs C p<0.05; B vs C p>0.05						

system, there are important changes in elution profiles. The peak that elutes at 6.93 min, and corresponds to vitamin A, is reduced by almost 30% when compared to the mixture of vitamin A $FeCl_3$. Likewise, there is a new peak at 14.75 min not seen when $FeCl_3$ or vitamin A are injected separately.

FIGURE 1
Elution profile of $FeCl_3$, vitamina A and $FeCl_3$ + vitamin A solutions in HPLC. Left column of each panel represents time in minutes, and second column represents the proportion of each peak

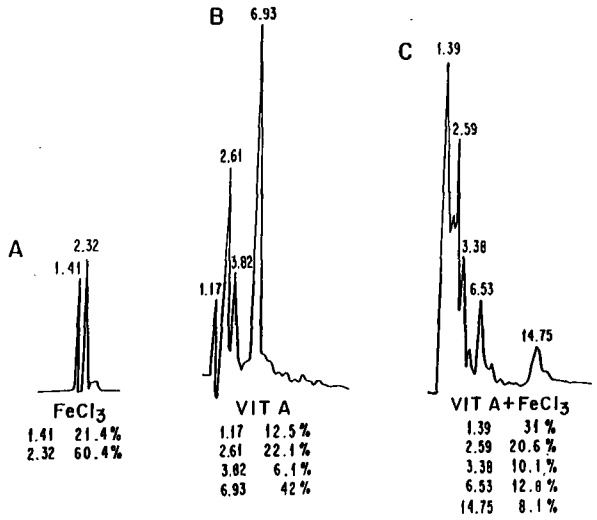
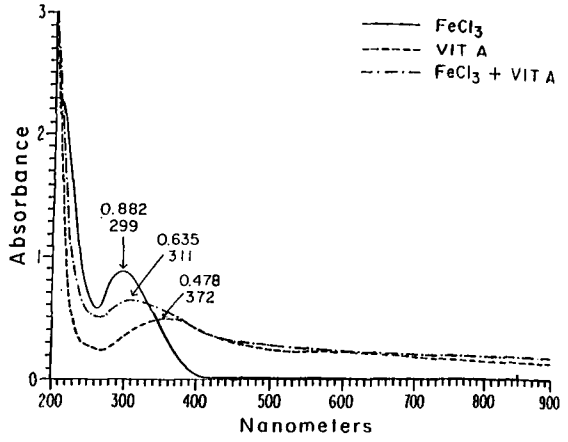


FIGURE 2
Spectrophotometric measurements of absorbance for $FeCl_3$, vitamin A and $FeCl_3$, + vitamin A solutions



Regarding spectrophotometric measurements, there were changes in absorbance when we measure $FeCl_3$ and vitamin A as compared to combinations of these two solutions (Figure 2). For example, $FeCl_3$ has a maximum absorbance at 299 nm and vitamin A at 372 nm; when both solutions are combined the new maximum is at 311 nm. The dialyzable iron from food according to the method of Miller did not produce convincing results (12). We are currently performing iron uptake studies using the Caco-2 cell system trying to elucidate the mechanism responsible for the increase in iron absorption by vitamin A and ß-carotene. The preliminary uptake studies using the Caco-cell system have produced promising results.

Other group of experiments were performed administering basal breakfasts made from either rice, corn or wheat and enriched with 1000, 2000 and 4000 IU of vitamin A. The results show that there is a two fold increase in iron absorption in the test with 1000 IU compared with the breakfast given alone, and increases in vitamin A doses from 2000 to 4000 IU, did not produce significant further increases in absorption from the three cereals. In the case of wheat breakfast with 1000 IU of vitamin A, this vitamin was dissolved in water and drinking slowly while eating the bread (13) (Table 4).

ß-carotene increases iron absorption from corn, wheat and rice compared to the breakfast given alone. However, increases in the dose from 1000 to 2000 IU ß-carotene/100 g cereal did not produce significant further increases in iron absorption. The effect of polyphenol on iron absorption, in presence of ß-carotene was also evaluated. It can be noticed that iron absorption is not modified by coffee when ß-carotene is added (Table 5).

Iron solubility with pH changes from 2 to 6, in presence of vitamin A was tested using different iron salts. There was a 75% decrease in iron solubility from ferrous fumarate when pH was raised from 2 to 6. When vitamin A was added to fumarate at pH 2 and then the pH raised to 6, iron solubility increased as a function of vitamin A concentration reaching a 78% of iron in solution when 3000 IU of vitamin A was added. Ferrous sulfate showed similar behavior. For EDTA and Ferrochel^R (iron-aminoacid chelate), it was not possible to observe an effect of vitamin A on iron solubility because iron was 100% soluble at pH 6 without any vitamin addition.

We performed the same solubility test to evaluate the effect of ß-carotene with pH changes. Either for ferrous fumarate or for ferrous sulfate virtually 100% of iron remained soluble at pH 6, when 3000 IU of ß-carotene was added. With no ß-carotene addition, only 26% of iron from ferrous fumarate and 36% from ferrous sulfate remained in solution when pH was raised to 6.

FINAL COMMENTS

Results from solubility experiments as well as human absorption studies show an important role for vitamin A and ß-carotene in improving iron absorption, specially from foods with a high content in inhibitors, which are the staple foods of many countries worldwide.

This unexpected behavior of vitamin A and ß-carotene in preventing the inhibition of iron absorption by phytates and polyphenols requires further studies to find out the mechanism for this peculiar reaction. The spectrophotometric results, elution patterns from HPLC and solubility of iron with vitamin A at pH 6, suggest that vitamin A binds iron liberated during the digestive process and forms a complex that acts as chelating agent preventing the inhibitory effect of phytates and polyphenols on non-heme iron absorption (10,11,13). It also

TABLE 4

Effect of increasing doses of vitamin A on iron absorption from rice, maize and wheat

Subjects and sex	Hb g/dL	Serum transferrim saturation %	Serum ferritin concent. µg/L	Iron absorption (%) A Food given alone	B Food+ 1000 IU vitamin A	C Food+ 2000 IU vitamin A	D Food+ 4000 IU vitamin A
1) 100 g polished rice							
6M 11F Mean	12.6	22.7	28.5	3.8	8.4	7.5	11.7
SEM	0.4	2.3	1.3	1.1	1.1	1.2	1.2
Statistics**: A vs B p<0.05; A vs C p<0.05; A vs D p<0.05; B vs C p>0.05; B vs D p >0.05							
2) Basal Breakfast: 100 g precooked maize flour + 50 g cheese + 10 g margarine							
6M 11F Mean	13.5	27.1	28.1	4.6	9.8	13.9	11.8
SEM	0.5	2.2	1.3	1.2	1.2	1.1	1.1
Statistics**: A vs B p<0.05; A vs C p<0.05; A vs D p<0.05; B vs C p>0.05; B vs D p >0.05							
3) Basal Breakfast: 100 g white wheat flour + 50 g cheese + 10 g margarine							
3M 17F Mean	13.0	29.0	31.5	4.2	7.4*	7.1	7.5
SEM	0.2	1.5	1.2	1.2	1.1	1.1	1.1
Statistics**: A vs B p<0.05; A vs C p<0.05; A vs D p<0.05; B vs C p>0.05; B vs D p >0.05							

* Vitamin A was administered as a beverage while eating the bread

** ANOVA with Bonferroni as a post-test

TABLE 5

Iron absorption from meals containing rice, maize or wheat given alone or administered with vitamin A or ß-carotene

Subjects and sex	Hb g/dL	Serum transferrim saturation %	Serum ferritin concent. µg/L	Iron absorption (%) A Basal breakfast give alone	B Basal breakfast + 2000 IU vitamin A	C Basal breakfast + 1000 IU ß-carotene.	D Basal breakfast + 2000 IU ß-carotene. + 8 g coffee
1) 100 g polished rice							
3M 8F Mean	12.9	28	21	1.4	4.2	5.5*	10.1
SEM	1.1	2	1	1.2	1.2	1.2	1.1
Statistics**: A vs B p<0.001; A vs C p<0.001; A vs D p<0.001; B vs C p>0.05; B vs D p <0.001; C vs D p<0.05							
2) Basal Breakfast: 100 g precooked maize flour + 50 g cheese + 10 g margarine							
4M 14F8 Mean	13.0	30	24	3.0	6.5	8.4	6.3
SEM	1.1	2	1	1.1	1.2	1.1	1.2
Statistics**: A vs B p<0.001; A vs C p<0.001; A vs D p<0.001; B vs C p>0.05; B vs D p >0.05; C vs D p>0.05							
3) Basal Breakfast: 100 g white wheat flour + 50 g cheese + 10 g margarine							
3M 16F Mean	13.2	25	18	3.4	5.5	8.3	8.4
SEM	1.1	2	1	1.1	1.1	1.1	1.1
Statistics**: A vs B p<0.05; A vs C p<0.001; A vs D p<0.001; B vs C p>0.05; B vs D p >0.05; C vs D p>0.05							

* Coffee infusion was administered in test C for the rice meal and in test D for the other two meals.

** ANOVA with Bonferroni as a post-test

supported the dramatic reduction of the prevalence of iron deficiency after one year of iron fortification program in Venezuelan population, in which one food vehicle, precooked corn flour, was fortified with vitamin A (9).

Since iron and vitamin A deficiencies are the two main deficiencies in many developing populations, it is recommended that both micronutrients should be used in fortification programs.

This observation agrees with the studies that show that the chemical compounds containing double bonds are capable of reacting with iron (14). This hypothesis also agrees with the results of Hodges et al. (2), who demonstrated interaction between vitamin A and iron metabolism. The dramatic reduction of the prevalence of iron deficiency shown in a recent Venezuelan survey is probably due to the effect of vitamin A preventing phytate inhibition, which is present in fortified precooked maize flour (9).

The poor socioeconomic population living in Asia, Africa, and Latin America have low iron bioavailability from their diets due to the high consumption of cereals, legumes, and tubers. The fortification with vitamin A not only prevents this vitamin deficiency, but also potentates the iron fortification.

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