# Sugar as a vehicle for iron fortification: further studies[1, 2]

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ABSTRACT The data presented confirm the advantages of sugar as a vehicle for iron fortification over other vehicles used in the past. The absorption comparison between ferric and ferrous salts added to sugar demonstrated that Fe (III)-EDTA complex and ferrous sulfate exhibited the highest absorption, while ferric ammonium citrate was poorly absorbed. It was also found that Fe (III)-EDTA reacts slowly with the tannin contained in tea; the color of the tea changes slightly in the first 2 hr after the addition of the fortified sugar. Iron absorption of sugar fortified with ferrous sulfate was tested in seven beverages. The mean absorption ratio from fortified sugar given with beverages to reference dose of iron ascorbate ranged between 0.42 and 0.70, that is, more than 4 times the absorption from fortified sugar when it is administered with a meal containing one or more vegetals. An absorption of between 0.25 and 0.80 mg of iron/soft drink sugar fortified with 3 mg of iron as ferrous sulfate can be expected in subjects with various degrees of iron deficiency. Thus, two soft drinks per day between meals would be enough to meet the iron requirement in more than 95% of menstruating women, even though the daily iron absorption from the diet is about 0.8 to 1.0 mg. *Am. J. Clin. Nutr.* 29: 274-279, 1976.

A previous report (1) on the utilization of sugar as a vehicle for iron fortification has shown several advantages over the early procedures in which bread or wheat products have been used. It showed that sugar does not interfere with the absorption of iron salt and that iron absorption from sugar enriched with ferrous sulfate administered with beverages such as Coca-Cola and Pepsi-Cola between meals is absorbed at a rate 4 times higher than when it is administered during a meal containing vegetals.

This paper provides further information on the iron absorption from sugar enriched with ferrous sulfate administered with other beverages and also the absorption from sugar enriched with several ferric iron salts. These studies demonstrated that practically all soft drinks do not strongly inhibit the iron absorption of fortified sugar and that sugar enriched with Fe (III)-EDTA complex is absorbed in the same proportion as sugar enriched with ferrous sulfate.

## Materials and methods

Seventy-seven adult peasants from rural areas of Venezuela volunteered to be tested for this study. These subjects were in apparent good health. For each person, in addition to iron absorption tests, blood hemoglobin concentration (2), serum iron concentration (3), and unsaturated iron-binding capacity (4) were also determined.

### *Source of labeled test material*

*Foods.* Black beans and maize were biosynthetically labeled with radioactive iron according to methods previously described (5).

*Radioactive iron salts.* Ferrous sulfate solution was prepared by mixing labeled ferrous sulfate with a specific activity of about 10 to 15 $\mu$Ci/$\mu$g Fe with a given amount of carrier ferrous sulfate.

Labeled ferric ammonium citrate solution was made by mixing a labeled ferric chloride solution of specific activity of 10 $\mu$Ci/$\mu$g with a solution containing the same carrier salt. It was precipitated with ammonium hydroxide, centrifuged, dissolved with citric acid, and adjusted to pH 6.5 with diluted ammonium hydroxide.

Labeled ferric chloride was made by mixing labeled ferric chloride solution of specific activity of 10 $\mu$Ci/$\mu$g with a solution containing the same carrier salt.

Labeled Fe (III)-EDTA complex was prepared by adding a solution containing labeled and carrier ferric chloride to EDTA disodium salt. Assays were carried out previously to determine the exact proportion in which EDTA and iron solution should be mixed by slowly adding EDTA disodium salt solution to ferric chloride

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solution at pH 2 to 3 using salicylic acid as the indicator (6).

*Preparation and mixing of labeled test material*

*Enriched sugar.* Sugar was mixed with labeled ferric or ferrous salt before the absorption test. At the time of administration the enriched sugar was given as a drink during meals or between meals. The effect of several beverages on the absorption of enriched sugar was tested. In addition to sugar, these beverages contain fruit flavor, preservatives, and other trade mark substances. The ascorbic acid contained was less than 0.5 mg/100 ml (7).

*Standard meal.* It contains: black beans (1.8 mg Fe), rice (0.3 mg Fe), maize (1.0 mg Fe), and meat (1.2 mg Fe). Details on the preparation are given elsewhere (8).

*Absorption tests*

Labeled iron salt was administered with food or with a soft drink in the morning after an overnight fast. No food or drink was allowed for 3 hr after the administration of the radioactive material. The next day the subjects were fed again with other radioactive materials labeled with different radioactive iron. Blood was drawn 15 days later to determine hematological characteristics and radioactivity. The subjects were fed again on the 15th and 16th days with new labeled material and blood was taken again on the 30th day. Approximately 0.6 $\mu$Ci of $^{59}$Fe and 2 $\mu$Ci of $^{55}$Fe were used in each test. Duplicate 10-ml blood samples were prepared for radioactive counting following the technique of Dern and Hart (9, 10). Radioactivity was measured in a Packard Tri-Carb scintillation counter. Triplicate standards of the labeled material were counted simultaneously with the blood samples. The iron absorption from each labeled material was calculated from the $^{59}$Fe and $^{55}$Fe activity incorporated into the subjects' red cells using an estimated blood volume based on sex, weight, and height (11).

*Statistical analysis*

As in previous studies (1, 5, 8), the mean absorption and standard deviation were calculated from the logarithm of the percentage of absorption and the results were retransformed as antilogariths to recover the original units. Statistical comparison between two absorption tests was determined in pairs by the Student *t* tests (12).

TABLE 1
Absorption of iron fortification as ferric ammonium citrate mixed with sugar and administered with a meal as a drink

| Identification | Hb (g/100 ml) | Transferrin saturation (%) | Iron absorption (%): Standard meal + sugar (10 g) + iron salt (5 mg Fe): Iron salt $^{59}$Fe | Standard meal + sugar (10 g) + iron salt (5 mg Fe): Black beans $^{55}$Fe | Ferric ammonium citrate alone (5 mg Fe) $^{59}$Fe | Iron ascorbate alone (3 mg Fe) $^{55}$Fe |
|---|---|---|---|---|---|---|
| *A) Sugar enriched with labeled ferric ammonium citrate administered in the middle of a meal as a drink (1st study)* | | | | | | |
| 1) D. A. | 12.4 | 29 | 0.4 | 0.5 | 7.3 | 58.2 |
| 2) I. T. | 14.7 | 39 | 0.9 | 1.6 | 2.3 | 24.5 |
| 3) V. M. P. | 17.7 | 41 | 1.1 | 1.3 | 2.1 | 14.7 |
| 4) E. H. | 14.1 | 29 | 1.3 | 2.0 | 6.2 | 15.7 |
| 5) R. B. | 15.2 | 24 | 1.4 | 2.1 | 3.8 | 21.1 |
| 6) I. D. | 14.0 | 26 | 1.5 | 2.1 | 2.1 | 13.0 |
| 7) T. B. | 16.7 | 12 | 1.8 | 4.4 | 5.7 | 5.6 |
| 8) R. C. | 14.3 | 20 | 2.3 | 2.8 | 12.5 | 54.1 |
| 9) V. C. | 14.0 | 20 | 3.7 | 5.1 | 13.1 | 45.3 |
| 10) G. C. G. | 14.7 | 20 | 4.7 | 5.3 | 11.2 | 44.1 |
| 11) E. M. | 12.7 | 15 | 8.8 | 11.6 | 27.9 | 61.6 |
| Mean | 14.6 | 25 | 1.8 | 2.6 | 6.2 | 25.8 |
| SEM | | | 1.3 | 1.3 | 1.3 | 1.3 |
| *B) Sugar enriched with labeled ferric ammonium citrate administered in the middle of a meal as a drink (2nd study)* | | | | | | |
| 1) E. M. | 14.7 | 33 | 0.4 | 0.9 | | |
| 2) A. G. | 12.6 | 21 | 2.1 | 3.3 | | |
| 3) R. P. | 15.2 | 47 | 3.7 | 6.6 | | |
| 4) G. V. | 11.6 | 10 | 3.7 | 6.0 | | |
| 5) C. V. | 12.2 | 51 | 3.9 | 5.4 | | |
| 6) C. V. | 13.1 | 32 | 4.2 | 2.8 | | |
| 7) C. T. | 12.7 | 12 | 6.2 | 7.0 | | |
| 8) F. D. | 12.0 | 12 | 8.0 | 16.2 | | |
| 9) T. B. | 10.3 | 7 | 9.5 | 17.4 | | |
| Mean | 12.7 | 25 | 3.5 | 5.3 | | |
| SEM | | | 1.4 | 1.4 | | |

## Results

### *Iron absorption from sugar fortified with ferric ammonium citrate*

Table 1 shows the effect of a standard meal on the absorption of both extrinsic and intrinsic iron. The extrinsic iron is represented by ferric ammonium citrate which has been incorporated into the sugar and administered in the middle of the meal as a drink. The intrinsic labeled iron is from black beans which have been biosynthetically labeled with $^{59}$Fe. In study A the geometrical mean iron absorption from iron salt tag was 1.8% lower than that observed from the black beans' tag (2.6%), so that the mean absorption ratio of extrinsic to intrinsic iron was 0.69. The low absorbability of ferric ammonium citrate was confirmed in study B.

### *Iron absorption from sugar fortified with ferric chloride and ferrous sulfate*

Table 2 shows the results of iron absorption from sugar enriched with either ferric chloride or ferrous sulfate administered as a drink in the middle of the meal. The mean absorption from ferric chloride was 1.1%, slightly higher than the absorption from vegetable iron (0.9%), but much lower than that observed from ferrous sulfate (2.5%). These results agree with previous studies, which demonstrated that the absorption of ferrous sulfate and ferric chloride are very similar to the absorption of vegetable iron when they are administered as iron fortification but that the absorption of ferrous sulfate administered as a drink with a meal was more than twice the absorption of the salt as iron fortification. The absorption of ferric salt was the same when the salt was administered in either circumstance (13).

When sugar enriched with these iron salts is administered with a beverage, the absorption from ferric chloride is still two-thirds the absorption from ferrous sulfate (Table 3).

### *Iron absorption from sugar fortified with EDTA*

Table 4 shows the iron absorption from sugar enriched with either Fe (III)-EDTA complex or ferrous sulfate and administered in the middle of a meal or with a beverage. It was a surprise to find out that EDTA in the ferric form showed an iron absorption similar to ferrous sulfate.

TABLE 2
Iron absorption from sugar fortified with either ferric chloride or ferrous sulfate and administered in the middle of a standard meal as a drink[a]

| Identification | Hb (g/100 ml) | Transferrin saturation (%) | Iron absorption (%) | | |
|---|---|---|---|---|---|
| | | | Meal + sugar enriched with ferric chloride | | Meal + sugar enriched with ferrous sulfate |
| | | | Ferric chloride $^{55}$Fe | Maize $^{59}$Fe | Ferrous sulfate $^{59}$Fe |
| 1) V. R. | 14.4 | 30 | 0.2 | 0.2 | 4.6 |
| 2) M. C. | 13.2 | 14 | 0.6 | 0.8 | 3.3 |
| 3) R. M. | 14.9 | 24 | 0.8 | 0.6 | 1.9 |
| 4) A. E. M. | 13.1 | 30 | 0.9 | 0.5 | 0.4 |
| 5) R. B. | 14.3 | 19 | 0.9 | 0.6 | 1.8 |
| 6) E. B. | 14.1 | 18 | 1.3 | 1.1 | 3.5 |
| 7) M. V. | 14.3 | 24 | 1.4 | 1.0 | 0.8 |
| 8) M. C. S. | 11.7 | 8 | 1.6 | 1.5 | 4.7 |
| 9) M. A. M. | 14.3 | 47 | 1.6 | 0.9 | 2.0 |
| 10) M. E. C. | 13.6 | 32 | 1.9 | 1.7 | 12.6 |
| 11) F. M. | 12.9 | 26 | 2.5 | 2.3 | 3.8 |
| Mean | 13.7 | 25 | 1.1 | 0.9 | 2.5 |
| SEM | | | 1.2 | 1.2 | 1.3 |

[a] Five grams of sugar enriched with iron salt (3 mg Fe) were administered in the middle of a standard meal as a drink.

TABLE 3
Iron absorption from sugar fortified with ferric and ferrous salts and administered with a beverage

| Identification | Hb (g/100 ml) | Serum iron (μg/100 ml) | Transferrin saturation (%) | Iron absorption (%) | | |
|---|---|---|---|---|---|---|
| | | | | Coca-Cola + $FeCl_3$ (3 mg Fe) $^{59}Fe$ | Coca-Cola + $FeSO_4$ (3 mg Fe) $^{59}Fe$ | Iron ascorbate (3 mg Fe) $^{59}Fe$ |
| 1) V. M. | 12.9 | 110 | 29 | 5.2 | 14.5 | 34.6 |
| 2) N. A. | 11.3 | 47 | 11 | 6.5 | 9.5 | 67.6 |
| 3) T. P. | 13.7 | 82 | 26 | 9.7 | 9.6 | 29.0 |
| 4) A. G. | 11.8 | 60 | 17 | 12.6 | 38.7 | 44.2 |
| 5) O. A. | 13.3 | 141 | 39 | 12.8 | 12.2 | 30.0 |
| 6) F. G. | 14.1 | 52 | 14 | 25.4 | 59.0 | 79.6 |
| 7) R. M. | 10.3 | 33 | 9 | 30.8 | 69.9 | 74.8 |
| 8) M. M. | 17.9 | 181 | 40 | 36.7 | 67.8 | 66.3 |
| 9) I. C. | 11.4 | 86 | 21 | 40.3 | 56.4 | 54.8 |
| 10) J. B. | 15.8 | 72 | 23 | 46.0 | 63.8 | 59.7 |
| 11) A. V. | 13.4 | 59 | 13 | 47.7 | 73.0 | 88.7 |
| 12) M. V. | 12.5 | 45 | 9 | 65.5 | 55.7 | 67.1 |
| 13) J. P. | 16.2 | 75 | 20 | 70.5 | 80.4 | 43.1 |
| Mean | 13.4 | 80 | 21 | 23.4 | 36.5 | 53.6 |
| SEM | | | | 1.3 | 1.3 | 1.1 |

TABLE 4
Iron absorption from sugar fortified with either ferrous sulfate or EDTA

| Identification | Hb (g/100 ml) | Transferrin saturation (%) | Iron absorption (%) | | | |
|---|---|---|---|---|---|---|
| | | | Meal + sugar enriched with Fe(III)-EDTA[a] $^{59}Fe$ | Meal + sugar enriched with ferrous sulfate[a] $^{59}Fe$ | Coca-Cola + sugar enriched with Fe(III)-EDTA[b] $^{59}Fe$ | Coca-Cola + sugar enriched with ferrous sulfate[b] $^{59}Fe$ |
| 1) P. A. | 14.7 | 22 | 0.7 | 0.8 | 9.0 | 5.7 |
| 2) T. R. | 14.0 | 27 | 1.0 | 0.7 | 5.4 | 5.6 |
| 3) S. A. | 15.4 | 39 | 1.6 | 6.5 | 12.2 | 14.0 |
| 4) T. P. | 12.7 | 25 | 4.7 | 6.4 | 28.3 | 24.9 |
| 5) F. P. | 16.0 | 55 | 7.5 | 8.1 | 30.9 | 23.1 |
| 6) B. G. | 13.3 | 14 | 7.6 | 7.9 | 9.6 | 11.1 |
| 7) E. M. | 13.8 | 22 | 18.2 | 9.4 | 18.1 | 25.8 |
| 8) P. C. | 14.3 | 16 | 25.3 | 16.3 | 45.4 | 58.3 |
| 9) R. P. | 10.0 | 7 | 28.5 | 16.4 | 12.6 | 39.3 |
| Mean | 13.8 | 25 | 5.4 | 5.4 | 15.5 | 17.7 |
| SEM | | | 1.6 | 1.5 | 1.3 | 1.3 |

[a] Five grams of sugar enriched with iron salt (3 mg Fe) were administered in the middle of the standard meal as a drink. [b] Five grams of sugar enriched with iron salt (3 mg Fe) were added to the dose of Coca-Cola.

### *Iron absorption from fortified sugar administered with beverages*

Several beverages of common use were tested in order to find out their inhibiting absorption effect on sugar fortified with ferrous sulfate (Table 5). The mean absorption ratio of each beverage to iron ascorbate absorption was between 0.40 and 0.47 with the exception of Naranja (Fanta), in which the ratio increased to 0.70. These results are in agreement with previous studies in which other beverages were tested (1).

## Discussion

The data presented here reinforce the results of previous studies which demonstrated

that sugar as a vehicle for iron fortification possesses several advantages over other vehicles previously utilized (1). However, it is yet necessary to find the most suitable iron salt to enrich the sugar. Ferrous sulfate as iron fortification does not induce adverse changes in color and taste of the sugar and the salt remains in the ferrous form for 1 year at least. The only adverse effect detected so far occurs when the sugar fortified with this salt is added to beverages containing high concentrations of tannin, such as tea; the color of the tea changes rapidly to black and iron is precipitated and poorly absorbed (14).

The results of testing three ferric iron salts indicate the poor absorbability of ferric ammonium citrate and the excellent absorbability of Fe (III)-EDTA complex, which showed iron absorption similar to that of ferrous sulfate. Similar results were also found in previous studies (15). It was also noticed that Fe (III)-EDTA complex shows a slow reaction with tea; the color of the tea infusion exhibits a slight change in the first 2 hr and iron is not precipitated for at least 24 hr. This preliminary information seems to indicate that this salt is suitable for iron fortification; however, further studies are necessary to demonstrate how the sugar enriched with this salt can be maintained in various climatic conditions, keeping its high absorbability without an adverse effect on the sugar.

The low inhibiting effect of some beverages on the absorption of iron in fortified sugar suggest their usefulness in iron nutrition, with the condition that they are taken between meals. Table 6 shows the iron absorption from enriched sugar added to eight soft

TABLE 5
Iron absorption from fortified sugar administered with soft drinks

| Identification | Hb (g/100 ml) | Serum iron µg/100 ml) | Transferrin saturation (%) | Iron absorption (%) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Seven-Up $^{59}$Fe | Ginger ale $^{59}$Fe | Chinotto $^{59}$Fe | Kola $^{59}$Fe | Sprite $^{59}$Fe | Naranja (Fanta) $^{59}$Fe | Iron ascorbate (3 mg Fe) $^{59}$Fe |
| 1st study | | | | | | | | | | |
| 1) C. C. | 14.0 | 141 | 41 | 3.0 | 3.8 | 10.1 | | | | 11.4 |
| 2) G. V. | 12.5 | 76 | 24 | 4.6 | 7.1 | 12.2 | | | | 30.3 |
| 3) P. P. | 15.6 | 80 | 24 | 5.8 | 16.3 | 31.5 | | | | 49.9 |
| 4) E. V. | 12.8 | 31 | 8 | 7.1 | 15.4 | 4.6 | | | | 15.5 |
| 5) H. G. | 13.4 | 82 | 25 | 9.2 | 17.9 | 29.6 | | | | 50.2 |
| 6) T. M. | 11.9 | 140 | 41 | 10.5 | 12.3 | 8.3 | | | | 17.8 |
| 7) A. R. M. | 15.4 | 148 | 41 | 20.1 | 17.6 | 12.6 | | | | 44.0 |
| 8) A. P. | 14.1 | 88 | 26 | 22.5 | 15.7 | 4.7 | | | | 19.7 |
| 9) M. S. | 14.2 | 78 | 25 | 27.5 | 34.7 | 22.9 | | | | 47.1 |
| 10) B. C. | 17.2 | 93 | 25 | 31.3 | 25.0 | 38.4 | | | | 79.7 |
| 11) M. M. | 12.4 | 69 | 16 | 46.2 | 17.7 | 45.2 | | | | 77.5 |
| 12) E. L. | 12.0 | 48 | 10 | 61.6 | 29.5 | 44.4 | | | | 60.2 |
| Mean | 13.8 | 90 | 26 | 14.1 | 15.5 | 16.7 | | | | 35.2 |
| SEM | | | | 1.3 | 1.2 | 1.3 | | | | 1.2 |
| 2nd study | | | | | | | | | | |
| 1) N. G. P. | 14.6 | 95 | 33 | | | | 3.9 | 5.8 | 6.1 | 10.5 |
| 2) G. G. | 14.2 | 107 | 35 | | | | 5.3 | 8.5 | 4.6 | 11.0 |
| 3) L. G. | | 137 | 37 | | | | 6.3 | 8.4 | 18.2 | 31.3 |
| 4) A. P. | 15.5 | 56 | 17 | | | | 6.8 | 10.5 | 19.1 | 22.8 |
| 5) E. P. | 14.0 | 43 | 15 | | | | 7.8 | 8.8 | 8.3 | 12.6 |
| 6) R. C. | 14.9 | 38 | 12 | | | | 7.9 | 4.8 | 11.6 | 24.8 |
| 7) J. P. | 11.5 | 70 | 21 | | | | 10.1 | 9.2 | 13.5 | 21.2 |
| 8) M. S. G. | 12.4 | 67 | 19 | | | | 10.7 | 35.0 | 62.2 | 73.1 |
| 9) E. G. | 14.5 | 80 | 19 | | | | 13.9 | 9.4 | 30.3 | 51.5 |
| 10) F. P. | 14.9 | 39 | 13 | | | | 24.8 | 24.7 | 22.3 | 27.3 |
| 11) D. M. | 12.5 | 54 | 14 | | | | 27.1 | 31.3 | 41.4 | 28.9 |
| 12) D. L. | 9.8 | 19 | 6 | | | | 45.9 | 30.6 | 69.1 | 63.8 |
| Mean | 13.3 | 67 | 0 | | | | 10.8 | 12.4 | 18.4 | 26.2 |
| SEM | | | | | | | 1.2 | 1.2 | 1.3 | 1.2 |

[a] Five grams of sugar containing 3 mg of iron as ferrous sulfate were added to each dose of soft drink.

TABLE 6
Calibration of absorption from sugar fortified with ferrous sulfate and administered with several beverages according to iron absorption from a reference dose

| Beverage | Calibrated iron absorption (%) | |
|---|---|---|
| | Beverages (mean) | Composite mean absorption from a reference dose of iron ascorbate |
| Seven-Up[a] | 12.5 | 31.2 |
| Kola[a] | 12.9 | |
| Ginger ale[a] | 13.7 | |
| Chinotto[a] | 14.8 | |
| Sprite[a] | 14.8 | |
| Coca-Cola (1st study)[b] | 16.7 | |
| Coca-Cola (2nd study)[b] | 20.2 | |
| Coca-Cola (3rd study)[a] | 21.2 | |
| Pepsi-Cola[b] | 20.8 | |
| Naranja (Fanta)[a] | 21.9 | |

[a] Present study. [b] Previous study.

drinks. It includes the results presented here and those published elsewhere (1).[6] The mean absorption ratio of fortified sugar when taken in a soft drink without food as compared to a reference dose of iron ascorbate ranges between 0.42 and 0.70, that is, more than 4 times the absorption of iron fortification when it is ingested with a meal containing one or more vegetable foods.

The data mentioned above suggest the possibility of using soft drinks containing fortified sugar for the prevention of iron deficiency anemia. An absorption of between 0.25 and 0.80 mg of iron/soft drink sugar fortified with 3 mg of iron as ferrous sulfate can be expected in subjects with various degrees of iron deficiency. Thus, two soft drinks/day between meals should meet the iron requirement in more than 95% of menstruating women, even though the daily iron absorption from diet is about 0.8 to 1.0 mg (16).

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[6] The results were calibrated multiplying the absolute mean absorption from each soft drink tested by the ratio between the composite mean absorption from the reference dose of iron ascorbate of all individuals and the mean absorption from the reference dose for the given study.