IDPAS #670

methods in nutrition

Fe(III)-EDTA complex as iron fortification[1,2]

Miguel Layrisse and Carlos Martínez-Torres

ABSTRACT Fe(III)-EDTA as iron fortification presents several advantages over the other iron salts previously used including ferrous sulfate. This iron compound exchange completely with vegetable food iron in the lumen of the gut but with the characteristics that the absorption from both, extrinsic and intrinsic food iron, is higher than that expected from other iron salts. The comparison between the iron absorption from Fe(III)-EDTA and ferrous sulfate as iron fortification indicates that the absorption from EDTA is about twice as high than that observed from ferrous sulfate. The data indicates that only 10 to 15 mg of iron as Fe(III)-EDTA as iron fortification would be necessary to prevent iron deficiency anemia in population relying their subsistence of vegetable food only and free of parasitic infection producing blood loss. *Am. J. Clin. Nutr.* 30: 1166–1174, 1977.

Reduced iron and several iron salts have been used in the past as iron fortification, however, not all are suitable for this purpose, in terms of iron absorption. While reduced iron, ferric chloride, and iron glycerophosphate show similar absorption as ferrous sulfate when wheat or maize or milk are used as a vehicle (1–3), ferric orthophosphate, sodium iron pyrophosphate, and ferric ammonium citrate are less well absorbed (2, 4). Recent studies on Fe(III)-EDTA complex have shown similar absorption as ferrous sulfate when sugar is fortified with this salt and administered with beverages or with a meal (4, 5).

This study provides information on iron absorption from increasing doses of Fe(III)-EDTA mixed with maize-soybean dough and administered with two standard meals. The results are compared with the absorption of ferrous sulfate administered in the same conditions.

Materials and methods

One hundred forty seven peasants from rural areas of Venezuela consented to collaborate with this study. Fifty were males and 97 were females. These subjects were in apparent good health; only a few of them suffered from iron deficiency anemia. Each individual participated in several iron absorption tests. Blood hemoglobin concentration (6), serum iron concentration (7), and unsaturated iron binding capacity (8) were determined in each case.

Source of labeled test material

Foods. Maize was biosynthetically labeled with radioactive iron according to a method previously described (9).

Iron salts. Labeled Fe(III)-EDTA complex was prepared by mixing mole to mole solutions of EDTA dissodium and solution of carrier ferric nitrate and tracer amount of labeled ferric chloride. Sodium bicarbonate is then added to the solution adjusting to pH 5. The Fe(III)-EDTA complex formed is precipitated by ethanol, the supernatant is discarded and the precipitate redisolved in water. The EDTA complex is precipitated again with ethanol and washed thrice with ethanol (10, 11).

Ferrous sulfate solution was prepared by mixing labeled ferrous sulfate with a specific activity of 10-15 μCi/μg Fe for ^{59}Fe and 35 μCi/μg Fe for ^{55}Fe with a given amount of carrier ferrous sulfate.

Iron ascorbate as a reference dose was prepared according to the method previously published (9).

Preparation of the standard meals

Meal A consists of four vegetable foods and a piece of meat. The amount of each food before cooking is as

---

[1] From the Departamento de Fisiopatología, Instituto Venezolano de Investigaciones Científicas, Caracas, Venezuela.

[2] Supported in part by the World Health Organization.

follows: 30 g of black beans, 50 g of maize-soybean flour, 35 g of rice, 50 g of plantain, and 75 g of meat. It contains 2.0 mg of meat iron, 5.7 mg of vegetable iron, 582 cal, 7.1 g of fat, and 30.9 of protein. Details of the preparation is previously reported (12). Meal B does not contain meat. The amount of each food before cooking is as follows: 30 g of black beans, 50 g of maize-soybean flour, 50 g of rice, and 50 g of plantain. It contains 5.9 mg of iron, 523 cal, 2.3 g of fat, 15.8 g of protein. Maize-soybean flour contains 8% of soybean. The maize dough was used as a vehicle for iron fortification.

Absorption studies

A meal labeled with radioactive iron was administered in the morning after overnight fast. No food or drink was allowed during 3 hr after the administration of the radioactive material. The next day another radioactive meal or iron salt was administered. Blood was drawn 15 days after the first administration of labeled material to determine hematological characteristics and radioactivity. The subjects were fed again on the day 15 and 16 with another radioactive material and blood was taken again on the day 30. Approximately 0.7 μCi of ^{59}Fe and 2μCi of ^{55}Fe was used in each test. Duplicate 10-ml blood samples were prepared for radioactive counting following the technique of Dern and Hart (13, 14). Radioactivity was measured in a liquid scintillation spectrometer. Triplicate standards of the food administered were counted simultaneously with the blood samples. The iron absorption from the foods or iron salts was calculated from the ^{59}Fe or ^{55}Fe activity in the subjects' blood using an estimate of blood volume based on sex, weight, and height (15). No correction was made to determine the total iron utilization.

Statistical analysis

The mean absorption and standard error were calculated from the logarithm of the percentage of absorption and the results were retransformed as antilogarithm to recover the original units. The comparison between the two absorption tests performed on the different meals calculated by the Student's *t* test in pair samples and the comparison between two absorption tests from the same meal by the least square method (16).

Results

Absorption of iron fortification as Fe(III)-EDTA complex

Several doses of iron as Fe(III)-EDTA labeled with ^{59}Fe were mixed with maize soybean dough biosynthetically labeled with ^{55}Fe and administered after cooking during the administration of meals A and B. Table 1 shows the absorption of iron fortification in the dose of 5, 25, and 50 mg of iron administered with meal A containing four vegetable foods and a piece of meat. It can be noticed that this iron compound completely exchange with the vegetable iron in the lumen of the gut, so that the mean absorption ratio of extrinsic to intrinsic iron is close to the unit in each test. The calibration of the absorption in each study by multiplying the observed absorption by the ratio of the composite mean absorption from the reference dose of iron ascorbate of all individuals and the mean absorption from the reference dose of iron ascorbate for the given study indicates that the percentage of iron absorption is not reduced when iron fortification is increased from 5 to 50 mg of iron. Thus, the iron absorptions from Fe(III)-EDTA was 4.5% and 4.1% with a dose of 5 and 50 mg of iron, respectively.

Similar dose of iron fortification were tested with the administration of the standard meal B containing no meat. Here again the mean absorption ratio was close to unit in each study and the mean absorption from iron fortification was not reduced when the dose was increased (Table 2).

Comparison between the absorption from Fe(III)-EDTA and ferrous sulfate as iron fortification

A dose of 25 mg of iron as Fe(III)-EDTA was mixed with a nonlabeled maize dough and administered in the same subject with meal B, meal B + 100 ml of orange juice and meal A (Table 3). There was not significant difference ($P > 1.10$) between the mean absorption from iron fortification administered with meal A and B. The addition of 100 ml of orange juice containing 50 mg of ascorbic acid did not significantly increased the absorption from iron fortification administered with meal B ($P > 0.20$). Similar studies with ferrous sulfate as iron fortification (Table 3, study b) showed similar results; the calibration of studies a and b according to iron ascorbate absorption indicates that the absorption from ferrous sulfate was distinctly lower than that obtained from EDTA. The mean absorption from EDTA was 5.0, 7.1, and 6.8 in the three tests while the mean absorption from ferrous sulfate was 2.1, 1.8, and 3.1, respectively.

TABLE 1
Absorption from different doses of iron fortification as Fe(III)-EDTA administered during the ingestion of the standard meal A

Identification	Hemoglobin (g/100 ml)	Transferrin saturation (%)	Iron absorption (%): Standard meal A: A Maize ^{55}Fe	Iron absorption (%): Standard meal A: B Fe(III)-EDTA ^{59}Fe	Iron absorption (%): C Iron ascorbate (3mg Fe) ^{55}Fe
A. Fe(III)-EDTA (5 mg Fe)					
1. VV	15.1	44	0.4	0.8	4.7
2. TV	13.9	29	1.1	1.4	14.0
3. CC	14.0	27	1.6	1.6	21.9
4. HB	14.3	27	1.3	1.8	25.7
5. SG	16.0	46	2.1	2.3	27.3
6. LC	14.5	24	2.2	2.7	11.4
7. LC	15.2	44	3.9	4.6	11.9
8. EL	13.3	31	5.4	5.8	40.2
9. VP	15.5	24	4.4	6.0	45.8
10. DC	13.8	17	6.0	6.6	65.7
11. BA	14.7	32	10.0	9.4	35.1
12. PC	14.0	20	14.7	17.1	50.5
13. LEP	15.6	13	27.6	31.3	72.9
Mean	14.6	29	3.4	4.1	25.7
SE of the mean			1.4	1.3	1.2
B. Fe(III)-EDTA (25 mg Fe)					
1. JLA	13.4	17	0.7	0.9	8.0
2. CN	14.9	22	3.0	3.5	5.6
3. CM	12.9	18	4.2	4.9	46.9
4. DL	12.4	22	4.9	5.5	23.6
5. LF	13.4	7	9.9	9.8	11.8
6. FF	13.2	12	11.1	11.8	48.9
7. JM	13.3	24	11.9	13.1	36.0
8. PB	13.1	8	12.9	15.9	54.4
9. EB	10.1	7	13.5	15.5	67.5
Mean	13.0	15	6.0	6.8	25.0
SE of the mean			1.4	1.4	1.4
C. Fe(III)-EDTA (50 mg Fe)					
1. BIS	16.7	15	1.5	1.9	13.2
2. RDS	16.4	26	1.6	1.8	29.7
3. CO	14.5	37	2.5	2.7	13.9
4. CS	12.8	20	3.5	3.1	33.9
5. FG	16.9	40	5.4	5.6	20.7
6. RG	15.0	8	6.4	7.5	66.4
7. JFS	16.4	42	7.8	8.8	68.9
8. JM	15.1	26	10.1	9.7	58.9
9. BV	14.9	23	10.8	11.0	59.8
10. JAP	15.6	16	14.0	15.7	69.5
Mean	15.4	25	4.9	5.3	36.6
SE of the mean			1.3	1.3	1.2

Dosage effect of iron fortification as ferrous sulfate

Table 4 shows the absorption of 5 and 50 mg of iron as ferrous sulfate administered with meal A and B. Apparently the difference between 5 and 50 mg of iron as ferrous sulfate mixed with maize and administered with meal was not enough to produce difference in absorption, so that the mean absorp-

TABLE 2
Absorption from different doses of iron Fe(III)-EDTA administered during the ingestion of the standard meal B

Identification	Hemoglobin (g/100 ml)
A. Fe(III)-EDTA (5 mg Fe)	
1. DM	13.1
2. ADV	13.4
3. MS	13.4
4. OR	12.9
5. FG	12.2
6. OS	13.6
7. MC	12.1
8. ER	16.0
9. EG	12.8
10. GC	11.7
11. JG	11.7
12. AG	12.1
13. JP	10.3
Mean	12.7
SE of the mean	
B. Fe(III)-EDTA (25 mg Fe)	
1. MH	13.6
2. AV	13.6
3. MT	13.4
4. VB	16.0
5. AA	14.0
6. LP	15.4
7. OR	12.2
8. MN	11.2
9. JR	12.2
10. HR	13.1
Mean	13.5
SE of the mean	
C. Fe(III)-EDTA (50 mg Fe)	
1. CB	13.5
2. MP	14.2
3. FG	14.0
4. AC	13.3
5. GV	12.9
6. GB	15.3
7. GA	14.1
8. GB	14.5
9. MH	14.9
10. MAP	13.8
11. MC	17.6
12. JA	14.6
13. AA	10.1
14. MP	13.1
15. LA	12.7
Mean	13.9
SE of the mean	

Iron absorption (%)	
andard meal A	
B Fe(III)-EDTA ^{59}Fe	C Iron ascorbate (3mg Fe) ^{55}Fe
0.8	4.7
1.4	14.0
1.6	21.9
1.8	25.7
2.3	27.3
2.7	11.4
4.6	11.9
5.8	40.2
6.0	45.8
6.6	65.7
9.4	35.1
17.1	50.5
31.3	72.9
4.1	25.7
1.3	1.2
0.9	8.0
3.5	5.6
4.9	46.9
5.5	23.6
9.8	11.8
11.8	48.9
13.1	36.0
15.9	54.4
15.5	67.5
6.8	25.0
1.4	1.4
1.9	13.2
1.8	29.7
2.7	13.9
3.1	33.9
5.6	20.7
7.5	66.4
8.8	68.9
9.7	58.9
11.0	59.8
15.7	69.5
5.3	36.6
1.3	1.2

A and B. Apparently the differ-
en 5 and 50 mg of iron as ferrous
ed with maize and administered
vas not enough to produce differ-
orption, so that the mean absorp-

TABLE 2
Absorption from different doses of iron fortification as Fe(III)-EDTA administered during the ingestion of the standard meal B

Identification	Hemoglobin (g/100 ml)	Transferrin saturation (%)	Iron absorption (%)		
			Standard meal B		C Iron ascorbate (3 mg Fe) ^{59}Fe
			A Maize ^{55}Fe	B Fe(III)-EDTA ^{59}Fe	
A. Fe(III)-EDTA (5 mg Fe)					
1. DM	13.1	38	1.2	1.4	13.4
2. ADV	13.4	19	1.4	1.9	24.1
3. MS	13.4	30	1.7	2.0	13.0
4. OR	12.9	15	2.6	3.3	28.9
5. FG	12.2	32	2.7	3.0	13.4
6. OS	13.6	39	4.0	5.4	11.8
7. MC	12.1	35	7.6	9.9	13.2
8. ER	16.0	42	8.0	8.7	26.3
9. EG	12.8	18	11.4	14.1	49.6
10. GC	11.7	15	11.5	13.5	19.0
11. JG	11.7	35	11.5	14.3	52.8
12. AG	12.1	24	11.6	13.8	26.8
13. JP	10.3	7	17.8	20.7	82.6
Mean	12.7	27	5.1	6.2	23.7
SE of the mean			1.3	1.3	1.2
B. Fe(III)-EDTA (25 mg Fe)					
1. MH	13.6	38	2.8	2.9	45.9
2. AV	13.6	35	4.1	4.3	50.5
3. MT	13.4	24	4.2	3.8	50.4
4. VB	16.0	27	4.4	5.0	46.0
5. AA	14.0	16	4.6	5.2	48.8
6. LP	15.4	15	4.9	4.6	90.1
7. OR	12.2	19	7.0	6.4	9.8
8. MN	11.2	5	15.1	14.2	75.8
9. JR	12.2	14	21.2	21.2	38.5
10. HR	13.1	12	24.8	26.1	71.9
Mean	13.5	21	7.0	7.0	46.6
SE of the mean			1.3	1.3	1.2
C. Fe(III)-EDTA (50 mg Fe)					
1. CB	13.5	30	1.2	1.1	20.4
2. MP	14.2	36	1.5	1.4	10.2
3. FG	14.0	26	2.3	2.1	9.1
4. AC	13.3	34	2.8	2.8	49.7
5. GV	12.9	22	2.9	2.6	4.9
6. GB	15.3	35	2.9	2.8	40.8
7. GA	14.1	31	3.1	3.1	14.5
8. GB	14.5	41	4.3	4.1	46.1
9. MH	14.9	31	4.4	4.4	12.8
10. MAP	13.8	28	4.5	4.4	47.3
11. MC	17.6	31	5.1	4.7	58.8
12. JA	14.6	16	8.9	9.7	87.7
13. AA	10.1	5	9.2	9.4	79.4
14. MP	13.1	8	15.6	23.7	98.5
15. LA	12.7	8	17.1	15.9	77.2
Mean	13.9	25	4.3	4.2	31.0
SE of the mean			1.2	1.2	1.3

TABLE 3
Comparison between the iron absorption from Fe(III)-EDTA and from ferrous sulfate as iron fortification and administered with different meals

Identification	Hemoglobin (g/100 ml)	Transferrin saturation (%)	Iron absorption (%)			
			A Meal B + salt (25 mgFe) ^{59}Fe	B Meal B + orange juice + salt (25 mgFe) ^{55}Fe	C Meal A + salt (25 mgFe) ^{59}Fe	D iron ascorbate ^{55}Fe
A. Fe(III)-EDTA as iron fortification						
1. CA	14.0	41	1.0		1.5	6.8
2. MR	12.9	10	2.4	5.7	19.9	66.2
3. BN	14.2	29	3.0	4.3	10.9	43.1
4. LG	13.4	23	3.8		9.3	45.8
5. IM	11.7	17	5.1		6.4	21.3
6. EP	15.3	26	5.6	11.4	6.7	47.5
7. AH	12.6	22	6.4		3.8	29.7
8. RH	12.8	28	8.0		3.3	18.8
9. PA	12.6	23	8.1	12.1	12.9	34.2
10. MB	13.7	26	9.2		11.7	38.8
11. ZM	12.1	29	12.6		17.6	62.7
12. PV	14.9	21	13.5		10.2	45.9
13. LO	13.3	12	16.1	12.0	15.2	57.6
14. NZ	10.8	5	18.0	12.6	15.7	48.1
Mean	13.2	22	6.3	8.9	8.5	35.6
SE of the mean			1.2	1.2	1.2	1.2
B. Ferrous sulfate as iron fortification						
1. MB	15.6	22	0.2	1.1	0.8	14.0
2. PAS	17.7	26	0.4	1.1	0.3	6.7
3. ET	14.0	40	0.5	1.4	2.8	18.2
4. FM	14.1	24	0.8	0.9	0.2	4.1
5. AA	14.7	20	1.3	0.5	1.6	12.6
6. AH	11.8	14	1.3		4.8	29.1
7. PU	14.8	14	1.4		4.0	21.3
8. NS	9.5	4	1.5		2.9	71.7
9. EA	12.3	8	1.8		2.2	25.8
10. AM	12.5	7	2.2	2.6	12.6	24.0
11. AU	12.6	13	2.7		0.8	13.4
12. CS	11.0	7	3.8		4.8	46.5
13. GM	12.5	31	3.9		5.2	20.8
14. RV	12.0	8	4.0		11.9	66.9
15. NU	15.4	30	4.0		2.4	47.5
16. EM	13.6	15	5.5		3.7	11.4
17. ALM	13.1	20	5.5	9.6	8.5	60.0
18. EU	13.6	14	7.2		4.4	84.6
Mean	13.4	18	1.8	1.5	2.6	23.8
Mean SE of the mean			1.3	1.4	1.3	1.2

tion from 5 and 50 mg of iron was 3.2 and 2.6% with meal A and 2.7 and 2.9% with meal B.

Effect of milk on the iron absorption from ferrous sulfate and Fe(III)-EDTA complex

Fourteen subjects were tested for the absorption from 5 mg of iron as ferrous sulfate and Fe(III)-EDTA administered with 200 ml of milk (Table 5). The iron absorption from EDTA was distinctly higher than that observed from ferrous sulfate in each subject tested and the mean absorption difference was statistically significant ($P < 0.001$).

Discussion

The studies on iron absorption carried out in the last 7 years specially those concerned with the interaction of foods and iron salts in

ABLE 4
bsorption from different doses of iron for
lfate administered during the ingestion
standard meals

Identification	Hemoglobin (g/100 ml)	Tr sa
A. Iron fortification with meal A		
1. SM	14.0	
2. RCV	12.4	
3. SC	8.7	
4. SM	13.4	
5. GG	12.6	
6. AM	9.8	
7. EZ	11.0	
8. SZ	13.6	
9. GM	12.9	
10. ER	10.6	
Mean	11.9	
SE of the mean		
B. Iron fortification with meal B		
1. ZP	15.0	
2. MP	14.6	
3. MTB	15.2	
4. VA	14.9	
5. JB	13.2	
6. JP	13.7	
7. YP	13.4	
8. FP	15.6	
9. CM	13.0	
10. JU	12.6	
11. MT	13.8	
12. MS	18.3	
13. JMP	17.8	
14. IP	14.1	
15. CP	9.9	
16. ER	15.3	
17. OP	14.4	
18. IR	12.5	
19. JJB	15.6	
20. JG	9.9	
21. JC	16.0	
Mean	14.2	
SE of the mean		

the lumen of the gut and the dev
the extrinsic label method allo
cise determination of the am
absorbed from fortified food.
demonstrated that most of t
either as ferric and ferrous salt
nonheme iron pool together w
and egg iron (1, 17–19). They
strate that increasing dose of i
to 60 mg mixed with vegetabl

Iron absorption (%)		
B Meal B + orange juice + salt (25 mgFe) ^{55}Fe	C Meal A + salt (25 mgFe) ^{59}Fe	D iron ascorbate ^{55}Fe
	1.5	6.8
5.7	19.9	66.2
4.3	10.9	43.1
	9.3	45.8
	6.4	21.3
11.4	6.7	47.5
	3.8	29.7
	3.3	18.8
12.1	12.9	34.2
	11.7	38.8
	17.6	62.7
	10.2	45.9
12.0	15.2	57.6
12.6	15.7	48.1
8.9	8.5	35.6
1.2	1.2	1.2
1.1	0.8	14.0
1.1	0.3	6.7
1.4	2.8	18.2
0.9	0.2	4.1
0.5	1.6	12.6
	4.8	29.1
	4.0	21.3
	2.9	71.7
	2.2	25.8
2.6	12.6	24.0
	0.8	13.4
	4.8	46.5
	5.2	20.8
	11.9	66.9
	2.4	47.5
	3.7	11.4
9.6	8.5	60.0
	4.4	84.6
1.5	2.6	23.8
1.4	1.3	1.2

A was distinctly higher than that
from ferrous sulfate in each sub-
d and the mean absorption differ-
statistically significant (P <

n

dies on iron absorption carried out
7 years specially those concerned
teraction of foods and iron salts in

ABLE 4
bsorption from different doses of iron fortification as ferrous
lfate administered during the ingestion
standard meals

Identification	Hemoglobin (g/100 ml)	Transferrin saturation (%)	Iron absorption (%) A Meal + ferrous sulfate (5 mgFe) ^{55}Fe	B Meal + ferrous sulfate (50 mgFe) ^{59}Fe	C Iron ascorbate (3 mg Fe) ^{55}Fe
A. Iron fortification with meal A					
1. SM	14.0	32	0.8	1.3	26.9
2. RCV	12.4	16	2.0	1.8	44.7
3. SC	8.7	5	2.2	4.0	70.3
4. SM	13.4	28	2.8	3.0	18.5
5. GG	12.6	37	3.4	1.9	26.2
6. AM	9.8	5	4.2	3.0	59.3
7. EZ	11.0	9	4.3	2.1	51.0
8. SZ	13.6	30	5.1	2.3	17.3
9. GM	12.9	15	5.6	5.8	77.6
10. ER	10.6	6	7.1	2.7	68.0
Mean	11.9	18	3.2	2.6	40.3
SE of the mean			1.2	1.1	1.2
B. Iron fortification with meal B					
1. ZP	15.0	39	0.3	0.2	8.7
2. MP	14.6	23	0.4	0.6	18.1
3. MTB	15.2	51	0.6	0.3	8.6
4. VA	14.9	28	0.8	2.6	11.9
5. JB	13.2	9	0.9	4.3	54.9
6. JP	13.7	19	1.0	1.2	57.8
7. YP	13.4	14	1.6	6.4	79.0
8. FP	15.6	37	1.6	1.3	41.5
9. CM	13.0	39	1.7	2.8	28.4
10. JU	12.6	16	2.1	1.0	15.1
11. MT	13.8	24	2.5	4.7	16.4
12. MS	18.3	26	2.5	1.7	21.4
13. JMP	17.8	23	3.0	5.5	65.4
14. IP	14.1	19	5.2	6.9	67.5
15. CP	9.9	8	5.7	14.8	81.9
16. ER	15.3	25	6.2	2.0	9.3
17. OP	14.4	30	9.0	6.3	45.3
18. IR	12.5	17	15.8	5.8	60.6
19. JJB	15.6	25	17.9	9.3	77.2
20. JG	9.9	8	19.3	9.2	58.4
21. JC	16.0	21	20.5	12.6	69.2
Mean	14.2	24	2.7	2.9	32.9
SE of the mean			1.3	1.3	1.2

the lumen of the gut and the development of the extrinsic label method allow a more precise determination of the amount of iron absorbed from fortified food. These studies demonstrated that most of the iron salts either as ferric and ferrous salt enter into the nonheme iron pool together with vegetable and egg iron (1, 17–19). They also demonstrate that increasing dose of iron from 0.1 to 60 mg mixed with vegetable food is absorbed to the same extent as the native iron (1). According to these results, food vehicles such as wheat, maize, and rice inhibit considerably the absorption of iron added to these foods.

Based on these studies, several students have recently tested other vehicles and several iron salts in order to find out the most suitable iron fortification that could be used in a country or in an area according to their

TABLE 5
Effect of milk on the iron absorption from ferrous sulfate and Fe(III)-EDTA

Identification	Hemoglobin (g/100 ml)	Transferrin saturation (%)	Iron absorption (%): A Milk (200 ml) + ferrous sulfate (5 mgFe) ^{59}Fe	B Milk (200 ml) + Fe(III)-EDTA (5 mg Fe) ^{55}Fe	C Iron ascorbate (3 mg Fe) ^{59}Fe
1. AM	13.6	13	0.6	1.7	10.5
2. AC	15.1	50	0.8	1.4	7.7
3. MC	13.4	29	4.9	7.6	17.1
4. MG	12.9	18	5.4	19.6	18.6
5. OZ	15.4	24	5.6	7.6	10.2
6. CS	12.9	17	7.9	16.2	26.2
7. BC	10.8	4	8.1	22.9	35.2
8. MR	14.9	27	11.4	21.8	37.6
9. CT	15.0	29	11.4	11.8	36.0
10. AC	17.2	39	12.7	13.7	62.0
11. CO	15.6	26	12.7	12.9	20.6
12. RP	11.3	6	16.3	19.3	59.6
13. JR	15.5		17.5	29.1	76.8
14. ED	10.8	6	27.2	33.1	84.7
	13.9	22	7.0	11.6	27.8
Mean SE of the mean			1.3	1.3	1.2

food habit and local facilities to enrich the vehicle selected. Common salt fortified with ferric orthophosphate and ascorbic acid has been recommended in countries where maize or rice are the staple food (20). EDTA added to fish sauce has been successfully used in a pilot study for iron fortification in Thailand (21). Sugar as a vehicle does not carry inhibiting iron absorption substances, and maintains ferrous sulfate in the ferrous form for at least a year (22). The absorption studies of fortified sugar administered with beverages indicated that the absorption is several times higher than the absorption from the same fortified sugar mixed with a meal. Either ferrous sulfate or EDTA is suitable to enrich sugar, but while iron from ferrous sulfate is precipitated and poorly absorbed when fortified sugar is added to beverages containing high concentration of tanin, such as tea (23), Fe(III)-EDTA reacts slowly with tea and iron is not precipitated for at least 24 hr (4).

The studies presented here provide further evidence on the characteristics of Fe(III)-EDTA absorption when it is administered as iron fortification. This iron compound seems to exchange completely with vegetable food iron in the lumen of the gut but with the characteristic that the absorption from both, extrinsic and intrinsic food iron, is higher than that expected from other iron salts used as iron fortification (1). This observation is confirmed comparing the absorption from different dose of iron in the form of ferrous sulfate and Fe(III)-EDTA prepared as iron fortification and administered with the same type of meals (Table 6). These absorption results were taken from those reported in Table 1 to 4 and calibrated according to the absorption from the reference dose of iron ascorbate. The data indicate that the absorption from both iron salts are not reduced when the dose of iron fortification is increased. Furthermore iron absorption from maize fortified with Fe(III)-EDTA is significantly distinct higher than that observed from maize fortified with ferrous sulfate in each instance.

The data also shows that meat in a proportion of 75 g per meal does not increase the absorption of iron fortification even when fortified food plus meal contained 11 mg of iron. This is in accord to previous studies, which demonstrated that more than 50 g of meat are necessary to increase the

ABLE 6
on absorption from maize enriched with
rrous sulfate or Fe(III)-EDTA
omplex administered
ith meals

Dose of iron fortification (mg)	Iron absorption (%): Maize enriched with ferrous sulfate (mean)	Maize enriched with Fe(III)-EDTA (mean)
Meal A		
5	2.5	4.9
25	3.4	8.4
		7.4
50	2.0	4.5
Meal B		
5	2.5	8.1
25	2.3	4.7
		5.5
50	2.7	4.2

absorption of about 4 mg of
(12, 24).
According to these results the
iron fortification that will be n
population that subsists on ve
only or with moderate meat int
vent iron deficiency anemia can
instance, in a population with
heme iron consumption of 1
the addition of 15 mg of iron f
EDTA, that is, 5 mg of iron
would determine a total dail
tion (dietary iron + iron fo
about 1 mg of iron in subj
ascorbate below 20% represe
with normal iron stores; abo
subjects with iron ascorbate a
tween 20 and 39%, represe
subjects and those with moder
ciency; and about 4 mg of iro
with iron ascorbate absorption
representing those with mark
ciency. The amount of iron
used in severe iron deficiency
iron fortification could be red
10 mg if such population is fr
infection producing blood loss
The data on EDTA present
apparently from studies publi
and Monsen (25) that dem
iron absorption from a stand
taining 4 mg of iron is reduce
half when 50 mg of EDTA i

TABLE 6
Iron absorption from maize enriched with ferrous sulfate or Fe(III)-EDTA complex administered with meals

Dose of iron fortification (mg)	Iron absorption (%)		
	Maize enriched with ferrous sulfate (mean)	Maize enriched with Fe(III)-EDTA (mean)	Composite mean absorption from a reference dose (3 mgFe) (mean)
Meal A			
5	2.5	4.9	
25	3.4	8.4	
		7.4	
50	2.0	4.5	31.0
Meal B			
5	2.5	8.1	
25	2.3	4.7	
		5.5	
50	2.7	4.2	

absorption of about 4 mg of vegetal iron (12, 24).

According to these results the amount of iron fortification that will be necessary in a population that subsists on vegetable foods only or with moderate meat intake, to prevent iron deficiency anemia can be seen. For instance, in a population with a daily nonheme iron consumption of 15 mg of iron, the addition of 15 mg of iron fortification as EDTA, that is, 5 mg of iron in each meal, would determine a total daily iron utilization (dietary iron + iron fortification) of about 1 mg of iron in subjects with iron ascorbate below 20% representing subjects with normal iron stores; about 1.4 mg in subjects with iron ascorbate absorption between 20 and 39%, representing normal subjects and those with moderate iron deficiency; and about 4 mg of iron in subjects with iron ascorbate absorption above 40%, representing those with marked iron deficiency. The amount of iron absorbed and used in severe iron deficiency is so high that iron fortification could be reduced to about 10 mg if such population is free of parasitic infection producing blood loss.

The data on EDTA presented here differ apparently from studies published by Cook and Monsen (25) that demonstrated the iron absorption from a standard diet containing 4 mg of iron is reduced to about one half when 50 mg of EDTA is added. However, the experiments were designed differently; while Cook and Monsen added an excess of EDTA in the proportion of 2 moles of EDTA per mole of iron in the diet, in the experiments presented here iron was incorporated into EDTA molecule in the proportion of mole to mole.

Three food vehicles: maize, milk, and sugar, enriched with Fe(III)-EDTA have been already tested for iron absorption. In each instance this salt, as iron fortification, has demonstrated so far, more advantages than that observed from other iron salts, including ferrous sulfate. EDTA is a chelating agent and its use in food technology to prevent oxidative damage of food has been restricted, however, the amount of EDTA necessary for 10 mg of iron fortification, about 60 mg, is comparable to the usual amount added to the diet.

References

1. LAYRISSE, M., C. MARTÍNEZ-TORRES, J. D. COOK, R. WALKER AND C. A. FINCH. Iron fortification of food. Its measurement by the extrinsic tag method. Blood 41: 333, 1973.
2. COOK, J., V. MINNICH, C. V. MOORE, A. RASMUSSEN, W. B. BRADLEY AND C. A. FINCH. Absorption of fortification iron in bread. Am. J. Clin. Nutr. 26: 861, 1973.
3. LAYRISSE, M., C. MARTÍNEZ-TORRES, M. RUPHAEL-DIVO, W. JAFFÉ AND J. E. TORRES-SUÁREZ. Iron absorption from skim milk enriched with iron glycerophosphate. Arch. Latinamer. Nutr. 23: 145, 1973.
4. LAYRISSE, M., C. MARTÍNEZ-TORRES AND M. RENZI. Sugar as a vehicle for iron fortification. Further Studies. Am. J. Clin. Nutr. 26: 274, 1976.
5. VITERI, F. WHO Meeting on Nutritional Anemia. Geneva, 1974.
6. CROSBY, W. H., J. L. MUNN AND F. W. FURTH. Standardizing a method for clinical hemoglobinometry. U. S. Armed Forces M. J. 5: 693, 1954.
7. International Committee for Standardization in Hematology. Proposed Recommendations for measurement of serum iron in human blood. J. Clin. Pathol. 56: 543, 1971.
8. IZAK, G., AND S. M. LEWIS. Studies on the Standardization of Serum iron and iron binding capacity assays. In: Modern Concepts in Hematology. New York: Academic Press, 1972, p. 69.
9. LAYRISSE, M., J. D. COOK, C. MARTÍNEZ-TORRES, M. ROCHE, I. N. KUHN AND C. A. FINCH. Food iron absorption: a comparison of vegetable and animal foods. Blood 33: 430, 1969.
10. SAWYER, D. T., AND J. M. MCKINNIE. Properties and infrared spectra of Ethylenediaminetetracetic acid complex. III. Chelates of high valent ions. J. Am. Clin. Soc. 82: 4191, 1960.

11. Flaschka, H. A. EDTA Titrations. Oxford: Pergamon Press, 1964, p. 85.
12. Layrisse, M., C. Martínez-Torres, M. González. Measurement of the total daily dietary absorption by the extrinsic tag model. Am. J. Clin. Nutr. 27: 152, 1974.
13. Dern, J. R., and W. L. Hart. Studies with doubly labelled iron. I. Simultaneous liquid scintillation counting isotopes of Fe^{55} and Fe^{59} as ferrous perchlorate. J. Lab. Clin. Med. 57: 322, 1961.
14. Dern, J. R., and W. L. Hart. Studies with doubly labelled iron. II. Separation of iron from blood samples and preparation of ferrous perchlorate for liquid scintillation counting. J. Lab. Clin Med. 57: 460, 1961.
15. Nadler, S. B., J. U. Hidalgo and T. Block. The Tulane table of blood volume in normal men. Surgery 51: 224, 1962.
16. Snedecor, G. W., and W. G. Cochran. Statistical Methods (6th ed.). The Iowa State University Press, p. 91, 1967.
17. Cook, J., M. Layrisse, C. Martínez-Torres, R. Walker, E. Monsen and C. A. Finch. Food iron absorption measured by an extrinsic tag. J. Clin. Invest. 51: 805, 1972.
18. Bjorn-Rasmussen, E., L. Hallberg and R. B. Walker. Food iron absorption in man. I. Isotopic exchange between food iron and inorganic salt added to food: Studies on maize, wheat and eggs. Am. J. Clin. Nutr. 25: 317, 1972.
19. Layrisse, M., and C. Martínez-Torres. Model for measuring dietary absorption of heme iron: test with a complete meal. Am. J. Clin. Nutr. 25: 401, 1972.
20. Sayers, M. H., S. R. Linch, R. W. Charlton, T. H. Bothwell, R. B. Walker and F. Mayet. The fortification of common salt with ascorbic acid and iron. Brit. J. Haematol. 28: 483, 1974.
21. Garby, L. Iron fortification of fish sauce in Thailand. Am. J. Trop. Med. Hyg. In press.
22. Layrisse, M., C. Martínez-Torres, M. Renzi, F. Velez and M. González. Sugar as a vehicle for iron fortification. Am. J. Clin. Nutr. 129: 8, 1976.
23. Disler, P. B., S. R. Linch, R. W. Charlton and T. H. Bothwell. Studies on the fortification of cane sugar with iron and ascorbic acid. Brit. J. Nutr. 34: 141, 1975.
24. Cook, J. D., and E. R. Monsen. Food iron absorption. I. Use of a semisynthetic diet to study absorption of non-heme iron. Am. J. Clin. Nutr. 28: 1289, 1975.
25. Cook. J. D., and E. R. Monsen. Food iron absorption in man. II. The effect of EDTA on absorption of dietary nonheme iron. Am. J. Clin. Nutr. 29: 614, 1976.

Vitamin A deficie
American childre

L. A. Majía, M.A., R. E. Hodg
B. Torún, M.D.

ABSTRACT In or
anemia in Central Ame
of Central America an
America and Panama
old, living between
dietary parameters re
between the ages of 5 an
and plasma retinol. Ch
all age groups there wer
saturation of transferri
Children with an adequ
nomic level, showed a
their serum. In contras
these findings, a poss
gested. *Am. J. Clin.*

Deficiency of vitamin A an
anemia have been shown to b
major nutritional problems,
children, not only in develop
(1-5) but also in highly develo
(6).

In most of these countries, a
intake of vitamin A appears to b
lying cause of low plasma levels
Nutritional anemia, on the othe
been traditionally associated wi
cies of iron, folate, vitamin B_{12},
It has also been thought that par
tation plays an important role
roles of vitamin E, vitamin B_6
microelements such as zinc and
not been fully studied in these
thus there is not enough evide
sider them of primary importan

Recent studies in humans an
mental animals by Hodges et
have demonstrated an apparent
between vitamin A deficiency a