Sodium iron NaFeEDTA as an iron fortification compound in Central America. Absorption studies[1, 2]

Fernando E. Viteri, Roberto García-Ibañez, and Benjamín Torún

ABSTRACT Studies were performed in seven children and 98 adults to compare the proportion of iron absorbed when administered as ferric sulfate ($Fe_2(SO_4)_3$), NaFeEDTA, hemoglobin (Hb), and ferrous ascorbate. Studies in children (mostly iron deficient) showed that when the compounds were given with a milk-rice-sugar formula totalling 5 mg Fe, iron from hemoglobin was absorbed best, followed by NaFeEDTA and by $Fe_2(SO_4)_3$ (mean percent absorption ± SD = 34.5 ± 1.5, 8.6 ± 1.9 and 3.3 ± 1.5, respectively). Studies in normal or iron deficient adults also demonstrated a better absorption of iron from NaFeEDTA than from $Fe_2(SO_4)_3$ whether these compounds were given in an aqueous solution (5 mg Fe) or with a standard meal consisting of beans, tortillas, bread, and coffee providing also a total of 5 mg Fe. Hb iron under the same conditions was absorbed in the same proportion to the reference iron ascorbate, always being higher than iron absorbed from the other compounds. $Fe_2(SO_4)_3$ and NaFeEDTA mixed in the same meal were absorbed in the same proportion as when NaFeEDTA alone was added to the meal and 2 to 3 times better than when $Fe_2(SO_4)_3$ alone was added to the meal. Addition of desferrioxamine depressed iron absorption from $Fe_2(SO_4)_3$ and NaFeEDTA, the latter being less affected. Addition of ascorbic acid increased absorption from both. When the compounds were added to the meal to provide 50 mg of iron, percent absorption was depressed in relation to the smaller iron dose in the case of $Fe_2(SO_4)_3$ and Hb but remained unaltered in the case of NaFeEDTA. Addition of 45 mg Fe as $Fe_2(SO_4)_3$ or NaFeEDTA to 0.4 mg Fe from Hb in the meal did not change Hb iron absorption. Addition of 45 mg Fe as Hb or NaFeEDTA to 0.4 mg Fe from $Fe_2(SO_4)_3$ in the meal enhanced iron absorption from the latter in the same proportions. Addition of 45 mg Fe as $Fe_2(SO_4)_3$ and Hb to 0.4 mg Fe as NaFeEDTA in the meal respectively depressed and enhanced iron absorption from NaFeEDTA. These studies indicate that NaFeEDTA, $Fe_2(SO_4)_3$ and nonheme food iron form a common pool different from the heme pool but which is changed in its characteristics by the presence of NaFeEDTA, resulting in a better absorption of iron. *Am. J. Clin. Nutr.* 31: 961–971, 1978.

Iron deficiency and ferropenic anemia are widespread among vulnerable groups in industrialized societies (1) and in the general population of tropical developing areas (2). In Central America, 15% of the total nonpregnant population and 24% of pregnant women at term are anemic, almost exclusively due to iron deficiency (3–6), based on the association found between anemia and low serum iron and percent saturation of total iron binding capacity, and on hematological responses to iron administration in population groups (5). Based on the same criteria, folate deficiency appears to play a very secondary role as a factor in nutritional anemias in Central America (5). Investigations to determine the causes of iron deficiency in Central America have strongly suggested that inadequate iron intake in terms of quantity and quality is the main cause (3, 5), hookwork infection being an important aggravating factor (7). Consequently, food fortification with iron should be an effective public health measure to combat iron deficiency in this region, provided that an adequate iron compound and a suitable vehicle or vehicles are found. Several iron compounds were studied in a

---

[1] From the Division of Human Nutrition and Biology, Institute of Nutrition of Central America and Panama (INCAP), Carretera Roosevelt, Zona 11, Guatemala City, Guatemala.

[2] Supported in part by the World Health Organization through a grant from its Nutrition Unit.

preliminary fashion at INCAP, aiming at selecting compounds that are stable under tropical environmental conditions, that will provide iron with a high biological availability when mixed with local diets and that will not cause significant undesirable changes in the food or dietary vehicle. The ferric sodium salt of NaFeEDTA fulfilled many of the desirable physical and chemical characteristics; moreover, iron versenate (NaFeEDTA) has proved effective in the treatment of iron deficiency anemia in adults (8) and children (9), the EDTA portion of the molecule being minimally absorbed (10), and has been successful as an iron fortifying agent added to fish sauce in a pilot trial in Thailand (11). However, very little data are available on the absorption of iron from this compound, particularly when added to food. Viteri (12) and Viteri et al. (13) indicated a better absorption of iron from NaFeEDTA than from $FeSO_4$ when these compounds were given with a rice-milk-sugar formula to children. While this manuscript was being reviewed, Layrisse and Martínez-Torres (14) published their results on absorption of iron from NaFeEDTA that also point to a better absorption of iron from this compound than from $FeSO_4$ when added to foods.

The present communication relates our findings in measuring the absorbability of iron from NaFeEDTA in adults and children under a variety of circumstances which included: (*1*) ingestion without and with food; (*2*) addition of different doses to food; (*3*) interactions with heme and nonheme iron added to food, and (*4*) effects of mixing with the food and inhibitor or a promoter of iron absorption (desferrioxamine and ascorbic acid, respectively).

Materials and methods

All the studies were approved by an ad hoc committee on the protection of human rights and were performed only after fully informed consent was obtained from the subjects or their parents in the case of children.

Seven well nourished children were the subjects in the evaluation of iron absorption from ferric sulfate ($Fe_2(SO_4)_3$), NaFeEDTA and hemoglobin added to 230 ml of a liquid formula that was administered after an overnight fast in a single feeding, warmed to body temperature. The formula contained 16.5 g of a rice-milk-sugar mixture (60:16:24) plus vitamins and $FePO_4$. The total amount of iron administered was 5 mg, 2.5 mg coming from the diet. The compounds were labeled either with ^{59}Fe or ^{55}Fe as follows: $Fe_2(SO_4)_3$ was prepared by adding labeled $FeCl_3$ (Amersham Searle) to a water solution containing $FeSO_4$, this solution was homogenized with the diet one hour later. Determination of ferrous/ferric ions in the water solution (15) demonstrated that by 10 min 87% of iron was in ferric form. Labeled NaFeEDTA was prepared by adding radioactive $FeCl_3$ to disodium EDTA according to the method of Sawyer and McKinnie (16); labeled hemoglobin was prepared biosynthetically in repeatedly bled rabbits. In each series of radioiron studies 5 mg of radioactive iron as ferrous ascorbate were also given in a solution containing 500 mg of ascorbic acid (Hoffman LaRoche) as a reference dose. The children received nothing by mouth for 4 hr after the administration of the test formula.

Absorption tests were performed on days 1 and 2 and again on days 16 and 17. Blood was obtained on days 16 and 32. Different radioiron isotopes were given in each of the tests performed in consecutive days. Each dose of ^{59}Fe or ^{55}Fe was less than 1 and 3 μCi, respectively. Radioactive iron incorporated into hemoglobin (a 100% incorporation of the absorbed dose was assumed) was measured together with a sample of the labeled formula following the method of Eakins and Brown (17) using a Nuclear Chicago ambient temperature liquid scintillation counter, adjusted to a counting error of less than 3%.

Iron absorption studies in adults were performed in normal male volunteers of the Guatemala Army, Military School, University of San Carlos Medical School, Residencia Universitaria de Ciudad Vieja and INCAP. Healthy adults (except for hookworm infection and iron deficiency anemia) from the Guatemalan lowlands (Hacienda Chocolá) also participated in these studies.

The general procedures used to measure radioactive iron absorption from the different compounds and under a variety of circumstances, were identical to those described in the studies with children. In every case, except where stated specifically, the radioactive compound was carefully homogenized with a component of the meal in the final stages of its preparation. When the meal contained a black bean porridge, the radioiron compound was homogenized into a common batch of this food. Food iron was determined in each meal component; the total amount of iron given took this iron into account. All studies were done by the extrinsic food-labeling technique (18). The radioactivity administered to adults per dose was less than 4 μCi of ^{59}Fe and 12 μCi of ^{55}Fe. Serum iron and total iron binding capacity were measured by the methods of Ramsay (19, 20).

Results are expressed both as arithmetic and geometric means (21). Tests of significance (22) included paired *t*-tests or sample *t*-tests, when comparisons involved different individuals. In this last case, absorption of iron from each subject for each compound was adjusted to the mean ferrous ascorbate absorption of the two groups of subjects.

Results

Studies in children

Table 1 shows the hemoglobin and serum iron concentrations, the saturation of total

TABLE 1
Iron absorption from ferric sulfate, iron-EDTA and hemoglobin mixed with a milk-rice-sugar formula, in preschool children

Child	Hemoglobin	Serum Fe	TIBC sat[a]	Iron absorption (%)			
				Compounds given with the formula			Ferrous ascorbate
				$Fe_2(SO_4)_3$	NaFeEDTA	Hemoglobin	
	g/dl	μg/dl	%				
JGS	11.3	33.5	10.3	3.7	12.7	54.1	76.8
EER	12.3	92.5	24.9	1.6	6.0	32.9	70.5
PRD	12.3	59.0	15.4	4.9	2.5	39.7	71.7
CHO	10.4	39.4	11.1	3.5	11.7	14.9	92.8
RET	11.4	49.2	13.2	3.0	9.4	30.5	70.7
SF	10.7	43.3	12.9	4.8	14.7	38.0	64.5
ALM	12.2	66.9	15.3	3.2	11.8	47.8	90.9
Arithmetic mean				3.5	9.8	36.8	76.8
SD				1.1	4.2	12.7	10.9
Geometric mean				3.3[b]	8.6[b]	34.5	76.2
SD				1.5	1.9	1.5	1.2

[a] Percent saturation of total iron binding capacity. [b] $P < 0.02$.

iron binding capacity and the iron absorption of children fed the milk-rice-sugar formula containing either $Fe_2(SO_4)_3$, NaFeEDTA, or hemoglobin. Four of the children were slightly anemic and iron deficient. Iron absorption from the reference ferrous ascorbate dose was more than 60% in all children, confirming their suboptimal iron nutritional status. Iron absorption from hemoglobin was also high, its geometric mean being 34.5%. Mean iron absorption from NaFeEDTA was 2.6 times that of $Fe_2(SO_4)_3$ ($P < 0.02$).

Studies in normal adults

The radioiron absorption from 5 mg of iron administered as $Fe_2(SO_4)_3$, NaFeEDTA, hemoglobin, and ferrous ascorbate in aqueous solutions to normal adults is presented in Table 2. Iron absorption from NaFeEDTA was higher, although not significantly different, than that from $Fe_2(SO_4)_3$; hemoglobin absorption was similar to that of ferrous ascorbate. In the following study (Table 3) $Fe_2(SO_4)_3$, NaFeEDTA, and hemoglobin were administered with a breakfast consisting of black bean gruel (120 g), four corn tortillas (120 g), a roll of bread (40 g), and a cup of coffee. The total amount of iron administered was 5 mg, 4.6 mg coming from the diet. $Fe_2(SO_4)_3$ and NaFeEDTA, labeled with different iron compounds, were administered together in the same meal mixed into the black bean gruel and heated before serving. The iron absorption from both of these sources was identical (amounting to 15.5% of that from ferrous ascorbate) and significantly lower than the iron absorption from hemoglobin mixed with the beans. The iron absorption from this last source was the same as that of ferrous ascorbate.

Studies in rural adult males

Twenty-one men in the Guatemalan lowlands were studied. Twelve had iron deficiency based on percent saturation of total iron binding capacity less than 20%, and 11 had anemia (23). Table 4 shows the percent of iron absorbed from $Fe_2(SO_4)_3$ and NaFeEDTA administered with the standard breakfast, and from the reference ferrous ascorbate. In contrast with the previous experiment, $Fe_2(SO_4)_3$ and NaFeEDTA were fed on different days mixed with the black bean gruel. Mean iron absorption from NaFeEDTA was 2.7 times higher than from $Fe_2(SO_4)_3$ ($P < 0.001$). Furthermore, none of the 11 anemic subjects absorbed more than 10% and only three absorbed more than 4.5% of the ferric sulfate iron, whereas 10 surpassed that level of absorption with NaFeEDTA, reaching values up to 19.9%.

Iron absorption in the presence of an inhibitor (desferrioxamine) and an enhancer (ascorbic acid) of iron absorption

The effect of adding 500 mg of desferrioxamine mixed with the meal and the labeled

TABLE 2
Radio-iron absorption from ferric sulfate, iron-EDTA and hemoglobin in normal adults. The compounds were given alone, in the fasting state

Subject	Weight	Hemoglobin	Serum Fe	TIBC sat.[a]	Iron absorption (%)			
					$Fe_2(SO_4)_3$	NaFeEDTA (5 mg)	Hemoglobin[b] (5mg)	Ferrous ascorbate (5 mg)
	kg	*g/dl*	*µg/dl*	*%*				
AS	85	15.8	143	37	0.4	3.1	28.7	16.7
AA	55	15.5	113	38	0.9	4.8	11.5	17.6
EU	64	16.1	75	21	0.9	2.5	11.2	7.3
DS	65	15.7	94	31	2.1	7.5	29.3	50.2
NS	84	15.7	190	57	3.2	3.5	5.4	35.7
HM	59	15.7	96	30	4.8	3.8	18.3	6.9
ER	61	16.4	137	49	4.8	7.6	40.3	15.3
AR	73	16.8	141	41	4.9	2.9	10.4	7.3
OL	80	17.3	105	33	7.2	3.7	8.6	17.1
PR	55	15.4	87	32	30.4	21.7	30.3	86.3
Arithmetic mean					5.9	6.1	19.4	26.0
SD					8.9	5.8	11.8	25.2
Geometric mean					2.6	4.8	16.1	18.4
SD					4.6	1.9	1.9	2.4

[a] Percent saturation of total iron binding capacity. [b] Administered with 200 ml of Pepsi Cola.

TABLE 3
Radio-iron absorption from ferric sulfate, iron-EDTA and hemoglobin in normal adults. Compounds were given with breakfast

Subject	Weight	Hemoglobin	Serum Fe	TIBC sat.[a]	Iron absorption (%)			
					Compounds given with a meal[b]			Ferrous ascorbate (5 mg)
					$Fe_2(SO_4)_3$ (5 mg Fe)	NaFeEDTA (5 mg Fe)	Hemoglobin (5 mg Fe)	
	kg	*g/dl*	*µg/dl*	*%*				
JRG	57	16.6	125	36	1.1	1.6	33.3	19.2
JCM	66	16.3	114	46	1.6	1.7	39.8	11.0
IC	64	15.6	139	42	2.4	4.0	18.4	23.7
MP	59	17.0	137	40	4.3	5.4	28.6	21.8
JP	59	16.5	96	35	6.3	5.4	46.7	32.1
CP	57	16.4	137	32	6.7	7.4	37.3	71.3
JC	66	15.0	63	15	8.9	7.3	48.4	68.3
MAJ	51	17.2	162	47	9.1	9.2	48.9	40.3
MM	64	17.5	45	17	12.0	12.1	38.5	45.4
ARR	54	17.9	145	39	13.2	11.9	32.3	50.2
Arithmetic mean					6.6	6.6	37.2	38.9
SD					4.3	3.7	9.6	21.3
Geometric mean					5.0	5.4	35.9	33.4
SD					2.4	2.0	1.3	1.8

[a] Percent saturation of total iron binding capacity. [b] 120 g black bean gruel + 4 tortillas + 1 roll + 1 cup of coffee.

iron compounds is presented in Table 5. This iron binding substance lowered iron absorption both from NaFeEDTA and $Fe_2(SO_4)_3$. However, iron from NaFeEDTA was absorbed 3 times more than that of ferric sulfate ($P < 0.001$). On the other hand, the ingestion of a drink containing 500 mg of ascorbic acid together with the meal enhanced iron absorption from both compounds and although the mean value for NaFeEDTA was greater than that for $Fe_2(SO_4)_3$, differences did not reach statistical significance.

Effect of dose of iron from different compounds and their interactions

The effects of two different amounts of iron ingested with the standard meal were studied by giving a total of 5 and 50 mg of iron coming from both the diet and either ferric sulfate, NaFeEDTA or hemoglobin. The interaction between the different compounds was explored by adding to the diet, containing a labeled compound and a total of 5 mg of iron, 45 mg of unlabeled extra iron from a water solution of either of the other two compounds. For clarity of exposition the results are presented grouped by the source of labeled iron. Tables 6, 7, and 8 show the percent absorption of iron from hemoglobin, $Fe_2(SO_4)_3$ and NaFeEDTA mixed with the standard meal and that of the reference fasting dose of ferrous ascorbate.

Absorption of the hemoglobin-iron added to the standard breakfast was identical to that of ferrous ascorbate when the total amount of iron was 5 mg. However, when the total amount of iron amounted to 50 mg, the percent absorption decreased significantly ($P < 0.001$). When the lower dose of hemoglobin iron was mixed with either 45 mg of cold NaFeEDTA or $Fe_2(SO_4)_3$, its absorption remained stable, again showing no differences from that obtained from ferrous ascorbate.

Absorption of iron from ferric sulfate was influenced by the amount of iron but in different ways depending on the source of iron added to the diet. As shown in Table 7, the percent of iron absorbed from 50 mg of iron as $Fe_2(SO_4)_3$ was half that from 5 mg (<0.001). Conversely, the addition of 45 mg of iron as cold NaFeEDTA or hemoglobin enhanced the percent absorption of the labeled $Fe_2(SO_4)_3$ iron by factors of 1.6 and 1.8, respectively ($P < 0.001$).

The fraction of iron absorbed as NaFeEDTA was 10 to 11% of ferrous ascorbate and remained constant whether the total

TABLE 4
Radio-iron absorption from ferric sulfate and iron-EDTA in a rural adult male population. Compounds were given with breakfast

Subject	Weight	Hemoglobin	Serum Fe	TIBC sat.[a]	Iron absorption (%): Compounds given with a meal[b]: $Fe_2(SO_4)_3$ (5 mg)	NaFeEDTA (5 mg)	Ferrous ascorbate (5 mg)
	kg	g/dl	µg/dl	%			
EP	51	16.4	138	35	0.6	8.3	14.1
JNR	58	14.9	120	33	1.0	7.6	26.9
FG	43	10.2[c]	30	6	1.1	4.6	64.6
MPY	52	13.1[c]	116	30	1.3	7.1	65.1
JGS	46	16.2	178	40	1.4	4.7	31.2
AC	49	14.6	110	26	1.5	5.2	38.6
JSC	49	13.3[c]	82	23	1.8	1.4	49.7
JB	57	14.4	157	33	1.8	4.9	56.9
PSG	59	12.4[c]	21	4	1.9	4.7	51.3
JCH	50	12.1[c]	45	10	2.5	2.6	61.9
JBA	47	13.7	53	14	2.7	6.0	47.8
FG	90	16.3	148	36	2.7	9.3	29.2
CS	50	12.8[c]	19	3	3.6	13.2	44.0
PC	51	6.3[c]	19	4	3.6	8.2	68.6
MA	47	9.2[c]	30	8	3.9	7.8	66.9
GG	45	15.3	64	13	4.4	5.4	58.7
AA	43	14.2	76	24	4.8	14.2	57.8
AC	47	12.1[c]	42	8	5.5	6.9	48.2
GY	51	14.2	74	17	7.3	6.2	63.3
JM	45	9.0[c]	30	6	7.7	7.6	65.1
VIO	46	11.8[c]	49	12	9.2	19.9	66.8
Arithmetic mean					3.3	7.4	51.3
SD					2.4	4.1	15.6
Geometric mean					2.4[d]	6.4[d]	48.2
SD					2.0	1.8	1.5

[a] Percent saturation of total iron binding capacity. [b] Black bean gruel + 4 tortillas + 1 roll + 1 cup of coffee. [c] Anemics. [d] $P < 0.001$.

TABLE 5
Radio-iron absorption from ferric sulfate and iron-EDTA mixed with desferrioxamine and ascorbic acid in normal adults. Compounds were given with breakfast

Subject	Weight	Hemoglobin	Serum iron	TIBC sat.[a]	Iron absorption (%) — Compounds given with a meal[b]: $Fe_2(SO_4)_3$ (5 mg) + desferrioxamine (500 mg)	NaFeEDTA (5 mg) + desferrioxamine (500 mg)	$Fe_2(SO_4)_3$ (5 mg) + ascorbic[c] acid (500 mg)	NaFeEDTA (5 mg) + ascorbic[c] acid (500 mg)
	kg	*g/dl*	*μg/dl*	%				
WMG	66	15.6	127	38	0.4	1.7	8.6	20.6
GJV	69	14.6	92	29	0.4	1.8	7.0	2.0
TJH	60	16.5	137	41	0.5	1.8	10.8	21.5
AM	58	16.8	137	37	0.6	1.6		
GR	56	16.3	145	43	0.7	2.2	3.7	1.8
CECC	62	16.5	125	32	0.7	1.7	12.3	12.5
RE	71	16.5	80	27	0.8	2.1	20.3	19.1
VRM	63	16.2	147	42	1.1	2.2	2.6	7.4
MIS	67	15.3	98	26	1.3	1.2	2.5	2.2
AL	52	14.8	137	39	1.4	2.6	7.9	21.6
Arithmetic mean					0.7	1.9	8.6	13.3
SD					0.3	0.4	6.0	8.6
Geometric mean					0.6[d]	1.8[d]	6.8	9.5
SD					0.1	0.1	2.5	2.8

[a] Percent saturation of total iron binding capacity. [b] 120 g black bean gruel + 4 tortillas + 1 roll + 1 cup of coffee. [c] Taken together with the meal as an orange flavored drink. [d] $P < 0.001$.

iron dose was 5 or 50 mg (Table 8). Percent iron absorption from NaFeEDTA was decreased to about half by the addition of 45 mg as $Fe_2(SO_4)_3$ ($P < 0.01$) and increased from 11 to 17% compared to that of ferrous ascorbate with the addition of 45 mg of cold hemoglobin iron (P:N.S).

Discussion

The studies presented are primarily aimed at comparing the percent absorption of iron from NaFeEDTA, $Fe_2(SO_4)_3$ and hemoglobin. We decided to use ferric sulfate as a comparable salt because unless strict reducing conditions are maintained, over 80% of the iron in a water solution of ferrous sulfate is rapidly oxidized to the ferric form, particularly during heating. Therefore, this would be the salt ctually ingested when ferrous sulfate is used for fortification. The results in children and adults indicate that the ferric salt of sodium EDTA is absorbed better than ferric sulfate and ferrous sulfate added to milk[3] or corn (14). This fact is evident even when $Fe_2(SO_4)_3$ and NaFeEDTA are mixed in the same meal (Table 3) because in this case iron absorption from both compounds is higher than that obtained in a similar group of subjects when $Fe_2(SO_4)_3$ is the only iron salt added to the meal (Table 7), being similar to the proportion absorbed when only NaFeEDTA was mixed with the diet (Table 8). In effect, when individual results are expressed in relation to the absorption of the reference dose of ferrous ascorbate, $Fe_2(SO_4)_3$ mixed with the diet is absorbed in a proportion of 7.9 ± 4.2% (mean ± SD) to that of ferrous ascorbate; when given together with NaFeEDTA this proportion increased to 16.6 ± 7.1%. Absorption of iron from NaFeEDTA mixed with the diet alone or together with $Fe_2(SO_4)_3$ amounts to 16.6 ± 26.2 and 17.4 ± 6.4% that of ferrous ascorbate, respectively.

This indicates that when given together, the iron from both compounds forms a

[3] In a collaborative study with the first author, Dr. Abraham Steckel from University of Chile has done some preliminary studies concerning iron absorption from fortified milk in infants which also show that labeled sodium iron EDTA provided by [illegible] absorbed 2.5 times better than iron in ferrous sulfate fortified milk: (F. E. Viteri and A. Steckel, paper in preparation). INCAP Publication I-948.

common pool which is absorbed better by the presence of NaFeEDTA. These data confirm the results of Cook and Monsen (24) who have indicated a common iron absorption pool for NaFeEDTA and nonheme iron be this food iron or extrinsic iron. However, we have found a beneficial effect of NaFeEDTA which is partially reversed when other nonheme iron is present in a very high proportion to NaFeEDTA iron (a ratio of 125 to 1, Table 8).

The iron in NaFeEDTA appears not only to be absorbed better than that in $Fe_2(SO_4)_3$, when both are administered alone but the addition of the former compound to the diet seems to improve the absorption of nonheme iron whether the amount of iron in NaFeEDTA is 0.4 or 45 mg. This finding is particularly important because the absorption of small amounts of ferric iron added to a meal reflect the absorption of food iron (extrinsic tagging) (18). The data recently provided by Layrisse and Martínez-Torres (14) using intrinsically labeled corn also show an improved absorption of corn iron when NaFeEDTA is mixed with experimental meals.

In the studies reported here another characteristic of the NaFeEDTA-iron pool is evident: when iron absorption is expressed

TABLE 6

Radio-iron absorption from hemoglobin administered at two doses and mixed with ferric sulfate and iron-EDTA in normal adults. Compounds were given with breakfast

Subject	Weight	Hemoglobin	Serum Fe	TIBC sat.[a]	Iron absorption (%) — Compounds given with a meal[b]: Hemoglobin (5 mg)	Hemoglobin (50 mg)	Hemoglobin (5 mg) + cold NaFeEDTA (45 mg)	Hemoglobin (5 mg) + cold $Fe_2(SO_4)_3$ (45 mg)	Ferrous ascorbate (5 mg)
	kg	*g/dl*	*μg/dl*	*%*					
LM	60	16.3	116	40	9.1	4.1	11.4		20.0
CRV	68	15.3	86	34	15.2	3.4	15.3		25.8
HCh	71	16.6	98	31	16.1	2.7	21.3		26.2
MP	55	14.2	86	24	25.5	12.7	30.9		25.3
RV	65	16.4	109	31	27.9	8.2	27.7		36.4
AB	79	16.4	128	33	42.1	15.8	29.2		24.4
AT	62	16.6	103	35	42.3	11.6	33.0		33.1
MB	63	15.7	85	27	42.9	11.5	43.8		23.5
AM	68	15.0	96	31	43.5	11.0	36.4		26.0
Arithmetic mean					29.4	9.0	27.7		26.7
SD					13.7	4.6	10.2		5.0
Geometric mean					25.9	7.7[c]	25.7		26.4
SD					1.8	1.9	1.5		1.2
JFFM	67	14.2	61	20				7.6	16.3
MFCZ	57	15.0	82	22				12.6	11.6
HRLT	59	16.2	76	31				12.6	16.2
GAGR	54	14.6	111	34				17.8	12.7
CATL	64	14.8	141	36				22.8	21.9
CEUM	56	14.0	82	27				23.3	5.6
HSLD	64	15.3	122	33				25.7	34.2
HHLA	74	15.8	97	28				25.8	57.0
BMG	59	15.1	116	33				27.6	31.5
NRM	65	14.1	109	40				33.8	18.5
ERMR	61	15.3	103	33				36.4	19.3
JFGC	57	13.4	153	39				37.2	16.8
EMS	64	15.1	86	29				41.9	36.0
EDSA	68	15.0	118	42				63.9	33.8
Arithmetic mean								27.8	27.3
SD								14.5	17.4
Geometric mean								24.3	22.6
SD								1.7	1.9

[a] Percent saturation of total iron binding capacity. [b] 120 g black bean gruel + 4 tortillas + 1 roll + 1 cup of coffee. [c] $P < 0.001$.

TABLE 7
Radio-iron absorption from ferric sulfate administered at two doses and mixed with iron-EDTA and hemoglobin in normal adults. Compounds were given with breakfast

Subject	Weight	Hemoglobin	Serum iron	TIBC sat.[a]	Iron absorption (%) — Compounds given with a meal[b]: $Fe_2(SO_4)_3$ (5 mg)	$Fe_2(SO_4)_3$ (50 mg)	$Fe_2(SO_4)_3$ (5 mg) + cold NaFeEDTA (45 mg)	$Fe_2(SO_4)_3$ (5 mg) + cold hemoglobin (45 mg)	Ferrous ascorbate (5 mg)
	kg	*g/dl*	*μg/dl*	*%*					
JGRP	52	15.3	87	31	0.6	0.8	1.4		19.0
JLAC	73	15.9	66	18	0.6	0.6	2.2		10.8
JJGC	54	16.6	119	36	1.2	0.8	2.5		27.8
JAMG	54	15.0	97	27	1.4	0.4	3.0		24.6
JAVF	58	16.6	93	24	1.4	0.4	1.7		13.2
VECO	69	15.6	121	33	1.5	0.8	2.6		16.9
JEVS	67	16.6	153	42	1.6	0.8	2.6		26.4
ABR	63	15.4	118	31	1.7	0.9	2.8		12.6
JRMR	67	15.7	95	25	1.8	0.4	2.8		41.3
EELA	54	15.5	116	32	1.8	0.4	1.4		20.2
HSL	58	14.1	85	27	2.0	1.8	4.1		44.1
MAPL	59	15.7	97	26	2.1	1.1	2.9		45.5
DWGA	64	14.4	91	22	2.8	0.6	2.5		22.2
JAHL	64	15.3	118	29	3.5	3.5	3.8		19.3
MAEF	54	15.4	133	33	4.5	1.9	4.1		54.9
Arithmetic mean					1.9	1.0	2.7		24.8
SD					1.0	0.8	0.8		15.3
Geometric mean					1.6[c]	0.8[c,d]	2.6[d]		23.7
SD					1.7	1.9	1.4		1.6
JFFM	67	14.2	61	20				1.5	16.3
HRLT	59	16.2	76	31				1.5	17.2
JFGC	57	13.4	153	39				1.8	66.8
NRM	65	14.1	109	40				2.1	18.5
GAGR	54	14.6	111	34				2.1	12.7
EDSA	68	15.0	118	42				2.3	33.8
ERMR	61	15.3	103	33				2.5	19.3
CATL	64	14.8	141	36				2.9	21.9
MFCZ	57	15.0	82	22				3.1	11.6
HSLD	64	15.3	122	33				3.3	34.2
CLUM	56	14.0	82	27				3.8	5.6
EMS	64	15.1	86	29				5.9	39.0
BMG	59	15.1	116	33				5.9	31.5
Arithmetic mean								3.3	27.3
SD								1.9	17.4
Geometric mean								2.9	22.6
SD								1.7	1.9

[a] Percent saturation of total iron binding capacity. [b] 120 g black bean gruel + 4 tortillas + 1 roll + 1 cup of coffee. [c] $P < 0.001$. [d] $P < 0.001$.

as percent of that of the reference ferrous ascorbate dose, the addition of the bean-tortilla-bread-coffee meal depresses absorption to 56% of that obtained when this compound is administered as a water solution. This reduction in percent iron absorption is, however, significantly less than the 75% reduction observed for $Fe_2(SO_4)_3$ under the same conditions.

In contrast to this behavior of iron in NaFeEDTA, hemoglobin iron absorption is not altered by food nor by the presence of either of the nonheme iron compounds tested, under any condition, indicating that heme iron forms a different pool. However, a similar enhancing effect on absorption of $Fe_2(SO_4)_3$ is noted when 45 mg of iron is added to the meal either as hemoglobin or NaFeEDTA. Therefore, the iron chelate favorably modifies the characteristics of the nonheme iron pool.

In an effort to explain these results, three hypotheses come to mind: *1*) sodium iron EDTA remains soluble at the normal pH in the sites of maximal iron absorption in the duodenum and upper jejunum: in effect, ethylenediaminotetraacetate has a very strong affinity constant to iron with a maximal efficiency at pH ranges between 3 and 6.5 (25). We have shown that NaFeEDTA remains fully soluble at a pH of 7.0 and near 50% remains in solution at pHs up to 7.4. *2*) Sodium iron EDTA impairs the formation of iron polymers which aggregate in the case of ferric salts at physiological pHs by hydrolytic polymerization and complex formation with other compounds in food including some inhibitors (26–28), and *3*) the iron chelate performs a function similar to a carrier in delivering the iron to the mucosa similarly to what has been described in studies with liver cells in vitro (27).

The "protective effect" of chelation by EDTA on iron absorption observed when this compound was administered with a meal and 500 mg of desferrioxamine could be explained by the last two hypothesis.

On the other hand, in normal subjects, the addition of large amounts of ascorbic acid to a meal containing iron as NaFeEDTA enhances iron absorption from a

TABLE 8
Radio-iron absorption from iron-EDTA administered at two doses and mixed with ferric sulfate and hemoglobin in normal adults. Compounds were given with breakfast

Subject	Weight	Hemoglobin	Serum iron	TIBC sat.[a]	Iron absorption (%) Compounds given with a meal[b] NaFeEDTA (5 mg)	NaFeEDTA (50 mg)	NaFeEDTA (5 mg) + cold $Fe_2(SO_4)_3$ (45 mg)	NaFeEDTA (5 mg) + cold hemoglobin (45 mg)	Ferrous ascorbate (5 mg)
	kg	g/dl	μg/dl	%					
EWBB	48	13.9	91	26	0.4	1.6	1.6		24.4
HEBR	69	16.1	57	21	0.8	1.5	0.6		25.0
MJFR	63	15.7	61	20	0.9	1.6	0.2		26.9
OEAC	66	15.3	83	30	0.9	1.7	0.8		17.6
EVBC	63	15.3	53	18	1.1	1.7	0.5		18.0
CAAL	61	15.3	106	31	2.1	1.8	1.4		13.6
JRCO	60	15.5	122	43	5.0	3.1	1.8		25.8
JFCD	54	15.0	72	22	6.2	3.2	2.5		58.9
UMCA	53	17.5	85	23	12.1	5.1	2.1		14.3
Arithmetic mean					4.3	2.8	1.8		24.9
SD					5.0	1.9	2.0		13.7
Geometric mean					2.2	2.4[c]	1.2[c]		22.6
SD					3.6	1.7	2.6		1.6
GAGR	54	14.6	111	34				1.8	12.7
CEUM	56	14.0	82	27				2.2	5.6
EDSA	68	15.0	118	42				2.0	33.8
JFFM	67	14.2	61	20				2.7	16.3
MFCZ	57	15.0	82	22				2.8	11.6
ERMR	61	15.3	103	33				3.0	19.3
HRLT	59	16.2	76	31				3.0	16.2
NRM	65	14.1	109	40				3.2	18.5
HSLD	64	15.3	122	33				4.0	34.2
JFGC	57	13.4	153	39				4.6	16.8
CATL	64	14.8	141	36				5.5	21.9
BMG	59	15.1	116	33				6.3	31.5
EMS	64	15.1	86	29				11.0	36.0
HHLA	74	15.8	97	28				16.2	57.0
Arithmetic mean								4.9	27.3
SD								4.0	17.4
Geometric mean								3.9	22.6
SD								1.9	1.9

[a] Percent saturation of total iron binding capacity. [b] 120 g black bean gruel + 4 tortillas + 1 roll + 1 cup of coffee. [c] $P < 0.01$.

value of around 2.3 to 9.5%, that is, a 4-fold increase. This effect of ascorbic acid is the same as that observed for $Fe_2(SO_4)_3$ under the same conditions (without ascorbic acid 1.6%, with ascorbic acid 6.8%).

Another important characteristic of the NaFeEDTA iron pool is that within the limits studied, its percent absorption when given with a meal remains constant independent of the total amount of iron administered. This is clearly different from the dramatic drop in percent absorption observed with both hemoglobin and $Fe_2(SO_4)_3$ when the total amount of iron was increased from 5 to 50 mg.

The percent absorption of iron from NaFeEDTA given with the standard meal in the rural adult male population which presented a high prevalence of iron deficiency and anemia was higher than that of the normal groups; however, when expressed as percent absorption of that from the reference dose of ferrous ascorbate remained relatively constant (means: 13.2 and 12.9, respectively). The same applies to $Fe_2(SO_4)_3$ (means: 6.8 and 5.0, respectively). Therefore, we conclude that the intestinal regulatory mechanisms operate in the case of NaFeEDTA as they do with dietary iron and most nonheme and heme iron sources administered in small doses.

In conclusion, NaFeEDTA can be considered as a very good compound to use in iron fortification in Central America, since its bioavailability is adequate and its absorption seems less hampered by inhibitors present in the staple foods consumed in this area. An added factor to be considered for the use of this compound is the essential absence of EDTA in the diet of this region. Based on Cook and Monsen's data (24) this iron compound may not be suitable for fortifying diets already containing EDTA as an additive.

The authors thank the volunteers who very enthusiastically participated as subjects in these studies and the personnel of the Division of Human Nutrition and Biology of INCAP, especially the Nursing Staff of the Clinical Center, Mrs. Cristina de Campos, Miss Carmen Escalante and Mr. Fabio Saldaña for technical assistance and to Dr. Noel Solomons for his valuable assistance, criticisms and collaboration. Also special thanks are given to Drs. C. Martinez-Torres and M. Layrisse at IVIC for validating our radioisotope counting methodology.

References

1. Finch, C. A., and E. R. Monsen. Iron nutrition and the fortification of food with iron. J. Am. Med. Assoc. 219: 1462, 1972.
2. Sood, S. K., L. Banerji and V. Ramalingaswami. Occurrence of nutritional anaemias in tropical countries. In: Occurrence, Causes and Prevention of Nutritional anaemias, edited by G. Blix. Symposia of the Swedish Nutrition Foundations VI. Uppsala: Almqvist and Wiksells, 1968, pp 36–49.
3. INCAP-OIR-Public Health Ministries of the Central American countries and Panama. Nutritional evaluation of the Central American Population, 1969.
4. Viteri, F. E., and M. A. Guzmán. Haematological status of the Central American population. Prevalence of individuals with haemoglobin levels below "normal." Brit. J. Haematol. 23: 723, 1972.
5. Viteri, F. E. Hematological Status of the Centra American Population: Iron and Folate Deficiencies. Washington, D.C.: Pan American Health Organization. 12th meeting of the advisory committee on Medical Research, 1973.
6. Cook, J. D., J. Alvarado, A. Gutnisky, M. Jamra, J. Labardini, M. Layrisse, J. Linares, A. Loria, V. Maspes, A. Restrepo, C. Reynafarje, L. Sánchez-Medal, H. Vélez and F. Viteri. Nutritional deficiency and anemia in Latin America: a collaborative study. Blood 38: 591, 1971.
7. Viteri, F. E., M. A. Guzman and L. Mata. Anemias nutricionales en Centro America. Influencia de infeción por uncinaria. Arch. Latinoam. Nutr. 23: 33, 1973.
8. Will, J. J., and R. W. Vilter. A study of the absorption and utilization of an iron chelate in iron deficient patients. J. Lab. Clin. Med. 44: 499, 1954.
9. Jiménez, F. E., B. R. Jiménez and F. E. Viteri. Tratamiento de la anemia ferropénica con nuevas sales de hierro. Rev. Med. Hosp. Nal. Niños (Costa Rica) 10: 105, 1975.
10. Foreman, H., and T. T. Trujillo. The Metabolism of C^{14} labeled ethylenediaminetetra-acetic acid in human beings. J. Lab. Clin. Med. 43: 566, 1954.
11. Garby, L., and S. Areekul. Iron supplementation in Thai fish-sauce. Ann. Trop. Med. Parasitol. 68: 467, 1974.
12. Viteri, F. E. Iron absorption from NaFeEDTA, $Fe_2(SO_4)_3$ and hemoglobin in children. Geneva: WHO Meeting on Nutritional Anemia, 1974.
13. Viteri, F. E., B. Torún and R. García. Iron fortification of sugar: advantages of NaFeEDTA over $FeSO_4$. In: Abstracts, Symposia and Free Communications, Xth International Congress of Nutrition, Kyoto, Japan: August 1975, No. 8211.
14. Layrisse, M. and C. Martínez-Torres. Fe (III)-EDTA complex as iron fortification. Am. J. Clin. Nutr. 30: 1166, 1977.
15. Theroux, F. R., E. F. Eldridge and W. L. R. Mallman. Laboratory Manual for Chemical and Bacterial Analysis of Water and Sewage. 3rd ed.

London: McGraw-Hill Book Co. Inc., 1943.

16. Sawyer, D. T., and J. M. McKinnie. Properties and infared spectra of Ethylenediaminetetraacetic acid complexes. III. Chelates of higher valent ions. J. Am. Chem. Soc. 82: 4191, 1960.
17. Eakins, J. D., and D. A. Brown. An improved method for the simultaneous determination of iron-55 and iron-59 in blood by liquid scintillation counting. Inter. J. Appl. Radiat. Isotopes 17: 391 1966.
18. Layrisse, M., C. Martínez-Torres and M. Gonzalez. Measurement of the total daily dietary absorption by the extrinsic tag model. Am. J. Clin. Nutr. 27: 152, 1974.
19. Ramsay, W. N. M. Plasma iron. Advan. Clin. Chem. 1: 1, 1958.
20. Ramsay, W. N. M. The determination of the total iron-binding capacity of serum. Clin. Chim. Acta 2: 221, 1957.
21. Cook, J. D., M. Layrisse and C. A. Finch. The measurement of iron absorption. Blood 3: 421, 1969.
22. Snedecor, G. W., and W. G. Cochran. Statistical Methods. The Iowa State University Press, Six Editition, 1967.
23. Viteri, F. E.,V. de Tuna and M. A. Guzmán. Normal haematological values in the Central American population. Brit. J. Haematol. 23: 189, 1972.
24. Cook, J. D., and E. R. Monsen. Food iron absorption in man. II. The effect of EDTA on absorption of dietary non-heme iron. Am. J. Clin. Nutr. 29: 614, 1976.
25. General information on chelation. Hampshire Chemical Division, W. R., Grace & Co. Poisson Ave., Nashua, New Hampshire, USA.
26. Furia, T. E. Sequestrants in Food. In: Handbook of Food Additives, edited by T. E. Furia. Cleveland, Ohio: The Chemical Rubber Co., 1968, p. 289.
27. Charley, P., M. Rosenstein, E. Shore and P. Saltman. The role of chelation and binding equilibria in iron metabolism. Arch. Biochem. Biophys. 88: 222, 1960.
28. Christopher, J. P., J. C. Hegenauer and P. D. Saltman. Iron metabolism as a function of chelation. In: Trace Element Metabolism in Animals-2, edited by W. G. Hoekstra, J. W. Suttie, H. E. Ganther and W. Mertz. Baltimore: University Park Press, 1974, pp. 133-145.