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26 *Prevention of Iron Deficiency by Means of Iron Fortification of Sugar*

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INTRODUCTION

Available evidence indicates that iron deficiency is widespread in both developed and developing areas. Important characteristics of such deficiency differ from one area to the other. Affluent societies have a nutritionally better and more varied diet, which contains enhancers of iron absorption and a higher proportion of heme iron. Because of these circumstances, there is a higher availability of dietary iron. Iron deficiency, therefore, affects only the vulnerable groups—women of reproductive age, adolescents and small children, and individuals who suffer from secondary anemia due to chronic blood loss. In contrast, iron deficiency is more widespread in populations whose diets are monotonous, based on cereals and legumes, contain little heme iron, and often limited intakes of ascorbic acid (1). Moreover, in most developing tropical areas, chronic blood loss due to *N. americanus*, *A. duodenale*, and *T. trichura* infections accentuate the deficiency of iron in older children and adult males, who are, otherwise, not particularly at risk from severe iron deficit (2). Even in the absence of anemia, a condition aggravated by iron deficiency, iron deficiency still has a series of functional consequences that make its eradication desirable (3–6).

These circumstances have made the nutrition scientist more aware of the necessity to increase the iron intake by essentially all population groups. This situation led several decades ago to the introduction of iron

fortified flour in developed countries Later on, iron was added to baby cereals and to milk preparations designed for infants. A common flaw in this practice was the poor attention given to evaluating the results. Some studies suggested that cereal fortification has had very little effect in improving iron nutrition These critical evaluations led to a reconsideration of the types of iron compounds used in the fortification of cereals. As a consequence, and based on new techniques that allow precise measurements of iron bioavailability, different forms of iron are being used in the fortification processes These new forms of iron have been thoroughly studied and should be more effective than those previously used However, to our knowledge, no evaluation of the effects of iron fortification on a broad population level has ever been carried out. In the pediatric population, positive effects have been clearly documented (7).

The design and implementation of a fortification program requires knowledge of a series of factors which, in the case of iron, are essential for the selection of the vehicle, the iron compounds to be used, and the amount that should produce the desired results This knowledge ought to lead to fortification programs that are tailored to the necessities and the conditions of different population groups. In other words, a specific compound and/or vehicle may be adequate for populations that consume a certain diet and inadequate for others.

This chapter describes the ongoing experience in the design and evaluation of iron fortification of sugar in Central America.

BACKGROUND CHARACTERISTICS OF CENTRAL AMERICAN POPULATIONS

A complete epidemiological description of the population was performed to establish the status of erythropoietic nutrients and the prevalence of anemia. From this work, it became evident that close to 25% of the Central American population presents some degree of anemia and that the iron deficiency is primarily due to dietary origin, aggravated by hookwork infection in the lowlands (8–11). In fact, iron deficiency is present in nearly one-half the Central American population, being more prevalent in women of reproductive age and in children. There were biochemical indications of inadequate folate nutrition, but no correlation was found between folate deficits and anemia at the population level (10) Iron deficiency was primarily due to dietary factors, characterized by (1) poor bioavailability of dietary iron (12) and by (2) inadequate amounts of iron intake in about 40% of the families in the rural areas (8,10)

Supplementation trials with orally administered $FeSO_4$ as the only intervention in lowland populations resulted in significant improvement in hematological and iron status. A more homogeneous improvement in hematological and erythropoietic nutrient status was obtained by means of oral administration of $FeSO_4$ + folic acid (10,13). These positive effects took place in spite of an unaltered diet and parasite loads, which included hookworm and trichuris.

This background clearly indicated that nutritional anemia in the lowlands might be drastically reduced if iron intake and bioavailability were enhanced. Improvements in folate nutrition, however, would result only in a slightly better hematological response in about 10% of the responding cases. This confirmed that iron deficit was the main cause of nutritional anemias. With this diagnosis of the problem and its cause the prevention of iron deficiency could be undertaken. Two approaches, not mutually exclusive, were possible: (1) increase the bioavailability of dietary iron, and (2) increase the amount of iron intake.

INCAP explored the possibility of preventing iron deficiency through iron fortification of an appropriate food vehicle. Previous studies suggested that sugar would be an adequate vehicle for iron because it has been used successfully in vitamin A fortification programs in Central America (14), and sugar does not interfere with iron absorption (15). Alternately, cereals and flours could be used in countries where these foods are a basic part of the staple diet.

SELECTION OF THE IRON SALT

The problem was to select a form of iron that would have adequate bioavailability when consumed with the staple diets of the region. Several studies and preliminary trials led to the selection of a chelated form of iron NaFeEDTA (sodium iron ethylenediaminetetraacetate) as the most promising iron compound to use. The theory was that, somewhat like heme, the iron would be protected from inhibitors of absorption in the diet. This was a novel idea that required investigations, which yielded the following information:

1. NaFeEDTA is stable (its reactivity is low), soluble in water at physiological pH's, yellow in color, and produces very little metallic taste. The salt contains an average of 13% iron and no toxic substances; it is over 99.5% pure. Contaminants are mostly metals present in any iron compound, copper and zinc being the most important.
2. NaFeEDTA can be easily added to sugar with simple technology. It

sticks to the crystals when sugar has over 1% moisture; thus, segregation is not a problem. When added in a 1:1000 proportion, it contributes a barely detectable yellowish tinge to sugar, noticeable only when directly compared with unfortified sugar of similar quality. The taste is indistinguishable from sugar in the form normally consumed in Central America.

3. The salt does not interfere at all with vitamin A fortification of the sugar and vice versa. Tests have been done with regard to stability and bioavailability of both compounds, yielding excellent results (16).

4. The iron in NaFeEDTA ingested orally is bioavailable for hemoglobin synthesis. In fact, it is as effective in treating iron deficiency as many inorganic and organic iron salts of common therapeutic use, such as ferrous sulfate and ferrous fumarate (17, 18).

5. When NaFeEDTA is ingested with representative Central American diets, the iron is 1.5 to 2.5 times more available than food iron (19,20). Furthermore, NaFeEDTA mixes with the non-heme iron of the diet and makes it about 2 times more available (19,21). The presence of inhibitors to iron absorption is less important when NaFeEDTA is present, and iron absorption tends to remain proportionally constant even when the amounts of NaFeEDTA added to the diet vary tenfold (from 5 to 50 mg of iron) (19). These specific qualities have important implications in terms of the possible effectiveness of NaFeEDTA as a fortifying compound to cereal–legume-based diets. A simple example will make this clear.

An adult female of reproductive age requires about 2 mg of iron per day absorbed from the diet. The mean iron intake from representative diets is 27 mg/day, but its absorption is only 4%. Therefore, she absorbs about 1.1 mg of iron/day. The addition of NaFeEDTA to the sugar in a proportion of 13 mg of iron/100 g of sugar provides her with 5.2 mg of additional iron (mean sugar consumption for her age–sex group is 40 g/day), but what is more important, the 32 mg of iron she is ingesting are now absorbed in about 8%. The total absorbed iron becomes 2.5 mg, thus satisfying her iron requirements.

6. The greater the iron deficit, the greater the proportion of iron absorbed from NaFeEDTA and vice versa. (19).

Up to now, the iron compound NaFeEDTA appears to be the ideal one for fortification of the Central American diet. This salt is clearly superior to other iron salts now being used for food fortification in the cereal industry primarily in (1) stability, low reactivity, and essentially undetectable flavor; (2) the greater bioavailability that it also confers on dietary non-heme iron; and (3) the relative constancy of its proportional

absorption within iron fortification levels ranging from 5 to 50 mg of iron.

NaFeEDTA potentially has certain disadvantages and possible undesirable characteristics, however, which are now under active research at INCAP. These include:

1. The higher cost relative to other possible iron fortification compounds. For example, NaFeEDTA is about 4 times as expensive as ferrous sulfate. However, its greater bioavailability (2 times) when consumed with Central American diets reduces its relative price to 2, and the greater bioavailability it confers to dietary food iron further reduces the price differential with $FeSO_4$. The approximate cost of fortifying 100 lb (46 kg) of sugar with 46 g of NaFeEDTA is about $0.16 (U.S. dollars). The price of 100 lb of sugar in Central America is close to $16.00. Thus the relative price increment is about 1%. In terms of costs per person per year, we have determined that the mean sugar consumption is 33 g per day. At present prices this represents $4.24 per year. One percent of the price of sugar, that is, $0.042, would be the cost of fortification per person per year. For 6 million inhabitants in Guatemala, this represents $254,400 per year. This cost seems reasonable, based on preliminary estimates of benefits from iron fortification.

2. The fact that NaFeEDTA has a strong chelating molecule (EDTA) could produce defects in absorption of other divalent cations such as Zn, Cu, Mg, Ca, Mn, and Co. Various forms of EDTA (disodium, disodium–calcium, tetrasodium) in the proportions to be used in the iron fortification process do not interfere with the absorption of such cations. On the contrary, the literature indicates that EDTA enhances their absorption (22–24). We are currently studying these effects. Our results in rats showed a 1.6-fold enhancement of Zn absorption from corn–bean diets containing constant amounts of iron when either Na_2 EDTA or NaFeEDTA are added (25). Zinc turnover is accelerated as expected from its greater absorption, but no more than normal. In humans (26), NaFeEDTA at fortification levels does not alter the rise in serum Zn consequent to the intake of $ZnSO_4$.

3. Very few studies have been done on the absorption and metabolism of EDTA, and none using NaFeEDTA as absorptive substrate. Studies in this area are needed, because even though only a small proportion (about 5%) of EDTA seems to be absorbed, it appears in the urine most probably chelated with divalent cations. This could mean that a more rapid loss of cations from the body might occur. Studies concerning Zn presently in progress at INCAP indicate that the rate of loss from the body is not accelerated by consumption of a diet containing up to 4 times

as much NaFeEDTA as would be used in fortification programs (25). On the other hand, part of the NaFeEDTA ingested appears to be absorbed intact, and this iron is most likely excreted in the urine as intact NaFeEDTA. Still, a large proportion of the iron absorbed from NaFeEDTA and transported to the bone marrow is freed in the intestine and is bioavailable in amounts that are superior to those from the dietary iron alone (27).

4. Finally, the U.S. Food and Drug Administration recently published a document in which, as a consequence of long-term studies in rodents, asserts that EDTA is a *safe* food additive (28). The level of EDTA intake through iron fortification of sugar in Central America would probably reach, at most, similar levels of EDTA intake now being consumed in developed societies.

In brief, a series of studies have been performed or are at present in progress that strongly suggest that NaFeEDTA is ideally suited for the fortification of diets based on cereals and legumes. All available evidence at present point to it as being safe.

OTHER CONSIDERATIONS

Three other important aspects of iron fortification programs should also be considered:

1. *Cost-effectiveness of iron fortification.* Efforts are being made to obtain the necessary information to estimate accurately the costs the government and the private sector would incur yearly in the treatment of iron deficiency anemia. A preliminary and conservative estimate of costs yields a yearly figure of $800,000 for Guatemala, that is, an average cost of $0.13 per capita per year. If fortification is effective, these costs should be reduced by about 80%. This would mean a savings of $640,000 per year in medical treatment for iron deficiency. The cost of fortification and the treatment of residual cases of anemia of other than dietary origin would amount to $414,400 per year. Fortification would have a net saving of $385,600 per year ($0.064 per capita per year).

2. *The possibility of iron overload.* This will be only briefly discussed here. Suffice it to say that theoretical considerations make it extremely improbable that through fortification the situation in Central America will change from one of rampant iron deficiency to one of iron overload. On the other hand, subjects in the population who are genetically susceptible to iron overload will, no doubt, have more chances of expressing their genotype if available iron in the diet increases. This phe-

nomenon would also occur if these subjects were to consume western-type diets with high iron bioavailability.

3. *Surveillance and evaluation.* Any fortification program must have surveillance and evaluation as an integral part of it. Monitoring the compliance with fortification regulations and a periodic sampling of the population to evaluate iron reserves (e.g., serum ferritin levels) should be part of the surveillance. Also, fortification programs must be dynamic in that they must be amenable to modification as a consequence of the results of periodic evaluations.

Having considered these aspects of an iron fortification program in Central America, the following questions still remained: Is iron fortification of sugar with NaFeEDTA effective at a population level under tropical and temperate conditions? Is it feasible? Will it be accepted and tolerated by the population? Will there be any unpredictable, undesirable side effects when NaFeEDTA is chronically consumed?

OBJECTIVES OF THE FORTIFICATION STUDY

There were five primary objectives in conducting a field evaluation of sugar fortification with iron:

1. To evaluate the feasibility of fortifying sugar with iron and to monitor its long-term acceptance by population groups.
2. In population groups where iron deficiency anemia is endemic, to determine the long-term effectiveness of iron fortified sugar in (a) improving iron nutrition, thus preventing future cases of iron deficiency anemia, and (b) reducing the prevalence of anemia.
3. To define the relative importance of variables that are known to influence iron nutrition (diet, specific morbidity, parasite loads, physiological and specific nutritional status) in modifying the effects of iron fortification on iron nutrition and hematological condition of population groups.
4. To estimate costs of sugar fortification and compare them with the costs incurred at present by the population in treating its cases of iron-deficiency anemia. These cases usually relapse under present conditions.
5. To determine and identify any side effects secondary to the chronic intake of NaFeEDTA—fortified sugar—especially the effects on folate, Vitamin B_{12}, and trace mineral nutrition (emphasis on zinc, copper, and magnesium).

DESIGN OF THE STUDY

Four communities, two in the lowlands and two in the highlands, were studied. The populations range from 1200 to 1800 inhabitants. The original design was for one community in each location to receive fortified sugar and the other to serve as control. However, after the baseline data was obtained in the four communities that had been selected from 38 initially screened it was evident that one of each pair could not serve as adequate control for the other. The prevalence and severity of anemia and of iron deficiency was different in each community (Table I).

The severity of anemia in the lowland community 13 and in the highland community 15 was the most similar; the prevelance was intermediate. From the standpoint of iron deficiency, the two lowland communities were clearly more deficient than the two in the highlands. Community 14 was definitely very iron deficient and had the highest prevalence of hookworm infection. The two highland communities were very similar with respect to iron deficiency and hookworm prevalence.

Because of this situation, several alternatives to the original study design were considered. We decided to keep one community as control and to fortify the other three. Community 15 was chosen as the control

TABLE I

	Lowlands		Highlands	
Community	13	14	15	16
Percentage prevalence of				
Low PCV[a]	30.9	43.1	34.9	21.3
Low sat. TIBC	33.6	57.5	12.0	22.6
Low Ferritin	51.8	71.6	37.0	34.1
High FEP	49.6	71.8	23.7	29.3
Severity of Hb deficit and of iron deficiency among those deficient				
Mean deficit in Hb (g/dl)	3.1	4.6	2.9	0.9
Mean sat. TIBC (%)	9.3	6.8	11.5	11.2
Mean serum ferritin (ng/ml)	7.0	5.2	9.0	9.5
Mean FEP (μg/dl RBC)	183.5	234.2	145.7	138.8

[a] PCV, packed cell volume. Low values are indicative of anemia. Sat TIBC, saturation of total iron-binding capacity, indicative of iron transport. Ferritin, serum ferritin, indicative of iron reserves. High levels of free erythrocyte protoporphyrin (FEP) indicate iron deficit during red cell formation (see Chapters 22 and 25, this volume).

because of its similarity with community 13 in prevalence and severity of anemia and with community 16 in prevalence and severity of iron deficiency. The fact that we had other studies in lowland communities where blood samples had been obtained over a period of time (without interventions) also influenced our decision. These communities could serve with some reservations as lowland controls, showing the natural changes that occurred over time. The three "fortified" communities, two in the lowlands and one in the highlands, had been supplied with iron-fortified sugar since June, 1977. Community 15, the control village, had also been supplied with unfortified sugar. The fortified sugar was produced in large batches under our direct supervision in a single sugar mill. The iron compound added was iron-chelated NaFeEDTA, kindly supplied by Grace and Co., United States, and by BASF, Germany.

The target level of iron in fortified sugar was 13 mg/100 g. Repeated analyses for iron in samples of the sugar (over 100 samples) yielded a mean and SD of 12.05 ± 2.0 mg/100 g, with a range between 8.4 and 16.5 mg/100 g. This compound is added by hand as a fine yellow powder, when the sugar is dropped (with between 1.5 and 3% humidity) from centrifuges. Then it is mixed, dried, and packed in 100-lb bags. The sugar is then stored in warehouses under our close supervision and is sold to the store keepers in the fortified villages at a price slightly lower than the market price of sugar. The entire production and distribution process is closely monitored. The consumption level of fortified sugar in homes is also monitored and accomplished by a qualitatively rapid test for iron done 5 times per year in every household.

Throughout the year, dietary intake, including sugar consumption, is surveyed periodically in a subsample of nearly 1400 subjects in the four communities; in-depth studies of iron nutrition were performed on these 1400 subjects periodically. Periodically throughout the year, census updating and morbidity data were obtained at the home level. Constant free medical attention is provided to the study communities through a government health post in each village. We were in charge of these posts for many reasons, one being to keep a constant surveillance on cases and treatment of anemia. Once a year a hematological survey is done between January and March to avoid seasonal variations. Two quantitative parasitological surveys have been done.

Thus far, hematological and erythropoietic nutrients data has been obtained 4 times, the last time after 32 months of fortification in communities 13, 14, and 16. Data is available for the 1979 survey, performed after 20 months of fortification. All of the community members who volunteered to have a PCV done constitute the *sample*, which is to be

followed throughout the study. A *subsample* was then selected to include the following three groups in each community:

Group I: All the severely anemics (PCV < 30% in the highland communities, and < 28% in the lowlands).

Group II: About 200 subjects whose PCV was greater than the above stated values but was below 1.5 SD from the mean for the normal population in each age–sex–altitude category (29).

Group III: About 150 subjects with PCV greater than 1.5 standard deviations below the normal mean for each age–sex–altitude category. These were considered normals.

All the subjects in the subsample had venous blood drawn to perform complete hematological determinations (PCV, Hb, RBC counts, and WBC counts) plus the following:

1. For evaluation of iron nutrition: serum iron, total iron-binding capacity (TIBC), transferrin saturation, free erythrocyte protoporphyrins (FEP), and serum ferritin.
2. For evaluation of folate nutrition: whole blood-folate level and RBC folate level.
3. For evaluation of Vitamin B_{12} nutrition: serum B_{12}.
4. For trace mineral nutrition: plasma, RBC, hair, and urinary Zn, Cu, and Mg.

After the baseline evaluation all subjects in Group I received $FeSO_4$ orally in amounts calculated to improve their PCV to that of subjects in Group II. From then on, any subject seeking medical care, whose PCV was below 30 or 28% in the highlands and lowlands, respectively, received the same treatment. Pregnant women attending the health post on a voluntary basis received 7.5 g $FeSO_4$ as tablets so that, assuming 20% absorption, it provides the equivalent of 300 mg of absorbed iron. This dose is administered during the second or third trimester of pregnancy following established practices of the Health Ministry of Guatemala.

RESULTS TO 20 MONTHS OF FORTIFICATION AND DISCUSSION

It must be realized that the results of the ongoing study here presented are only partial in terms of the areas covered and preliminary also because of the time at which the available hematological and biochemical information were collected. Results will be available shortly on the

changes observed after 32 months of fortification; these data will be the final for the study.

Four data collection areas will be presented in some detail as indicative of the types of outcomes of the study: (1) fortified sugar intake; (2) dietary intake; (3) hematological changes, and (4) iron nutrition.

Fortified Sugar Intake

The means of six surveys covering a 2–7 year period are presented in Table II. Sugar intake is expressed as grams/person/day within household (the unit of observation is the family, in this case). It is evident that the means and standard deviations among the four populations are very similar over time. However, the variability of individual sugar intakes

TABLE II

Sugar Intake in the Study Communities in Six Surveys[a]

Survey		Communities			
		13	14	15	16
1	Mean	39	36	45	42
	SD	22	31	32	24
	No.[b]	246	360	204	338
2	Mean	32	34	33	34
	S.D.	22	24	15	16
	No.	248	372	213	346
3	Mean	30	33	30	36
	S.D.	21	27	13	14
	No.	263	401	235	375
4	Mean	31	32	30	31
	S.D.	23	24	19	17
	No.	267	392	238	388
5	Mean	33	34	29	29
	S.D.	23	27	16	17
	No.	270	410	236	391
6	Mean	31	30	30	30
	S.D.	16	22	16	16
	No.	271	412	239	400
All	Mean	33	33	33	34
	S.D.	21	26	20	18
	No.	1565	2347	1365	2238

[a] Data for 2 years, g/person/day.

[b] Number of families.

was high (coefficient of variation > 50% in over 80% of the surveys) and could be significant in terms of iron intake throughout time if families were consistently high or low consumers. The families were distributed in each community by terciles of sugar intake at each survey, in order to evaluate their stability in sugar intake. The limits of sugar intake between terciles ranged from 23 to 27 g/person/day and from 36 to 41 g/person/day for the lower and upper terciles, respectively, in the different surveys. The majority of families (between 50 and 63% of them) changed terciles during the 2-year period. Between 19 and 22% of families moved between the mid-upper or mid-lower terciles; only 2 and 5% of families consistently were either in the upper or lower terciles, respectively.

Sugar intake varied within households depending on the age of the family members. However, as shown in Table III, mean intakes are similar except for those for infants which were about one-half to three-fourth those of older age groups. Hence children will consume more iron from fortified sugar per kilogram body weight than adults.

The next important point in terms of fortified sugar intake is the compliance of the different families within the communities with the fortification program. Families were free to purchase their sugar at the regular price at the village stores. We provided the stores with fortified sugar. In the lowland communities there was filtration in of unfortified sugar from workers in nearby sugar mills who received a sugar quota

TABLE III

Sugar Intake (g/Person/Day) by Age Groups in the Study Communities in a Representative Survey

Communities		Age groups (years)							
		<1	1–4	5–8	9–12	13–16	17–20	21–49	50+
13	Mean	17	33	31	35	40	36	41	47
	SD	10	23	23	27	33	27	32	43
	No.	(15)	(126)	(115)	(98)	(83)	(57)	(271)	(131)
14	Mean	25	31	31	37	40	38	44	49
	SD	18	21	18	23	25	20	25	25
	No.	(26)	(239)	(214)	(158)	(145)	(95)	(482)	(183)
15	Mean	28	30	34	38	38	37	39	40
	SD	9	12	12	15	14	11	15	14
	No.	(10)	(127)	(148)	(145)	(97)	(55)	(257)	(130)
16	Mean	29	34	36	39	42	44	43	41
	SD	26	14	10	15	16	17	15	13
	No.	(23)	(175)	(165)	(167)	(139)	(89)	(439)	(185)

per day worked. In spite of all efforts to encourage the consumption of only fortified sugar in these communities (Nos. 13 and 14), compliance was less than optimal as shown in Fig. 1, which presents the cummulative plot of compliance per community. The highland fortified community (No. 16), for example, had an excellent compliance as judged by the number of times the test for iron in sugar was positive at the household level; more than 75% of families had over 80% positive tests. This percentage in the lowlands families was only 30%; however, 70% of lowland families complied with the program over 50% of the time.

These results stress the need of a close surveillance of pilot studies in free-living communities. It is obvious that the final analysis of the data will have to take into account each family's compliance record.

Dietary Intake

Nutrient intake by individuals has been obtained by repeated 24-hr recall surveys, with proper quality control. A summary of results of two surveys prior to the fortification program, contrasting the mean adequacy of nutrient intakes of nonanemic subjects with the anemic subjects is presented in Fig. 2. Differences between means are significant

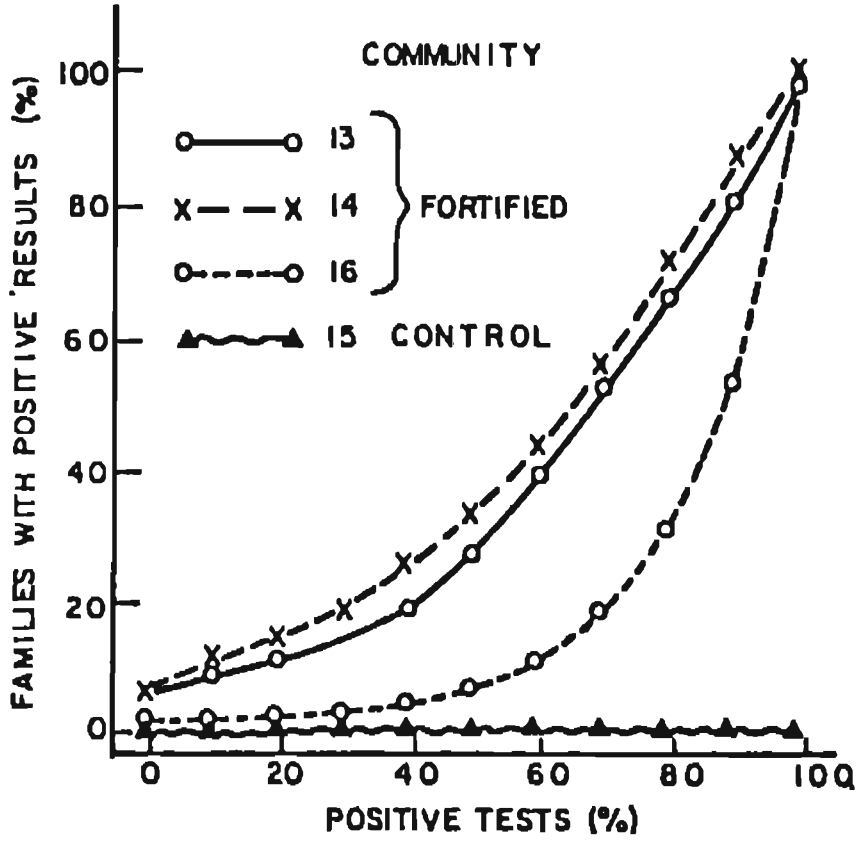

Fig. 1. Cummulative plot of the detection of fortified sugar in the households in each of four communities. Data from 6 surveys per community.

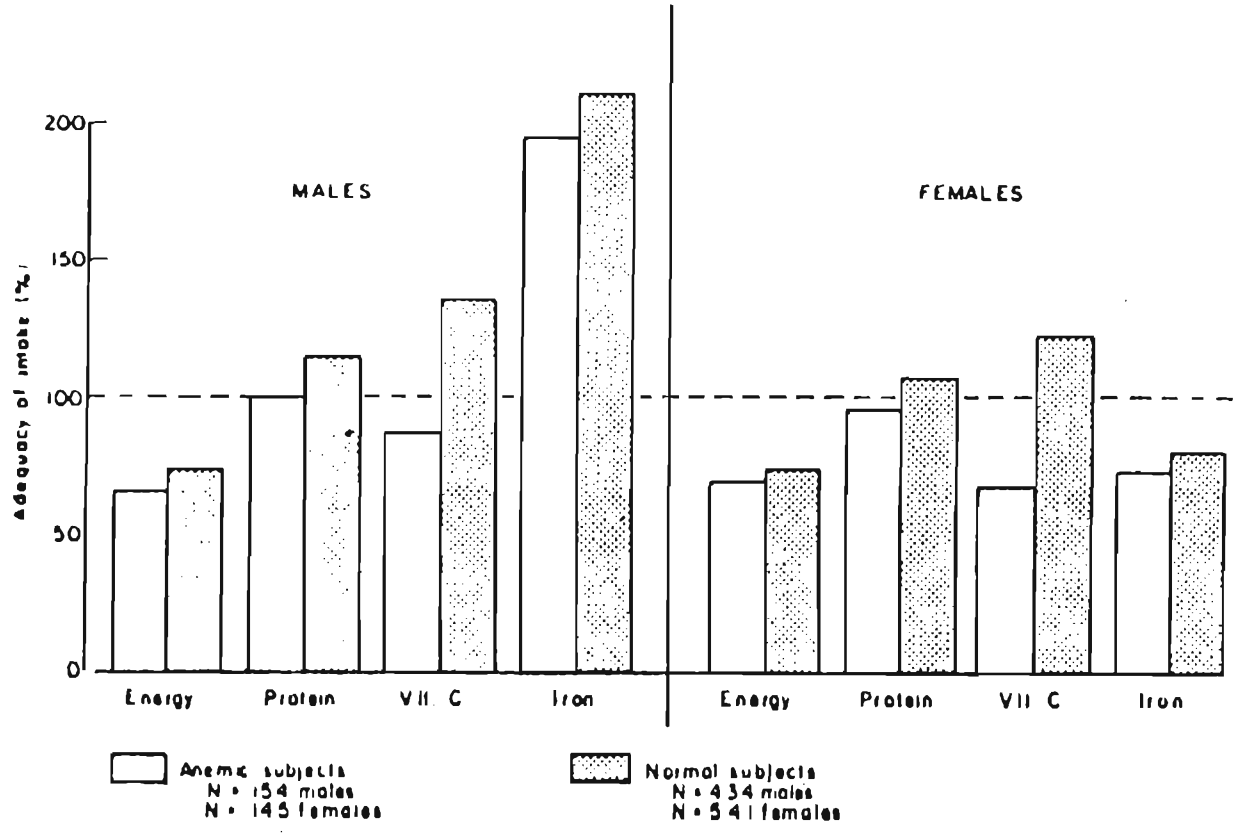

Fig. 2. Average adequacy of nutrient intake of subjects in each of the four communities studied.

except for energy intakes, and illustrate that energy intake is lower than desirable in both sexes and independent of hematological status. Without doubt the anemic female eats very poorly; mean adequacy is barely satisfactory only for total protein intakes. The most significant difference between anemic and normal subjects is vitamin C intake. This finding underscores the possible essential role of ascorbic acid intake on iron nutritional status at the population level where staple diets are predominantly cereal-pulse combinations. In the case of iron, an additional intake of 5 mg per person per day would bring adequacy to near 95%.

With regard to the types of iron ingested, the anemic subjects consumed less iron from animal sources than the nonanemic subjects. This was very evident in terms of heme iron: the range of mean intakes for anemic subjects was 2.7 and 3.6% of total iron intake, whereas for the normal population the range of mean intake was 3.8–5.7%.

These findings confirm the primary importance of the diet in determining the iron status of populations in developing countries.

Hematological Changes

First, the hemoglobin concentrations (Hb) in g/dl and the hematocrit (packed cell volume or PCV) in percentages and the changes in indi-

viduals in each community from initial to the 20 months evaluation are presented for children 5 to 12 years of age; for nonpregnant, nonlactating females older than 21 years of age and for males 21 years and over. These specific age and sex groups are analyzed separately because their expected changes in 20 months of observations are the least, compared to other age-sex groups (small children and adolescents) where age-sex related changes in Hb and PCV are predictably taking place. This first type of analysis ignores the initial hematological condition of the subjects, which has a definite influence on the degree of response expected from correction of iron deficits.

Second, the population as a whole in each community, independent of age and sex is divided into two groups, normal or low, based on cut-off points defined for Hb and PCV as 1.5 standard deviations from the Central American norms (29) below the normal mean value for each age–sex-altitude category. In each group the g Hb/dl and percentage PCV by which each individual differs from the normal mean for his/her corresponding age–sex–altitude category is expressed as Hb Units (HbU) or PCV Units (PCVU). Means and standard deviations are computed for HbU and PCVU initially and after 20 months evaluation, as well as for the individual changes which occurred in that lapse of time. This approach centers upon the differential responses obtained from normal and "anemic" individuals with Fe fortification.

Paired *t* tests of the individual changes between the two evaluations in the various categories described above were done to define whether the changes were significant within each community and category. This is designated t(a) in the tables. The mean changes in the various categories in each of the fortified communities (No. 13, 14, and 16) was then compared with the changes observed in the control community (No. 15) and is designated t(b) in the tables.

This type of statistical analysis is the simplest and provides only a baseline from which interpretation of the observed effects can be initiated. More complex analyses, including linear and nonlinear regressions, covariance analyses and multiple correlation analyses will be performed when the final data are available.

Results are presented in summary form in Tables IV to VII.

Hb increased in all communities and age-sex groups (Table IV), except for the adult males in the control community (No. 15). The increments in the fortified communities 13 and 16 are greater than those in the control, reaching significant *t* values in the child population. In community 14, the most anemic and iron-deficient one, children's Hb response was less relative to the control, suggesting the persistence of other factors which mask the response to iron fortification. A similar trend is observed in

TABLE IV

Mean Values in Hb by Age–Sex Groups and Communities and Changes between Baseline and 20 Months Evaluations

	13			14			16			15		
Group	N[a]	Basal[b]	20[b]	N	Basal	20[b]	N	Basal	20[b]	N	Basal	20[b]
Females > 21 years	33	13.1 ± 1.3	13.8 ± 1.8	36	11.1 ± 3.0	12.2 ± 1.7	54	13.7 ± 2.0	14.8 ± 1.5	58	13.3 ± 1.6	14.1 ± 1.2
Males > 21 years	25	14.7 ± 2.0	15.5 ± 1.2	30	12.5 ± 3.4	14.0 ± 2.3	29	16.1 ± 1.1	16.5 ± 1.5	28	15.5 ± 3.3	15.8 ± 1.5
Children 5–12 years	37	11.6 ± 1.4	13.8 ± 1.0	26	11.8 ± 1.7	12.2 ± 1.8	55	12.8 ± 1.7	14.9 ± 0.8	57	12.7 ± 1.3	14.2 ± 0.9
		Δ20-B[c]	t(a)		Δ20-B	t(a)		Δ20-B	t(a)		Δ20-B	t(a)
Females > 21 years		0.75 ± 1.86	2.32[d]		1.09 ± 2.42	2.70[d]		1.12 ± 2.10	3.92[d]		0.75 ± 1.55	3.69[d]
Males > 21 years		0.77 ± 1.41	2.73[d]		1.51 ± 2.70	3.06[d]		0.33 ± 0.91	N.S. (1.95[d] one tail)		0.32 ± 2.79	N.S.
Children 5–12 years		2.23 ± 1.65	8.22[d]		0.39 ± 1.55	N.S.		2.15 ± 1.53	10.42[d]		1.56 ± 1.24	9.50[d]
		13 vs. 15	t(b)		14 vs. 15	t(b)		16 vs. 15	t(b)			
Females > 21 years			N.S.			N.S.			N.S.			
Males > 21 years			N.S.			N.S.			N.S.			
Children 5–12 years			2.11[d]			−3.39[d]			2.24[d]			

[a] N, number of subjects.

[b] B, mean SD for baseline evaluation; 20 = mean ± SD for 20 months evaluation.

[c] Δ20-B, mean ± SD change between evaluations; t(a), t value for change within community; t(b), t value for comparison with community 15 (control).

[d] Statistically significant.

TABLE V

Mean Values and Changes in PCV by Age–Sex Groups and Communities and Change between Basal and 20 Months evaluations[a]

	13			14			16			15		
Group	N[a]	B[b]	20[b]	N	B	20	N	B	20	N	B	20
Female > 21 years	41	37.9 ± 4.6	37.8 ± 5.7	45	33.0 ± 7.5	34.2 ± 4.9	60	39.4 ± 4.8	41.0 ± 3.8	65	39.8 ± 4.1	39.1 ± 4.1
Male > 21 years	28	43.3 ± 5.6	42.1 ± 4.8	40	34.8 ± 9.6	37.5 ± 7.1	32	45.5 ± 4.0	45.8 ± 4.0	39	42.4 ± 6.1	44.5 ± 4.9
Children 5–12 years	117	35.6 ± 3.1	38.3 ± 2.8	221	34.8 ± 3.5	36.4 ± 4.1	242	38.6 ± 3.0	40.6 ± 2.3	192	37.6 ± 3.2	40.4 ± 2.8

	Δ20-B[c]	t(a)	Δ20-B	t(a)	Δ20-B	t(a)	Δ20-B	t(a)
Female > 21 years	−0.12 ± 6.00	N.S.	1.22 ± 7.03	N.S.	1.59 ± 4.84	2.54[d]	−0.65 ± 4.50	N.S.
Male > 21 years	−1.22 ± 1.75	−3.69[d]	2.69 ± 8.37	2.03[d]	0.28 ± 3.52	N.S.	2.00 ± 6.81	N.S.
Children 5–12 years	2.65 ± 3.86	7.43[d]	1.59 ± 4.72	5.01[d]	1.98 ± 3.29	9.36[d]	2.83 ± 3.44	11.40[d]

	13 vs. 15	t(b)	14 vs. 15	t(b)	16 vs. 15	t(b)
Female > 21 years		N.S.		N.S.		2.67[d]
Male > 21 years		−2.58[d]		N.S.		N.S.
Children 5–12 years		N.S.		−3.08[d]		−2.61[d]

[a] N, number of subjects.

[b] B, mean ± SD for baseline evaluation; 20, mean ± SD for 20 months evaluation.

[c] Δ20-B, mean ± SD change between evaluations; t(a), *t* value for change within community; t(b), *t* value for comparison with community 15 (control).

[d] Statistically significant.

TABLE 447

Mean Values and Changes in HbU by "Normality" Groups—Baseline and 20 Months Evaluations

	13			14			16			15		
	N[a]	B[b]	20[b]	N	B[b]	20	N	B[b]	20	N	B[b]	20
Normal	91	0.20 ± 0.87	0.51 ± 1.38	85	0.17 ± 1.06	−0.38 ± 1.34	137	0.08 ± 0.29	0.15 ± 0.53	146	0.12 ± 1.41	0.37 ± 1.04
Low	62	−3.13 ± 1.89	−0.01 ± 1.57	124	−4.56 ± 2.26	−1.84 ± 2.18	64	−0.90 ± 0.40	0.01 ± 0.33	75	−2.86 ± 1.38	−0.38 ± 1.54
Total	153	−1.15 ± 2.14	0.30 ± 1.47	209	−2.64 ± 2.98	−1.24 ± 2.02	201	−0.23 ± 0.56	0.10 ± 0.48	221	−0.89 ± 1.99	0.12 ± 1.28
	Δ20-B[c]		t(a)	Δ20-B[c]		t(a)	Δ20-B[c]		t(a)	Δ20-B[c]		t(a)
Normal	0.31 ± 1.38		2.29[d]	−0.54 ± 1.46		−3.41[d]	0.06 ± 0.50		N.S.	0.25 ± 1.45		2.08[d]
Low	3.12 ± 2.07		11.87[d]	2.72 ± 2.46		12.31[d]	0.91 ± 0.50		14.56[d]	2.48 ± 1.56		13.77[d]
Total	1.45 ± 2.18		8.23[d]	1.39 ± 2.65		7.58[d]	0.33 ± 0.64		7.31[d]	1.00 ± 1.82		8.17[d]
	13 vs. 15		t(b)	14 vs. 15		t(b)	16 vs. 15		t(b)			
Normal			N.S.			−3.98[d]			N.S.			
Low			2.01[d]			N.S.			−8.23[d]			
Total			2.10[d]			N.S. (1.77[d] one tail)			−5.13[d]			

[a] N, number of subjects.

[b] B, mean ± SD for baseline evaluation; 20, ± mean SD for 20 months evaluation.

[c] 20Δ-B, mean ± SD change between evaluations; t(a), *t* value for change within community; t(b), *t* value for comparison with community 15 (control).

[d] Statistically significant..

TABLE VII

Mean Values and Changes in PCVU by "Normality" Groups—Baseline and 20 Months Evaluations

	13			14			16			15		
	N[a]	B[b]	20[b]	N	B[b]	20[b]	N	B[b]	20[b]	N	B[b]	20[b]
Normal	93	0.39 ± 2.49	−1.44 ± 4.26	62	0.42 ± 2.55	−3.51 ± 3.68	114	−0.02 ± 2.26	0.05 ± 3.40	113	−0.02 ± 2.48	−1.53 ± 3.88
Low	68	−7.01 ± 1.87	−2.51 ± 4.62	59	−7.52 ± 2.31	−5.09 ± 5.11	96	−6.16 ± 1.80	−0.66 ± 2.95	82	−6.46 ± 1.94	−1.81 ± 4.13
Total	161	−2.73 ± 4.30	−1.89 ± 4.43	121	−3.45 ± 4.29	−4.28 ± 4.49	210	−2.83 ± 3.69	−0.28 ± 3.21	195	−2.73 ± 3.91	−1.65 ± 3.98
	Δ20-B[c]		t(a)	Δ20-B[c]		t(a)	Δ20-B[c]		t(a)	Δ20-B[c]		t(a)
Normal	−1.83 ± 4.32		−4.09[d]	−4.06 ± 4.09		−7.82[d]	0.07 ± 3.55		N.S.	−1.51 ± 3.54		−4.53[d]
Low	4.49 ± 4.18		8.88[d]	2.43 ± 5.75		3.25[d]	5.50 ± 3.37		15.99[d]	4.65 ± 4.55		9.25[d]
Total	0.84 ± 4.44		2.40[d]	−0.90 ± 5.92		N.S.	2.55 ± 4.40		8.40[d]	1.08 ± 5.02		3.00[d]
	13 vs. 15		t(b)	14 vs. 15		t(b)	16 vs. 15		t(b)			
Normal			N.S.			−4.13[d]			3.36[d]			
Low			N.S.			−2.46[d]			N.S.			
Total			N.S.			−3.05[d]			3.12[d]			

[a] N, number of subjects.

[b] B, mean ± SD for baseline evaluation; 20, ± mean SD for 20 months evaluation.

[c] Δ20-B mean ± SD change between evaluations; t(a), *t* value for change within community; t(b), *t* value for comparison with community 15 (control).

[d] Statistically significant.

PCV (Table V), although results are more variable. In general, adult males and females did not show significant changes in iron status with fortification since they were near normal values initially.

These results and preliminary interpretations are further substantiated when "normality" groups in HbU and PCVU are considered (Tables VI and VII). At this point in time it appears that it may be too early to detect clear changes in hematological condition in the fortified communities although a trend toward normalization is evident. This possibility has been complicated further, by an improvement in hematological conditions of the control community. This was not clearly noted in

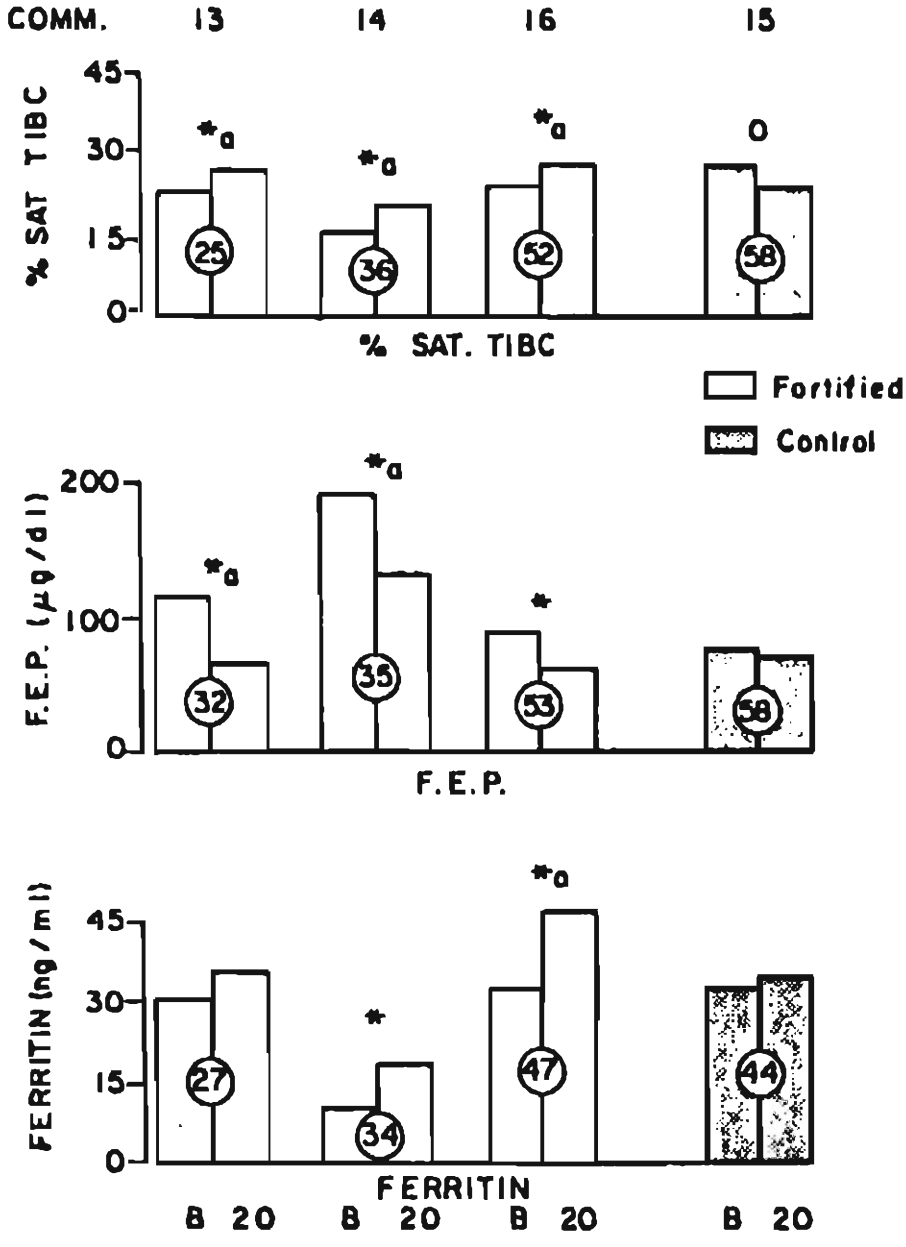

Fig. 3. Mean values of indicators of iron nutrition at the baseline and 20 months evaluations for adult females. * Significantly better; (0) significantly worse; (a) significantly better than community 15; (b) significantly worse than community 15; circled numbers indicate number of subjects.

previous evaluations. The reasons for these changes are not apparent but may be due to delayed effects of vitamin A fortifications which started one year before the initial evaluation of iron status (30). Further studies will most probably clarify this unexpected situation.

Iron Nutrition

Essentially the same type of analyses as those for Hb and PCV have been done for percent saturation TIBC (% sat TIBC), FEP, and ferritin. The mean values and their changes are presented for age-sex categories in Figs. 3 to 5 and for "normality" categories and total populations in Figs. 6 to 8.

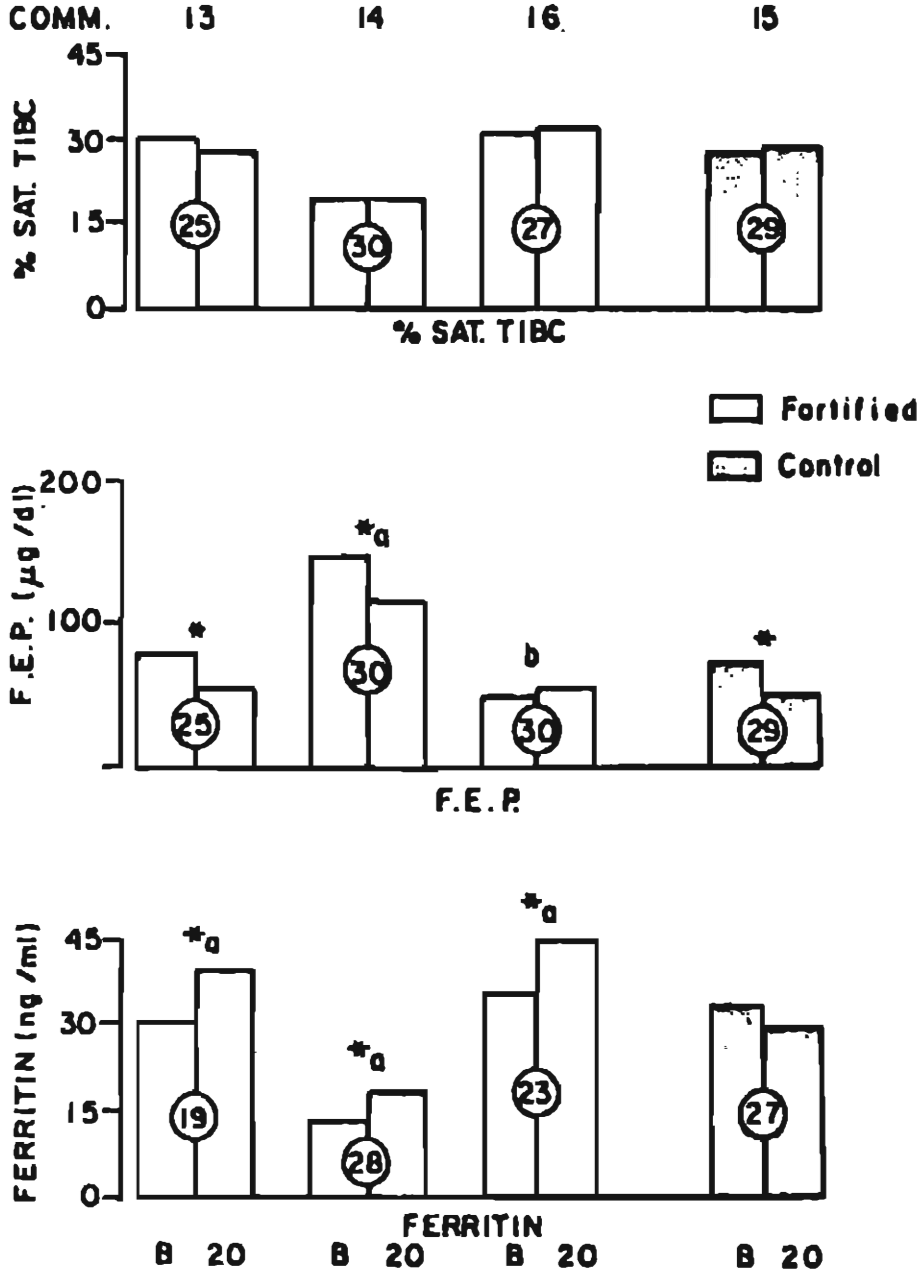

Fig. 4. Mean values of indicators of iron nutrition at the baseline and 20 months evaluations for adult males. See Fig. 3 for definition of symbols.

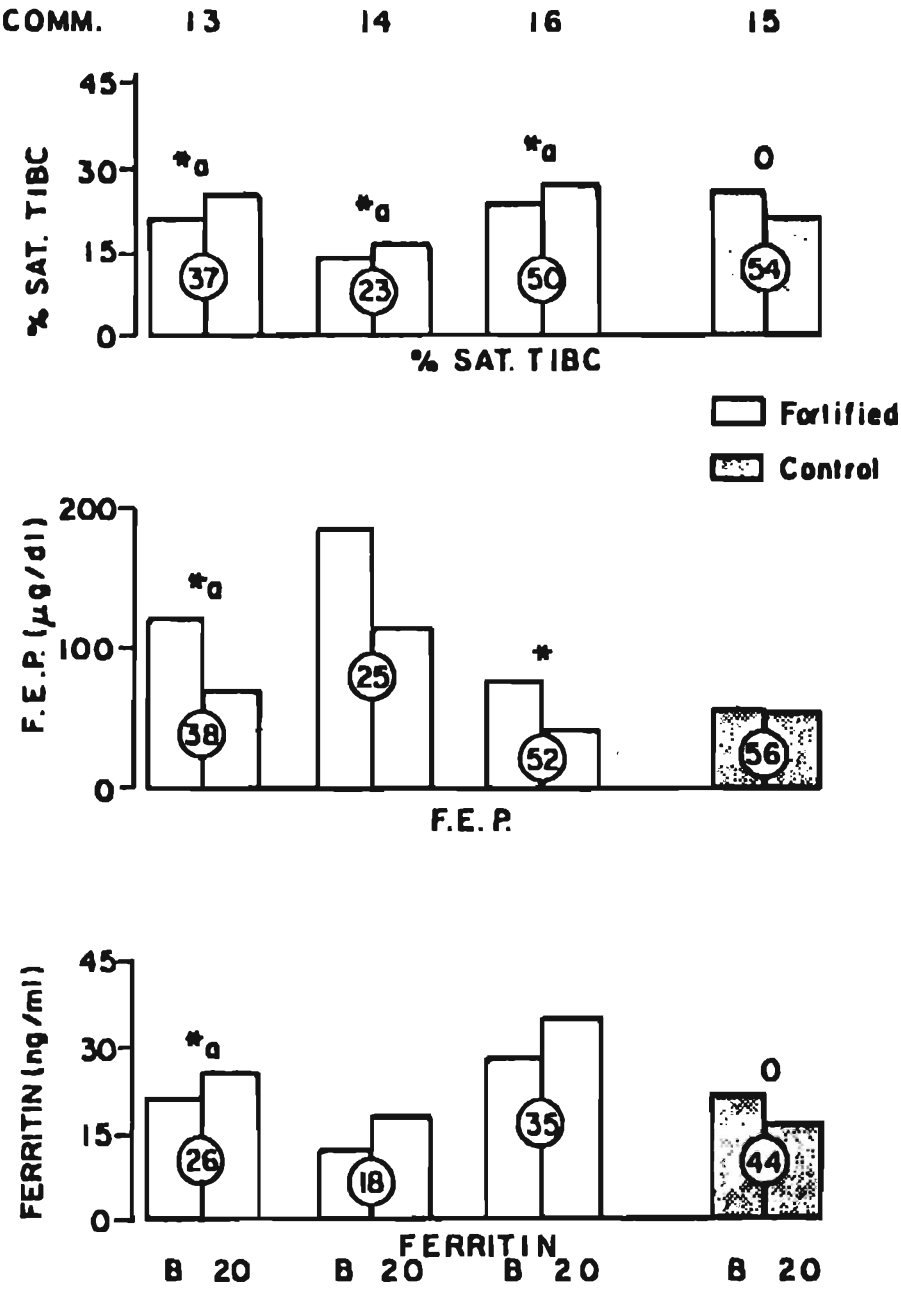

Fig. 5. Mean values of indicators of iron nutrition at the baseline and 20 months evaluations for children 5–12 years of age. See Fig. 3 for definition of symbols.

The changes observed with fortification follow a predicted pattern compatible with an improvement in iron nutrition: adult females significantly improve their iron transport (% sat. TIBC) as well as their iron availability during erythropoiesis (FEP), and iron reserves (ferritin) (Fig. 3). Adult males primarily improve iron reserve (ferritin) and iron availability for Hb synthesis (FEP), while iron transport is essentially unchanged (Fig. 4).

Children behave very similarly to adult females (Fig. 5). It is important to notice that the control community (No. 15) showed a slight and often not significant deterioration in iron nutrition in essentially all variables

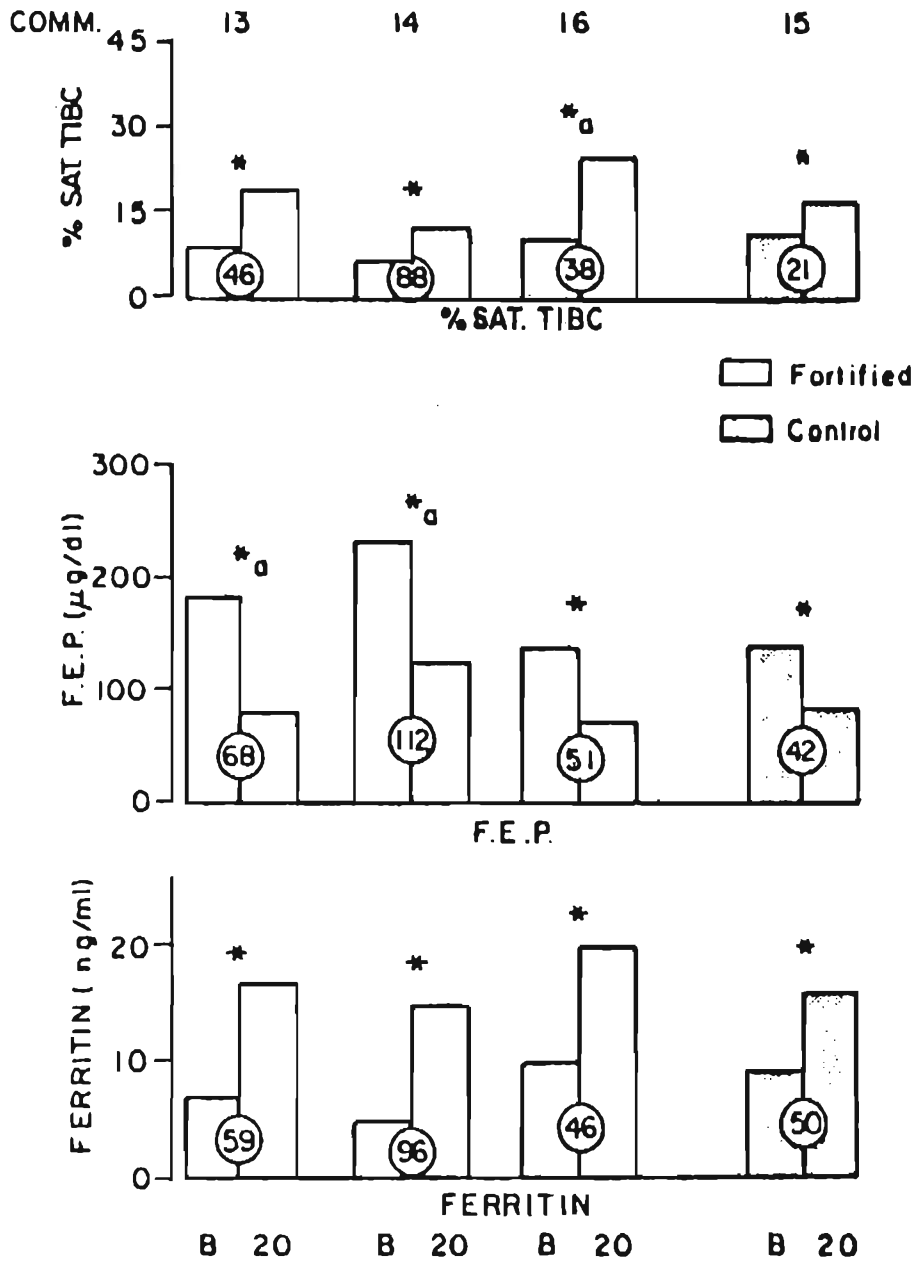

Fig. 6. Mean values of indicators of iron nutrition at the baseline and 20 months evaluations for deficient subjects.

measured, particularly in children and adult females, which constitute the "at risk" population. The results by "normality" groups present, again, what is expected. First, that naturally and due to the phenomenon of regression to the mean, the "defficient" population tends to improve, whereas the "normal" populations tend to deteriorate (31, 32). However, often the changes in the fortified communities reflect a greater improvement in the "deficient" group (Fig. 6) and less of a deterioration in the "normal" group than the control community (Fig. 7). The overall result is that clearly the iron nutrition of the fortified population appears significantly improved (Fig. 8).

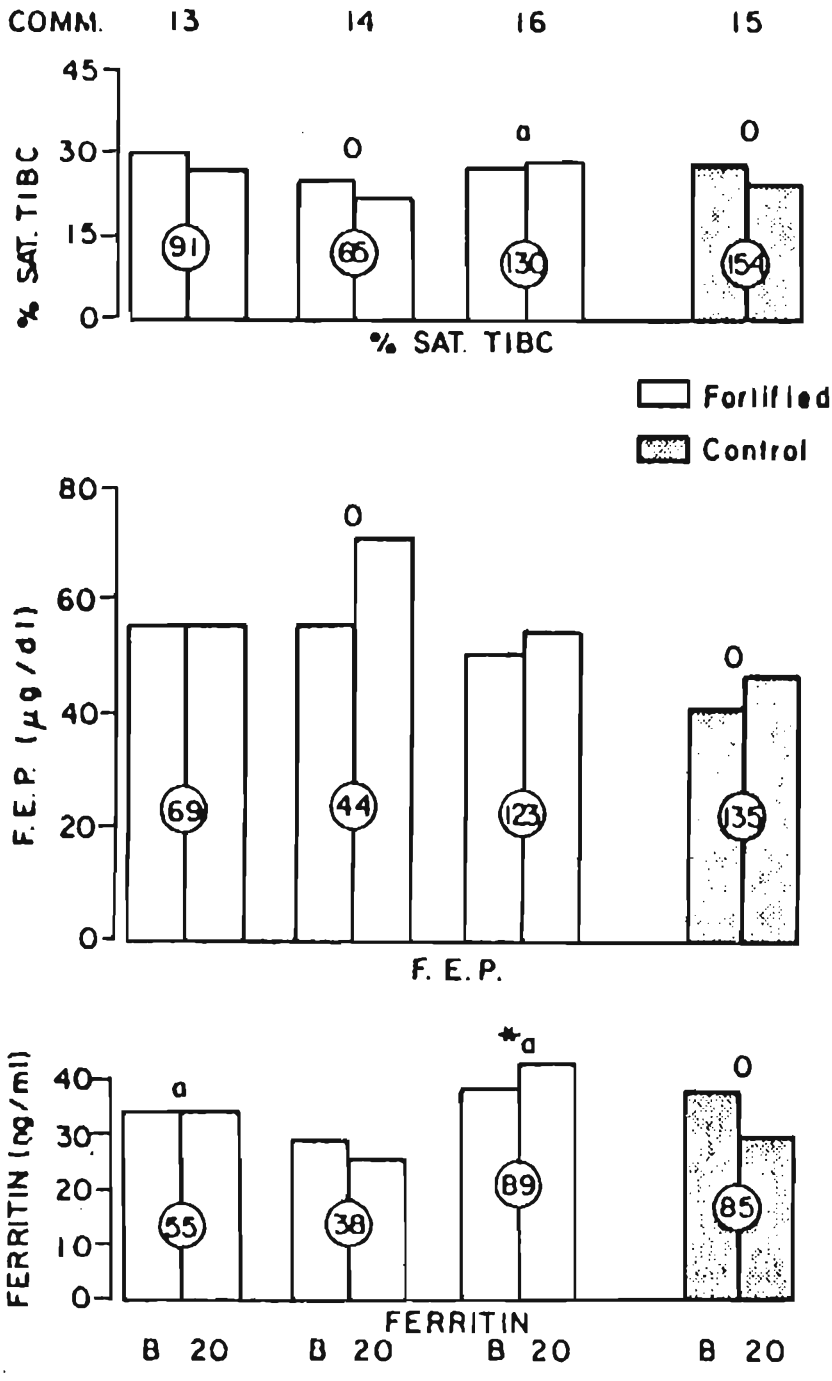

Fig. 7. Mean values of indicators of iron nutrition at the baseline and 20 months evaluations for normal subjects.

The magnitude of changes observed in iron nutrition were often greater than predicted, the opposite of what was observed in hematological condition. The fact is that there are no data with which to compare the sequential changes at the population level of fortification trials. It may be that the responses to very small increments in iron availability differ substantially from the responses to iron supplement which provide over 20 times more iron than food fortification. Interactions with other environmental factors such as repeated and/or chronic infections, vitamin A intake and compliance with the fortification are undoubtedly influenc-

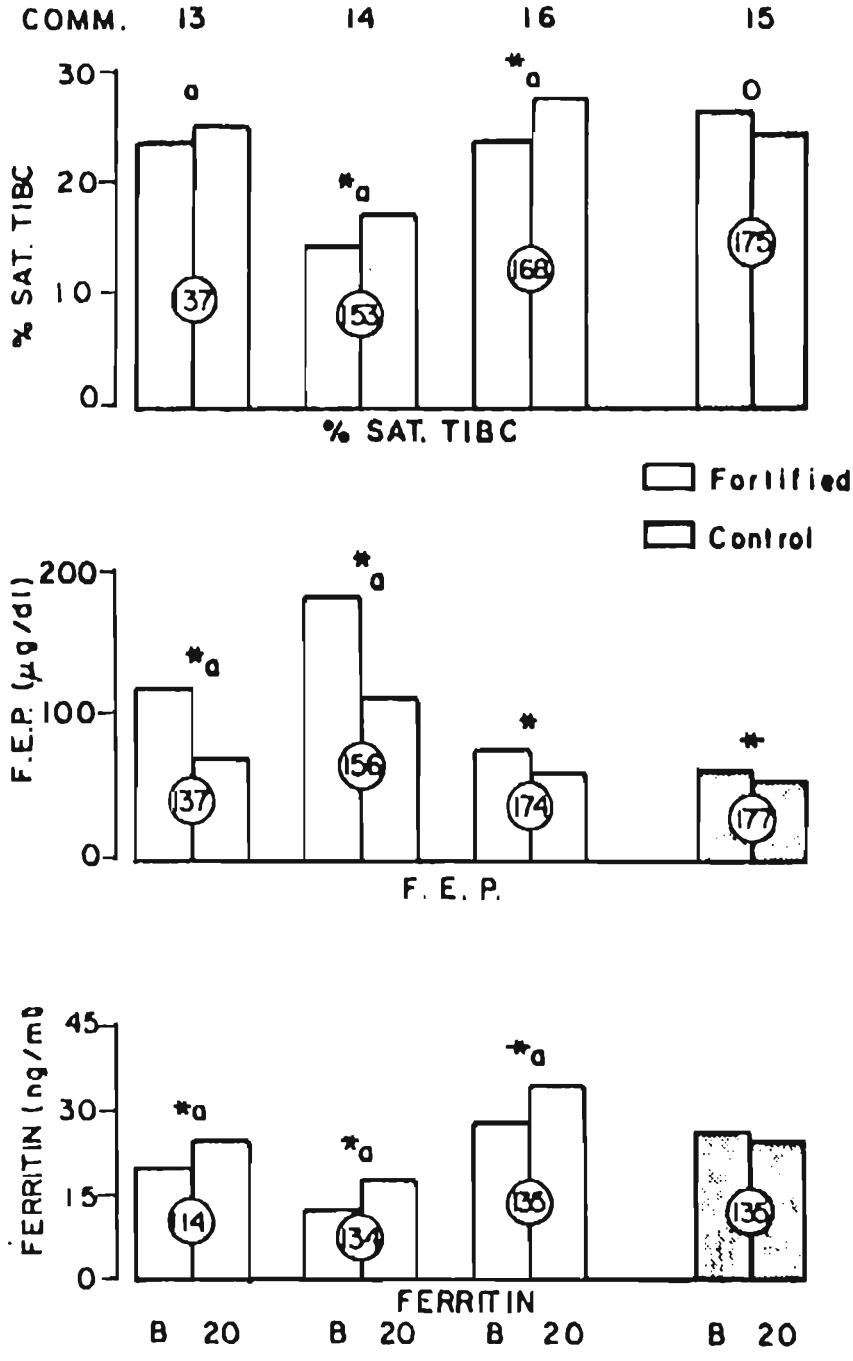

Fig. 8. Mean values of indicators of iron nutrition at the baseline and 20 months evaluations for the total population.

ing results. The continued observation and quantification of these and other variables for a longer time span of fortification will improve our appreciation of the series of phenomena which influence the response to small changes in dietary iron intake.

Finally, the evaluation of supplementation and fortification trials must consider two interrelated outcomes which demand different evaluative concepts. On the one hand, the effect of the intervention must be quantitated against the baseline situation of the population being studied, as well as against the "natural" changes which take place in "control"

communities. The second aspect which must be evaluated is that of the efficacy of the intervention, that is, how effective the intervention is in bringing the population to an optimal condition. The comparison in this case is against a set of norms and distributions recognized adequate and characteristic of healthy populations. At 20 months of sugar fortification with NaFeEDTA at a level of 1:1000, the fortified communities are demonstrating a clear positive *effect* in iron nutrition. As expected the efficacy of fortification trials must be evaluated in long term programs. We expect that 3 years will allow us to estimate certain aspects of the *efficacy* of fortification.

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