The effect of vitamin A fortification of sugar on iron metabolism in preschool children in Guatemala[1-3]

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ABSTRACT The effect of improvement in vitamin A nutriture on biochemical indicators of iron nutrition during national vitamin A fortification of sugar was investigated longitudinally. Four "paired-comparison-subgroups" of preschoolers were studied before fortification (survey I) and, respectively, at 6 months (survey I versus II), at 1 yr (survey I versus III), at 1½ yr (survey I versus IV), and at 2 yr (survey I versus V) after fortification began. Comparing I versus II gave a positive correlation ($p < 0.001$) between changes in serum retinol or retinol-binding protein and changes in iron, total iron binding capacity, and percentage transferrin saturation. In contrast, changes in serum ferritin correlated negatively ($p < 0.05$). Comparing V with I, retinol, retinol-binding protein, iron, and percentage transferritin saturation increased, but ferritin also increased ($p < 0.05$). Consequently, the distribution of serum iron and ferritin values of the children improved ($p < 0.05$). Because dietary iron did not change through the study period, the results suggest that vitamin A fortification had a favorable effect on iron metabolism and nutritional status. *Am J Clin Nutr* 1982;36:87–93.

KEY WORDS Vitamin A, iron, vitamin A and iron, vitamin A fortification

Introduction

Since 1922, several isolated studies have suggested that the lack of vitamin A in humans and experimental animals may result in anemia (1–9). More recently, Hodges et al. (10) and Mejía et al. (11–13) have indicated that there is a biological interaction between hypovitaminosis A, iron metabolism, and hematopoiesis. Their work suggests that vitamin A deficiency alters the metabolism of iron that, in turn, may lead to anemia. An association between hypovitaminosis A and anemia in Indian children has also been observed by Mohanram et al. (14) and Jagadeesan and Reddy (15).

Due to the high incidence of hypovitaminosis A and iron deficiency that prevails in some areas of Central America (16, 17), we believed it imperative to study their possible interaction. In Guatemala, a program of vitamin A fortification of sugar at the national level has significantly improved vitamin A nutriture (18, 19). This offered a unique opportunity to examine the effect of the changes observed in vitamin A nutritional status on the biochemical indicators of iron nutrition and metabolism in the same population. The result of this evaluation is the subject of the present report.

Methods

Data gathered during the evaluation of the effectiveness of vitamin A fortification of sugar in Guatemala (18) were used in the study. This evaluation program began in October–November 1975 and ended in October–November 1977. Within this period, five nutrition surveys were carried out in representative samples of preschool children, age 1 to 5 yr, in 12 small rural communities. A basal survey (survey I) was carried out before fortification followed by four subsequent ones (surveys II to V) at 6-month intervals, after the vitamin

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A intervention began. On completion of this evaluation, blood serum aliquots from the study subjects were stored at −20°C and protected from light. In this study, the statistical approach for the random selection of the sample of children in each community was cross-sectional each time. However, due to the small size of the communities, there were children, who at random, appeared in the sample in more than one survey. This situation provided the opportunity to study individual children longitudinally between pairs of surveys, in terms of their change in vitamin A, as was already reported previously (19). Using essentially the same approach, the present study was conducted to investigate longitudinally the changes in iron nutritional parameters in relation to the previously observed changes in serum retinol. Thus, comparisons were made in 77 children examined in surveys I and II, 75 in surveys I and III, 46 in surveys I and IV, and 51 in surveys I and V, corresponding, respectively, to 6 months, 1, 1½, and 2 yr after initiation of vitamin A fortification.

In the blood serum samples, the following biochemical levels were determined.

1) As indicators of vitamin A nutriture: *a*) Total retinol by the method of Bessey et al. (20). These data were already available from the overall evaluation of the fortification of sugar with vitamin A (18). *b*) Retinol-binding protein (RBP) by radial immunodiffusion as described by Mancini et al. (21).

2) As indicators of iron nutriture: *a*) Iron, total iron binding capacity (TIBC), and percentage saturation of transferrin (% ST) by the method of Ramsay (22). *b*) Ferritin, to be used as an index of iron stores (23), by the two site radioimmunometric assay described by Addison et al. (24) as modified by Miles et al. (25).

In addition to data on vitamin A intake reported in a previous publication (19), data on iron intake were also obtained from the same study population during the periods October–November 1975 and October–November 1977. These data were used to evaluate the stability of iron intake throughout the 2 yr. This dietary information was obtained by the method described by Flores et al. (26) and the adequacy of intake was calculated by comparison with the FAO/WHO dietary allowances (27, 28) adapted by INCAP to Central America and Panama (29).

The biochemical data were analyzed as follows: linear correlations were made between the changes observed in the levels of vitamin A and iron parameters in each of the groups of children studied in surveys I and II, I and III, I and IV, and I and V, respectively. These changes were determined for each child as positive or negative difference after substracting the value obtained in survey I from the corresponding value obtained in the other survey. Furthermore, the magnitude of these changes, expressed as the positive or negative units of net change, were evaluated by paired *t* tests between the two statistically dependent members of each pair of comparisons made. In addition, a comparison was made of the distribution of cases, by categories of iron parameters, between surveys I and V and its statistical significance tested by χ^2.

No hematological data were contemplated in the vitamin A fortification study; therefore, this type of information was not available.

Results

Figure 1 shows the relationships observed between changes in serum retinol and changes in each of the other biochemical parameters in the children studied in surveys I and II, i.e., the effect of 6 months of vitamin A fortification. There were highly significant positive correlations ($p < 0.001$) between the change in the level of serum retinol and the change in the serum levels of iron, TIBC, and % ST. In contrast, the change in serum retinol correlated negatively ($p < 0.05$) with the change in the level of serum ferritin. Correlations of the same magnitude and direction were found between changes in serum RBP and those in iron parameters. In these children, the correlation coefficient for the changes in serum levels of retinol versus RBP was 0.74 ($p < 0.001$).

Using the same approach, no significant correlations were found between the change in the level of serum retinol and the change in the serum levels of iron parameters of children studied in surveys I to III, I to IV, and I to V. The change in serum retinol, however, correlated significantly in all instances with the change in serum RBP ($p < 0.001$).

Table 1 shows the direction and extent of the average net units of change in serum retinol and iron parameters when comparing, by paired *t* tests, children studied in surveys I to II, I to III, I to IV, and I to V, respectively. This data representation, however, does not intend to imply a longitudinal sequence of changes observed through the 2 yr of evaluation. This clarification is made in view that the four comparisons made between pairs of surveys belong to separately selected groups of children. It can be observed that children followed between surveys I and II (6 months after initiation of vitamin A fortification) experienced an increase in the serum levels of retinol, iron, and TIBC. In contrast, serum levels of ferritin decreased. The slight increase in % ST was not significant. Children followed between surveys I and III (1 yr after initiation of fortification) experienced a significant elevation in serum retinol but no significant changes in iron parameters. It is interesting to note, however, the tendency of serum iron and % ST to decrease and of

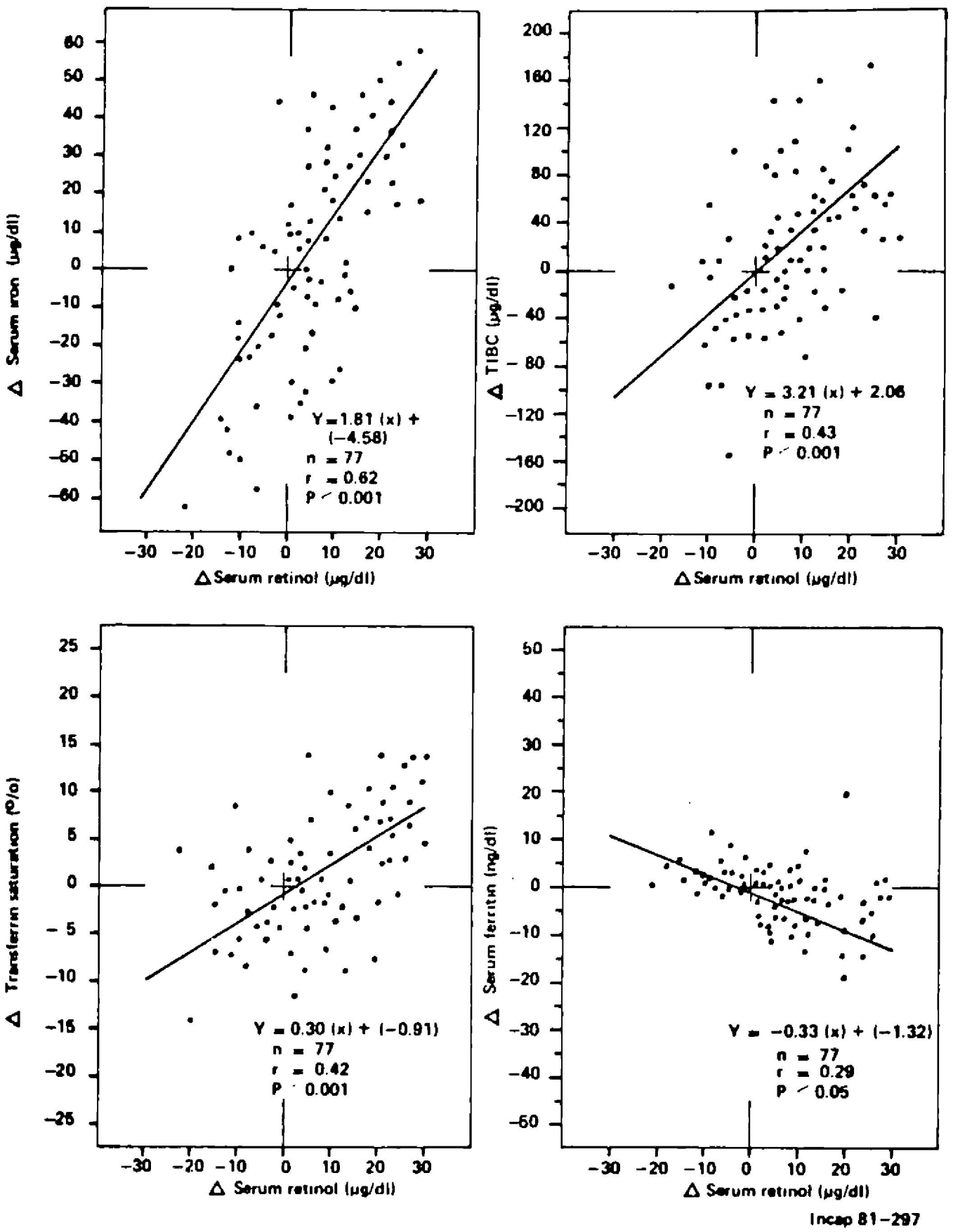

FIG. 1. Correlations between changes in serum retinol and changes in serum levels of iron parameters after 6 months of vitamin A fortification (Δ: change).

TABLE 1
Average net units of change in serum retinol and iron parameters between pairs of surveys

	I vs II	I vs III	I vs IV	I vs V
	(n = 77)	(n = 75)	(n = 46)	(n = 51)
Retinol (μg/dl)	+5.1 ± 10.2*	+5.6 ± 10.5*	+3.6 ± 10.7*	2.5 ± 10.0*
Iron (μg/dl)	+4.5 ± 21.5*	−3.6 ± 30.9	+13.1 ± 36.9*	+9.1 ± 32.5*
TIBC (μg/dl)	+18.3 ± 76.1*	−8.8 ± 67.9	−7.8 ± 65.0	−13.6 ± 68.8
% ST	+0.6 ± 7.4	−1.0 ± 7.7	+3.8 ± 10.6*	+2.2 ± 9.0*
Ferritin (ng/ml)	−3.0 ± 11.7*	+2.1 ± 11.0	+5.5 ± 9.9*	+4.9 ± 19.7*

* Statistically different ($p < 0.05$ or better).

serum ferritin to increase. Children compared between surveys I and IV (1½ yr) and I and V (2 yr after initiation of fortification) showed significant increases in the serum levels of retinol, iron, % ST. and ferritin. Their TIBC decreased but the change did not reach statistical significance.

The distribution of cases, by categories of iron parameters, in the 51 children studied in surveys I and V is presented in **Table 2**. After 2 yr of vitamin A fortification, an improved distribution of biochemical levels of iron parameters in the children studied indicated an improved iron nutritional status. Very similar results, both in direction and magnitude of change, were observed in survey IV after 1½ yr of vitamin A fortification, as illustrated by the fact that in this survey, the proportion of serum iron values higher than 75 μg/dl was 50% and the proportion of serum ferritin levels higher than 20 ng/ml was 28%, compared to the figures of 26% for iron and 13% for ferritin in the same children in survey I (before fortification).

The dietary data revealed that in comparison with the prefortification survey, the implementation of sugar fortification resulted in a significant 3-fold increase in the average daily intake of retinol equivalents, as reported in a separate publication (19). As shown in **Table 3**, the average intake of iron and its adequacy did not change significantly throughout the period of evaluation. It was also evident, considering all surveys, that on the average, only 37% of the families met the recommended level of intake for this essential mineral. Although the food consumption data were obtained at the family level, previous epidemiological studies (30) for this type of population indicate that the dietary characteristics of the children are a direct reflection of those of their families, which justifies the assumption that the iron intake of the preschool children studied did not improve throughout the 2-yr period.

TABLE 2
Percentage distribution of cases in surveys I and V by categories of iron parameters

	Survey I*	Survey V†
	%	%
Serum iron (μg/dl)		
<50	43.1	25.5‡
50–75	27.5	39.2
>75	29.4	35.3
Serum TIBC (μg/dl)		
<250	5.9	2.0
250–350	37.3	49.0
>350	56.9	49.9
% ST		
<15	39.2	27.5
15–20	33.3	33.3
>20	27.5	39.2
Serum ferritin (ng/ml)		
<10	64.7	21.6‡
10–20	23.5	58.8
>20	11.8	19.6

* Basal survey.
† Two years after initiation of vitamin A fortification.
‡ The distributions are significantly different ($p < 0.05$ or better).

TABLE 3
Daily average per capita intake of iron and distribution of percent dietary adequacy from October–November 1975 through October–November 1977

Survey period	Iron intake	Percentage adequacy		
		<50	50–99	≥100
	mg			
I (basal)	13	24*	47	29
II	14	20	43	37
III	15	18	40	42
IV	14	19	40	42
V	14	18	45	37

* Percentage of families. The distribution of adequacies between the different surveys is not statistically different ($\chi^2 = 5.16$, df = 8).

Discussion

The results indicate that in the children studied there was a relationship between biochemical indicators of vitamin A and iron nutriture. They also show that vitamin A fortification of sugar had a favorable effect on the metabolism of iron.

After 6 months of vitamin A fortification, there was a clear positive association between improvement in vitamin A nutrition, as defined by serum levels of retinol or RBP, and the levels of serum iron. A significant positive correlation between serum levels of retinol and iron was shown previously by Mejía et al. (11) in Central American children using a cross-sectional analysis. This finding was later confirmed by Wenger et al. (31) who found the same association in elderly subjects. In addition, it has been demonstrated in a group of experimental children that a

high daily oral dose of 8000 μg of retinyl palmitate, with no change in dietary iron intake, caused a significant increase in the plasma levels of this mineral (14). These observations are confirmed by the present study, which in addition reveals a positive correlation of retinol and RBP, with % ST, although to a lesser degree.

It is interesting to note that during the 6-month period, the elevation of serum levels of iron was accompanied by a simultaneous elevation in TIBC. Since TIBC indirectly measures approximately 90% of serum transferrin, this phenomenon suggests that vitamin A may affect the levels of this iron-carrier glycoprotein, knowing that the synthesis of glycoproteins can be impaired by the lack of vitamin A (32). It is interesting to note that in Mohanram's study of Indian children given a high dose of vitamin A for 2 wk, there was a slight, but statistically insignificant increase in the levels of TIBC from 452.6 ± 29.7 to 478 ± 25.1, despite an elevation in plasma iron (14).

Another interesting phenomenon observed during the initial period of 6 months is the negative relationship between the changes in serum retinol or RBP levels and the change in serum ferritin. Serum ferritin levels have been shown to reflect the amount of stored iron (23). Thus, the present finding suggests that children with low levels of serum retinol or RBP also have elevated amounts of iron in storage, provided that iron in the diet is not the first limiting factor. This stored iron would be mobilized by improving the vitamin A nutritional status.

Blackfan and Wolbach (5) have reported that a common finding in the vitamin A-deficient infant, when studied postmortem, is hemosiderosis of the liver and spleen, and a description is made in the same publication indicating that a similar phenomenon occurs in experimental animals. More recently, a greater concentration of iron has been found in liver and spleen of vitamin A-deficient rats by either chemical analysis (10, 13) or by measuring the incorporation of radioactive iron after ^{59}Fe administration (12). These observations are in agreement with the finding of the present study that in hypovitaminosis A stored iron is increased.

After more prolonged periods of vitamin A intervention, however, the effect of the vitamin seems to be different, as has been shown in Table 1.

If a longitudinal extrapolation among the four different pair-groups of children were possible, several speculations could be made for interpreting the sequential changes observed throughout the study. First, the tendency of serum iron to decrease after 1 yr of fortification, although not significant, is probably due, on the one hand, to the previous depletion of stored iron and, on the other hand, to a possible enhancement of iron utilization for red cell formation. Although the direct effect of hypovitaminosis A on iron utilization for hematopoiesis per se cannot be evaluated in this study, previous studies have revealed that in the vitamin A-deficient rat (12), as well as in vitamin A-deficient children (Mohanram M, Reddy V, unpublished data), there is a significant reduction in ^{59}Fe incorporation into erythrocytes. Thus, the elevation in iron stores observed after 1 yr, but particularly after 1½ and 2 yr of fortification, is probably due to an enhancement of dietary iron absorption triggered by both the initial depletion of iron reserves and the already discussed increment of iron utilization. As a consequence, in the long run TIBC levels decrease as may be expected in normal iron metabolism. What is clear, however, is that after longer periods of vitamin A fortification there was an overall significant improvement of the iron nutritional parameters in these children. Namely, their serum iron, % ST and, more important, their iron reserves became significantly elevated and their TIBC decreased. These positive changes are also evidenced by the distribution of cases by categories of iron parameters illustrated when comparing surveys I and V (Table 2). Siimes et al. (33) have shown that in healthy children the levels of serum ferritin remain constant from 6 months to 16 yr of age; therefore, the observed favorable change in iron reserves cannot be attributed to the fact that the children were 2 yr older at the end of the study.

The fact that the intake of iron did not change significantly in this population during the 2-yr period of evaluation precludes the possibility that the observed changes in iron parameters could have been the result of improved dietary adequacy of this mineral. However, vitamin A intake increased significantly as a result of fortification, suggesting

that the changes in iron nutrition and metabolism were effected by the vitamin.

The design of the present study does not allow us to speculate whether the described effect of vitamin A is direct or indirect. Regardless of the mechanism, however, it is clear that the improvement of vitamin A nutriture in a population, for example through fortification, may have an important positive impact on iron nutrition and metabolism. When planning nutritional interventions, proper consideration should be given to this interaction.

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