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Response to an iron fortification programme in relation to vitamin A status in 6–12-year-old school children

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Plasma retinol and indices of iron status were measured in 148 school children (6–12 years) receiving a soup fortified with iron and vitamin C for a period of 15 weeks. The most significant change in serum iron (P = 0.0005) and transferrin saturation (P = 0.0002) was seen in subjects with plasma retinol ≥ 40 μg/dl, while subjects with plasma retinol < 20 μg/dl showed no response. Serum ferritin improved most in the retinol categories < 40 μg/dl, suggesting that the absorption of iron was not impaired by marginal vitamin A status, but that it was rather the mobilisation of iron from stores that was affected. Changes in vitamin A status correlated positively and significantly with changes in serum iron (r = 0.37; P = 0.0001) transferrin saturation (r = 0.27; P = 0.004) and haemoglobin (r = 0.21; P = 0.03), but negatively with serum ferritin (r = –0.28; P = 0.003). The presence of marginal vitamin A deficiency in a community may limit the effectiveness of an iron intervention programme and vitamin A status should therefore also be considered when such programmes are planned.

Introduction

The role of vitamin A in iron metabolism has in recent years received increased attention (West & Roodenburg, 1992). Not only have several studies demonstrated an association between vitamin A status and iron or haematological status (Mejía *et al.*, 1977; Mohanram *et al.*, 1977; Bloem *et al.*, 1989), but it has also been found that supplementation with vitamin A led to a concomitant improvement in iron status (Mejía & Arroyave, 1982; Bloem *et al.*, 1990). In a study carried out about 20 years ago, where vitamin A deficiency was experimentally induced in eight healthy male volunteers, it was found that, despite an adequate intake of iron, five of the subjects developed a mild anaemia. The anaemia did not respond to iron therapy, but improved only when the vitamin A deficiency was corrected (Hodges *et al.*, 1978). In two recent studies, anaemic children (Mejía & Chew, 1988) and anaemic pregnant women (Suharno *et al.*, 1993) were supplemented with either vitamin A alone, iron alone, a combination of vitamin A and iron or a placebo for a period of 2 months. In both studies the haematological parameters responded to all three non-placebo treatments, though the best response was obtained when vitamin A and iron were given simultaneously.

This interaction between vitamin A and iron status may have implications for iron inter-

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vention programmes, particularly when undertaken in areas where vitamin A deficiency, or even mild vitamin A deficiency, may be prevalent. A recent national survey of 4283 6–71-month-old children in South Africa showed that 33% had low serum vitamin A concentrations (< 20 μg/dl) and that 3% were vitamin A deficient (serum concentrations < 10 μg/dl) (South African Vitamin A Consultative Group, 1995). Anaemia is also a common health problem in South Africa and the prevalence in primary school children ranges from 4 to 43% (Lamparelli *et al.*, 1988; Kruger *et al.*, 1996).

In a recent study carried out by our group, the feasibility of using soup as a vehicle for iron fortification to improve the iron status of primary school children was evaluated (Badenhorst *et al.*, unpublished). This gave us the opportunity to also assess the vitamin A status of these children and to determine whether there was a relationship between vitamin A status and response to the iron fortification programme (IFP).

Methods

Study population

The study was carried out in a primary school in Worcester, a town situated ±100 km north-east of Cape Town, South Africa. The school serves a community characterised by a low socio-economic status and is part of the existing Peninsula School Feeding Association school feeding scheme. The study population comprised 148 Coloured children between the ages of 6 and 12 years. Data on vitamin A status was available of 130 children at the pre-intervention assessment, 148 children at the post-intervention assessment and of 113 children at both assessments. Written informed consent was obtained from the parents or guardians of all participants prior to the study. The study was approved by the Ethics Committee of the Medical Research Council and permission was also obtained from the Department of Education and the Peninsula School Feeding Association.

Fortification

The soup supplied to the school by the Peninsula School Feeding Association was used as a vehicle for fortification. The fortification was done by the manufacturer of the soup (Funa Foods, Springs, South Africa). One portion (160 ml) of the fortified soup contained 60 mg ferrous fumarate (supplying 20 mg elemental iron or 1 mg absorbable iron, the amount recommended for 1–12-year-old children, WHO, 1970) and 100 mg vitamin C to enhance the absorption of iron. The soup was distributed daily during the school week (5 days) for a period of 15 weeks. Records on compliance kept by the class teacher showed that participants consumed the soup for an average of 92% of the total number of soup days during the study period.

Design

Iron, haematological and vitamin A status was assessed at baseline and again after 15 weeks of iron intervention. The relationship between vitamin A and iron status was analysed separately for the pre-intervention and post-intervention assessments in a cross-sectional manner. To examine the relationship between vitamin A status and respone to the IFP, iron status was studied longitudinally. Post-intervention plasma vitamin A levels were used for this purpose, because it was regarded a better reflection of vitamin A nutriture *during* the intervention period. Serum albumin was measured at both assessments in a subsample of 107 children to serve as an indicator of hydration status (Walmsley & Guerin, 1984).

Laboratory methods

Blood (5 ml) was obtained by venipuncture. A full blood count which included haemoglobin (Hb), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) was done by flow cytometry and cell counting on a haematology auto-analyzer, the Technicon H2 (Technicon Instruments Ltd, Dublin, Ireland) within 6 h of blood collection. The rest of the blood was processed and stored at –80°C until assayed.

Serum ferritin was determined by an immunoradiometric assay (Ferritin MAb Solid Phase Component System, Becton Dickinson and Company, Orangeburg, New York), using an Auto Gamma 500C counting system from United Technologies Packard, USA. Serum iron was determined spectrophotometrically with a Technicon RA-1000 automated system, using a colorimetric method without deproteinization (Boehringer Mannheim, Mannheim, Germany).

Total iron binding capacity (TIBC) was determined, using the same method, after saturation of transferrin with iron and precipitating uncomplexed iron with magnesium carbonate (Boehringer Mannheim, Mannheim, Germany). Transferrin saturation (TS) was calculated by expressing total serum iron as a percentage of TIBC. The SimulTRAC-SNB Radioassay Kit for Vitamin $B_{12}[^{57}Co]$/Folate[^{125}I] (Becton Dickinson and Company, Orangeburg, New York) was used to determine RBC folate. Serum albumin was measured spectrophotometrically, using a kit from Boehringer Mannheim (Mannheim, Germany).

A slightly modified version of the reversed-phase HPLC method described by Catignani & Bieri (1983) was used to determine plasma retinol. For the purpose of this study severe vitamin A deficiency was defined as plasma retinol concentrations below 10 µg/dl and marginal vitamin A deficiency as plasma concentrations between 10 and < 30 µg/dl. Plasma concentrations equal to and above 30 µg/dl were considered adequate.

Dietary intake

The dietary intake of a randomly selected subsample of 38 children in their third year of schooling (age ranging from 8 to 12 years) was obtained by means of the 24 h recall method for a Sunday and two weekdays at both the pre- and post-intervention assessments. Three-dimensional plastic food models, household measures, as well as the Food Quantities Manual of the MRC (Langenhoven *et al.*, 1991a), were used to estimate portion sizes. Each food item was coded afterwards, using the MRC Food Composition Tables (Langenhoven *et al.*, 1991b) and processed by computer to obtain the mean daily intakes of energy, macro- and micronutrients. The 1989 Recommended Dietary Allowance (RDA) for 7–10-year-old children (National Research Council, 1989) was used as a reference to compare intake with.

Anthropometry

Weight was measured (in light clothing) to the nearest 0.1 kg on an electronic load cell scale, and height (without shoes) to the nearest 0.1 cm, using a metal tape measure instrument fitted to the wall.

Statistical analysis

The Wilcoxon signed rank test was used to compare pre- and post-intervention values with each other. To test for significant differences between the means of more than two vitamin A categories, non-parametric Tukey-type multiple comparisons at a significance level of 0.05 were used. The Wilcoxon two-sample test was used when two groups were compared. Spearman correlations were used to test for the association between change in vitamin A status and changes in the indicators of iron status.

Results

The haematological and biochemical data of the pre- and post-intervention assessments are summarised in Table 1. Only the children for whom data on iron and haematological status at both the pre- and post-intervention assessments and vitamin A status at the post-intervention assess-

Table 1. Haematological and biochemical data (mean ± SD) at the pre- and post-intervention assessments

	n	*Pre*	*Post*	*P-values*
Haemoglobin (g/l)	125	133 ± 10	122 ± 8	0.0001
Haematocrit (%)	125	42.0 ± 2.6	36.9 ± 2.1	0.0001
s iron (µmol/l)	135	11.7 ± 5.2	13.8 ± 6.9	0.0018
%TS	130	19.3 ± 9.0	24.6 ± 12.5	0.0001
s ferritin (µg/l)	134	21.0 ± 19.1	27.0 ± 23.0	0.0001
s albumin (g/l)	107	50.3 ± 2.3	47.4 ± 2.2	0.0001
MCV (fl)	125	84.7 ± 4.6	82.6 ± 4.5	0.0001
MCH (pg)	125	26.8 ± 1.9	27.3 ± 1.8	0.0001
MCHC (g/dl)	125	31.7 ± 0.9	33.1 ± 0.8	0.0001

s = serum; TS = transferrin saturation; MCV = mean corpuscular volume; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration.

Table 2. The vitamin A status of the study population

	Pre (winter)	*Post (summer)*
n	113	113
Plasma vitamin A (μg/dl)	37.5 ± 8.8[a]	31.9 ± 8.2[b]
% children		
<10 μg/dl	0	0
<20 μg/dl	1.8	7.2
<30 μg/dl	23.7	46.7

[a]Mean ± SD; [b]$P = 0.0001$ compared to pre-intervention.

ment were available, are included in this table. Mean serum iron, transferrin saturation, serum ferritin, MCH and MCHC improved significantly, while there was a significant decrease in MCV, haematocrit, haemoglobin and serum albumin.

The vitamin A status of the study population is given in Table 2. Mean plasma vitamin A was significantly lower at the post-intervention assessment ($P = 0.0001$). None of the children were severely deficient (plasma concentrations below 10 μg/dl) at either assessment. However, 23.7% of the children at the pre- and 46.7% of the children at the post-intervention assessment had plasma retinol concentrations below 30 μg/dl and were therefore considered to be marginally vitamin A deficient.

The dietary intake of vitamin A, iron, vitamin C, folic acid, vitamin B_{12} and energy, determined in a subsample of 38 subjects, is shown in Table 3. The mean intake of vitamin A at the post-intervention assessment in December was significantly lower than that at the pre-intervention assessment in August ($P < 0.05$) and below the RDA for the child 7–10 years old. The mean intake of iron and vitamin C was, due to the consumption of the fortified soup, significantly higher at the post-intervention assessment ($P < 0.0001$). The mean intake of folic acid and vitamin B_{12} exceeded the RDA at both assessments.

Table 4 shows the indicators of iron status grouped according to plasma vitamin A concentrations. The mean serum iron and %TS at the pre-intervention assessment were the lowest in children with plasma retinol concentrations below 30 μg/dl, increased with increments in vitamin A status, and was significantly higher in children with plasma retinol concentrations ≥40 μg/dl. Above 40 μg/dl, however, there was no further increase in serum iron and %TS. Mean haemoglobin in the children with plasma retinol concentrations ≥30 μg/dl was significantly higher than the mean haemoglobin in children with plasma retinol concentrations <30 μg/dl. Serum ferritin, in contrast to the other parameters, tended to be higher in the children with plasma retinol <30 μg/dl. This

Table 3. Dietary intake[a] (mean ± SD) of a subsample of the study population ($n = 38$)

Nutrient	*Pre (winter)*	*Post (summer)*	*RDA*[b]
Energy (kJ)	7807 ± 2536	6779 ± 2551[c]	8400
Vitamin A (μg RE)	1026 ± 1435	600 ± 591[c]	700
Iron (mg)	8.0 ± 2.8	20.3 ± 9.7[d]	10
Vitamin C (mg)	43.0 ± 34.6	95.1 ± 52.1[d]	45
Folic acid (μg)	159 ± 64	148 ± 109	100
Vitamin B_{12} (μg)	5.3 ± 12.2	2.8 ± 2.8	1.4

[a]Three 24 h recalls (two weekdays and a Sunday).
[b]Recommended dietary allowance for the child 7–10 years (1989).
[c]$P < 0.05$; [d]$P < 0.0001$, compared to pre-intervention.

Table 4. Indicators of iron status (mean ± SD) grouped according to plasma retinol concentrations

Retinol (μg/dl)	*n*	*s Iron (μmol/l)*	*TS (%)*	*Haemoglobin (g/l)*	*s Ferritin (μg/l)*
Pre-intervention					
<30	30	8.6 ± 4.4	14.2 ± 6.9	130 ± 7	25.0 ± 28.1
30–39.9	41	10.9 ± 5.4	18.2 ± 9.5	133 ± 9 [a]	20.0 ± 17.7
40–44.9	37	13.5 ± 4.0 [b]	21.8 ± 6.5 [b]	133 ± 9	21.5 ± 16.1
≥45	22	12.3 ± 4.3	20.3 ± 7.4	136 ± 10	18.1 ± 15.0
Post-intervention					
<20	9	7.0 ± 2.9	11.9 ± 4.1	121 ± 8	21.3 ± 17.5
20–29.9	62	12.2 ± 6.3	22.1 ± 11.3	120 ± 8	28.2 ± 26.6
30–39.9	49	14.0 ± 5.0	24.1 ± 9.8	125 ± 7 [a]	26.5 ± 14.8
≥40	28	18.3 ± 8.8[c]	31.7 ± 15.5[d]	124 ± 6	27.3 ± 25.3

s = serum.
[a]Significantly different from <30 μg/dl category [Wilcoxon two-sample test, $P = 0.0403$ (pre), $P = 0.0065$ (post)]; [b]significantly different from <30 μg/dl vitamin A category, [c]significantly different from <20 and 20–29.9 μg/dl vitamin A categories, [d]significantly different from <20 μg/dl vitamin A category (Tukey-type multiple comparisons at $P = 0.05$).

difference was, however, not statistically significant. No association was evident with haematocrit, serum transferrin, TIBC, RBC folate, MCV, MCH or MCHC.

At the post-intervention assessment (Table 4) serum iron and %TS were the lowest in children with plasma retinol <20 μg/dl and again increased with increments of vitamin A status. Serum iron in the ≥40 μg/dl retinol category differed significantly from the <20 and 20–29.9 μg/dl categories, while %TS in the ≥40 μg/dl vitamin A category differed significantly from the <20 μg/dl category. Again, children with plasma retinol concentrations ≥30 μg/dl had a significantly higher mean haemoglobin than the children with plasma retinol <30 μg/dl. No association was seen with haematocrit, serum transferrin, TIBC, RBC folate, MCV, MCH, MCHC or serum ferritin. None of the children at the pre-intervention assessment and only one child at the post-intervention assessment had RBC folate concentrations below the cut off value of 120 ng/ml.

The mean changes in the indicators of iron status, categorised according to post-intervention vitamin A status, are presented in Figs 1–3. The mean change in serum iron (Fig. 1a) was insignificant or negative in all the plasma retinol categories <40 μg/dl and it was only in the children with plasma retinol concentrations ≥40 μg/dl that the mean serum iron improved significantly from pre-intervention levels ($P = 0.0005$). A similar pattern was seen with the %TS (Fig. 2a); children with plasma retinol below 20 μg/dl showed a negative mean change in %TS, %TS increased significantly in the 20–29.9 and 30–39.9 μg/dl vitamin A categories, but the most significant change was observed in the children with plasma retinol concentrations ≥40 μg/dl ($P = 0.0002$). Serum ferritin (Fig. 3a), which is an indicator of iron stores, showed an opposite pattern, improving significantly in the 20–29.9 μg/dl and 30–39.9 μg/dl vitamin A categories. The greatest improvement in serum ferritin was observed in the children with plasma retinol concentrations <20 μg/dl, but due to the small numbers ($n = 8$), statistical significance could not be shown. The mean change in haemoglobin was negative in all the vitamin A categories.

As the absorption of iron is influenced by existing iron status, and, according to Cook & Skikne (1989), individuals with serum ferritin concentrations >60 μg/l rarely respond to iron therapy, we decided to exclude 7 subjects with baseline serum ferritin concentrations >60 μg/l and to also analyse the data of subjects with initial low concentrations of serum iron separately from the data of the rest of the group. These data are presented in Figs 1b and c; and 2b and c. In Fig. 3b and c children were divided according to baseline serum ferritin concentrations. Though the increase in serum iron, %TS and ferritin was greater in the group with initial

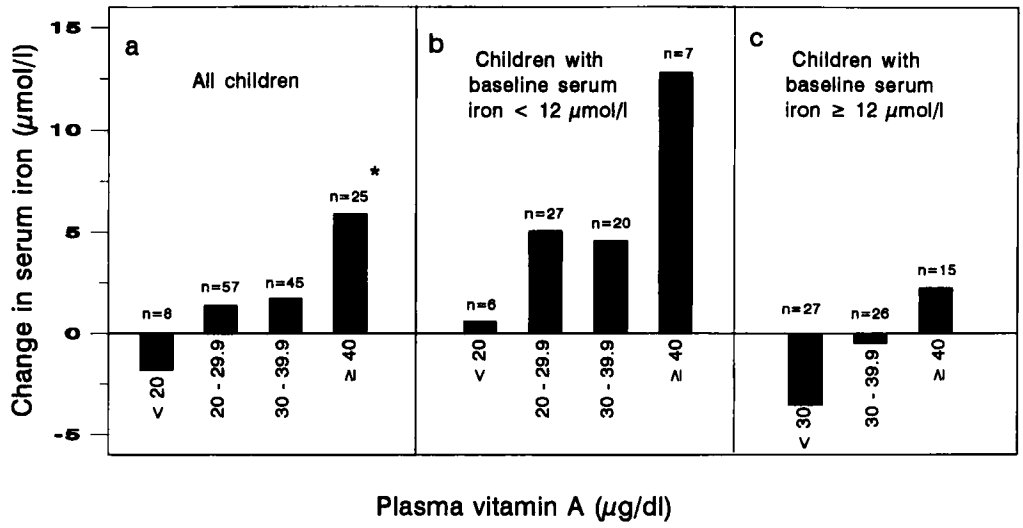

Figure 1. The mean changes in serum iron after 15 weeks on the iron fortification programme, grouped according to vitamin A status; **P* = 0.0005 compared to pre-intervention values; (7 children with baseline serum ferritin >60 μg/l excluded in b and c).

low serum concentrations of iron or ferritin, a similar pattern was seen in both groups (i.e. serum iron and %TS showing the greatest response in children with plasma retinol concentrations ≥40 μg/dl; and serum ferritin improving most in the retinol categories <40 μg/dl). Due to insufficient numbers in certain categories no statistics were performed on these data.

Because there was a significant deterioration in vitamin A status during the study period, we also correlated the change in vitamin A status with changes in iron status. The former correlated positively and significantly with changes

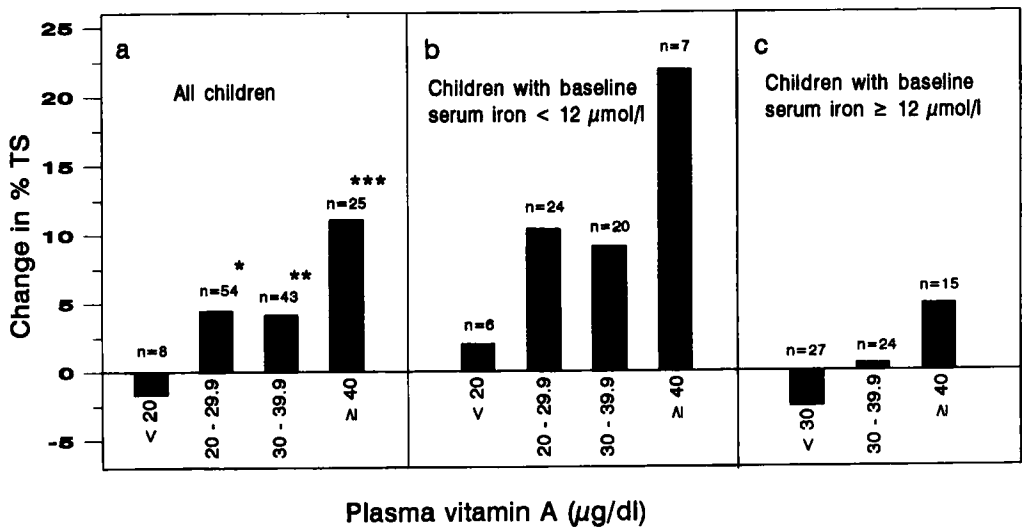

Figure 2. The mean changes in transferrin saturation (%TS) after 15 weeks on the iron fortification programme, grouped according vitamin A status; **P* = 0.0139, ***P* = 0.0270, ****P* = 0.0002 compared to pre-intervention values; (7 children with baseline serum ferritin >60 μg/l excluded in b and c).

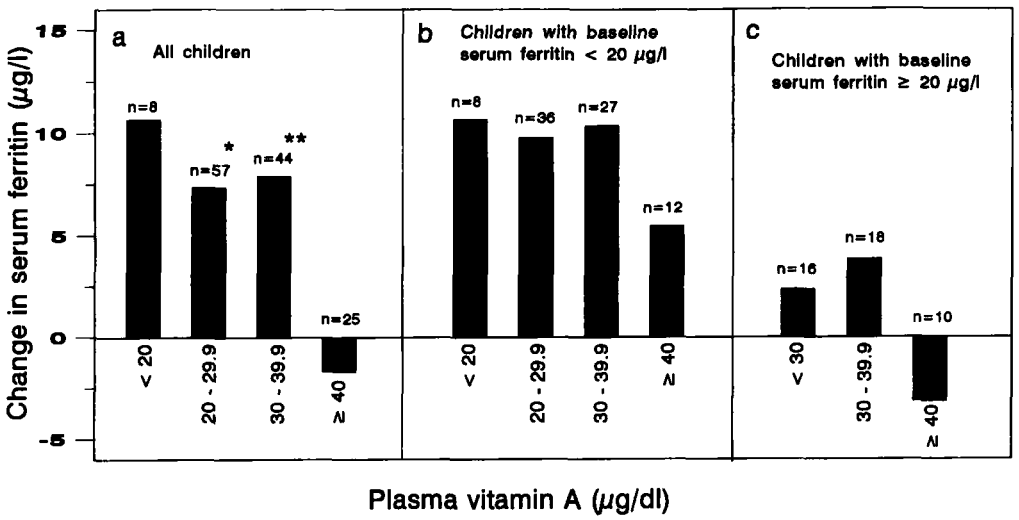

Figure 3. The mean changes in serum ferritin after 15 weeks on the iron fortification programme, grouped according to vitamin A status; $*P = 0.0114$, $**P = 0.0009$ compared to pre-intervention values; (7 children with baseline serum ferritin >60 µg/l excluded in b and c).

in serum iron, %TS and haemoglobin, but negatively with serum ferritin (Table 5).

Stunting (height-for-age *Z*-scores <–2 SD of the NCHS median) and underweight (weight-for-age *Z* scores <–2 SD of the NCHS median) were found in 17.2% and 16.6% of children, respectively. There was, however, no association between vitamin A status and anthropometric status or age.

Discussion

This study has shown that children with marginal vitamin A status have significantly lower levels of serum iron, %TS and haemoglobin than children with optimal vitamin A status, both before and after 15 weeks on an iron fortification programme.

Table 5. Correlation coefficients for the association between change in vitamin A status and changes in indicators of iron status

	n	*r*	*P*
Serum iron	113	0.37	0.0001
%TS	111	0.27	0.004
Haemoglobin	106	0.21	0.03
Serum ferritin	113	–0.28	0.003

Several other studies, in humans and in experimental animals, have shown an association between vitamin A and iron status (Mejía *et al.*, 1977; Mohanram *et al.*, 1977; Hodges *et al.*, 1978; Mejía *et al.*, 1979). It has also been shown that response to iron supplementation is greater when vitamin A is given in conjunction with iron. This is, however, to our knowledge the first time that the response to iron supplementation is looked at in relation to the vitamin A status of the subjects. Improvement in serum iron and %TS after 15 weeks on the programme was most marked in the children with plasma vitamin A concentrations ≥40 µg/dl and more than double the response seen in the 20–29.9 and 30–39.9 µg/dl vitamin A categories. Children with plasma vitamin A concentrations <20 µg/dl showed a negative response. As the absorption of iron from the intestinal tract depends on existing iron status, we decided to also analyse the response to the IFP of those with low iron status at baseline separately from the response of the rest of the group and also exclude those with baseline serum ferritin values >60 µg/l. As expected, a greater response was seen in the group with initial low iron status. However, in relation to vitamin A status, the same trend was observed in both groups.

Due to the consumption of the fortified soup, our subjects had an adequate intake of iron.

Folate and vitamin B_{12} status were also adequate (as assessed by RBC folate and dietary intake of folic acid and vitamin B_{12}). One would therefore have expected a similar improvement in the indicators of iron status of all the subjects exposed to the IFP. Yet, even when initial iron status was taken into account, the children with marginal vitamin A status did not respond as well to the IFP as those with optimal vitamin A status.

Serum ferritin, an indicator of iron stores, showed an opposite pattern and improved significantly in the marginally vitamin A deficient group. This implies that the absorption of iron was not impaired by the marginal vitamin A status. The significantly lower levels of serum iron and %TS in the marginally vitamin A deficient group, as well as their smaller response to the IFP, despite a significant improvement in serum ferritin, suggests that it is the mobilisation of iron from the storage depots that is affected by the inadequate vitamin A status.

Though the mechanism involved is not completely understood and our study was not designed to answer this question, the results of several other studies also suggest that vitamin A may be involved in the regulation of iron release from the stores. In rats fed a vitamin A deficient diet, Mejía *et al.* (1979) demonstrated an increase in liver iron which was accompanied by a decrease in plasma iron and haemoglobin levels. An increase in haemoglobin, serum iron and transferrin saturation has been reported in vitamin A supplementation studies, with no significant change in serum ferritin (Mejía & Chew, 1988; Bloem *et al.*, 1990). Mejía & Arroyave (1982) found that the changes in serum retinol in children, exposed to a vitamin A fortification programme for 6 months, correlated positively and significantly with the changes in serum iron, %TS and TIBC, but showed a significant negative correlation with the changes in serum ferritin. It has been hypothesised that the main effect of vitamin A in iron metabolism is to maintain adequate levels of iron in the plasma so that erythropoiesis is favoured (Mejía & Chew, 1988; Bloem *et al.*, 1989). A similar pattern in the indicators of iron status, i.e. adequate or increased levels of serum ferritin, accompanied by a decrease in serum iron and %TS, is seen during states of infection (Gibson, 1990). The association between vitamin A and infection is also well recognised (Begum *et al.*, 1992). It is therefore not impossible that the underlying mechanisms of these two interactions are interlinked, as has also been suggested by Thurnham (1993). In our study only one child at the pre-intervention assessment and none of the children at the post-intervention assessment had raised leucocyte counts. More sensitive indicators of infection such as α_1-acid glycoprotein (Filteau *et al.*, 1993) have, however, not been measured.

The decrease in HCT and Hb values is difficult to explain in the absence of a control group and one can only speculate on the reasons for this unexpected observation. The significant increase in the MCH and MCHC suggests that haematological status did improve and that the drop in Hb and HCT may rather be due to other factors, such as haemodilution. Haemoconcentration during winter and haemodilution during summer, resulting in raised Hb, HCT and erythrocyte counts in winter and decreased levels during summer, have been reported by Lee *et al.* (1987). The post-intervention assessment of our study did take place during summer and the significantly lower levels of serum albumin at this assessment further suggests that a degree of haemodilution might have been present.

The significant difference in plasma vitamin A levels between the pre- and post-intervention assessments can most probably be attributed to a seasonal difference in intake. The dietary intake of these children showed that less cooked carotene-rich vegetables (e.g. carrots and pumpkin) were consumed at the post-intervention assessment (summer) than during the winter months when the baseline assessment took place.

The cut off values for vitamin A deficiency recommended by the WHO (< 10 µg/dl = deficient; < 20 µg/dl = low) are based on experience with xerophthalmia. To accommodate for the role of marginal vitamin A deficiency in child health and mortality, revision of these cut off values has been suggested (Begum *et al.*, 1992; Hussey & Labadarios, 1992; Flores, 1993). The findings of our study support this suggestion. With regard to iron status, the optimal level of vitamin A in the plasma appears to be a concentration of 40 µg/dl. Serum iron and %TS at both the pre- and post-intervention assessments increased with increasing levels of vitamin A status, but appeared to reach a plateau at 40 µg/dl. Response to the IFP was also the most significant at this level. Further studies and also

in different population and age groups are, however, needed to confirm this observation.

In conclusion, the results of our study suggest that the presence of marginal vitamin A deficiency in a population may limit the effectiveness of an iron intervention programme. It is therefore important that the vitamin A status of the population is also considered when such programmes are planned.

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