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Micronutrient and iron supplementation and effective anti-malarial treatment synergistically improve childhood anaemia

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Summary The control of childhood anaemia in malaria holoendemic areas is a major public health challenge for which an optimal strategy remains to be determined. Malaria prevention may compromise the development of partial immunity. Regular micronutrient supplementation has been suggested as an alternative but its effectiveness remains unsettled. We therefore conducted a randomised placebo-controlled intervention trial with 207 Tanzanian children aged 5 months to 3 years on the efficacy of supervised supplementation of low-dose micronutrients including iron (Poly Vi-Sol with iron) three times per week, with an average attendance of ≥ 90%. The mean haemoglobin (Hb) level increased by 8 g/l more in children on supplement (95% CI 3–12) during the 5-month study. All age groups benefited from the intervention including severely anaemic subjects. The mean erythrocyte cell volume (MCV) increased but Hb in children ≥ 24 months improved independently of MCV and no relation was found with hookworm infection. The data therefore suggest that micronutrients other than iron also contributed to Hb improvement. In the supplement group of children who had received sulfadoxine-pyrimethamine (SP) treatment, the mean Hb level increased synergistically by 22 g/l (95% CI 13–30) compared to 7 g/l (95% CI 3–10) in those without such treatment. Supplementation did not affect malaria incidence. In conclusion, micronutrient supplementation improves childhood anaemia in malaria holoendemic areas and this effect is synergistically enhanced by temporary clearance of parasitaemia.

keywords *Plasmodium falciparum* malaria, anaemia, iron, micronutrients, sulfadoxine-pyrimethamine, child, Tanzania

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Introduction

Childhood anaemia represents a major health problem in sub-Saharan Africa. It may impair mental and motor development in infants and children (Idjradinata & Pollitt 1993), and transfusion-requiring anaemia is a challenge to health facilities in areas where both malaria and HIV infection are prevalent (Schellenberg *et al*. 1999). Anaemia is multifactorial and the result of a synergism of different causes (Fleming & Werblinska 1982; Newton *et al*. 1997). Malaria and iron deficiency are known major contributors to anaemia (Trowbridge *et al*. 1993; Greenwood 1997). However, nutritional deficiencies of micronutrients other than iron may also affect haemoglobin (Hb) status (Northrop-Clewes *et al*. 1996). In malaria holoendemic areas, the main aetiologies vary according to age, e.g. malaria being most important in small children, low dietary iron availability manifesting during the weaning period (Tatala *et al*. 1998), and blood loss due to hookworm infection occurring in older children (Brooker *et al*. 1999).

Oral iron supplementation is a worldwide practice to prevent anaemia in children, although the absorption of daily iron supplements is inefficient and decreases logarithmically over time (Viteri *et al*. 1995). Iron supplementation twice weekly is as efficacious as daily supplementation in improving Hb levels in preschool children with low iron status (Schultink *et al*. 1995). Spacing of iron doses leads to greater compliance (Palupi *et al*. 1997), lower costs, and is associated with a reduction of side effects (Angeles-Agdeppa *et al*. 1997). Nutritional deficiency of vitamin B2 (riboflavin) imposes limitations on the absorption and use of iron and is common in tropical areas (Powers & Bates 1987). Iron

absorption is facilitated by vitamin C (Mao & Yao 1992); maximum Hb levels were achieved by supplementation with a combination of iron and vitamin A in Guatemalan children (Mejia & Chew 1988).

In children with malaria, however, regular iron supplementation may exert little or no effect on Hb levels (Vaughan *et al*. 1977; Oppenheimer *et al*. 1986; Schneider *et al*. 1995; van den Hombergh *et al*. 1996), and studies in tropical areas have raised concerns that iron supplementation may increase susceptibility to infections, particularly malaria and pneumonia (Smith *et al*. 1989; van den Hombergh *et al*. 1996; Nwanyanwu *et al*. 1996; Oppenheimer 1998). Supplementation with both vitamins and iron was associated with a trend towards higher parasitaemias in Gambian children (Bates *et al*. 1987), whereas other studies could not demonstrate a similar increased susceptibility to infection (van Hensbroek *et al*. 1995; Menendez *et al*. 1997). Exacerbation of malaria infection may be an early response to iron treatment (Murray *et al*. 1978), or may be detected later when iron stores are repleted (Oppenheimer *et al*. 1986). The conflicting results on malaria-related effects by oral iron supplementation may depend on the dose and duration of iron treatment (Oppenheimer 1998). Age, inherited disorders of globin genes, diet, previous exposure to malaria, malaria transmission and efficacy of antimalarials have also been implicated as interacting factors (Oppenheimer 1998).

Combined vitamin–mineral supplements are widely used in industrialized countries and have also been recommended to children and pregnant women in tropical areas (Alnwick 1998; Tatala *et al*. 1998). Children aged 6–24 months are particularly at risk of micronutrient deficiencies, being dependent on complementary food containing low amounts of bioavailable micronutrients and phytate, a micronutrient absorption inhibitor (Gillooly *et al*. 1983; Thu *et al*. 1999). We wanted to assess the effect of prophylactic supplementation with micronutrients and iron on Hb levels in an holoendemic area, and if, and how, prompt malaria treatment promoted or interacted with such an effect. The impact of supplementation on malaria morbidity was also assessed. This randomised, placebo-controlled community-based study investigated the impact of oral prophylactic supplementation three times a week on Tanzanian children aged 5 months to 3 years.

Methods

Study population

The study was conducted in Fukayosi village, Bagamoyo district of coastal Tanzania, from June to November 1995, during the seasonal peak of perennial malaria transmission. Members of the village government (the 10 cell leaders) conducted a census of all children aged 5 months to 3 years. Of 220 children enumerated, the mothers of 217 children attended a public meeting where the objectives and methodology of the study were described. At the time of study registration, the parents of 211 children agreed to participate and provided oral informed consent. Formal exclusion criteria for enrolment were migration plans, the presence of congenital malformations and Hb concentration < 50 g/l at baseline, requiring immediate treatment. After exclusion, a cohort of 207 children were enrolled. Each child was given an identity number and demographic details were noted and confirmed from the Mother and Child Health Card. The children were randomly allocated to the supplement group (group A) or the placebo group (group B) by a computer-generated number table.

Study design

Children of group A received the micronutrient preparation Poly Vi-Sol with Iron (Mead Johnson Nutritional Group, a Bristol-Myers Squibb Company), the contents of which are shown in Table 1. The supplement was directly imported from the US manufacturer and did not require refrigeration. One ml was given three times a week on alternate days under supervision. Group B received a placebo preparation of one ml, corresponding to one mg, promethazine hydrochloride (Elys Ltd, Dar es Salaam, Tanzania) in an identical dosing regimen. The supplement and placebo had different colours to facilitate correct administration. However, neither the research assistants involved in the project nor the mothers of the children knew the treatment code. All children were to receive a total of 56 doses over 5 months administered during home visits by six research assistants who were assigned 30–35 children each. Attendance of children during home visits was recorded and any perceived adverse effects were noted. The two principal investigators supervised the performance of the research assistants. On a few occasions during temporary travel, the mothers gave the doses to their children.

Two cross-sectional surveys, at study start and end, determined baseline and outcome data. All children were then clinically examined by one of the principal investigators, including axillary temperature, body weight and spleen size. Capillary blood (40 μl) was collected for thick blood films and haematological measurements. For active case detection of clinical malaria episodes, all children were seen fortnightly by the research team at the village dispensary for axillary temperature measurement. Capillary blood for analyses of Hb S and HIV-antibodies and a stool specimen were collected at two of these regular visits. For passive malaria case detection, children with a history of fever would report to the dispensary either on the mother's initiative or by referral from a

Table 1 Contents of micronutrient supplementation to group A (Poly Vi-Sol with Iron drops*) The dose given was 1 ml three times a week†

	Poly Vi-Sol with Iron drops, per one ml dose	Recommended Daily Allowance, percent‡	
		< 1 year	1–4 years
Vitamin A, IU	1500	100	60
Vitamin D, IU	400	100	100
Vitamin E, IU	5	100	50
Vitamin C, mg	35	100	85
Vitamin B1, mg	0.5	100	70
Vitamin B2, mg	0.6	100	75
Niacin, mg	8	100	90
Vitamin B6, mg	0.4	100	60
Iron, mg §	10	67	100

*Due to instability in solution, neither folic acid nor vitamin B12 was added.
†Recommended dose is one ml daily by the manufacturer, Mead Johnson Nutritional Group, a Bristol-Myers Squibb Company.
‡According to the manufacturer.
§Elemental iron, from 27 mg ferrous sulphate.

research assistant during home visits on alternate days. One team medical assistant and one nurse were on duty at the village dispensary around the clock, 7 days a week. At least one of the principal investigators was present daily.

Clinical malaria episodes and follow-up

In all children with a measured axillary temperature ≥ 37.5 °C, a standardized medical examination was performed to rule out fever conditions other than malaria and to evaluate the clinical severity of the fever episode. Capillary blood was collected for immediate preparation and examination of a thick blood film, with an additional 40 μl prediluted for haematological measurements. The prediluted specimen was transported in an insulated container for one hour before being analysed and the result was available at the dispensary the following day. A *Plasmodium falciparum* asexual parasite density > 4000 per μl, estimated at immediate slide examination in the field, was considered indicative of a clinical malaria episode requiring chloroquine treatment.

Treatment with chloroquine syrup (Elyquine® by Elys Ltd, Dar-es-Salaam, Tanzania) was given in accordance with Ministry of Health guidelines. A team nurse supervised the intake of 25 mg/kg over three days and the child was observed at the dispensary for 20 min after each dose. In case of vomiting, a full dose was repeated. The axillary temperature was measured daily and, 3 days after starting treatment, capillary blood was again collected for a thick blood film and haematological parameters. Additional treatment with sulfadoxine-pyrimethamine (SP) was given if a child showed clinical signs of treatment failure (WHO 1996), i.e. if the condition of the patient deteriorated while on chloroquine treatment or if recrudescence of symptoms occurred within 14 days of starting treatment. A follow-up capillary blood specimen for haematological analysis and a bloodslide were collected after each clinical malaria episode, at the regular fortnightly dispensary visit or immediately after day 14. Clinical malaria episodes appearing from day 14 onwards were assumed to be due to re-infection and were treated as new episodes and handled as described.

If a child was diagnosed with a bacterial infection, antibiotics were provided that would not affect parasitaemia.

Laboratory methods

Blood slides were stained with 5% Giemsa stain. The asexual parasite densities were estimated by counting parasites against 200 white cells, assuming a standard leukocyte count of 8000/μl. If less than 10 parasites were recorded per 200 white cells, estimation was made against another 300 leucocytes. The final examination of all slides was performed by one of the principal investigators.

Capillary blood collection was standardized and performed by only two team members. For haematological measurements, an automated haematological analyser was used (Cell-Dyn 610™, Abbott laboratories). Daily commercial quality controls of Hb and mean erythrocyte cell volume (MCV) were performed. The coefficient of variation for the within-sample precision of the Hb estimate was $< 1\%$, both for immediately run samples and after a 6-h delay in analysis. As an indicator of iron status, MCV was used rather than serum ferritin and erythrocyte protoporphyrin due to the influence of repeated malaria episodes on the latter estimates (Stoltzfus *et al*. 1997). The MCV was not corrected for reticu-

locyte count, since an Hb steady state level was assumed to have been reached after 5 months' supplementation. Haemoglobin S was determined by a solubility test (Cook & Raper 1971) and a positive result was confirmed by haemoglobin electrophoresis. HIV infection was determined by an ELISA test for HIV antibodies (Behring) and confirmed by Western blot when positive. Results of the HIV test were blinded and only available after study termination. Stool examination for geohelminths was qualitative and performed by the formalin-aether concentration technique (Ash & Orihel 1987).

Statistical methods

Randomization and data entry were performed with Epi-Info™ 6.2; data were analysed in JMP™ 3.1 (SAS Institute Inc.) and STATISTICA™ 5.5. (StatSoft Inc.). We used Pearson's chi-square test to compare proportions, and the paired *t*-test or analysis of variance to compare normally distributed continuous data. The Wilcoxon rank-sum test was employed for sparse data. Multiple linear regression analysis was performed on the change in Hb concentration during the study as dependent variable with baseline Hb added as a covariate. Data transformation was performed on correlated independent variables. The change in MCV was analysed in a similar way.

Children were not excluded from analyses after treatment for a clinical malaria episode. Attention was thus given to the frequency of antimalarial treatment, since a potential higher treatment rate in one group could lead to subsequent parasite suppression by residual blood concentration of antimalarials. Severe anaemia was defined as an Hb concentration < 80 g/l, and Hb levels > 110 g/l were considered normal (CDC 1989). Red cell microcytosis was defined according to age, as MCV < 70 in children < 24 months and MCV < 73 in children ≥ 24 months (Oski 1993).

Ethical considerations

Ethical clearance for this study was obtained from the Ethical Committee of Karolinska Institutet in Stockholm (#95 152) and from the Muhimbili University College of Health Sciences in Dar-es-Salaam. The supplement Poly Vi-Sol with Iron was a gift from Mead Johnson Nutritional Group, but the study had no commercial affiliations.

Results

Study population

Of 211 children registered, four were excluded due to their initial Hb < 50 g/l. Baseline characteristics of the 207 randomized children are displayed in Table 2. Twelve children did not finish the study (six in each group) due to refusal (five) or migration (seven). Baseline characteristics of these 12 children, including age and Hb, were similar to those completing the study. Hence analysis comprised the 195 children who completed the study: 98 children in group A (supplement group) and 97 children in group B (placebo group).

Children of both group A and B received a median of 55 of the scheduled 56 doses of supplementation or placebo (interquartile range (IQR) for group A: 52–56, and for group B: 53–56). Only eight children (two in group A and six in group B) received less than 42 (75%) doses. The mean iron dose to infants from group A was 1.4 mg per kg body weight (mean body weight at baseline 7.2 kg). The children > 24 months received 0.9 mg iron per kg and dose (mean body weight 11.6 kg). There were no side-effects due to the intervention in either group. Median attendance at the fortnightly cross-sectional visits was 100% in both groups. The overall attendance during home visits three times per week was 93% in group A and 90% in group B ($P = 0.14$). During the study period, 143/367 (39%) of fevers (from malaria and other causes) were recorded during active case detection, and 224 (61%) were found by passive reporting. Three children were admitted to hospital for gastro-enteritis or malaria but none had a blood transfusion and they continued to be followed up during and after hospital admission.

Haemoglobin AS was detected in 46 (24%) of all subjects but no child had the HbSS genotype. Hookworm was diagnosed in 25 (13%) children and 19 of these were ≥ 18 months old. None was HIV positive.

Table 2 Baseline characteristics of study participants

	Supplement group A ($n = 104$)	Placebo group B ($n = 103$)
Mean age (months) ±SD	19 ± 9	20 ± 8
Mean weight (kg) ±SD	9.4 ± 2.2	9.7 ± 2.0
Mean Hb (g/L) ±SD	86 ± 17	90 ± 16
Mean MCV (fl) ±SD	69 ± 8	68 ± 8
Prevalence of parasitaemia	93/104 (89%)	93/103 (90%)
Median parasite density (per μl)	3600 (IQR 800–9600)	2600 (IQR 800–8300)

Table 3 Haemoglobin concentration (Hb) and mean cell volume (MCV) by age, before and after five months supplementation (group A) or placebo (group B)

			Mean Hb (g/l) ±SD			Mean MCV (fl) ±SD		
Age (months)*	Group	N	Start	Finish	Difference	Start	Finish	Difference
5–11	A	29	73 ± 14	90 ± 17	17 ± 18	67.9 ± 7.5	71.0 ± 7.3	3.1 ± 5.2
5–11	B	15	78 ± 17	86 ± 18	8 ± 16	65.3 ± 8.0	65.5 ± 8.5	0.2 ± 2.9
12–23	A	38	88 ± 16	94 ± 15	6 ± 16	67.1 ± 7.9	73.1 ± 7.5	6.0 ± 7.0
12–23	B	49	88 ± 14	91 ± 13	3 ± 14	67.5 ± 7.4	68.2 ± 7.1	0.7 ± 3.2
24–36	A	31	95 ± 13	102 ± 15	7 ± 12	73.5 ± 7.1	74.0 ± 6.0	0.5 ± 4.6
24–36	B	33	98 ± 15	97 ± 12	−1 ± 12	70.3 ± 7.1	70.1 ± 6.9	−0.2 ± 3.9
All	A	98	86 ± 16	95 ± 16	10 ± 16	69.4 ± 8.0	72.8 ± 7.0	3.4 ± 6.2
All	B	97	90 ± 16	92 ± 14	2 ± 14	68.1 ± 7.5	68.4 ± 7.3	0.3 ± 3.4

*Age at study start

Effect on haematological values

Haemoglobin levels before and after the intervention are displayed in Table 3. At baseline, 174 (89%) children were anaemic (Hb < 110 g/l) and 58 (30%) were severely anaemic (Hb < 80 g/l). The mean Hb concentration increased by 10 g/l in group A but only by 2 g/l in group B (mean difference 8 g/l (95% confidence interval, CI, 3–12)). The mean Hb level in children with severe anaemia at baseline improved more in group A (20 g/l) than in group B (12 g/l), $P = 0.03$. No child with a normal baseline Hb became severely anaemic after supplementation.

The baseline Hb concentration was linearly related to age (Spearman rank correlation coefficient 0.50 ($P < 0.001$)), and 29 (50%) of all severe anaemia cases were recorded in children < 12 months who constituted 23% of the study population. Of 1254 Hb estimates, the results of three were < 50 g/l.

Red cell microcytosis was recorded in 117 (60%) children at baseline (Table 3). The MCV was linearly related to age (Spearman rank correlation coefficient 0.31 ($P < 0.001$)). During the study, MCV increased 3.4 fl in group A and 0.3 fl in group B (mean difference 3.1 fl (95% CI, 1.7–4.5)). In a multiple linear regression model, both the intervention and low MCV at baseline were highly significant explanatory variables for the increase in MCV during the study. The addition of age or body weight did not contribute significantly to the fit of the regression equation.

A regression model of the increase in Hb concentration during the study is presented in Table 4. After adjusting for baseline Hb level, variables that were tested for significance ($P < 0.10$) but not retained in the model were: number of doses of supplement/placebo; MCV; increase in MCV; incidence of clinical malaria episodes; chloroquine treatment; weight; and age. In contrast, intervention group and antimalarial treatment with sulfadoxine-pyrimethamine (SP) were highly significant dependent variables. In the final model, the two-way interaction term between intervention group and SP treatment was significant (Table 4). Hence, comparisons at each level of intervention group and SP treatment were made (Table 5). Within the group of SP-treated children, group A and group B children did not differ in age ($P = 0.28$) or mean baseline Hb ($P = 0.75$). SP treatment increased Hb by 6 g/l (95% CI − 0.3–13) in unsupplemented children and by 15 g/l (95% CI 8–22) in supplemented subjects (Table 5). The total increase in mean Hb in

Table 4 Multiple linear regression model on change in haemoglobin concentration between study start and end. Whole model test, $R^2 = 0.40$, $P < 0.001$. Baseline Hb was added as a covariate

Explanatory variable	Regression coefficient (95% CI)	Significance level, p
Group A/Group B	3.3 (–0.6–7.2)	0.10
SP-treatment	2.7 (–3.4–8.8)	0.38
Interaction term		
SP-treatment * Group A/B	9.9 (1.5–18.3)	0.02
Baseline Hb	−0.5 (–0.4 to –0.6)	< 0.001
Intercept	44.2 (34.1–54.3)	< 0.001

Table 5 Increase in haemoglobin concentration (g/l) ±SD, i.e. difference in mean Hb between study start and end, in children with or without second-line sulfadoxine-pyrimethamine (SP) treatment and supplementation (group A) or placebo (group B) N = number of subjects

	Group A	Group B
SP treatment	22 ± 19 ($n = 21$)	7 ± 20 ($n = 20$)
No SP treatment	71 ± 3 ($n = 77$)	1 ± 12 ($n = 77$)

supplemented children who had received SP treatment was 22 g/l (95% CI 13–30) (Table 5). The subgroups were not randomised, however, and the children who received SP treatment were on average 6 months (95% CI 3–9) younger and their mean baseline Hb was 7 g/l (95% CI 1–12) lower than in those without such treatment. The synergistic effect of micronutrient supplementation and SP treatment was, however, confirmed after adjusting for age and baseline Hb in a regression model.

SP was administered in 49 clinical malaria episodes with chloroquine treatment failure to 21 children of group A and 20 children of group B (Table 5). In group A children, one course of SP improved Hb by 15 g/l (95% CI 4–26) if it was administered during the last three study months, and by 5 g/l (95% CI −5–15) if it was given during the first two months. The increase in Hb by the supplement was statistically not related to haemoglobin genotype (not shown). The haemoglobin level was unrelated to hookworm infection.

Effects on malaria

We recorded 271 clinical malaria episodes, 149 in group A and 122 in group B. The mean clinical malaria incidence rate in group A was 0.28 episodes/person-month *vs*. 0.24 in group B ($P = 0.25$). Similarly, there was no difference between group A and B in the incidence of fever episodes not due to malaria, $P = 0.95$ ($n = 96$). Neither the geometric mean parasite density at the time of malaria diagnosis, nor the geometric mean asymptomatic parasitaemia during follow-up of clinical malaria episodes were statistically different between the two groups ($P = 0.75$ and $P = 0.35$, respectively). There was no difference between the two groups in parasitaemia at the study end, including the prevalence of parasitaemia and the proportion parasitaemias $> 4000/\mu l$ and $> 10000/\mu l$ (data not shown). Adjusting for the sickle cell trait did not affect the lack of difference in clinical malaria incidence rate between the two groups.

Discussion

Public health interventions to prevent childhood anaemia in malaria holoendemic areas need to combine effectiveness with feasibility and sustainability. This study was undertaken to investigate whether spacing the administration of low-dose micronutrients and iron to young children with anaemia retained its efficacy on long-term Hb concentrations, despite the presence of malaria parasitaemia, and to evaluate the interactive influence of malaria case management regimen on Hb levels (Tanzania 1997). The supplementation and the malaria case management procedures were both considered feasible and potentially sustainable measures for the prevention of childhood anaemia, addressing the nutritional deficiency state as well as the malaria infection.

Haemoglobin levels were improved by the supplementation and no adverse effects were recorded. All age groups, i.e. up to three years of age, benefited from the intervention, including severely anaemic subjects (Table 3). In severely anaemic children, even a modest increase in Hb may make a difference between a potentially lethal and non-lethal malaria episode. However, the relatively small study group and close follow-up did not allow investigation of the possible prevention of transfusion-requiring anaemia. The extensive rise in mean Hb in supplemented infants and a recorded Hb improvement in infants of the placebo group suggested that, in addition to the intervention effect on Hb, a statistical 'regression to the mean' of Hb levels occurred with age (Table 3) (Nwanyanwu *et al*. 1996).

Improved iron status, as measured by the increase in MCV, was observed in supplemented subjects < 24 months (Table 3). Increase in MCV was related to a low baseline MCV, predominantly occurring in the youngest children and indicating past iron deficit and therefore an increased absorption of iron during the study period (Hulten *et al*. 1995). Infants were given a higher iron dose per kg body weight than toddlers because iron requirements are considerably higher during infancy than in older children (Table 1) (Dallman 1986; National Research Council 1989). However, body weight did not contribute significantly to the regression model on the increase in MCV, suggesting that the iron dose received per kg body weight may not have been crucial for MCV improvement.

The absorption of iron is a function of the quantity and predominant form (haem or non-haem) of iron in the food, the interaction between iron and other dietary components, and the regulation of iron absorption. A cereal diet low in vitamin C, which enhances the absorption of low bio-availability iron, is a major cause of anaemia in Tanzania (Hallberg *et al*. 1986; Tatala *et al*. 1998). As body stores diminish, there is a compensatory increase in the absorption of iron, where high bio-availability iron may double its uptake whilst the absorption of non-haem iron increases only slightly (Dallman *et al*. 1993). However, when iron in a well-absorbed form such as ferrous sulphate is added to foods, the amount absorbed is inversely proportional to its concentration (Saarinen & Siimes 1977). Moreover, iron intake has an immediately blocking effect on further absorption, so that intermittent supplementation timed to gastrointestinal cell renewal may be more efficient than a daily supplement schedule (Fairweather-Tait *et al*. 1985). Therefore, the intermittent administration of iron in a low dose together with vitamin C most likely promoted absorption.

Our results are in contrast to those by Bates *et al*. (1987), who failed to detect an improvement in haematological indices in Gambian children by high doses of iron and

vitamin B and C. In The Gambia, the Hb drop associated with heavy seasonal malaria transmission may be difficult to reverse without the aid of antimalarial chemoprophylaxis (McGregor *et al*. 1966). A further point may be that the supplement used did not contain vitamin A which improves Hb concentration (Suharno *et al*. 1993). The authors suggested that more frequent dosing of micronutrients may have improved the outcome, but the implementation of large-scale daily supplementation programmes seems unrealistic (Palupi *et al*. 1997).

The mean Hb level of group A children ≥ 24 months improved independently of MCV (Table 3). This suggests that the Hb improvement in older children may have been due to effects of micronutrients other than iron. The anaemia of vitamin A deficiency is similar to iron deficiency with low MCV and low serum iron levels (Mohanram *et al*. 1977). Simultaneous administration of vitamin A and iron to children with deficiencies of both nutrients produces a better response with respect to serum iron, transferrin saturation and Hb than administration of vitamin A or iron supplementation alone (Mejia & Chew 1988; Suharno *et al*. 1993; Kolsteren *et al*. 1999). A diet deficient in vitamin B2 leads to red cell aplasia (Lane & Alfrey 1963), and hypochromic anaemia responding to vitamin B6 has been described in malnourished patients (Foy & Kondi 1958). It is still unclear whether vitamin C also has a direct role in haematopoiesis, or if the anaemia in subjects with scurvy is a result of the interactions of ascorbic acid with iron and folic acid metabolism. Dietary iron deficiency in children often coexists with dietary ascorbic acid deficiency, and children with scurvy frequently require both iron and vitamin C to correct a microcytic anaemia (Cohen *et al*. 1981). Scurvy itself may cause iron deficiency as a consequence of external bleeding but poor iron absorption is more important (Mao & Yao 1992; Sharma & Mathur 1995). Finally, vitamin D deficiency rickets has been associated with myelofibrosis and anaemia (Yetgin *et al*. 1989). Hence, several micronutrients may have improved Hb in children ≥ 24 months, but it could not be determined which of these may have been most important.

Despite iron supplementation, microcytosis was still prevalent in most children at the end of the study (Table 3), possibly indicating the presence of undiagnosed thalassaemia which is common in sub-Saharan Africa (Hill 1992; Mockenhaupt *et al*. 1999).

The clinical malaria incidence rate and malaria parasitaemia were not affected by the supplement. However, undiagnosed thalassaemia may have protected against an increase in malaria infection caused by the supplement (Oppenheimer *et al*. 1987). The supplement also contained vitamin A which is known to enhance immune functions, even in individuals with adequate vitamin A levels (Ross 1992; Ribaya-Mercado 1997). Folate was not included in the supplement as it is unstable in solution, and hence did not reduce the antimalarial effects of SP (van Hensbroek *et al*. 1995). Since the frequency of antimalarial treatment was similar in both groups, there was no differential parasite suppression due to residual blood concentrations of antimalarials.

Second-line antimalarial treatment with SP to unsupplemented children moderately increased long-term Hb levels although the improvement did not reach statistical significance. Previously, short-term Hb levels have been shown to recover on first-line SP treatment (van Hensbroek *et al*. 1995; van den Hombergh *et al*. 1996; Verhoeff *et al*. 1997), as well as on second-line SP treatment (Ekvall *et al*. 1998). However, to maintain Hb levels over time, repeated antimalarial doses may be needed. In pregnant women, presumptive SP treatment prevented the development of severe anaemia (Shulman *et al*. 1999), but monthly SP administration gave no haematological relief to Kenyan children exposed to intense malaria transmission (P. Bloland, unpublished data). Weekly malaria chemoprophylaxis with dapsone-pyrimethamine to Tanzanian infants was more protective against severe anaemia than daily iron supplementation (Menendez *et al*. 1997). Hence, for long-term effect on Hb, regular antimalarial intake appears to be required. However, this may impair the development of semi-immunity, with rebounds of both clinical malaria and severe anaemia occurring after stopping chemoprophylaxis (Greenwood *et al*. 1995; Menendez *et al*. 1997). It has been suggested that spaced doses of antimalarials, i.e. incomplete chemoprophylactic parasite suppression, may improve Hb levels and at the same time still be able to stimulate an immune response to malaria (Greenwood *et al*. 1988). Incomplete chemoprophylactic suppression of parasitaemia has produced a 5% increase in haematocrit in children from holoendemic areas (Björkman *et al*. 1986; Menon *et al*. 1990), whereas complete parasite suppression was associated with a 10% haematocrit improvement (McGregor *et al*. 1956; Bradley-Moore *et al*. 1985).

In the present study, second-line antimalarial treatment with SP to supplemented children synergistically improved the long-term Hb response (Table 5). Supplementation with iron after antimalarial treatment is controversial, and not all studies have shown a beneficial effect on Hb (Nwanyanwu *et al*. 1996; van den Hombergh *et al*. 1996). Although iron improved short-term haematological recovery after antimalarial therapy in Gambian children, there was no synergy between the two treatments (van Hensbroek *et al*. 1995). Menendez *et al*. (1997) did not detect any synergetic effect on severe anaemia between weekly antimalarial chemoprophylaxis and 4 months of iron supplementation. The synergy we observed may have been related to the effects of micronutrients other than iron. After the temporary clearance of parasitaemia and thus removal of the haematopoietic sup-

pression (Kurtzhals *et al*. 1997), pre-existing nutritional deficiencies may limit the Hb restitution, making vitamin supplements essential for a complete bone marrow response.

Synergy was only obtained with SP treatment, i.e. when malaria treatment was effective and parasitaemia eliminated or substantially reduced. The synergistic effect on Hb was not found after treatment with chloroquine, a much less effective antimalarial in this setting (Ekvall *et al*. 1998). Furthermore, the long half-life of SP may have promoted the Hb recovery by an extended period of aparasitaemia. Subsequent analysis of the data suggested that the synergistic effect faded when parasitaemia recurred, although the statistical interpretation was limited due to the small number of patients. In our study, the mean Hb concentration improved by 26% over five months in children receiving both supplement and SP treatment. These data suggest that a spaced supplement regimen containing micronutrients and iron together with SP treatment for malaria may improve childhood anaemia in holoendemic areas. The synergy between the two treatments leads to a rapid Hb improvement, and the further use of supplement secures a continuous rise in Hb in-between SP treatments, during intervals when the antiparasitic effect may be fading and the Hb curve flattening out. The duration and treatment intervals under such a regimen remain to be determined, but should for practical reasons be linked to the Integrated Management of Childhood Illnesses, or the Mother and Child Health Programme and Expanded Programme on Immunization (EPI +) (Alonso González *et al*. 2000; Nicoll 2000).

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