

# Ⓜ Intermittent administration of iron and sulfadoxine-pyrimethamine to control anaemia in Kenyan children: a randomised controlled trial

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## Summary

**Background** Iron supplementation is recommended for children at high risk of anaemia, but its benefits may not outweigh the associated risk of malaria in areas of seasonal transmission. We investigated the effect on haemoglobin concentrations of intermittent administration of iron supplements and sulfadoxine-pyrimethamine in symptom-free children under intense health surveillance.

**Methods** In a trial of two by two factorial design, 328 anaemic Kenyan children were randomly assigned either iron or placebo and sulfadoxine-pyrimethamine or placebo (82 to each group). Primary outcomes were haemological indicators of iron status and inflammation at the end of the follow-up, and occurrence of malaria attacks. Morbidity surveillance consisted of medical examinations every 4 weeks, continuous passive case detection, and visits twice a week to community health-workers. Analyses were by intention to treat.

**Findings** After 12 weeks, the groups assigned iron plus sulfadoxine-pyrimethamine, iron alone, or sulfadoxine-pyrimethamine alone had higher haemoglobin concentrations than the group assigned placebo (treatment effect adjusted for prognostic factors at baseline: 11.1 g/L [95% CI 7.5 to 14.7]; 10.7 g/L [7.1 to 14.3]; and 3.1 g/L [-0.5 to 6.7]). Administration of iron plus sulfadoxine-pyrimethamine also lowered the proportion with anaemia from 100% at baseline to 36% at 12 weeks, and of iron deficiency from 66% at baseline to 8% at 12 weeks. Survival analysis showed no evidence of substantially increased risk of malaria after iron supplementation.

**Interpretation** Iron supplementation gives substantial health benefits, which may outweigh possible inherent risks caused by malaria. A larger study than ours is needed to assess benefits and risks of intermittent administration of sulfadoxine-pyrimethamine in reducing the incidence of malaria attacks in areas of seasonal malaria transmission.

*Lancet* 2002; **360**: 908–14. Published online August 20, 2002. <http://image.thelancet.com/extras/01art9014web.pdf>

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## Introduction

About three-quarters of children younger than age 5 years in eastern Africa have anaemia, defined as haemoglobin concentrations below 110 g/L.<sup>1</sup> Iron deficiency, when severe enough to lead to anaemia, has been associated with impaired physical growth<sup>2,3</sup> and mental development,<sup>4</sup> decreased appetite,<sup>3</sup> reduced physical activity,<sup>5</sup> and possibly with increased incidence of some infections.<sup>6</sup>

Routine iron supplementation of children living in communities with a high prevalence of anaemia has been advocated,<sup>7</sup> but under certain, undefined, conditions could lead to increased risk of malaria.<sup>6,8,9</sup> Oral iron supplementation (2 mg/kg) given daily to infants aged 8–24 months in an area of high, perennial transmission did not increase the incidence of malaria.<sup>10</sup> Under these conditions, however, children born to immune mothers remain partly protected for 3–6 months after birth. In areas of seasonal malaria or in children aged 6 months to 5 years, protective immunity is less likely, and the risk of malaria or malaria-related outcomes associated with iron supplementation might therefore be higher. Comparison of evidence from trials in infants and preschool children in areas of very seasonal malaria<sup>11,12</sup> and highly endemic areas<sup>10,13</sup> seems to support this hypothesis. Thus, the benefits of iron supplementation may not outweigh the risk of adverse effects in populations with low immunity against malaria.

Although mild to moderate anaemia often arises in asymptomatic malaria,<sup>14</sup> little is known about its functional importance. Such malaria infections are probably associated with increased risk of malaria attacks<sup>15</sup> but are needed to gain protective immunity against severe disease and subsequent death, which develops much faster than immunity against fever and parasite density.<sup>16</sup> Thus, the effect of treatment on all-cause mortality remains unknown. Chemoprophylaxis reduces malaria morbidity and all-cause mortality but is no longer advocated in endemic areas<sup>10,17,18</sup> mainly for fear of accelerated spread of resistance and of increased risk of malaria attacks after the intervention ceases.<sup>10,19</sup>

The potential gains in haemoglobin concentrations of iron supplementation and malaria control in children in areas with seasonal malaria remain uncertain. Hence, with the aim of obtaining the maximum beneficial effects of iron supplementation and chemoprophylaxis and the minimum possible adverse risks, we assessed the efficacy in improving iron status of intermittent administration of iron and sulfadoxine-pyrimethamine, when given alone or in combination, to Kenyan children in conditions of seasonal malaria. We also explored the effects of interventions on the risk of a malarial attack and the time until its first occurrence.

## Methods

### Area and population

The study was done during rainy seasons in the period 1998–2000 among randomly selected children living in Mtito Andei Division, Eastern Province, Kenya. Malaria transmission is highly seasonal in this area.<sup>20</sup> An earlier survey among children aged 2–36 months in this area showed frequencies of malaria, anaemia, *Ascaris lumbricoides* infestation, and *Trichuris trichiura* infestation of 31%, 72%, 3%, and 5%, respectively (HV, unpublished). Reported malaria infections were exclusively due to *Plasmodium falciparum*. No hookworm or schistosomiasis was found. A 14-day in-vivo drug-sensitivity study was done in 1997 among children (mean age 3·2 years) in the outpatient department of a nearby health centre. Of 35 children studied who were treated with sulfadoxine-pyrimethamine, 30 (86%) were sensitive to treatment.<sup>21</sup>

The present study was facilitated by community health-workers who had been trained to provide health education and promotion, but who are not routinely involved in treatment of illnesses. A research clinic was established in the area and staffed by a clinical officer who was on duty 24 h per day, 7 days per week, throughout the study. Children were recruited from neighbouring communities in three groups, each at the start of a rainy season in the period 1998–2000. The study received ethical approval from the African Medical and Research Foundation and the Kenya Medical Research Institute. Informed consent was obtained from community leaders and local government officials, and from parents of participating children. Children included in the study and their siblings received free medical care for common childhood illnesses.

Children were randomly sampled and invited for study from an up-to-date population census of communities neighbouring the research clinic. At screening, children were judged eligible for the study when they met the following criteria: the haemoglobin concentration was 60–110 g/L; the child was aged 2–36 months; the axillary temperature was below 37·5°C; there were no symptoms suggestive of malaria or anaemia, or any systemic illness occurring in combination with a blood dipstick test result indicating current or recent malarial infection; the parents intended to stay in the study area during the intervention period and gave their consent; no allergy to sulfa drugs was reported; and no sulfa drugs had been used in the previous 3 weeks. Children with a positive malaria dipstick test result but without symptoms of systemic illness were included.

### Design and procedures

The study was a double-blind, placebo-controlled trial with a two by two factorial design and an intervention period of 12 weeks. Children were randomly assigned to one of four groups intermittently receiving: sulfadoxine-pyrimethamine and iron supplement; sulfadoxine-pyrimethamine and iron placebo; sulfadoxine-pyrimethamine placebo and iron supplement; sulfadoxine-pyrimethamine placebo and iron placebo. Children in all groups were under intense health surveillance throughout the intervention period.

Malaria, anaemia, and other factors known or suspected to be geographically or temporally clustered might be prognostic for the outcomes considered. Balanced block randomisation was expected to give an even distribution of these factors over the experimental treatment groups. Thus, recruitment continued until eight children had been randomly selected per community. Interventions were randomly allocated in duplicate within 41 of these blocks. The allocation schedule was generated

by one of us (HV) for each block, by means of tables with randomised permutations, and only after acceptance of all children making up a block. The order of the children listed in each block was concealed from the person generating the allocation schedule.

Both placebos and active compounds were administered as suspensions that were indistinguishable in taste and appearance; they were prepared under supervision by an experienced pharmacologist (G van der Meer, Gelderse Vallei Hospital, Ede, Netherlands). Bottles were colour-coded, but none of the field investigators was aware of the code until after crude analysis and a plan for further analysis had been prepared.

Iron was administered by community health-workers twice per week as ferrous fumarate in a 6·25 g/L suspension at a target dose of 6 mg elemental iron per kg bodyweight weekly. They used syringes on which amounts of suspension, colour code for each suspension, and the name of the child had been preindicated. Compared with ferrous sulphate suspension, ferrous fumarate suspension has the advantages of increased dosing accuracy and a longer shelf-life (up to 6 weeks after the bottle is opened). Ferrous fumarate suspension may cause fewer side-effects than ferrous sulphate tablets, and the iron is absorbed at a similar rate. No suspension was used beyond 6 weeks after bottles were opened.

Sulfadoxine-pyrimethamine was administered by the clinical officer employed by the project once every 4 weeks at therapeutic doses (25 mg and 1·25 mg per kg bodyweight, respectively), by means of syringes to dispense the correct volume of suspension. When given intermittently to infants alongside routine vaccinations against childhood diseases, sulfadoxine-pyrimethamine can substantially reduce the frequencies of occurrence of malaria attacks and severe anaemia in areas of high endemicity.<sup>22</sup>

The primary endpoint for iron status was the haemoglobin concentration at the end of follow-up (12 weeks). To increase the precision of measurements, haemoglobin concentrations at baseline and 12 weeks were calculated as the mean of duplicate measurements on the same blood sample. Another primary endpoint was the proportion of children with at least one malaria attack during follow-up. A malaria attack was defined as the presence of fever (axillary temperature  $\geq 37\cdot5^\circ\text{C}$ ) and a positive dipstick test result, indicating current or recent infection. Additional analyses were done on the proportion of children with anaemia (haemoglobin concentration below 110 g/L) or iron deficiency (serum ferritin concentrations below 12  $\mu\text{g/L}$ ), the frequency of adverse drug reactions, and the time until first occurrence of a malaria attack.

We expected that a sample size of 75 children per treatment group would be required for us to measure a difference between groups in haemoglobin concentration within 5 g/L of the true value ( $\alpha=0\cdot05$ ) with the assumption of a drop-out rate of 20%. This sample size also allowed us to estimate a difference between groups in the risk of a malaria attack to within 18% of the true difference ( $\alpha=0\cdot05$ ), on the basis of an expected group risk of 50% and a drop-out rate of 20%.

Children were medically examined at baseline, and after 4 weeks, 8 weeks, and 12 weeks. At each of these visits, samples of capillary blood from each child were tested by malaria dipstick, and haemoglobin concentration was measured. For each day of the recruitment period, the community health-worker and mothers from one community were asked to bring invited children to the research clinic. A questionnaire was administered to

collect vital information, and the child was measured. Children were then photographed for future identification, and their mothers and community health-workers received a treatment schedule and an identification card. The distance between the home of each child and the research clinic was measured in duplicate by a global positioning system (Model 12, Garmin International, Olathe, KS, USA).

Community health-workers each supervised one block of eight children within their own community through meetings twice per week with each child. For each of the meetings, the mothers were asked to bring the child to the community health-worker; the experimental iron or iron placebo suspensions were administered as described above. In addition, these visits were used for surveillance and monitoring of illness episodes or adverse effects. Thus, the community health-workers also recorded axillary temperature on a one-page questionnaire, together with information about whether the child had been ill on previous days, and whether adverse drug reactions had occurred. Questionnaires were handed in and perused within a day after completion. Compliance of the work of community health-workers was also checked, without their awareness, by inspection of whether residue of suspension was present in syringe tips, and during arbitrary visits to the children's homes.

Parents were asked to report immediately to the clinic with their child when suspected fever or other illnesses arose during the intervention period.

Children were withdrawn and treated appropriately if haemoglobin concentrations were below 50 g/L, if they met one or more criteria of severe and complicated malaria, or if they had manifestations of other severe disease.

Children reported to be sick were medically examined; blood samples were obtained and assayed by malaria dipstick test, and haemoglobin concentration was measured. Children with malaria attacks, defined by the occurrence of both fever confirmed by thermometer and a positive result on the dipstick test, were treated with amodiaquine or, if this was unsuccessful, with halofantrine under supervision. Afebrile children with a reported history of recent fever were tested repeatedly by thermometer and dipstick assay every few hours until the result was positive or a diagnosis of malaria could be excluded. For febrile children with a negative dipstick test, the blood slide was examined within 24 h at the nearest facility, 50 km away. Children were always promptly treated for common illnesses, or referred if necessary. During the intervention period, treatment with sulfa antibiotics was avoided. Sick and treated children remained in the study to contribute to the analysis of the treatment effects on haemoglobin concentration. Those with anaemia at the end of the intervention period were treated with iron, and those with positive results of dipstick tests for malaria with amodiaquine and sulfadoxine-pyrimethamine.

Axillary temperature was measured by electronic thermometer (Model HP 5316; Philips, Groningen, Netherlands). Haemoglobin concentrations were

measured by photometer (HemoCue, Ängelholm, Sweden). Dipstick tests (Model ML02; AMRAD/ICT, Sydney, NSW, Australia) were used for rapid detection in blood of antigens specific to *Plasmodium falciparum*.<sup>23</sup> In patients with manifestations of malaria, the sensitivity and specificity of this test is estimated to be more than 95% in detecting parasitaemia, as ascertained by microscopy.<sup>24,25</sup> A negative test result is highly predictive of the absence of malarial infection, but a positive test result probably has less predictive value for the presence of infection.<sup>26</sup> Resources for routine microscopic examination of blood smears were not available.

Procedures for collection and handling of blood samples have been described elsewhere.<sup>20</sup> Ferritin concentrations were measured by immunoassay (Roche, Mannheim, Germany) on an Elecsys electrochemiluminescence analyser. Concentrations of C-reactive protein were measured as an indicator of inflammation in an assay (Orion Diagnostics, Espoo, Finland) adapted to allow measurement in low volumes of ranges from zero to very high values. The results with this method correlated well with those by standard methods (Beckman Coulter, Brea CA, USA; Roche Diagnostics, Mannheim, Germany).

#### Statistical analysis

Anthropometric indices were calculated by Epi Info (version 6.04b). Data cleaning and standard analysis were undertaken with SPSS (version 7.5.2) on an intention-to-treat basis. Iron deficiency was measured in children who concurrently had negative results when their blood was assayed by malaria dipstick test and serum concentrations of C-reactive protein of 15 mg/L or less. Lower cut-off

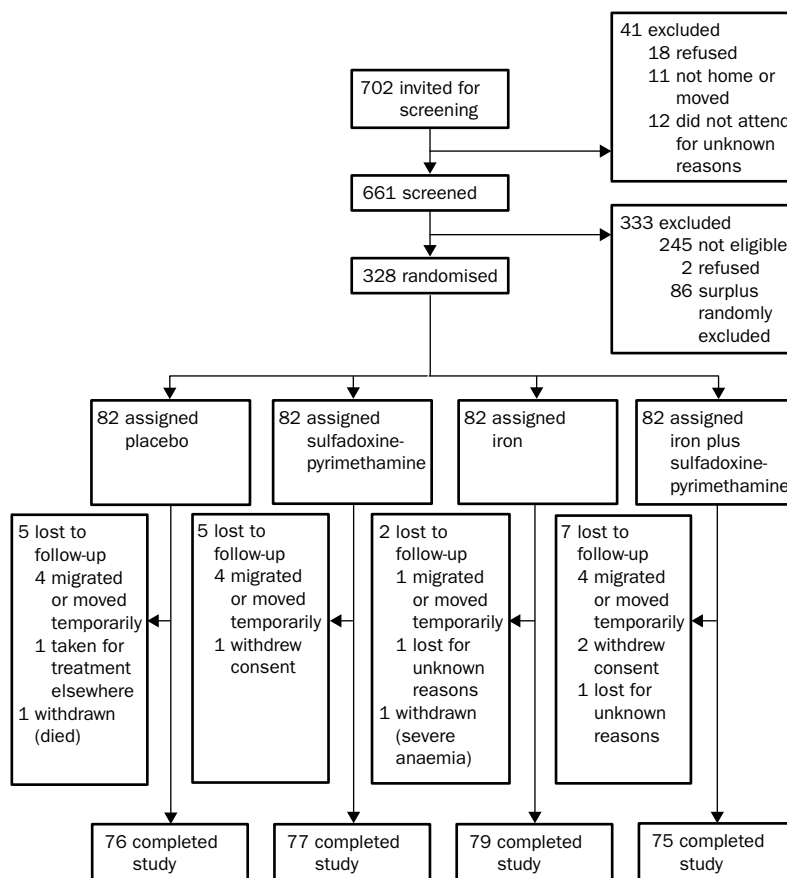


Figure 1: Trial profile

	Intervention group			
	Placebo (n=82)	Sulfadoxine-pyrimethamine (n=82)	Iron (n=82)	Iron plus sulfadoxine-pyrimethamine (n=82)
<b>Demography</b>				
Boys/girls	41/41	48/34	46/36	45/37
Age (mean, SD) (months)	19.6 (9.3)	17.8 (9.5)	17.3 (9.8)	18.4 (9.7)
Number of siblings (mean, SD)	1.9 (1.5)*	1.8 (1.4)	2.2 (1.7)	2.1 (1.8)
Distance between home and clinic (mean, SD) (km)	3.8 (2.8)	3.8 (2.7)	4.0 (2.9)	4.0 (3.0)
<b>Clinical and laboratory data</b>				
Haemoglobin concentration (mean, SD) (g/L)	97.6 (9.3)	94.4 (11.8)	93.0 (11.1)	95.4 (10.9)
Serum CRP concentration (median, IQR) (mg/L)	17.0 (14–20)†	17.0 (14–22)‡	18.0 (13–25)†	17.0 (14–21)§
Serum ferritin concentration (median, IQR) (µg/L)	16.0 (5.8–33.5)¶	12.1 (5.3–49.2)¶	15.1 (4.8–37.7)	15.8 (5.4–43.7)**
Number with malarial infection††	26 (32%)	23 (28%)	26 (32%)	25 (31%)
<b>Anthropometry (mean, SD)</b>				
Height-for-age (z score)	-1.47 (1.27)	-1.56 (1.11)	-1.70 (1.09)	-1.64 (1.10)
Weight-for-height (z score)	-0.25 (1.03)*	-0.09 (1.01)	-0.18 (1.14)	-0.28 (1.00)§
Weight-for-age (z score)	-1.09 (1.29)*	-1.10 (1.11)	-1.22 (1.24)	-1.28 (1.24)§
Mid upper-arm circumference (cm)	14.95 (1.10)	14.82 (1.27)	14.73 (1.18)	14.54 (1.10)

CRP=C-reactive protein. \*n=81. †n=78. ‡n=80. §n=79. ¶n=76. ||n=77. \*\*n=75. ††As indicated by a dipstick test result.

Table 1: **Baseline characteristics**

values of serum C-reactive protein concentrations did not result in appreciably different prevalence estimates.

The effects of interventions on iron status were assessed as the difference in mean haemoglobin concentrations at the end of the intervention period between each of the intervention groups and the placebo group. Additional analyses were done to adjust for block effects so as to take the study design into account, and to adjust for imbalances in prognostic factors at baseline. Interaction effects were examined directly by multiple regression to assess whether the effects on haemoglobin concentration at 12 weeks depended on haemoglobin concentration at baseline or age class.

Interventions were compared for their effect on the risk of malaria attacks, defined by a positive malaria dipstick test result with fever. In this analysis, we also stratified by dipstick test result at baseline. In children with a negative test result at baseline, such a case was thought to result from a newly acquired infection. In their counterparts with a positive test result at baseline, such a case may also indicate recrudescence of a patent infection or fever unrelated to malarial infection. Fisher's exact test was used to test for significance of risk differences, and corresponding confidence intervals were estimated on the assumption of a normal approximation of the binomial distribution. Differences in survival time were assessed by inspection of Kaplan-Meier curves and pairwise log-rank tests. Multivariate Cox regression models were used to assess further the effects of administration of iron and sulfadoxine-pyrimethamine, and to assess and adjust for potential confounding by prognostic factors at baseline.

Response indicator	Number of participants	Difference in mean haemoglobin concentration (g/L) from that of the placebo group (95% CI)		
		Sulfadoxine-pyrimethamine	Iron	Iron plus sulfadoxine-pyrimethamine
Crude	307	1.5 (-2.4 to 5.4)	8.5 (4.6 to 12.4)	9.1 (5.2 to 13.0)
Adjusted*	296	3.1 (-0.5 to 6.7)	10.7 (7.1 to 14.3)	11.1 (7.5 to 14.7)

\*Adjusted for block effects and prognostic factors at baseline (haemoglobin concentration, age class, sex, current or recent malarial infection as indicated by dipstick test, and z scores for height-for-age and weight-for-height).

Table 2: **Effects of interventions on haemoglobin concentration assessed 12 weeks after baseline (end of follow-up)**

Chronic inflammation unrelated to malaria could be an important cause of anaemia. This possibility was examined by measuring, in children who received placebo and whose blood sample was negative when assayed by malaria dipstick tests at all surveys (baseline, 4 weeks, 8 weeks, and 12 weeks), the proportion who had serum concentrations of C-reactive protein of 10 mg/L or higher at baseline and at 12 weeks.

#### Role of the funding source

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

## Results

702 children were invited to take part in the study, and 416 of 661 children screened met the eligibility criteria (figure 1). The parents of two children refused further participation after screening. Because the number of eligible children per community generally exceeded the required number per block, a total of 86 eligible children were randomly excluded. Of 328 children undergoing randomisation, 307 (94%) completed the trial and 21 (6%) did not (migrated or moved temporarily from the

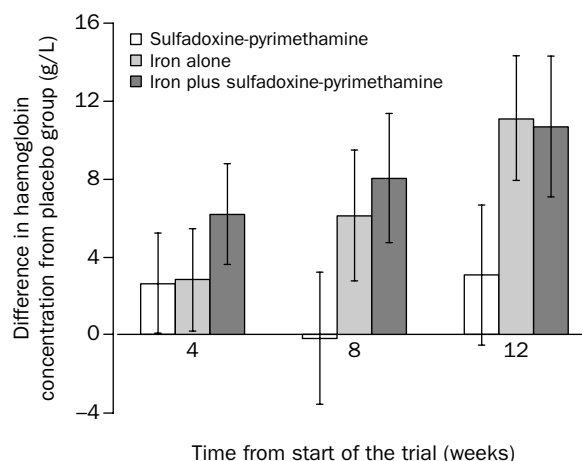


Figure 2: **Haemoglobin response to interventions during trial**

Treatment response was measured as the difference at 12 weeks in mean haemoglobin concentration between treatment groups and the placebo group, adjusted for block effects to take study design into account, and for prognostic factors at baseline (haemoglobin concentration, age class, sex, current or recent malarial infection as indicated by dipstick test, and z scores for height-for-age and weight-for-height). Error bars indicate 95% CI.

Time	Proportion (%) with iron deficiency (95% CI)			
	Placebo	Sulfadoxine-pyrimethamine	Iron	Iron plus sulfadoxine-pyrimethamine
Baseline	61 (41 to 81)	70 (53 to 88)	67 (48 to 86)	66 (48 to 83)
12 weeks	77 (53 to 94)	60 (42 to 78)	10 (0 to 24)	8 (0 to 19)

\*Measured in children with both a negative result in the malaria dipstick test and serum C-reactive protein concentration  $\leq 15$  mg/L.

Table 3: Effect of interventions on the frequency of iron deficiency, defined by serum ferritin concentration  $<12$   $\mu\text{g/L}$ \*

study area, 13; parents withdrew consent, three; developed severe anaemia, one; died, one; developed malaria but treated elsewhere, one; unknown reasons, two). Of all iron or iron-placebo doses scheduled until the time of censoring, 99% were administered. This estimate was the same whether censoring was due to completion of the trial or to being withdrawn from the study or lost to follow-up. All sulfadoxine-pyrimethamine or its placebo doses scheduled until the time of censoring were administered. No severe skin reactions were reported in 3824 child-weeks of follow-up.

The intervention groups were similar in terms of the distribution of baseline characteristics (table 1). At 12 weeks, the difference in haemoglobin concentration between the group assigned combined iron supplementation and intermittent administration of sulfadoxine-pyrimethamine and the placebo group haemoglobin concentration was 9.1 g/L (table 2). Measured in the same way, the effect of iron supplementation alone was 8.5 g/L and that of intermittent administration of sulfadoxine-pyrimethamine alone 1.5 g/L. Adjustment for block effects and differences in prognostic factors at baseline led to similar effect estimates (11.1 g/L, 10.7 g/L, 3.1 g/L; table 2). There was no evidence that the effect of combined iron supplementation and intermittent administration of sulfadoxine-pyrimethamine differed from the summed effects of the individual interventions (difference 2.7 g/L [95% CI -7.8 to 2.5];  $p=0.31$ ).

Results from the repeated surveys suggested that iron supplementation, alone or with sulfadoxine-pyrimethamine, continued to increase haemoglobin concentrations over the entire intervention period (figure 2). Despite these gains, anaemia had not resolved at the end of the intervention in 51% of children who had received iron alone, and in 36% of children who had received iron plus sulfadoxine-pyrimethamine. The corresponding prevalences of anaemia in children who received sulfadoxine-pyrimethamine only or placebo were 73% and 71%. Iron supplementation, alone or with sulfadoxine-pyrimethamine, resulted in a substantial reduction in the proportion of children of iron

	Number with malaria attacks/total			
	Placebo	Sulfadoxine-pyrimethamine	Iron	Iron plus sulfadoxine-pyrimethamine
<b>All children</b>	23/82 (28%)	14/82 (17%)*	24/82 (29%)	20/82 (24%)
<b>Malaria status at baseline as assessed by dipstick test</b>				
No infection	10/56 (18%)	4/59 (7%)†	10/56 (18%)	10/57 (18%)
Current or recent infection	13/26 (50%)	10/23 (44%)	14/26 (54%)	10/25 (40%)

Children who did not complete follow-up of 12 weeks were kept in the analysis. Sulfadoxine-pyrimethamine vs placebo, Fisher's exact test: \* $p=0.13$ ; † $p=0.09$ .

Table 4: Effect of interventions on risk of malaria attacks

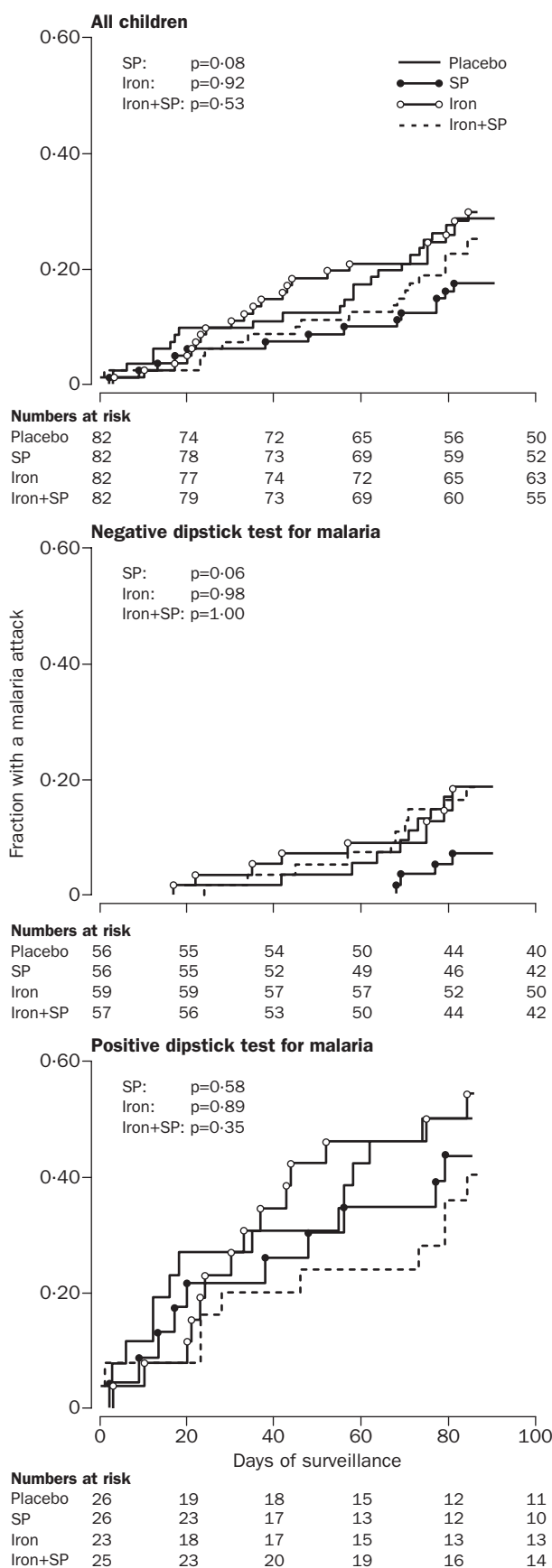


Figure 3: Kaplan-Meier curves showing probability of malaria attacks during 12 weeks of surveillance

SP=sulfadoxine-pyrimethamine.  $p$  values indicate results of pairwise log-rank tests to compare survival time of intervention groups and the placebo group.

	Hazard ratio (95% CI)					
	Sulfadoxine-pyrimethamine		Iron		Iron plus sulfadoxine-pyrimethamine	
	Crude	Adjusted*	Crude	Adjusted*	Crude	Adjusted*
<b>All children (n=328)</b>	0.58 (0.30 to 1.14)	0.59 (0.29 to 1.20)	1.08 (0.60 to 1.92)	1.18 (0.65 to 2.13)	0.82 (0.44 to 1.52)	0.76 (0.40 to 1.42)
<b>Malaria status at baseline as assessed by dipstick test</b>						
No infection (n=228)	0.35 (0.11 to 1.12)	0.33 (0.09 to 1.23)	0.99 (0.41 to 2.37)	1.20 (0.48 to 3.01)	1.00 (0.42 to 2.40)	0.91 (0.36 to 2.29)
Current or recent infection (n=100)	0.85 (0.37 to 1.96)	0.82 (0.33 to 2.04)	1.13 (0.52 to 2.45)	1.19 (0.53 to 2.63)	0.65 (0.27 to 1.55)	0.66 (0.27 to 1.63)

\*Adjusted for factors at baseline: age class (<6 months; 6–12 months; 12–24 months; >24 months); sex; z scores for height-for-age and weight-for-height; distance between home of child and research clinic; and dipstick test result.

Table 5: Comparison by hazard ratio for a malaria attack between groups who received interventions or placebo

deficiency (table 3). Sulfadoxine-pyrimethamine did not substantially affect this proportion. In addition, we saw no evidence that iron supplementation led to changes in serum C-reactive protein concentrations (data not shown).

Malaria attacks occurred in 17% of children in the sulfadoxine-pyrimethamine group compared with 28% in the placebo group (table 4; difference 11% [95% CI –2 to 24];  $p=0.13$ ). When analysis was restricted to children without malarial infection at baseline, the proportions with malaria attacks were 7% and 18%, respectively (difference 11%, 95% CI not calculated because few cases arose in children who received sulfadoxine-pyrimethamine;  $p=0.09$ ). In this restricted dataset, the intervention groups were also similar in their distribution of baseline characteristics (not shown). There was no evidence that iron supplementation, either alone or in combination with sulfadoxine-pyrimethamine, substantially increased the risk of a malaria attack.

Kaplan-Meier analysis suggested that malaria attacks happened later in the sulfadoxine-pyrimethamine group than in the placebo group (figure 3). This protective effect of sulfadoxine-pyrimethamine against attacks caused by newly acquired infections was unlikely to be due to chance (figure 3;  $p=0.06$ , log-rank test). There seemed to be no or little effect on survival time of administration of iron, either alone or in combination with sulfadoxine-pyrimethamine (figure 3).

Cox regression analysis showed no or marginal confounding due to imbalances between intervention groups in prognostic factors assessed at baseline (table 5). There was no evidence of an interaction between the effects of iron and sulfadoxine-pyrimethamine on malaria ( $p=0.25$ ), nor for interaction between the effects on malaria of either iron or sulfadoxine-pyrimethamine and the result of the malaria dipstick test at baseline. When survival time was modelled exclusively as a function of iron and sulfadoxine-pyrimethamine treatment, the hazard ratios of malaria attacks for iron and sulfadoxine-pyrimethamine were 1.20 (95% CI 0.77 to 1.87,  $p=0.41$ ) and 0.68 (0.43 to 1.06,  $p=0.09$ ), respectively. When this model was restricted to children with a negative dipstick test result at baseline, the corresponding estimates were 1.50 (0.76 to 2.96,  $p=0.25$ ) and 0.67 (0.34 to 1.32,  $p=0.25$ ).

Of 35 children who received placebo and whose blood was negative for malaria at all surveys (baseline, 4 weeks, 8 weeks, 12 weeks), 30 (86% [95% CI 74 to 97]) had serum C-reactive protein concentrations of 10 mg/L or higher both at baseline and at 12 weeks.

## Discussion

Our findings show that iron supplementation results in a striking improvement of haemoglobin concentrations and substantially lowers the frequency of both iron deficiency and anaemia; there was no evidence that it was

accompanied by a substantially increased risk of malaria. Intermittent administration of sulfadoxine-pyrimethamine alone yielded only a marginal increase in haemoglobin concentrations but might substantially protect against malaria. Administration of sulfadoxine-pyrimethamine in addition to iron supplementation did not result in further improvement of haemoglobin concentrations.

The sample selected was representative of children meeting the selection criteria in the study area. The intervention groups had similar characteristics at baseline, and the number of children lost to follow-up during the study was low. Hence, these factors are unlikely to have produced substantial bias. Compliance with iron supplementation was excellent, and there were no indications that children missed a substantial number of scheduled doses. Similar haemological responses can be expected when these interventions are implemented in children aged 2–36 months with mild to moderate anaemia living in areas of seasonal malaria.

Malaria dipstick tests are highly sensitive in detecting parasitaemia, but the specificity can be lower because positive results can be obtained for some time after clearance of parasitaemia.<sup>26</sup> A previous study showed that in 70% of cases, the dipstick test used gave negative results within 7 days of the start of curative chemotherapy.<sup>27</sup> As a result, the effect of iron supplementation on reported survival time until first occurrence of a malaria attack may have been underestimated in children with a positive test result at baseline (figure 3), which would also lead to an underestimate of effect of iron supplementation in all children. In children with a negative test result at baseline, however, the reported survival curves validly show the risk of a malaria attack due to a newly acquired infection. In these children, both the risk analysis (table 4) and the Kaplan-Meier analysis suggested little or no effect of iron on malaria (figure 3). Cox regression analysis indicated a 20% increase in the hazard ratio with iron supplementation, with 95% CI compatible with a three-fold increase in the risk (table 5; adjusted values). Thus, a substantial increase is unlikely but cannot be ruled out. A larger trial would be needed to assess this effect more precisely. Serum concentrations of C-reactive protein were not affected by iron supplementation, indicating that it did not lead to increased inflammation.

We have shown previously that worm infections are rare in children of similar age to those in the study, in the same area.<sup>20</sup> Iron status should therefore be improved if the intake of absorbable iron is increased. The feasibility of iron-supplementation programmes for preschool children should be considered. In addition, children in rural Africa might benefit from agronomic interventions aimed at increasing the iron concentration of plant-based foods while decreasing the concentration of absorption-inhibiting factors in grains, such as phytates and polyphenols.

As indicated by the proportion with anaemia at 12 weeks, our interventions were not as effective as expected. The efficacy might be improved by use of longer intervention periods or by increasing the frequency of iron administration.<sup>28</sup> Other possible causes of anaemia should also be taken into account, including deficiencies in vitamin A and other micronutrients and  $\alpha$ -thalassaemia. We noted that most (86%) children who received placebo and who remained free of malaria throughout the trial had high serum concentrations of C-reactive protein both at baseline and at 12 weeks. This finding suggests that chronic disease due to infections other than malaria might be another cause of anaemia.<sup>29–31</sup>

The age-group studied probably represents the range at highest risk of anaemia due to either iron deficiency or malaria. The interventions might have been less effective in older children. Only one instance of severe anaemia was observed. Therefore, intermittent administration of sulfadoxine-pyrimethamine is unlikely to have a role in future public-health programmes to control mild or moderate anaemia in areas with similar rates of transmission. It might be used to reduce the incidence of malaria attacks or—in areas with high, perennial transmission—to prevent severe anaemia.<sup>22</sup> The current switch of African countries to sulfadoxine-pyrimethamine for first-line treatment of uncomplicated malaria justifies concerns, however, that parasite resistance against this drug combination could rapidly become such a problem that the drug will no longer be effective.

We conclude that iron supplementation (twice a week at 6 mg per kg bodyweight) gives substantial health benefits that may outweigh the associated risk of adverse effects caused by malaria. Intermittent administration of sulfadoxine-pyrimethamine in addition to iron supplementation does not result in further improvements in haemoglobin concentrations; further studies are warranted, however, to investigate its possible benefits in reducing the incidence of malaria attacks. Future research should assess the benefits and risks of more frequent administration of iron for longer intervention periods.

#### Contributors

H Verhoef, C West, and F Kok were responsible for study design and interpretation of results. H Verhoef did data analysis with assistance from S de Vogel and R van der Valk. H Verhoef, S Nzyuko, S de Vogel, R van der Valk, M Wanga, A Kuijsten, and J Veenemans obtained the data.

#### Conflict of interest statement

None declared.

#### Acknowledgments

This study was supported by a grant from the Netherlands Foundation for the Advancement of Tropical Research (number WV93-273).

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