

Efficacy of iron-fortified whole maize flour on iron status of schoolchildren in Kenya: a randomised controlled trial

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Summary

Background Sodium iron edetic acid (NaFeEDTA) might be a more bioavailable source of iron than electrolytic iron, when added to maize flour. We aimed to assess the effect, on children's iron status, of consumption of whole maize flour fortified with iron as NaFeEDTA or electrolytic iron.

Methods 516 children, aged 3–8 years, from four schools in Marafa, Kenya, were randomly assigned to four groups. All were given the same amount of porridge five times a week. The porridge for one group was made from unfortified whole maize flour; for the other three groups it was fortified with either high-dose NaFeEDTA (56 mg/kg), low-dose NaFeEDTA (28 mg/kg), or electrolytic iron (56 mg/kg). Concentrations of haemoglobin, plasma ferritin, and transferrin receptor were analysed in samples taken at baseline and at the end of the 5-month intervention. The primary outcome was iron-deficiency anaemia. We analysed data on an intention-to-treat basis. This trial is registered with ClinicalTrials.gov, number NCT00386074.

Findings The prevalence of iron-deficiency anaemia in children given unfortified flour was 10%. Compared with placebo, the prevalence of iron-deficiency anaemia in children given flour fortified with high-dose NaFeEDTA, low-dose NaFeEDTA, and electrolytic iron changed by –89% (95% CI –97% to –49%), –48% (–77% to 20%), and 59% (–18% to 209%), respectively. Consumption of high-dose NaFeEDTA improved all measured iron-status indicators. Low-dose NaFeEDTA decreased the prevalence of iron deficiency but did not noticeably change the prevalence of anaemia. Electrolytic iron did not improve any of these iron-status indicators. Children who were iron-deficient at baseline benefited more from high-dose and low-dose NaFeEDTA than those with sufficient iron at baseline.

Interpretation Consumption of whole maize flour fortified with NaFeEDTA caused modest, dose-dependent improvements in children's iron status. Fortification with electrolytic iron did not improve their iron status. Therefore, in high-phytate flours, NaFeEDTA is more suitable than electrolytic iron for supplementation of iron in the diet.

Introduction

Fortification of staple cereal flours could be a cost-effective, sustainable way to improve iron status in developing countries. It could be achieved by addition of a suitable substance (or fortificant), either on a large scale to centrally processed flour or on a small scale, in local communities.¹ International efforts to advocate flour fortification are now beginning to move the process forward. 49 countries routinely add iron to flour (compared with two countries in 1990).² Thus about 15% of output from the world's flour mills is fortified with iron.² In sub-Saharan Africa, Nigeria and South Africa have made fortification of flour with iron mandatory, and countries such as Cape Verde, Côte d'Ivoire, Guinea, Ghana, and Kenya are planning flour-fortification programmes.² In eastern Africa, maize and wheat flours are suitable fortification vehicles because they are consumed as part of the typical diet.

Elemental iron powders are the most widely used nutrient vehicle for fortification of flour. In an authoritative review, Hurrell and colleagues³ concluded that electrolytic iron is the only form of elemental iron that can be recommended as an iron fortificant in cereal flours. Fortification of wheat and maize flour with electrolytic iron is mandatory by law in South Africa (at

minimum concentrations of 44·28 mg/kg and 50·40 mg/kg for sifted and unsifted flour, respectively).⁴ However, although electrolytic iron is inexpensive, its bioavailability is questionable because it can bind to phytates in cereals. Only two randomised trials have assessed whether consumption of sifted, low-phytate flour, fortified with electrolytic iron, affects iron status.^{5,6} The first trial⁵ was not conclusive; in the second trial, the iron status of participants improved.⁶

In developing countries, however, the typical diet includes whole grain flour, which has a much higher content of phytate than low-extraction white flour. In such cases, NaFeEDTA might be a better fortificant than electrolytic iron for supplementation of iron, because EDTA chelates iron, and might prevent it from binding to phytates. Isotope studies suggest that iron absorption from NaFeEDTA might be two to three times higher than from electrolytic iron.⁷ This substance might not only improve absorption of added iron, but also of non-haem iron in food.⁸ When given in fortified condiments, NaFeEDTA improved iron status.^{9–12} It is well absorbed when added to corn masa flour,¹³ which contains inhibitors of iron absorption. Therefore NaFeEDTA is potentially suitable for iron fortification in high-phytate foods. We aimed to assess the effect of consumption of

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whole maize flour fortified with high and low doses of NaFeEDTA, and with electrolytic iron, on children. We expected that the efficacy of iron fortification might be affected by children's iron status at baseline.

Methods

Participants

Our study was based at four schools in Marafa, in the hinterland of Malindi district, in the semiarid coastal lowlands of Kenya. We did all fieldwork between May and November, 2004, to reduce non-compliance due to holidays in April and August, and from December to early January. Because of this constraint, our trial period coincided with the two rainy seasons (March–May and October–December), in which malaria transmission is highest. Most malaria episodes in the region are due to *Plasmodium falciparum*.¹⁴

Local families are mostly poor subsistence farmers from Mijikenda tribal groups. Their diet is monotonous and predominantly based on maize, with a low content of animal products. Children sometimes receive one daily meal through government-funded feeding programmes that are operated by schools in periods of drought.

We selected children who were enrolled in nursery and the first year of primary school. 4 weeks before the intervention we began to give children a target daily amount of cooked unfortified flour to assess the acceptability of the fortification vehicle and compliance

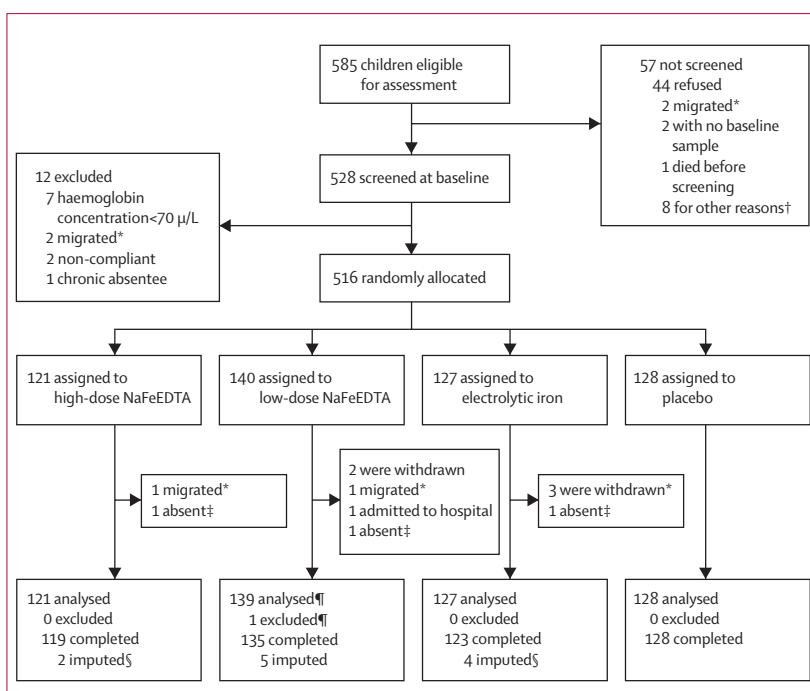
with consumption of the target quantity. 3 weeks before the trial we screened 528 children for eligibility. Our criteria for inclusion were age 3–8 years and consumption of at least 50% of the target amount of cooked unfortified flour during the month-long run-in period. Children with chronic disease, overt severe malnutrition, mental disability, or haemoglobin concentrations lower than 70 g/L were excluded. All children were examined by a clinical officer. Those who had fever or other signs of malaria and who tested positive for malaria were given sulfadoxine-pyrimethamine before the start of the intervention. Children who became ill during the intervention period were treated or referred according to guidelines from the Kenyan Ministry of Health. Ethical approval was granted by the ethical review committee of Kenya Medical Research Institute (KEMRI). We obtained signed informed consent from the parents of all enrolled children.

We took samples of venous blood at baseline into containers with Na-heparin (Becton-Dickinson; Temse, Belgium), and transferred blood into bottles with EDTA for haematological analysis and to detect *P falciparum* malaria antigenaemia by dipstick test. Heparinised blood was separated in the field within 1 h of collection; plasma was kept and transported in a cool box for 2–6 h, and subsequently stored in liquid nitrogen (-196°C) and below -70°C . We also preserved blood sediment in buffer (AS1; Qiagen, Valencia, CA, USA) at ambient temperatures for subsequent α -thalassaemia genotyping. We measured children's height to the nearest 1 mm and weight to the nearest 0.1 kg (Leicester stadiometer and Tanita BWB800 scale, respectively; Chasmors, London, UK). The scales were calibrated daily with standard weights.

Procedures

The fortification vehicle consisted of *uji*, a porridge of maize flour cooked in water and sweetened with sugar. The target daily intake was 700 mL *uji* (containing 100 g flour) for children aged 3–5 years and 1000 mL *uji* (containing 150 g flour) for children aged 6–8 years. This target amount was provided daily to every child in two equally divided portions. We estimated that addition of iron at a low dose of 28 mg/kg flour and a high dose of 56 mg/kg flour would provide 20% and 40%, respectively, of the daily iron requirements for children aged 3–5 years and 18% and 36%, respectively, of the requirements for children aged 6–8 years. In this calculation, we assumed that 5% of the added iron would be absorbed.

Both fortified and unfortified whole maize flour for *uji* were produced and coded at Unilever Kenya, Nairobi. Flour was fortified with NaFeEDTA (Akzo Nobel, Netherlands) at 56 mg or 28 mg iron per kg flour, or electrolytic iron (Food Chemicals Codex V-grade; Industrial Metal Powders; Pune, India) at 56 mg/kg flour. The electrolytic iron fortificant was identical to that specified for mandatory addition to flour in South



	High-dose NaFeEDTA	Low-dose NaFeEDTA	Electrolytic iron	Placebo
n	121	140	127	128
Age (years)	5·9 (1·4)	5·9 (1·5)	5·8 (1·4)	6·0 (1·4)
Sex (female)	65 (54%)	70 (50%)	63 (50%)	62 (48%)
Haemoglobin concentration (g/L)	109·9 (11·6)	110·8 (10·9)	110·9 (11·3)	114·0 (10·7)
Plasma ferritin concentration ($\mu\text{g}/\text{L}$)				
All children*	30·0 (20·5–45·0)	28·2 (16·0–40·8)	26·0 (16·0–44·0)	34·0 (20·0–53·8)
Children without inflammation*††	28·0 (19·5–44·0)	27·0 (15·0–39·0)	24·0 (15·0–41·5)	32·0 (19·0–53·0)
Plasma soluble transferrin receptor concentration (mg/L)*	2·7 (2·2–3·4)	2·7 (2·2–3·3)	2·6 (2·2–3·3)	2·5 (2·1–3·1)
Plasma C-reactive protein concentration (mg/L)*	1·0 (0·0–3·0)	1·0 (0·0–3·0)	0·0 (0·0–2·0)	0·5 (0·0–3·0)
Inflammation‡	8 (6·6%)	13 (9·3%)	6 (4·7%)	9 (7·0%)
Anaemia§	71 (58·7%)	84 (60·4%)	75 (59·1%)	60 (46·9%)
Iron deficiency¶	18 (14·9%)	27 (%)	21 (16·5%)	12 (9·4%)
Iron-deficiency anaemia	12 (9·9%)	19 (13·6%)	15 (11·8%)	8 (6·3%)
Malaria antigenaemia	62 (51·2%)	65 (46·4%)	62 (48·8%)	65 (50·8%)
Helminth infestation				
Ascaris	3 (2·5%)	1 (1·0%)	2 (2·0%)	0
Trichuris	0	0	2 (2·0%)	2 (2·0%)
Hookworm	14 (12·0%)	16 (11·0%)	11 (9·0%)	13 (10·0%)
Any infection	16 (13·2%)	17 (12·1%)	14 (11·0%)	15 (11·7%)
Positive sickling test result	21 (17·4%)	31 (22·1%)	26 (20·5%)	21 (16·4%)
α -thalassaemia genotype				
Data not available	3 (2·5%)	10 (7·2%)	6 (4·7%)	6 (4·7%)
Normal	42 (34·7%)	42 (30·0%)	29 (22·8%)	37 (28·9%)
Heterozygous	57 (47·1%)	66 (47·1%)	71 (55·9%)	66 (51·6%)
Homozygous	19 (15·7%)	22 (15·7%)	21 (16·5%)	19 (14·8%)
Height for age (Z score)	-1·4 (1·4)	-1·5 (1·3)	-1·1 (1·3)	-1·4 (1·2)
Weight for height (Z score)	-0·8 (0·7)	-0·8 (0·7)	-0·7 (0·8)	-0·7 (0·6)
Weight for age (Z score)	-1·5 (1·0)	-1·5 (1·0)	-1·3 (0·9)	-1·4 (0·9)

Data are mean (SD) or number (%) unless indicated otherwise. *Median (interquartile range) †n=113, 126, 121, and 119, respectively. ‡Inflammation defined as C-reactive protein >10 mg/L in plasma. §Anaemia defined as haemoglobin <110 g/L and <115 g/L for children aged <5 years and ≥5 years, respectively; ¶Iron deficiency defined as plasma ferritin concentration <12 µg/L and <15 µg/L for children aged <5 years and ≥5 years, respectively. ||Iron-deficiency anaemia defined as concurrent anaemia and iron deficiency.

Table 1: Baseline characteristics of study participants, by intervention group

Africa.⁴ Flour fortified with iron was also supplemented with a nutritional premix (Roche; Basel, Switzerland) containing vitamin A (2500 µg/kg), thiamin (3·5 mg/kg), riboflavin (4·0 mg/kg), and niacin (45·0 mg/kg). We measured the mean particle diameter of electrolytic iron particles along three orthogonal axes by laser beam diffraction (Rhodos, Sympatec, Clausthal-Zellerfeld, Germany); the median particle size was 34 µm (10th–90th percentiles: 14–62 µm). Webfigure 1 shows the morphology of the electrolytic iron particles under scanning electron microscopy.

Each flour type was labelled with colour-coded packaging. This code was withheld from participants and investigators until all data had been collected and statistical analyses had begun. Flour types could not be visually distinguished, even in the cooked product. Eligible children were randomly allocated to one of four treatment groups, which were colour coded to correspond to the flour packaging. The allocation code was generated by simple randomisation with a table of random numbers

by an investigator (HV) who did not participate in the screening or enrolment of children.

The intervention started for all children on the same day, immediately after the month-long run-in period. Children consumed *ujji* in graduated mugs, at school, 5 days a week for 5 months. Teachers and fieldstaff supervised preparation of the porridge, and ensured that there was no crossover between groups and that children did not share *ujji*. The amount of *ujji* consumed by every child was recorded after every meal.

We measured haemoglobin concentration in blood samples in a haematology analyser (KX-21, Sysmex Corporation, Japan). At baseline, we used a sickling test, with the slide method and sodium metabisulphite as a reducing agent. We tested for current or recent malaria infection with a rapid, qualitative dipstick assay (Paracheck Pf, Orchid Biomedical Systems, Goa, India) that detects *P falciparum*-specific histidine-rich protein-2. We chose this test in preference to conventional microscopic examination of blood-films because it has a reported

See Online for webfigure 1

	High-dose NaFeEDTA	Low-dose NaFeEDTA	Electrolytic iron	Placebo
n	121	139	127	128
Haemoglobin concentration (g/L)*	117.2 (8.5)	114.7 (8.8)	112.2 (9.9)	115.7 (9.7)
Crude effect	1.6 (-0.7 to 3.8)	-0.7 (-2.9 to 1.5)	-3.5 (-5.7 to -1.2)	Reference
Adjusted effect†	4.0 (2.3 to 5.6)	1.3 (-0.3 to 2.8)	-1.3 (-2.9 to 0.3)	Reference
Plasma ferritin concentration ($\mu\text{g/L}$)‡	35.0 (24.5 to 47.0)	28.0 (20.0 to 39.0)	23.0 (13.0 to 32.0)	23.0 (16.0 to 36.0)
Crude effect§	54% (33% to 78%)	23% (7% to 41%)	-3% (-16% to 12%)	Reference
Adjusted effect†§	67% (49% to 89%)	36% (-21% to 53%)	6% (-5% to 20%)	Reference
Plasma soluble transferrin receptor concentration (mg/L)‡	2.3 (1.9 to 2.7)	2.4 (2.0 to 2.8)	2.6 (2.1 to 3.2)	2.5 (2.1 to 3.1)
Crude effect§	-11% (-17% to -5%)	-8% (-14% to -2%)	4% (-3% to 11%)	Reference
Adjusted effect†§	-15% (-19% to -11%)	-12% (-16% to -8%)	0% (-4% to 5%)	Reference
Anaemia¶	38 (31.4%)	58 (41.7%)	65 (51.2%)	48 (37.5%)
Crude effect	-16 (-45 to 28)	11 (24 to 63)	36 (-6 to 98)	Reference
Adjusted effect†	-36 (-58 to -1)	-2 (-33 to 44)	12 (-23 to 63)	Reference
Iron deficiency**	3 (2.5%)	14 (10.0%)	33 (26.0%)	27 (21.1%)
Crude effect	-88% (-96 to -61)	-52% (-75 to -9)	23% (-26 to 105)	Reference
Adjusted effect†	-91% (-97 to -49)	-70% (-85 to -40)	1% (-40 to 69)	Reference
Iron-deficiency anaemia††	2 (1.7%)	12 (8.6%)	27 (21.3%)	13 (10.2%)
Crude effect	-84% (-96 to -28)	-15% (-61 to 86)	109% (8 to 306)	Reference
Adjusted effect†	-89% (-97 to -49)	-48% (-77 to 20)	59% (-18 to 209)	Reference

Values are mean [95% CI] or number (%) unless indicated otherwise. Treatment effects are measured as group differences (continuous outcomes) relative to placebo. All analyses are by intention to treat. *Mean (SD). †Effect of intervention adjusted for baseline factors: haemoglobin concentration, plasma concentrations of ferritin, soluble transferrin receptor and C-reactive protein, and malaria antigenaemia. ‡Median (IQR). §Values indicate difference between groups, expressed as a percentage relative to the placebo group, obtained by exponentiation of effect estimates from log-transformed data. ¶Anaemia defined as haemoglobin concentration <110 g/L for children aged <5 years and <115 g/L for those ≥5 years. ||Values indicate percentage difference in prevalence as compared with placebo [95% CI], obtained by conversion of prevalence ratios from Cox regression with constant time at risk. **Iron deficiency defined as plasma ferritin concentration <12 µg/L for children aged <5 years and <15 µg/L for those ≥5 years. ††Iron deficiency anaemia defined as concurrent anaemia and iron deficiency.

Table 2: Effect of consumption of iron-fortified flour on iron status, compared with placebo

sensitivity and specificity of more than 90%.^{15–17} Plasma concentrations of C-reactive protein, ferritin, and soluble transferrin receptor were measured in the Netherlands (Meander Medical Centre, Amersfoort) on a Behring nephelometer (BN-Prospec, Dade-Behring, Marburg, Germany), with Behring kits and calibration and assay procedures. We used concentration of C-reactive protein as a marker of inflammation, since infection-induced inflammation can increase ferritin concentrations independently of iron status.¹⁸ Genotyping for α-thalassaemia was done by PCR.¹⁹ All children received malaria chemotherapy 14 days before final blood collection to avoid inflammation-induced effects on iron status indicators.

The primary outcome was iron-deficiency anaemia. Secondary outcomes were iron deficiency; anaemia; and difference in haemoglobin concentration and plasma concentrations of ferritin and soluble transferrin receptor in the two treatment groups. We used WHO Guidelines²⁰ to define anaemia as haemoglobin concentration of less than 110 g/L or 115 g/L for children younger or older than 5 years, respectively; iron deficiency as plasma ferritin concentration of less than 12 µg/L or 15 µg/L for children younger or older than 5 years, respectively; iron deficiency-anaemia as concurrent anaemia and iron deficiency; and inflammation as 10 mg/L or more of C-reactive protein in plasma.

Statistical analysis

We do not report sample size calculations because we interpreted a completed study; all information about precision is indicated by the 95% CIs of our effect estimates. We analysed anthropometric Z scores for all children with Epi-Info 2005 software (version 3.3.2), and analysed other data with SPSS software (version 12.0). The total amount of *ujif* consumed per group was calculated as a proportion of the amount assigned to every group during the intervention period.

Analysis was by intention to treat, except that we retrospectively excluded one child with abnormal iron status and sickle-cell anaemia. Data were assessed for normality by visual examination of distribution plots, and were normalised as appropriate by log transformation. We obtained geometric means and corresponding CIs for absolute concentrations by calculation of exponents for log-transformed data.

Missing data were imputed blindly, before we started primary analysis, as the mean or median values from children without missing values in the same group. Because numbers of missing values were low, results from this simple approach are comparable with better but more complicated methods of imputation.²¹ The method assumes that children whose data are imputed are representative for their group and are not systematically different from children for whom a full set of data was obtained.²²

We estimated treatment effects as group differences at the end of intervention relative to placebo. We used multiple linear regression to adjust for baseline concentrations of haemoglobin, ferritin, soluble transferrin receptor and C-reactive protein, and malaria antigenaemia. We decided a priori that adjusted estimates would be more relevant than crude estimates because even minor differences in prognostic factors at baseline could still lead to confounding.

For binary outcomes, we estimated prevalence ratios and corresponding CIs with Cox regression analysis and constant time at risk.^{23,24} This method is generally used for survival analysis; we used it to produce valid point estimates of prevalence ratios when adjusting for baseline factors.²⁵ However, we were aware that it can produce inflated estimates of SEs and CIs.²⁵ We converted prevalence ratios obtained by Cox regression and effect estimates obtained from log-transformed data to percentage differences relative to placebo. We analysed subgroups to assess whether the magnitude of the treatment effect was affected by iron deficiency or iron-deficiency anaemia at baseline, malaria antigenaemia at baseline, α -thalassaemia genotype, or the result of the sickling test. This trial is registered with ClinicalTrials.gov, number NCT00386074.

Role of the funding source

Unilever Food and Research Institute, Vlaardingen, and Akzo Nobel Chemicals, Arnhem, Netherlands, provided funds. Unilever participated in study design and data interpretation. Authors from Wageningen University had full access to all data in the study and had final responsibility for the decision to submit for publication.

Results

505 children completed the study (figure 1). Values for iron-status indicators were imputed for one child whose baseline plasma sample was lost and for 11 children who either discontinued the intervention or were absent at the end of intervention. We did not know the α -thalassaemia genotype for 25 children, because two blood samples were spilled during transportation, five samples were lost, seven were not clearly labelled, and 11 were not obtained because children were absent at the final survey.

Table 1 shows the baseline characteristics of children in the four groups. Haemoglobin concentrations were highest in the placebo group. Almost half the children (49%) had current or recent malaria infection. 290 (56%), 78 (15%), and 54 (11%) children had anaemia, iron deficiency, and iron-deficiency anaemia, respectively. The prevalence of children who were heterozygous and homozygous for α -thalassaemia was 260 (49%) and 81 (16%), respectively. 19% of all study participants had a positive result for the sickling test. Of the three worm infections assessed, hookworm was the most often detected.

At baseline, both malaria antigenaemia and a homozygous genotype for α -thalassaemia were associated

with low haemoglobin concentrations (mean difference -5.2 g/L , 95% CI -3.4 to -7.0 g/L) and $(-8.8\text{ g/L}, -6.0$ to -11.6 g/L), respectively. A heterozygous genotype for α -thalassaemia was not associated with noticeably low concentrations of haemoglobin (data not shown).

Groups assigned to receive *uji* fortified with high-dose NaFeEDTA, low-dose NaFeEDTA, electrolytic iron, and unfortified flour consumed 92%, 89%, 90%, and 93%, respectively, of the total amount given to them during the intervention period. At the end of the intervention few children had inflammation, which was of low degree: the median concentration of C-reactive protein in all groups was 0 mg/L , with IQRs of 0.0 – 1.0 mg/L in the two NaFeEDTA groups, 0.0 – 2.0 mg/L in the electrolytic iron group, and 0.0 – 0.8 mg/L in the placebo group.

The prevalence of iron-deficiency anaemia in children who consumed flour fortified with high-dose NaFeEDTA was almost 90% lower than in controls (table 2). The prevalence of iron-deficiency anaemia in children given low-dose NaFeEDTA was about half that in those given placebo, although the 95% CI was compatible with a 20% increase in iron-deficiency anaemia. Iron-deficiency anaemia did not change in the group assigned flour fortified with electrolytic iron.

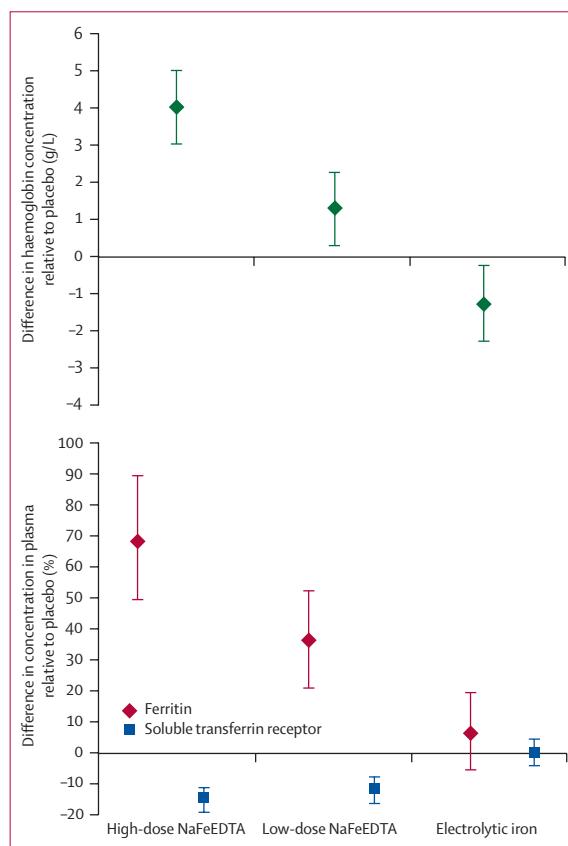


Figure 2: Effect of consumption of iron-fortified flour on concentrations of haemoglobin, plasma ferritin, and plasma soluble transferrin receptor
Flour was fortified with 56 mg/kg NaFeEDTA (high-dose), 28 mg/kg NaFeEDTA (low-dose), and 56 mg/kg electrolytic iron. Line bars indicate 95% CIs.

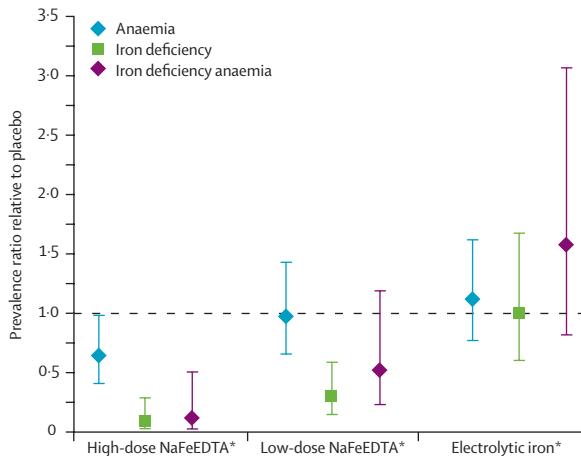


Figure 3: Effect of consumption of iron-fortified flour on anaemia, iron deficiency, and iron deficiency anaemia

*Flour was fortified with 56 mg/kg NaFeEDTA (high-dose) and 28 mg/kg NaFeEDTA (low-dose), and 56 mg/kg electrolytic iron. Dotted line indicates no-effect (prevalence ratio=1). Line bars indicate 95% CIs.

Consumption of flour fortified with high-dose and low-dose NaFeEDTA caused modest improvements in haemoglobin concentration compared with placebo (figure 2). In both groups given NaFeEDTA, plasma concentrations of ferritin increased and soluble transferrin receptor decreased (figure 2). By contrast, fortification with electrolytic iron did not affect any of these three iron-status indicators (figure 2).

Prevalence of anaemia was reduced only for the group given high-NaFeEDTA (figure 3). Prevalence of iron deficiency was 91% lower in the high-dose NaFeEDTA group and 70% lower in the low-dose NaFeEDTA group, but did not change for children given electrolytic iron. Prevalence of iron-deficiency anaemia was reduced by 89% in the high-dose NaFeEDTA group and by 48% in the low-dose NaFeEDTA group, but was more prevalent in children given electrolytic iron than those given placebo.

Children with iron deficiency or iron-deficiency anaemia at baseline benefited more from fortification with NaFeEDTA than children without such deficiencies (figure 4). In children with iron-deficiency anaemia at baseline, consumption of high-dose NaFeEDTA, low-dose NaFeEDTA, and electrolytic iron changed haemoglobin concentration by 11.7 g/L, 8.5 g/L, and -0.4 g/L, respectively, whereas for other children haemoglobin changed by 3.4 g/L, 0.5 g/L, and -1.2 g/L (differences in effect estimates, 95% CI; 8.4 g/L, 2.3–14.4; 8.0 g/L, 2.5–13.6; 0.7 g/L, -5.1 to 6.6). We noted similar effect modification for plasma ferritin and soluble transferrin receptor. Neither α -thalassaemia genotype, sickling test result, nor malaria antigenaemia at baseline seemed to alter the effect of fortified flour consumption. We did not record any adverse events associated with NaFeEDTA consumption.

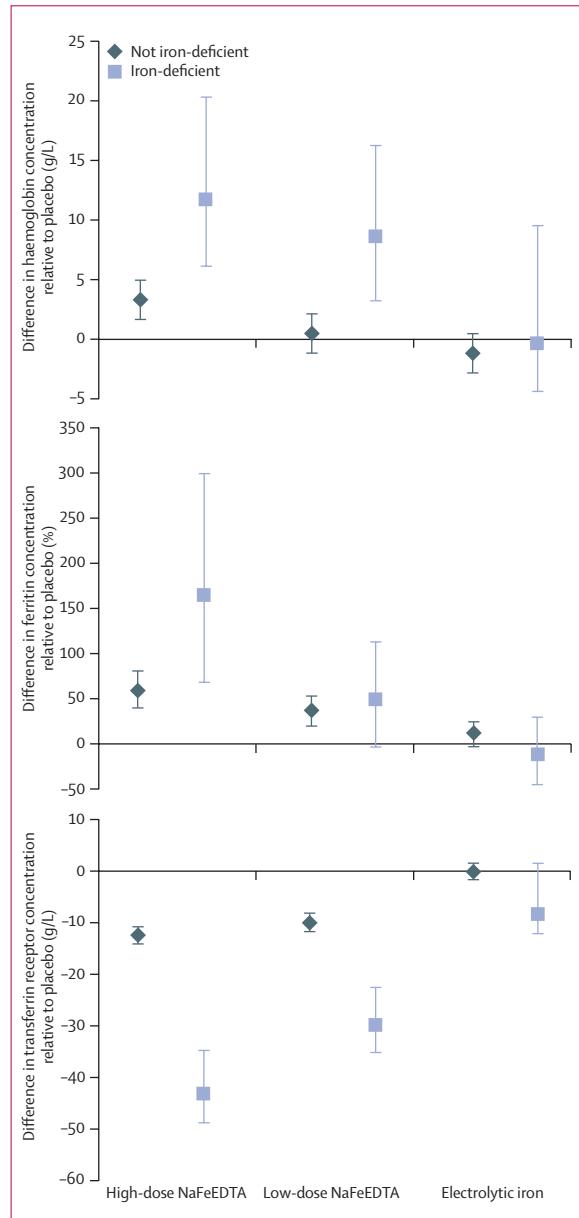


Figure 4: Effect of consumption of iron-fortified maize flour fortification on concentrations of haemoglobin, plasma ferritin, and plasma soluble transferrin receptor, by degree of iron sufficiency in children at baseline. Flour was fortified with 56 mg/kg NaFeEDTA (high-dose) and 28 mg/kg NaFeEDTA (low-dose) and 56 mg/kg electrolytic iron. Line bars indicate 95% CIs.

Results of an additional per-protocol complete-case analysis did not differ from those obtained with imputed values as reported here (data not shown).

Discussion

Consumption of whole maize flour fortified with high-dose NaFeEDTA reduced iron-deficiency anaemia, iron deficiency, and anaemia in Kenyan children. Our findings show that low-dose NaFeEDTA conferred protection against iron deficiency, but not against iron-

deficiency anaemia or anaemia. Electrolytic iron did not confer protection against any of these disorders. Consumption of both high-dose and low-dose NaFeEDTA improved the iron status of children, as indicated by their increased concentrations of haemoglobin and plasma ferritin, and decreased concentrations of soluble transferrin receptor. Treatment effects were most pronounced in children who had iron deficiency and iron-deficiency anaemia at baseline.

Haematological measurements at the end of the intervention were valid indicators of iron status because all children received malaria chemotherapy 2 weeks before assessment to avoid inflammation-induced effects on iron-status indicators. We also regarded our effect estimates as accurate indicators of the true values, since we achieved good precision, small loss to follow-up (2%), good compliance, and low likelihood of crossover of treatments.

The lower than expected prevalence of iron deficiency in our study population did not bias our results. Because only a small proportion of anaemic children in our study were iron-deficient, protection against iron deficiency and iron-deficiency anaemia were more pronounced than protection against anaemia. Malaria and α -thalassaemia at baseline were also associated with reduced haemoglobin concentrations, although these factors, in combination with iron deficiency, still did not fully account for the high prevalence of anaemia.

Iron status at baseline modified the effect of NaFeEDTA. In children with iron-deficiency anaemia, the effect of high-dose NaFeEDTA on haemoglobin concentration was more than three times greater than the effect in iron-replete children. Our findings accord with stable isotope studies that suggest that iron absorption becomes more effective as iron stores become depleted.²⁶ By contrast, even in the subgroup analysis, the treatment effect of electrolytic iron on iron status was negligible.

Malaria could have affected our findings in three ways. First, we regarded baseline infection as a potential confounder of treatment effects. Evidence for such confounding was absent, however, because adjustment for malaria antigenaemia did not change effect estimates. Second, baseline infection could have modified the magnitude of the treatment effect. Although we had no evidence for such effect modification, our study design did not allow us to exclude this possibility. Third, malaria episodes during the intervention could have mediated treatment effects—iron interventions can increase susceptibility to malaria²⁷ and conversely malaria might reduce absorption of iron because of inflammation.^{28,29} If malaria reduces iron absorption, then iron interventions would improve iron status more in populations without malaria, and be less effective in populations with high endemic malaria.

We concluded that fortification of whole maize flour with electrolytic iron does not improve iron status, at least in the concentration and form used in this study.

But this does not necessarily contradict the finding that electrolytic iron can improve iron status when consumed with low-extraction wheat flour.⁶ Extraction reduces the phytate content of flour,³⁰ which might increase bioavailability of iron. Discrepancies in results can furthermore be explained by differences in the morphology, but not the size, of iron particles. We used iron particles with a median diameter of 34 μm (10th–90th percentile: 14–62 μm); those used by Zimmermann and co-workers⁶ were of a similar size (28 μm [10–56 μm]; Zimmermann, Swiss Federal Institute for Technology and Wageningen University; personal communication). Although iron particles used in our study had similar morphological characteristics to those used by Zimmermann and co-workers (see webfigures 1 and 2), we cannot exclude the possibility that the surface area to weight ratio was different.

With continuous iron interventions, iron stores increase rapidly and after 2–3 years reach plateau values that depend on the absorbable iron that is supplied.³¹ Thus we think that continued intervention beyond 5 months would eventually have led to an even greater discrepancy between the treatment effects associated with NaFeEDTA and electrolytic iron.

The most relevant potential adverse effect of protracted administration of large quantities of an EDTA complex is zinc deficiency,³² as reported in studies with daily intakes exceeding 1 g EDTA per kg bodyweight as Na₂EDTA.³³ However, fortification of food with NaFeEDTA seems to have no detrimental effect on the metabolism of zinc, copper, calcium, or magnesium, and might even enhance zinc status by improving zinc absorption.^{32,34,35} The safety of NaFeEDTA at the doses used in our trial will need to be confirmed.

Contributors

HV, SJMO, and CEW participated in the conception and design of the study; PEAA, RA, DLM, HV, and SJMO executed the study; CAW ensured the quality control of the flour; RK did biochemical analyses; and PEAA and HV analysed data and wrote the manuscript, with input from all authors (HV, SJMO, CEW, PEAA, RA, DLM, HV, CAW, RK, and FK). CEW died on August 27, 2004; he is deeply missed by friends and colleagues, to whom he was an inspiration.

Conflict of interest statement

CAW is an employee of Akzo Nobel Chemicals, the Netherlands; SJMO is an employee of Unilever, Netherlands. Other authors (DLM, RA, RK, FK, and HV) declare that they have no conflicts of interest.

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See Online for webfigure 2

References

- 1 Philar R, Johnson Q. Small scale mill fortification manual. Ranum P, Wesley AS, eds. Ottawa, Canada: The Micronutrient Initiative, 2005.
- 2 Micronutrient Initiative/Unicef. Vitamin and mineral deficiencies: a global progress report. Ottawa, Canada: The Micronutrient Initiative, 2004. <http://www.micronutrient.org/resources/publications/VMD/VMd%20GPR%20English.pdf> (accessed Jan 16, 2007).
- 3 Hurrell R, Bothwell T, Cook JD, et al. The usefulness of elemental iron for cereal flour fortification: a SUSTAIN Task Force report. *Nutr Rev* 2002; **60**: 391–406.
- 4 Department of Health South Africa. Foodstuffs, cosmetics and disinfectants Act, 1972 (Act No. 54 of 1972). Regulations relating to the fortification of certain foodstuffs. Pretoria, South Africa, 2003. <http://www.doh.gov.za/docs/regulations/foodstuff/fortification.html> (accessed Jan 25, 2007).
- 5 Nestel P, Lalubola R, Sivakaneshan R, Wickramasinghe A, Atukorala S, Wickramanayake T. The use of iron-fortified wheat flour to reduce anaemia among the estate population in Sri Lanka. *Int J Vit Nutr Res* 2004; **74**: 35–51.
- 6 Zimmermann M, Winichagoon P, Gowachirapant S, et al. Comparison of the efficacy of wheat-based snacks fortified with ferrous sulphate, electrolytic iron, or hydrogen-reduced elemental iron: randomized, double-blind, controlled trial in Thai women. *Am J Clin Nutr* 2005; **82**: 1276–82.
- 7 Hurrell RF. How to ensure adequate iron absorption from iron-fortified food. *Nutr Rev* 2002; **60**: 7–15.
- 8 Hurrell RF, Reddy M, Burri J, Cook JD. An evaluation of EDTA compounds for iron fortification of cereal-based foods. *Br J Nutr* 2000; **84**: 903–10.
- 9 Lamparelli R, MacPhail A, Bothwell T, et al. Curry powder as a vehicle for iron fortification: effects on iron absorption. *Am J Clin Nutr* 1987; **46**: 335–40.
- 10 Garby L, Areekul S. Iron supplementation in Thai fish-sauce. *Ann Trop Med Parasitol* 1974; **68**: 467–76.
- 11 Ballot D, MacPhail A, Bothwell T, Gillooly M, Mayet F. Fortification of curry powder with NaFe(III)EDTA in an iron-deficient population: report of a controlled iron-fortification trial. *Am J Clin Nutr* 1989; **49**: 162–69.
- 12 Huo J, Sun J, Miao H, et al. Therapeutic effects of NaFeEDTA-fortified soy sauce in anaemic children in China. *Asia Pac J Clin Nutr* 2002; **11**: 123–27.
- 13 Davidsson L, Dimitriou T, Boy E, Walczyk T, Hurrell RF. Iron bioavailability from iron-fortified Guatemalan meals based on corn tortillas and black bean paste. *Am J Clin Nutr* 2002; **75**: 535–39.
- 14 Mbogo C, Snow R, Kaburu E, et al. Low-level *Plasmodium falciparum* transmission and the incidence of severe malaria infections on the Kenyan coast. *Am J Trop Med Hyg* 1993; **49**: 245–53.
- 15 Mboera LE, Fanello CI, Malima RC, et al. Comparison of the Paracheck-Pf test with microscopy, for the confirmation of *Plasmodium falciparum* malaria in Tanzania. *Ann Trop Med Parasitol* 2006; **100**: 115–22.
- 16 Van den Broek I, Hill O, Gordillo F, et al. Evaluation of three rapid tests for diagnosis of *P. falciparum* and *P. vivax* malaria in Colombia. *Am J Trop Med Hyg* 2006; **75**: 1209–15.
- 17 Guthmann JP, Ruiz A, Priotto G, Kiguli J, Bonte L, Legros D. Validity, reliability and ease of use in the field of five rapid tests for the diagnosis of *Plasmodium falciparum* malaria in Uganda. *Trans Roy Soc Trop Med Hyg* 2002; **96**: 254–57.
- 18 Wieringa FT, Dijkhuizen MA, West CE, Northrop-Clewes CA, Muhilal. Estimation of the effect of the acute phase response on indicators of micronutrient status in Indonesian infants. *J Nutr* 2002; **132**: 3061–66.
- 19 Liu YT, Old JM, Miles K, Fisher CA, Weatherall DJ, Clegg JB. Rapid detection of α-thalassaemia deletions and α-globin gene triplication by multiplex polymerase chain reactions. *Br J Haematol* 2000; **108**: 295–99.
- 20 WHO. Iron deficiency anaemia assessment, prevention and control: a guide for programme managers. Geneva, Switzerland: World Health Organization, 2001.
- 21 Van der Heijden GJ, Donders AR, Stijnen T, Moons KG. Imputation of missing values is superior to complete case analysis and the missing-indicator method in multivariable diagnostic research: a clinical example. *J Clin Epidemiol* 2006; **59**: 1102–09.
- 22 Unnebrink K, Windeler JW. Intention-to-treat: methods for dealing with missing values in clinical trials of progressively deteriorating diseases. *Stat Med* 2001; **20**: 3931–46.
- 23 Lee J, Chia K. Estimation of prevalence rate ratios for cross-sectional data: an example in occupational epidemiology. *Br J Ind Med* 1993; **50**: 861–62.
- 24 Lee J. Odds ratio or relative risk for cross-sectional data? *Int J Epidemiol* 1995; **24**: 464–65.
- 25 Barros AJ, Hirakata VN. Alternatives for logistic regression in cross-sectional studies: an empirical comparison of models that directly estimate the prevalence ratio. *BMC Med Res Methodol* 2003; **3**: 21.
- 26 Hallberg L. Perspectives on iron absorption. *Ann Rev Nutr* 2001; **21**: 1–21.
- 27 Sazawal S, Black RE, Ramsan M, et al. Effects of routine prophylactic supplementation with iron and folic acid on admission to hospital and mortality in preschool children in a high malaria transmission setting: community-based, randomised, placebo-controlled trial. *Lancet* 2006; **367**: 133–43.
- 28 Ganz TT. The role of hepcidin in iron sequestration during infections and in the pathogenesis of anemia of chronic disease. *Isr Med Assoc J* 2002; **4**: 1043–45.
- 29 Nemeth E, Ganz T. Regulation of iron metabolism by hepcidin. *Ann Rev Nutr* 2006; **26**: 323–42.
- 30 Feil B. Phytic acid. *J New Seeds* 2001; **3**: 1–35.
- 31 Hallberg L, Hulthen L, Garby L. Iron stores and haemoglobin iron deficits in menstruating women. Calculations based on variations in iron requirements and bioavailability of dietary iron. *Eur J Clin Nutr* 2000; **54**: 650–57.
- 32 International Nutritional Anemia Consultative Group. Iron EDTA for food fortification. Washington DC, USA: The Nutrition Foundation, INACG Secretariat, 1993.
- 33 Swenerton H, Hurley LS. Teratogenic effects of a chelating agent and their prevention by zinc. *Science* 1971; **173**: 62–64.
- 34 Davidsson L, Kastenmayer P, Hurrell RF. Sodium iron EDTA [NaFe(III)EDTA] as a food fortificant: the effect on the absorption and retention of zinc and calcium in women. *Am J Clin Nutr* 1994; **60**: 231–37.
- 35 Davidsson L, Ziegler E, Zeder C, Walczyk T, Hurrell R. Sodium iron EDTA [NaFe(III)EDTA] as a food fortificant: erythrocyte incorporation of iron and apparent absorption of zinc, copper, calcium, and magnesium from a complementary food based on wheat and soy in healthy infants. *Am J Clin Nutr* 2005; **81**: 104–09.