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Maize Porridge Enriched with a Micronutrient Powder Containing Low-Dose Iron as NaFeEDTA but Not Amaranth Grain Flour Reduces Anemia and Iron Deficiency in Kenyan Preschool Children[1–3]

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Abstract

Few studies have evaluated the impact of fortification with iron-rich foods such as amaranth grain and multi-micronutrient powder (MNP) containing low doses of highly bioavailable iron to control iron deficiency anemia (IDA) in children. We assessed the efficacy of maize porridge enriched with amaranth grain or MNP to reduce IDA in Kenyan preschool children. In a 16-wk intervention trial, children (n = 279; 12–59 mo) were randomly assigned to: unrefined maize porridge (control; 4.1 mg of iron/meal; phytate:iron molar ratio 5:1); unrefined maize (30%) and amaranth grain (70%) porridge (amaranth group; 23 mg of iron/meal; phytate:iron molar ratio 3:1); or unrefined maize porridge with MNP (MNP group; 6.6 mg iron/meal; phytate:iron molar ratio 2.6:1; 2.5 mg iron as NaFeEDTA). Primary outcomes were anemia and iron status with treatment effects estimated relative to control. At baseline, 38% were anemic and 30% iron deficient. Consumption of MNP reduced the prevalence of anemia [−46% (95% CI: −67, −12)], iron deficiency [−70% (95% CI: −89, −16)], and IDA [−75% (95% CI: −92, −20)]. The soluble transferrin receptor [−10% (95% CI: −16, −4)] concentration was lower, whereas the hemoglobin (Hb) [2.7 g/L (95% CI: 0.4, 5.1)] and plasma ferritin [40% (95% CI: 10, 95)] concentrations increased in the MNP group. There was no significant change in Hb or iron status in the amaranth group. Consumption of maize porridge fortified with low-dose, highly bioavailable iron MNP can reduce the prevalence of IDA in preschool children. In contrast, fortification with amaranth grain did not improve iron status despite a large increase in iron intake, likely due to high ratio of phytic acid:iron in the meal. J. Nutr. 142: 1756–1763, 2012.

Introduction

Iron deficiency anemia (IDA)[8] is a major public health problem in young children in developing countries and is associated with impaired cognitive development and morbidity (1). Supplementation has successfully been used to improve iron status in Kenya (2,3). However, following the findings that supplementation with iron and folic acid may be associated with adverse effects in iron-replete children (4), the WHO advised that in malaria endemic areas, iron supplementation with doses such as the entire reference nutrient intake should be administered only to those who are at risk of iron deficiency (ID) (5). This recommendation extends to the untargeted use of multi-micronutrient powders (MNP) containing dosages in the range of the iron reference nutrient intake so as to avoid the potential adverse effects of a large bolus of iron taken in a single dose/meal (5). Screening for ID is, however, not always logistically feasible in resource-poor settings (6). Iron fortification of foods has been shown to be an effective strategy to alleviate IDA among school-age children and infants (7–10). Providing iron at low dosages in forms that are more bioavailable or increasing iron intake through dietary interventions are 2 promising strategies to overcome this problem.

Home fortification with MNP can be incorporated into the existing feeding practices and ready-to-eat foods, such as

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[3] This trial was registered at clinicaltrials.gov as NCT01224535.
[8] Abbreviations used: Hb, hemoglobin; ID, iron deficiency; IDA, iron deficiency anemia; MNP, multi-micronutrient powder; PF, plasma ferritin; RDT, rapid diagnostic test; TfR, soluble transferrin receptor.
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porridge, just before consumption. MNP with either a low (11,12) or high (13–15) dose of iron have been shown to improve iron status in children with or without malnutrition (16). Iron fortification with low dosages of iron require optimized bioavailability; however, cereal- and legume-based porridges used as children's foods are rich in phytate. The addition of exogenous phytase to low-dose, highly absorbable iron MNP has been shown to improve iron status (11). Similarly, the use of NaFeEDTA has been shown to be effective in improving iron status when used to fortify high phytate foods (9). Home fortification with MNP with low dosages of bioavailable iron as NaFeEDTA without phytase may therefore be a promising strategy to improve iron status among children.

Dietary diversification can promote the use of locally available and acceptable mineral-rich foods (17). Diets enriched with animal-source foods have been shown to improve growth (18) and iron status (19). However, to our knowledge, limited evidence exists (20) on the effectiveness of iron-rich, plant-source foods in improving iron status, even if there have been promotional activities on their use. The amaranth grain (*Amaranthus cruentus*) contains high iron concentrations and is a drought-resistant, fast-maturing crop suitable for cultivation in semi-arid areas (21). Amaranth grain-enriched maize porridge was found to be acceptable at a ratio of 70% amaranth:30% maize (22). Food diversification with amaranth grain, which has been promoted in Kenya as a nutrient-dense food, may potentially improve iron status among children. The aim of this study was therefore to investigate the efficacy of consumption of maize porridge enriched with amaranth grain or a low dose iron-containing micronutrient powder on anemia and iron status of Kenyan preschool children. It is expected that this data may assist in the informed decision-making process on the appropriate strategies to combat ID in a semi-arid rural Kenya.

Participants and Methods

Study design, site, and participants. The study was a randomized, partially blinded, controlled trial conducted over a period of 16 wk. The study was conducted from October 2010 to February 2011 in the Migwani Division, Mwingi District, Kenya. Migwani, situated 200 km northeast of Nairobi, was purposively selected, because it falls within an agro-ecological zone in a semiarid area that experiences food shortage for most parts of the year. Children aged 12–59 mo were assigned to either unrefined plain maize porridge (control group), unrefined maize porridge enriched with amaranth grain flour at the ratio of 30% maize flour:70% amaranth (amaranth group), or unrefined maize porridge fortified at the time of consumption with a low dose of iron from a MNP containing 2.5 mg iron in the form of NaFeEDTA and other micronutrients such as vitamin A, vitamin C, vitamin B-12, and folic acid; MNP group) (**Table 1**). Informed verbal and written consent was obtained from the principal caretaker/parent. The study was approved by the ethical review committees of Kenyatta National Hospital and Wageningen University. A research permit was obtained from the Ministry of Higher Education, Science and Technology, Kenya.

Sampling procedure and sample size calculation. Children in 2 of 6 randomly chosen administrative locations within the Migwani Division, Migwani and Nzauni, were selected using the random walk method (23). The inclusion criteria were: age between 12 and 59 mo, apparently healthy at the time of entry into the study, and having lived in the village for at least 6 mo prior to the intervention and continuing to live there for the next 1 y. A sample size of 90/group was sufficient to detect an increase in hemoglobin (Hb) concentration of 4 g/L with an SD of 9 (9), assuming a power of 80% and an attrition of 12% (24). A total of 306 children invited into the study were screened and 279 qualified for the study. The 279 children were randomly allocated using block randomization by age and sex generated with Excel (Microsoft) by one investigator not involved in recruitment and data collection to 1 of the 3 treatment groups (**Fig. 1**).

TABLE 1 Composition of the micronutrient powder used in the intervention study

Nutrients	Amount per 1-g sachet
Retinyl palmitate, *μg RE*	100
Cholecalciferol, *μg*	5
1-α tocopheryl acetate, *mg TE*	5
Phylloquinone, *μg*	30
Thiamin, *mg*	0.5
Riboflavin, *mg*	0.5
Pyridoxine, *mg*	0.5
Folic acid, *μg*	90
Niacin, *mg*	6
Vitamin B-12, *μg*	0.9
Vitamin C, *mg*	60
Iron (as NaFeEDTA), *mg*	2.5
Zinc, *mg*	2.5
Selenium, *μg*	17
Copper, *mg*	0.34
Iodine, *μg*	30

Screening procedure. A questionnaire was used to obtain background information for all the children. All the children underwent a clinical examination and anthropometric measurements were taken. Those who had not been dewormed within the last 3 mo prior to the start of the study were dewormed. Children aged ≥2 y received 20 mL of Zentel containing 400 mg albendazole (Glaxo Smithkline), whereas children <2 y received one-half that dose. Only those children who met the inclusion criteria and provided blood samples were included in the study.

Intervention procedures. Both maize and amaranth grain flour were produced and packed into 2-kg packets by East Africa Nutraceuticals. DSM Nutritional Products produced the MNP (Table 1). The target daily intake was 350 mL of porridge for all the children, considered to be an amount that they could comfortably consume in one session. Each serving of unrefined maize porridge contained ~60 g flour. A serving of unrefined, amaranth-enriched maize porridge contained 24 g maize flour and 56 g amaranth flour. Both flours were precooked, extending their shelf life and reducing the cooking time in the field to 15 min. The 2 types of porridge were cooked at 3 different cooking centers from where they were distributed in thermo flasks to 7 additional centers for feeding. Before distributing the porridge to the feeding centers, one teaspoon (5 g) of sugar was added to all the serving bowls labeled with the child's name and identification number. In addition, the MNP was also added to the bowls for those children in MNP group. Similar serving cups equivalent to 350 mL of the porridge were used to serve in all the centers. The porridge was served between 0800 and 1100 h. Attendance and leftovers were recorded daily.

Iron and phytic acid concentration determination. Two samples of maize/amaranth flour and 3 for plain maize flour were sampled from the flour batches for iron and phytic acid concentration determination. In the laboratory, ~10 g for iron and 10 g for phytate analysis were taken from each packet, put in an airtight container, and stored at −20°C until analysis at Wageningen University. The iron concentration was determined using microwave digestion atomic emission spectrometry (VISTA-PRO CCD Simultaneous ICP-AES, Varian) as described by Novozamsky et al. (25). The phytic acid concentration was analyzed as described by Makower (26) with modifications (27). The phosphate concentration was measured according to van Veldhoven et al. (28) and the results are expressed as grams total phytic acid/100 g dry matter (27). The difference of duplicate sample relative to the mean value was <10%.

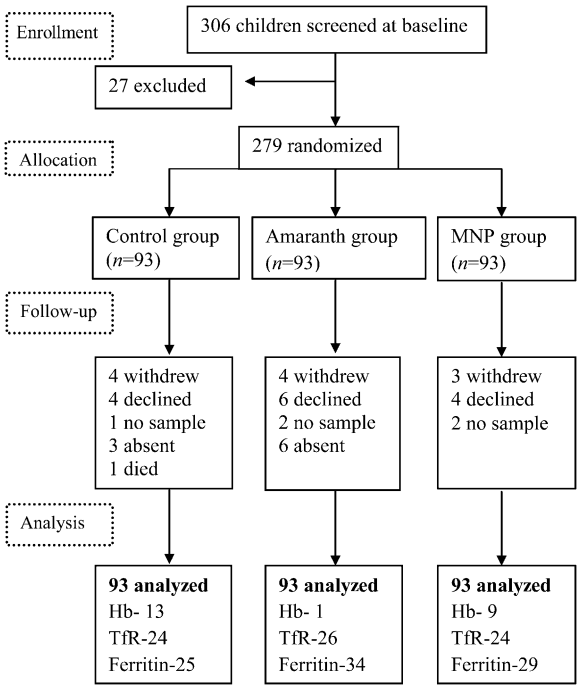

FIGURE 1 Trial profile and group allocation of the participating preschool children. Amaranth group is maize porridge enriched with amaranth grain flour at the ratio of 30% maize flour: 70% amaranth and MNP group is maize porridge fortified with a low iron-containing MNP (2.5 mg iron in the form of NaFeEDTA). Excluded children fulfilled the inclusion criteria, but no blood samples could be withdrawn. Values for Hb, TfR, and ferritin in the different groups represent the number of imputed missing values either at baseline or postintervention, including children who had samples for one or more biochemical analysis but insufficient blood samples to run all biochemical analysis. MNP, multi-micronutrient powder; TfR, soluble transferrin receptor.

Anthropometric measurements. Weight measurements were taken to the nearest 0.1 kg with a UNICEF Salter scale or a digital bathroom scale (Ashton Meyers Model no 7752) with a capacity of 25 and 150 kg, respectively. The bathroom scale was only used if a child refused to put on the provided weighing pants or if they did not fit. Standing height (children >2 y) or recumbent length (children <2 y) was measured to the nearest 0.1 cm on a wooden height/length board (UNICEF model, item no. 0114500). Weight and height measurements were done twice and the average used for further analysis. Age was calculated from the birth date reported on clinic cards or, in rare cases, by parents/principal caretaker recall. Anthropometric Z-scores were calculated using the 2006 WHO growth standards (WHO_ANTHRO, version 3.2.2). Stunting, underweight, or wasting was defined by Z-score < −2 SD for anthropometric indices (29). One child was excluded from the nutrition status analysis due to a biologically implausible height value at baseline (30).

Malaria test. All children were tested for malaria infection at baseline with a rapid diagnostic parasite lactate dehydrogenase test kit (OptiMAL-IT). Due to supply problems, 3 different rapid diagnostic test (RDT) kits (OptiMAL-IT, Carestart, or Carestart PF/VOM Combo) were used at endpoint, but only one type of the test kit was used per child, which was dependent on the date of final sample collection. Thick blood smear slides were also prepared for all samples at endpoint and microscopy to examine the presence or absence of malaria parasites was performed at the Kenya Medical Research Institute, Nairobi.

Laboratory analysis. Nonfasting venous blood samples were collected by venipuncture. The Hb concentration was determined using the Sysmex hematological analyzer (KX-21, Sysmex). Heparinized blood samples (tubes by Becton-Dickinson) were stored in cool boxes with ice packs in the field. Within 6 h after collection, the samples were centrifuged at 3000 × *g* for 10 min and separation was done at Migwani hospital. Plasma was then stored in a freezer at −20°C for 2–3 d before transportation to Nairobi for further analysis. Plasma concentrations of ferritin (PF), C-reactive proteins (CRP), and soluble transferrin receptor (TfR) were analyzed using fully automated Cobas Integra analyzers (Roche Diagnostics) at the Pathologists Lancet Kenya laboratory, Nairobi. The laboratory assay CV were <10% with daily internal quality controls and monthly quality control performed by thistle.

Anemia was defined as an Hb concentration <110 g/L (31). To adjust for anemia prevalence at an altitude of 1250 m, 2.4 g/L of Hb was subtracted from each individual (32). ID was defined as a PF concentration <12 μg/L in the absence of elevated CRP (31) or a TfR concentration >8.3 mg/L (test kit reference value). IDA was defined as concurrent anemia and ID. Body iron (in mg Fe/kg body weight) was calculated using a published algorithm (33). For body iron calculation, we first converted the TfR concentrations to ELISA assay equivalents using the regression equation by Pfeiffer et al. (34). Elevated acute phase protein was defined as CRP >5 mg/L (35). A correction factor of 0.67 for those with elevated CRP was used to yield an adjusted PF concentration representative for a child without acute inflammation (35).

Statistical analysis. Analysis was done by intention to treat. Data were analyzed using PASW Statistics version 18.0.3 (IBM) and PROC MIANALYZE in SAS version 9.2 (SAS Institute). Missing data values of Hb, ferritin, and TfR were imputed before primary analysis using multiple imputations. The data were imputed 5 times using the fully conditional specification method with the default PASW Statistics initialization value (36). Treatment group, number of days attended, sex, age, baseline, and postintervention weight, height, Hb, PF, and TfR concentrations were used as predictors in the imputation model. Pooled estimates from the imputed data are reported.

ANCOVA with planned contrasts was used to estimate treatment effects relative to control group for continuous outcomes, because it has more power in a model that includes covariates to detect true effects (37). Cox regression with robust variance estimates was used to estimate treatment effects relative to control group for binary outcomes with constant time at risk (38). The Cox regression was preferred to logistic regression, because the prevalence ratio is easier to interpret than OR and the robust variance estimate reduces the width of the CI resulting from the use of prevalence data that follows a binomial distribution (38). Exponents of the prevalence ratios obtained by Cox regression as well as effect estimates obtained from log-transformed data were converted to percentage differences relative to the control group. Adjusted estimates were analyzed, including baseline Hb, PF, and TfR concentrations as covariates. A post hoc subgroup analysis was done to assess whether the magnitude of the treatment effect was influenced by ID at baseline. Chi-square and *t* tests were used to assess the differences between and within groups. We also performed per-protocol analysis to check whether the results of imputation and cases with complete data differed. $P < 0.05$ (95% CI) was considered significant.

Results

A total of 239 children completed the study, equivalent to 86% of the children randomized at baseline. Eleven children withdrew within the first month of the study and one child died from causes unrelated to the study as judged by the ethics committee. The endpoint measurement for biochemical indicators was not done for 19 children, because either their veins could not be detected ($n = 5$) or their caretakers declined ($n = 14$). Nine children were absent for the end measurement (**Fig. 1**). The baseline characteristics of these children did not differ from those of the children who completed the study (data not shown).

The iron and phytic acid concentration in the amaranth-fortified maize flour was 29 and 960 mg/100 g dry matter, respectively, with a phytate:iron molar ratio of 3:1, whereas that of maize flour only was 7 and 418 mg/100 g dry matter,

respectively, with a phytate:iron molar ratio of 5:1. The amaranth porridge was estimated to provide an additional 18.9 mg of iron/meal and the MNP provided an additional 2.5 mg of iron/meal to the 4.1 mg iron/meal provided by plain maize porridge with a phytate:iron molar ratio 2.6:1. The total number of feeding days ranged from 92 to 96, depending on the serving station, with an average attendance of 83.7%. On average, 345 mL of porridge was eaten during the feeding session, 6 d/wk in a period of 16 wk across all the groups.

In the baseline characteristics, only the incidence of elevated CRP differed in the amaranth and MNP groups compared with control ($P < 0.05$) (**Table 2**). The prevalence of elevated CRP at endpoint was not significantly higher for both the amaranth (13.9%) and MNP (9.4%) groups and did not differ across groups (data not shown). No malaria was reported at baseline, whereas at endpoint, microscopy showed that 3.8% of the children had malaria, which did not differ across the groups. About one-quarter (24.7%) of the children had been dewormed 6 mo prior to commencing the study.

Postintervention, the prevalence of underweight was lower in both the amaranth (12.7%) and MNP (17.9%) groups but did not differ relative to the control group (21.3%). Hb and PF concentrations significantly increased, thereby reducing the prevalence of anemia, ID, and IDA across all the groups (data not shown). The increments in Hb ($P < 0.05$) and PF ($P < 0.01$) concentrations and the decrease in TfR concentrations ($P < 0.01$) in the MNP group, but not the amaranth group, significantly differed from those in the control group (**Table 3**). The reduction in prevalence of anemia, ID, and IDA in the MNP group ($P < 0.05$), but not the amaranth group, also significantly differed from those in the control group.

Relative to control, Hb increased whereas TfR concentrations decreased ($P < 0.01$) among children with ID at baseline in the MNP group (**Fig. 2**). PF concentrations increased by 49% among children in the MNP group who were not iron deficient at baseline ($P < 0.05$). The Hb concentration modestly increased among children who were iron deficient at baseline in the amaranth group but did not differ from the control group. A per-protocol, complete-case analysis revealed a similar trend to the imputed data analysis for all results presented.

Discussion

The addition of amaranth grain flour to maize-based porridge did not have a significant treatment effect on anemia and iron status among children. Relative to control, Hb and PF concentrations significantly increased, whereas TfR concentrations significantly decreased in the MNP group. The prevalence of anemia, ID, and IDA also significantly decreased in the MNP group.

Although the amaranth porridge iron concentration was high compared with that of maize flour, it did not affect anemia or iron status. The amaranth porridge phytate:iron molar ratio was high compared with the preferred <0.4:1 in meals without enhancers and could explain the low iron absorption from the porridges (39). Phytate removal, addition of an enhancer (ascorbic acid), or phytic acid degradation by adding exogenous phytase has been suggested to improve iron absorption from high phytic acid-containing foods (39–41). However, the addition of a phytase for home-prepared complementary foods may be limited due to legislation issues on the use of genetically modified organisms (41). Fermentation could also not be used, because a previous study showed that fermented porridge was not acceptable in the population (22).

The ID and IDA prevalence in the MNP group was 75% lower than in the control group. This finding concurs with a meta-analysis concluding that MNP are as effective as supplementation at reducing anemia prevalence (42), a study among South African children where ID decreased by 30% after using an MNP (11), and other home and centralized fortification efficacy studies in Africa (9,13,43). Porridge with MNP did improve Hb and TfR concentrations, particularly in iron-

TABLE 2 Baseline characteristics of Kenyan preschool children by intervention group[1]

	Control	Amaranth group	MNP group
n	93	93	93
Sex (males), *n (%)*	46 (49.5)	40 (43)	48 (51.6)
Age, *mo*	37.3 ± 12.3	37.5 ± 14.1	35.8 ± 10.1
Height for age, *Z-score*	−2.0 ± 1.2	−1.8 ± 1.0	−1.9 ± 1.2
Weight for age, *Z-score*	−1.2 ± 1.0	−1.2 ± 1.0	−1.3 ± 1.0
Weight for height, *Z-score*	−0.2 ± 1.1	−0.3 ± 1.0	−0.3 ± 1.2
Stunted,[2] *n (%)*	51 (54.8)	40 (43)	43 (47.3)
Underweight,[2] *n (%)*	20 (21.5)	21 (22.6)	23 (24.7)
Wasted,[2] *n (%)*	5 (5.4)	2 (2.2)	2 (2.2)
Hb, *g/L*	112 ± 11	112 ± 12	111 ± 11
TfR, *mg/L*	5.0 (4.6, 5.4)	5.3 (4.9, 6.7)	5.3 (4.9, 5.7)
PF, *μg/L*	17.6 (14.9, 20.8)	18.1 (15.3, 21.4)	16.1 (13.6, 19)
Body iron,[3] *mg/kg*	0.9 (0.02,1.9)	0.8 (−0.1, 1.7)	0.4 (−0.5, 1.3)
Inflammation (CRP >5 mg/L), *n (%)*	15 (16.5)	7 (7.7*)	3 (3.4*)
Anemia,[4] *n (%)*	33 (35.5)	36 (38.7)	38 (40.9)
ID,[5] *n (%)*	23 (24.7)	30 (32.3)	30 (32.3)
IDA,[6] *n (%)*	14 (15.1)	22 (23.7)	24 (25.8)

[1] Values are mean ± SD, geometric mean (95% CI), or *n* (%).*Different from control, $P < 0.05$. ID, iron deficiency; IDA, iron deficiency anemia; Hb, hemoglobin; MNP, multi-micronutrient powder; PF, plasma ferritin; TfR, soluble transferrin receptor.
[2] Defined as Z-score < −2.
[3] To convert body iron in mg/kg to mmol/kg, multiply by 0.0171 (11).
[4] Defined as Hb concentration <110 g/L and adjusted for altitude (31,32).
[5] Defined as PF concentration <12 μg/L (31) or TfR >8.3 mg/L and adjusted for presence of infection (35).
[6] Defined as concurrent anemia and ID.

TABLE 3 Effects of amaranth and MNP-fortified porridge consumption on iron status among preschool children in Kenya[1]

	Effect estimates of biomarkers concentration		
	Control	Amaranth group	MNP group
n	93	93	93
Endpoint Hb, *g/L*	115 ± 11	115 ± 11	117 ± 9
Crude	Reference	0.59 (−2.5, 3.7)	2.1 (−1.0, 5.2)
Adjusted[2]	Reference	1.12 (−1.2, 3.4)	2.7 (0.4, 5.1)*
Endpoint PF,[3] *μg/L*	25.5 (20.3, 32)	26.8 (21.2, 34)	36 (29.4, 44)
Crude[4]	Reference	5 (−27, 48)	41 (4, 90)*
Adjusted[2,4]	Reference	4 (−28, 49)	40 (10, 95)*
Endpoint TfR,[3] *mg/L*	4.8 (4.4, 5.2)	5.0 (4.6, 5.3)	4.4 (4.1, 4.7)
Crude[4]	Reference	4 (−6, 15)	−7 (−15, 2)
Adjusted[2,4]	Reference	0.4 (−8, 10)	−10 (−16, −4)*
Anemia,[5] *n (%)*	30 (32.3)	31 (33.3)	18 (19.4)
Crude[6]	Reference	3 (−33, 57)	−41 (−66, 3)
Adjusted[2,6]	Reference	−8 (−36, 32)	−46 (−67, −12)*
ID,[7] *n (%)*	16 (17.2%)	14 (15.1)	5 (5.4)
Crude[6]	Reference	−13 (−62, 101)	−67 (−90, 4)
Adjusted[2,6]	Reference	−17 (−65, 100)	−70 (−89, −16)*
IDA,[8] *n (%)*	11 (11.8)	9 (9.7)	4 (4.3)
Crude[6]	Reference	−14 (−68, 130)	−69 (−92, 25)
Adjusted[2,6]	Reference	−29 (−75, 101)	−75 (−92, −20)*

[1] Values are mean ± SD and effect estimate (95% CI) unless otherwise indicated. *Different from control, $P < 0.05$. Hb, hemoglobin. ID, iron deficiency; IDA, iron deficiency anemia; Hb, hemoglobin; MNP, multi-micronutrient powder; PF, plasma ferritin; TfR, soluble transferrin receptor.
[2] Adjusted for baseline Hb, TfR, and PF.
[3] Values are geometric mean (95% CI).
[4] Values indicate difference between groups expressed as percentage relative to the control group obtained after exponentiation of effect estimates from log-transformed data.
[5] Defined as Hb concentration <110 g/L and adjusted for altitude.
[6] Values indicate percentage difference in prevalence estimates obtained by conversion of prevalence ratios from Cox regression with robust estimates and constant time at risk.
[7] Defined as PF concentration <12 μg/L or TfR >8.3 mg/L, corrected for inflammation if CRP >5 mg/L.
[8] Defined as concurrent anemia and ID.

deficient children. Iron absorption exponentially increases in individuals with ID and the higher effect of the treatment on Hb and TfR in iron-deficient children may be related to their iron status, because newly available iron may be preferentially used for erythropoiesis (44). In contrast, the fortified porridge significantly improved PF concentrations in iron-sufficient children at baseline, indicating that in iron-sufficient participants, newly available iron will be preferentially used to increase iron stores, because erythropoiesis may be downregulated. Findings from subgroup analyses should, however, be interpreted with caution, because the chance of bias is higher (45).

Although the presence of other micronutrients in the fortificant, particularly vitamin A (46), vitamin B-12, and folic acid (47), may have contributed to the increased Hb, the complex interactions and their role in iron absorption are not sufficiently understood (47). Vitamin A may mobilize hepatic iron stores and increase erythropoiesis, resulting in an increased Hb and reduced PF (48). However, in our study, PF increased in the MNP group, especially among anemic children. Furthermore, the evidence supporting the public health importance of vitamin B-12 and folic acid is limited (47) and although not quantified in our study, their effect is likely to be of a smaller magnitude than the effect of EDTA on enhancing iron bioavailability.

Hb and PF concentrations significantly improved in the control group, reducing anemia and ID prevalence. This may be due to a reduction of helminthe burden due to deworming, the availability of mangoes during the study period, and additional native iron, energy, and protein provided by the maize porridge. Intestinal parasite infections, especially hookworms, increase the risk of anemia (49). Deworming is shown to have an effect on iron status and reduces IDA but only when the infection was severe (50). Our baseline data suggest that 75% of children were not dewormed within the preceding 6 mo. Nonetheless, the magnitude of the deworming effect on Hb and iron status is uncertain, because we did not collect information on the parasite load in the study population. A previous study in the same area and season showed that mangoes contributed to 30% of total vitamin C intake and 90% of children had energy intakes below the estimated average requirement (51). The provision of maize porridge could therefore have increased iron intake, whereas the vitamin C from mangoes could have enhanced iron absorption in all the groups (40).

This study has some limitations. First, although the participants were randomly allocated into 3 groups, the study design was partially blinded. The maize-only or maize with MNP porridge were identical in appearance and taste, but it was evident that amaranth porridge was different due to the beige color of amaranth and thinner consistency (22). This difference did not affect the compliance, because no significant difference was shown in attendance and the amount of porridge eaten. Although we did not take samples across the centers during the life of the intervention to analyze for differences in consistency or nutrient profile, no differences were expected, because the cooks were well trained on porridge preparation, which was done in the presence of the research assistant in charge of the center. Furthermore, we did not expect a difference between groups due to not blinding, because the assistants were well

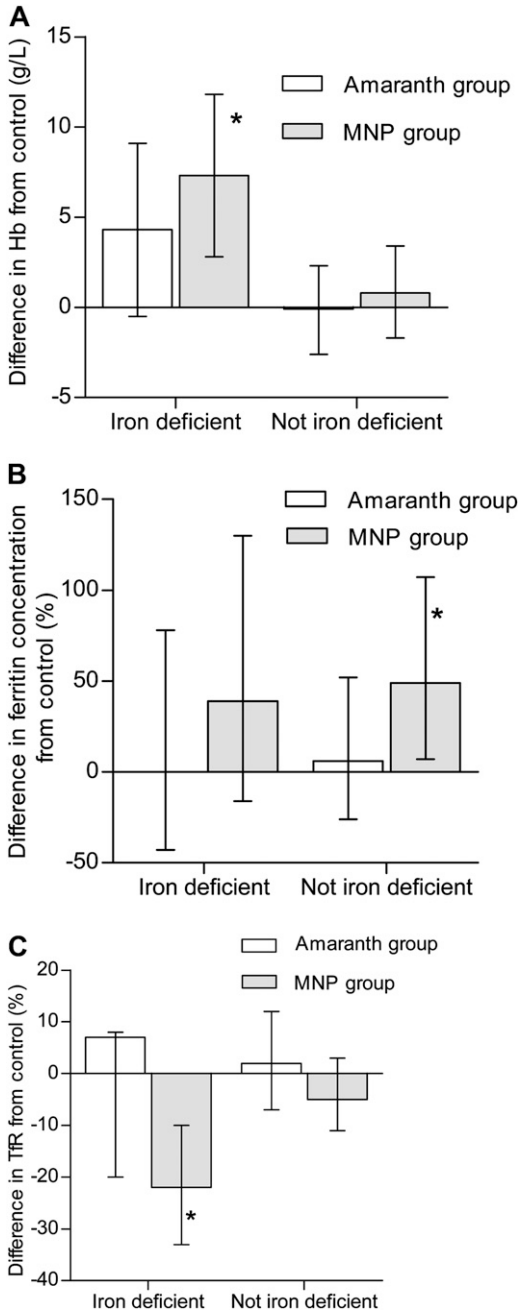

FIGURE 2 Difference from controls in circulating Hb (*A*), ferritin (*B*), and TfR (*C*) concentrations in Kenyan preschool children who consumed porridge fortified with amaranth or MNP for 16 wk and were not iron deficient at baseline. Values are effect estimates adjusted for baseline Hb, PF, and TfR concentrations, with error bars indicating 95% CI, $n = 93$/group. *Different from control $P < 0.05$. Hb, hemoglobin; MNP, multi-micronutrient powder; PF, plasma ferritin; TfR, soluble transferrin receptor.

trained and the supervisors ensured that all absent children were followed-up equally and the laboratory personnel performing the analysis were not aware of the treatment allocations. Second, our study duration was rather short for a food diversification intervention to show significant effects (52). This was particularly true for the amaranth group and we think that with longer duration and a larger sample size, it may be possible that amaranth grain may significantly improve iron status.

Difficulties in collecting blood samples resulted in several missing values, necessitating the imputation of data. The pooled outputs of multiple imputations were used, because they are considered to be better statistical inferences than those provided by a single imputation or complete case analysis (53,54). We used 3 different RDT tests for malaria at endpoint with small differences in sensitivity and specificity but testing a different range of malaria parasites. Although all the RDT results were negative, concurrent microscopy showed that 3.8% of children were positive for malaria infection. This slide-positive/RDT-negative discordance has been reported at low parasite densities (55,56). In this study, only CRP currently recommended by WHO as an independent indicator of the acute phase response (57) was used. It is shown to explain serum ferritin variance better than other markers of inflammation (58). We corrected the PF values for inflammation using correction factors that are better than adjusting the cutoff value upwards or excluding those with inflammation (35,57). Uncertainties on the impact of inflammation may still remain, because the correction retains all cases without completely explaining the variance in inflammation (59).

This study provides evidence that enriching maize porridge with MNP containing 2.5 mg iron has the additional benefit of reducing anemia and ID within a short period. Such a low dose of iron can probably only be effective when in the form of NaFeEDTA. The WHO currently recommend the use of elemental iron for home fortification for children 6–23 mo, while the use of NaFeEDTA is limited to clinical trials (60). Recently, NaFeEDTA has been added to the list of vitamin and mineral substances allowed as food additives in the EU (61). In areas with an underweight prevalence of ~30%, 2 mg of iron as NaFeEDTA is suggested for children 6–24 mo of age (62). The need for legislation in countries such as Kenya is therefore evident to allow the use of NaFeEDTA in children's food, because its efficacy has been shown.

Although this study shows that amaranth grain has the potential to improve iron status, it also confirms that the addition of plant-based foods to the existing diet even when having a high iron concentration may not show significant improvement without the reduction of phytic acid. The addition of MNP in maize porridge is more suitable than dietary diversification with grain amaranth for improving iron status among children and its effectiveness could be further evaluated in large-scale programs.

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Literature Cited

1. Shaw JG, Friedman JF. Iron deficiency anemia: focus on infectious diseases in lesser developed countries. Anemia. 2011;2011:10.1155/2011/260380.
2. Friis H, Mwaniki D, Omondi B, Muniu E, Thiong'o F, Ouma J, Magnussen P, Geissler PW, Michaelsen KF. Effects on haemoglobin of multi-micronutrient supplementation and multi-helminth chemotherapy: a randomized, controlled trial in Kenyan school children. Eur J Clin Nutr. 2003;57:573–9.

3. Verhoef H, West C, Nzyuko S, de Vogel S, van der Valk R, Wanga M, Kuijsten A, Veenemans J, Kok F. Intermittent administration of iron and sulfadoxine-pyrimethamine to control anaemia in Kenyan children: a randomised controlled trial. Lancet. 2002;360:908–14.
4. Sazawal S, Black RE, Ramsan M, Chwaya HM, Stoltzfus RJ, Dutta A, Dhingra U, Kabole I, Deb S, Othman MK, 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.
5. WHO. Conclusions and recommendations of the WHO consultation on prevention and control of iron deficiency in infants and young children in malaria endemic areas. Food Nutr Bull. 2007;28:S621–7.
6. Ojukwu JU, Okebe JU, Yahav D, Paul M. Cochrane review: oral iron supplementation for preventing or treating anaemia among children in malaria-endemic areas. Evid Based Child Health J. 2010;5: 967–1183.
7. Zimmermann MB, Wegmueller R, Zeder C, Chaouki N, Rohner F, Saïssi M, Torresani T, Hurrell RF. Dual fortification of salt with iodine and micronized ferric pyrophosphate: a randomized, double-blind, controlled trial. Am J Clin Nutr. 2004;80:952–9.
8. Moretti D, Zimmermann MB, Muthayya S, Thankachan P, Lee T-C, Kurpad AV, Hurrell RF. Extruded rice fortified with micronized ground ferric pyrophosphate reduces iron deficiency in Indian schoolchildren: a double-blind randomized controlled trial. Am J Clin Nutr. 2006;84:822–9.
9. Andang'o PEA, Osendarp SJM, Ayah R, West CE, Mwaniki DL, De Wolf CA, Kraaijenhagen R, Kok FJ, Verhoef H. Efficacy of iron-fortified whole maize flour on iron status of schoolchildren in Kenya: a randomised controlled trial. Lancet. 2007;369:1799–806.
10. The Chilenje Infant Growth Nutrition and Infection Study Team. Micronutrient fortification to improve growth and health of maternally HIV-unexposed and exposed Zambian infants: a randomized controlled trial. PLoS ONE. 2010;5:e11165.
11. Troesch B, van Stuijvenberg ME, Smuts CM, Kruger HS, Biebinger R, Hurrell RF, Baumgartner J, Zimmermann MB. A micronutrient powder with low doses of highly absorbable iron and zinc reduces iron and zinc deficiency and improves weight-for-age Z-scores in South African children. J Nutr. 2011;141:237–42.
12. Ndemwa P, Klotz CL, Mwaniki D, Sun K, Muniu E, Andango P, Owigar J, Rah JH, Kraemer K, Spiegel PB, et al. Relationship of the availability of micronutrient powder with iron status and haemoglobin among women and children in the Kakuma Refugee Camp, Kenya. Food Nutr Bull. 2011;32:286–91.
13. Adu-Afarwuah S, Lartey A, Brown KH, Zlotkin S, Briend A, Dewey KG. Home fortification of complementary foods with micronutrient supplements is well accepted and has positive effects on infant iron status in Ghana. Am J Clin Nutr. 2008;87:929–38.
14. Varma JL, Das S, Sankar R, Mannar MGV, Levinson FJ, Hamer DH. Community-level micronutrient fortification of a food supplement in India: a controlled trial in preschool children aged 36-66 mo. Am J Clin Nutr. 2007;85:1127–33.
15. Osei AK, Rosenberg IH, Houser RF, Bulusu S, Mathews M, Hamer DH. Community-level micronutrient fortification of school lunch meals improved vitamin A, folate, and iron status of schoolchildren in Himalayan villages of India. J Nutr. 2010;140:1146–54.
16. Lemaire M, Islam QS, Shen H, Khan MA, Parveen M, Abedin F, Haseen F, Hyder Z, Cook RJ, Zlotkin SH. Iron-containing micronutrient powder provided to children with moderate-to-severe malnutrition increases haemoglobin concentrations but not the risk of infectious morbidity: a randomized, double-blind, placebo-controlled, non-inferiority safety trial. Am J Clin Nutr. 2011;94:585–93.
17. Thompson B. Food-based approaches for combating iron deficiency. In: Kraemer K, Zimmermann MB, editors. Nutritional anaemia. Basil (Switzerland): Sight and Life; 2007.
18. Grillenberger M, Neumann CG, Murphy SP, Bwibo NO, van't Veer P, Hautvast JGAJ, West CE. Food supplements have a positive impact on weight gain and the addition of animal source foods increases lean body mass of Kenyan schoolchildren. J Nutr. 2003;133:S3957–64.
19. Szymlek-Gay EA, Ferguson EL, Heath A-LM, Gray AR, Gibson RS. Food-based strategies improve iron status in toddlers: a randomized controlled trial. Am J Clin Nutr. 2009;90:1541–51.
20. Haas JD, Beard J, Murray-Kolb L, del Mundo A, Felix A, Gregorio G. Iron bio-fortified rice improves the iron stores of non anaemic Filipino women. J Nutr. 2005;135:2823–30.
21. Pedersen B, Kalinowski LS, Eggum BO. The nutritive value of amaranth grain (Amaranthus caudatus). Plant Foods Hum Nutr. 1990;40:61–71.
22. Macharia-Mutie CW, Van de Wiel AM, Moreno-Londono AM, Mwangi AM, Brouwer ID. Sensory acceptability and factors predicting the consumption of grain amaranth in Kenya. Ecol Food Nutr. 2011;50:375–92.
23. UNICEF. Monitoring progress toward the goals of the world summit for children: a practical handbook for multiple-indicator surveys. New York: Programme Division, United Nations Children's Fund; 1995.
24. Gibson R, Ferguson E. An interactive 24-hour recall for assessing the adequacy of iron and zinc intakes in developing countries. Technical Monograph Series 8, 2008. Washington, DC: HarvestPlus.
25. Novozamsky I, van Eck R, Houba V, van der Lee JJ. Solubilization of plant tissue with nitric acid-hydrofluoric acid-hydrogen peroxide in a closed-system microwave digestor. Commun Soil Sci Plant Anal. 1996;27:867–75.
26. Makower RU. Extraction and purification of phytic acid in beans (Phaseoulus vulgaris). Cereal Chem. 1970;47:288–95.
27. Egli I, Davidsson L, Juillerat MA, Barclay D, Hurrell RF. The influence of soaking and germination on the phytase activity and phytic acid content of grains and seeds potentially useful for complementary feeding. J Food Sci. 2002;67:3484–8.
28. Van Veldhoven PP, Mannaerts GP. Inorganic and organic phosphate measurements in the nanomolar range. Anal Biochem. 1987;161:45–8.
29. WHO. Physical status: the use and interpretation of anthropometry. Report of a WHO Expert Committee, Technical Report Series 854. Geneva: WHO; 1995.
30. CDC. SAS program for the CDC growth charts; 2007 [cited 11 February 2012]. Available from: http://www.cdc.gov/nccdphp/dnpa/growthcharts/resources/BIV-cutoffs.pdf.
31. WHO. Iron deficiency anaemia: assessment, prevention and control: a guide for programme managers. Geneva: WHO; 2001.
32. Sullivan KM, Mei Z, Grummer-Strawn L, Parvanta I. Haemoglobin adjustments to define anaemia. Trop Med Int Health. 2008;13:1267–71.
33. Cook JD, Flowers CH, Skikne BS. The quantitative assessment of body iron. Blood. 2003;101:3359–64.
34. Pfeiffer CM, Cook JD, Mei Z, Cogswell ME, Looker AC, Lacher DA. Evaluation of an automated soluble transferrin receptor (sTfR) assay on the Roche Hitachi analyzer and its comparison to two ELISA assays. Clin Chim Acta. 2007;382:112–6.
35. Thurnham DI, McCabe LD, Haldar S, Wieringa FT, Northrop-Clewes CA, McCabe GP. Adjusting plasma ferritin concentrations to remove the effects of subclinical inflammation in the assessment of iron deficiency: a meta-analysis. Am J Clin Nutr. 2010;92:546–55.
36. SPSS Inc. PASW Missing Values 18 [cited 15 October 2011]. Available from: www.sussex.ac.uk/its/pdfs/PASWMissingValues18.pdf.
37. Vickers AJ, Altman DG. Statistics notes: analysing controlled trials with baseline and follow up measurements. BMJ. 2001;323:1123–4.
38. Barros AJD, 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.
39. Hurrell RF, Reddy MB, Juillerat M-A, Cook JD. Degradation of phytic acid in cereal porridges improves iron absorption by human subjects. Am J Clin Nutr. 2003;77:1213–9.
40. Hurrell R, Egli I. Iron bioavailability and dietary reference values. Am J Clin Nutr. 2010;91:S1461–7.
41. Troesch B, Egli I, Zeder C, Hurrell RF, de Pee S, Zimmermann MB. Optimization of a phytase-containing micronutrient powder with low amounts of highly bioavailable iron for in-home fortification of complementary foods. Am J Clin Nutr. 2009;89:539–44.
42. Dewey KG, Yang Z, Boy E. Systematic review and meta-analysis of home fortification of complementary foods. Matern Child Nutr. 2009;5:283–321.
43. Faber M, Kvalsvig JD, Lombard CJ, Benade AJS. Effect of a fortified maize-meal porridge on anaemia, micronutrient status, and motor development of infants. Am J Clin Nutr. 2005;82:1032–9.
44. Cook JD. Defining optimal body iron. Proc Nutr Soc. 1999;58:489–95.
45. Sun X, Briel M, Walter SD, Guyatt GH. Is a subgroup effect believable? Updating criteria to evaluate the credibility of subgroup analyses; 2010 [cited 10 November 2011]. Available from: http://www.bmj.com/content/340/bmj.c117. BMJ.

46. Semba RD, Bloem MW. The anemia of vitamin A deficiency: epidemiology and pathogenesis. Eur J Clin Nutr. 2002;56:271–81.
47. Fishman SM, Christian P, West KP. The role of vitamins in the prevention and control of anaemia. Public Health Nutr. 2000;3:125–50.
48. Zimmermann MB, Biebinger R, Rohner F, Dib A, Zeder C, Hurrell RF, Chaouki N. Vitamin A supplementation in children with poor vitamin A and iron status increases erythropoietin and hemoglobin concentrations without changing total body iron. Am J Clin Nutr. 2006;84:580–6.
49. Brooker S, Peshu N, Warn P, Mosobo M, Guyatt H, Marsh K, Snow R. The epidemiology of hookworm infection and its contribution to anaemia among pre-school children on the Kenyan coast. Trans R Soc Trop Med Hyg. 1999;93:240–6.
50. Le Huong T, Brouwer ID, Nguyen KC, Burema J, Kok FJ. The effect of iron fortification and de-worming on anaemia and iron status of Vietnamese schoolchildren. Br J Nutr. 2007;97:955–62.
51. Macharia-Mutie CW, Brouwer ID, Mwangi AM, Kok FJ. Complementary feeding practices and dietary intake among children 12-23 months in Mwingi district, Kenya. Intl J Food Safety Nutr Public Health. 2010;3:45–56.
52. Ruel MT. Can food based strategies help reduce vitamin A and iron deficiencies? A review of recent evidence. Washington, DC: International Food Policy Research Institute; 2001. p. 46–7.
53. van der Heijden GJMG, Donders ART, Stijnen T, Moons KGM. 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;59:1102–9.
54. Janssen KJM, Donders ART, Harrell FE Jr, Vergouwe Y, Chen Q, Grobbee DE, Moons KGM. Missing covariate data in medical research: to impute is better than to ignore. J Clin Epidemiol. 2010;63:721–7.
55. Drakeley C, Reyburn H. Out with the old, in with the new: the utility of rapid diagnostic tests for malaria diagnosis in Africa. Trans R Soc Trop Med Hyg. 2009;103:333–7.
56. Erdman LK, Kain KC. Molecular diagnostic and surveillance tools for global malaria control. Travel Med Infect Dis. 2008;6:82–99.
57. WHO. Assessing the iron status of populations: including literature reviews. 2nd ed. Geneva: WHO; 2007.
58. Ayoya MA, Spiekermann-Brouwer GM, Stoltzfus RJ, Nemeth E, Habicht J-P, Ganz T, Rawat R, Traoré AK, Garza C. α1-Acid glycoprotein, hepcidin, C-reactive protein, and serum ferritin are correlated in anemic schoolchildren with Schistosoma haematobium. Am J Clin Nutr. 2010;91:1784–90.
59. Grant FKE, Suchdev PS, Flores-Ayala R, Cole CR, Ramakrishnan U, Ruth LJ, Martorell R. Correcting for inflammation changes estimates of iron deficiency among rural Kenyan pre-school children. J Nutr. 2012; 142:105–11.
60. WHO. Guideline: use of multiple micronutrient powders for home fortification of foods consumed by infants and children 6–23 months of age. Geneva: WHO; 2011.
61. European Union. Commission Regulation (EU) No 1161/2011 of 14 November 2011 amending Directive 2002/46/EC of the European Parliament and of the Council, Regulation (EC) No 1925/2006 of the European Parliament and of the Council and Commission Regulation (EC) No 953/2009 as regards the lists of mineral substances that can be added to foods. EURLex. 2011;54:29–30.
62. Yang Z, Siekmann J, Schofield D. Fortifying complementary foods with NaFeEDTA: considerations for developing countries. Matern Child Nutr. 2011;7:123–8.