Multi-micronutrient Sprinkles including a low dose of iron provided as microencapsulated ferrous fumarate improves haematologic indices in anaemic children: a randomized clinical trial

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Abstract

Home-fortification of complementary foods with micronutrients (including iron) as Sprinkles is a new strategy to control iron deficiency and anaemia in developing countries. However, the most effective dose and form of iron is not known. The purpose of this study was to compare the efficacy of various doses (12.5, 20 or 30 mg) and treatment methods (multi-micronutrient Sprinkles vs. ferrous sulphate drops) on haemoglobin (Hb) concentration after 8 weeks of treatment in anaemic children. In total, 133 anaemic Ghanaian children (Hb 70–99 g L⁻¹) aged 6–18 months were randomly assigned to one of five daily interventions for 8 weeks. Out of the five interventions, four used Sprinkles, and one used iron drops. Of the four Sprinkles groups, three included 12.5, 20 or 30 mg of iron as ferrous fumarate, and one included 20 mg of iron as ferric pyrophosphate. The iron drops group included 12.5 mg of iron as liquid ferrous sulphate. Hb concentrations were measured at baseline, week 3 and week 8. The primary outcome measure was Hb concentration at 8 weeks after treatment. We compared differences in Hb and ferritin concentrations and prevalence of iron deficiency anaemia (Hb < 100 g L⁻¹ and soluble transferrin receptor concentrations >8.5 mg L⁻¹) from baseline to 8 weeks within and between groups. Adherence and reporting of side effects (staining of the teeth, ease of use, diarrhoea and darkening of stools) were compared between groups. Mean change in Hb was 1.4 g L⁻¹ (SD = 1.8) ($P = 0.0001$). Change in Hb concentrations from baseline to 8 weeks was significant in all groups ($P = 0.0001$ – 0.0007), with no differences across groups. Geometric means of serum ferritin varied from 18.6 to 44.0 µg L⁻¹ at baseline. At week 8, these means were in the interval of 48.0–78.3 µg L⁻¹, with no group differences. Prevalence of iron deficiency anaemia decreased significantly from baseline to 8 weeks in all groups with the exception of the iron drops group, with no group differences. Adherence was lower in the drops group (64%) as compared with Sprinkles groups (84%). Greater staining of the teeth and less ease of use were reported in the drops group as compared with Sprinkles groups. A dose as low as 12.5 mg of iron as ferrous fumarate when provided as Sprinkles may be effective in anaemic children.

Keywords: iron dose, ferrous fumarate, ferric pyrophosphate, ferrous sulphate, Sprinkles, randomized clinical trial, iron deficiency anaemia.

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Introduction

Iron deficiency anaemia (IDA) among infants and young children is a health priority of the WHO and UNICEF (WHO/UNICEF 2001; Zelee Hill *et al.* 2004). Recently, of 38 proposals for action, the Copenhagen Consensus ranked interventions to increase the availability of micronutrients as the second highest priority in advancing the lives of people living in developing countries (Behrman *et al.* 2004). In particular, the reduction of IDA through food supplements was considered to have an exceptionally high ratio of benefit to cost. 'Sprinkles' are a 'complementary food supplement' containing micronutrients in powder form that are packaged in single-dose sachets (Zlotkin *et al.* 2005). They are sprinkled onto food to increase the micronutrient density of the food. Sprinkles were developed to improve adherence, acceptability and ease of use over conventional iron supplements.

Initially, two dosing regimens of Sprinkles were tested: 80 mg of elemental iron provided as encapsulated ferrous fumarate daily for 2 months (Zlotkin *et al.* 2001); and 40 mg of the same form of iron daily for 6 months (Zlotkin *et al.* 2003a) as treatment and maintenance regimens, respectively. Although these doses are suitable in a clinic setting, lower doses are preferred in a community setting where individualized screening and monitoring of the recipient children is not possible. In addition, a lower dose would likely have fewer side effects. There is also some evidence to suggest that higher doses of iron may negatively affect the absorption of zinc (Lind *et al.* 2003). For a community setting, Nestle & Alnwick (1997) recommended a daily dose of 12.5 mg of iron [as ferrous sulphate (FS) drops] for children 6–24 months

old. However, the absolute amount of absorbed iron varies with the dose of iron, the form of iron (FS, ferrous fumarate or ferric pyrophosphate) and different modes of delivery (drops, tablets, iron-fortified foods). There is no available information on the efficacy of lower doses of Sprinkles when added to complementary foods. Therefore, the aim of the present study was to determine the lowest possible effective dose and optimal method of treatment for improving Hb concentration in anaemic children using a clustered randomized clinical trial.

Materials and methods

Study area, population and recruitment

The study took place from April to September of 2003 in the field study area of Kintampo Health Research Centre, located in the Kintampo district of Ghana. This is a malaria-endemic area where the principal complementary food is an unfermented maize-based porridge, which is low in bioavailable iron and zinc (Lartey *et al.* 1999). The prevalence of anaemia varies from 65% to 70% in young children (under 24 months of age) and is predominantly due to iron deficiency (Quarshie & Amoafu 1998; The Micronutrient Initiative 2004). Families live in housing compounds and share food and other aspects of daily living. We identified young children from an existing birth database managed by the Kintampo Research Center, and recruited them over a 2-week period from 1 April to 15 April 2003. Eligibility criteria included: age between 6 and 18 months at the time of recruitment; axillary temperature less than or equal 37.5°C, no history of iron supplementation within 2 weeks prior to the day of recruitment, willingness

to stay within the study area for at least 2 months, parental consent, ingesting a semi-solid weaning food and Hb concentrations in the interval of 70–99 g L⁻¹. Children who had Hb concentrations <70 g L⁻¹ were not included in the trial and were treated.

The study was a clustered randomized clinical trial. Housing compounds (clusters containing no more than four participants) were randomly assigned to one of the five intervention groups using a random digit generator. Out of the five interventions, four used Sprinkles (sachets were identical except for a printed group number), and one used iron drops. Of the four Sprinkles groups, three provided iron as microencapsulated ferrous fumarate (Descote Ferrous Fumarate 60, Particle Dynamics Inc., St Louis, MO, USA), at doses of 12.5 (FF12.5), 20 (FF20) or 30 mg (FF30), and one provided iron as micronized ferric pyrophosphate (SunActive FeTM, Taiyo International, Takaramachi, Japan) at a dose of 20 mg (MFP). The iron drops group included 12.5 mg of iron as FS drops (approximately 1 mL; Fer-in-SolTM, Mead Johnson, Ottawa, Canada). Caregivers and the field staff in the Sprinkles' groups were unaware of the dose and form of iron in the Sprinkles sachets. These sachets also included 30 mg ascorbic acid, 300 RE vitamin A (as acetate), 5 mg zinc (as zinc gluconate), 7.5 µg vitamin D (as cholecalciferol) and 160 µg folic acid. Caregivers were instructed to add the contents of a Sprinkles sachet to their children's meal serving (after the meal was cooked) once daily for an 8-week period at any mealtime. Those in the FS group were instructed to give 1 mL of liquid iron daily between meals during the same 8-week period (Fig. 1).

The primary outcome was Hb concentration at 8 weeks from baseline. Secondary outcomes included: IDA and serum ferritin concentrations at the end of 8 weeks; and adherence, ease of use and side effects (diarrhoea, staining of the teeth and darker stools) during the 8-week study period. IDA was defined as Hb <100 g L⁻¹ and soluble transferrin receptor (sTfR) concentrations >8.5 mg L⁻¹.

We randomized by housing compounds to minimize cross contamination due to food sharing. Those performing the statistical analyses were unaware of group assignments until the code was broken after the completion of data analyses.

Data collection

Baseline assessments took place at a health clinic and included a questionnaire to collect demographic information. Length and weight were measured as previously described (Zlotkin *et al.* 2001). Hb concentration was measured from a drop of blood obtained from a finger prick using a portable HEMOCUE B-Hb photometer (Hemocue, Angelholm, Sweden) by trained technicians using standardized techniques (Cohen & Seidl-Friedman 1988). Capillary blood samples (500 µL) were obtained using aseptic techniques and collected into microtube containers (SARSTEDT, Aktiengesellschaft & Co., Nümbrecht, Germany). Blood samples were preserved in ice-lined cold boxes and returned to the base station within 6 h of collection. The serum was separated by centrifugation (10 min at 12 000 g) before storage at -40°C. Serum ferritin, sTfR and C-reactive protein (CRP) concentrations were assayed in duplicate by a commercial ELISA (Ramco Laboratories, Houston, TX, USA) as previously described (Miles 1974; Zlotkin *et al.* 2001; Le Hung *et al.* 2005). A rapid immunodiagnostic antigen test, 'Parasight-F-test', was used at baseline to detect the presence of antigens to *Plasmodium falciparum* to determine malaria positivity. This test has shown good sensitivity (86.7% to 93.4%) and specificity (98.2% to 99.3%) (Sandeep *et al.* 2003).

Field workers visited participants in their homes weekly after the baseline visit for a total of seven follow-up visits. At each visit, a questionnaire regarding adherence, ease of use and side effects (diarrhoea, darkening of stools and staining of teeth) over the preceding week was completed. At each visit, field workers provided caregivers with verbal educational support to maximize adherence to the intervention and provided advice regarding any concerns. Caregivers were also asked whether they shared any of the assigned sachets or drops with other family members, and were instructed to give all sachets or drops to the participating child only.

At 3 weeks from baseline, Hb concentration was reassessed. Weekly monitoring continued as before. At 8 weeks, anthropometric and biochemical measurements were repeated.

Fig. 1. Trial profile. CRP, C-reactive protein; sTfR, soluble transferrin receptor.

Sample size calculations

Sample size was calculated using Bonferroni's correction for multiple comparisons with an alpha error of 0.05, beta error of 0.20 (80% power), and standard deviation of 8 g L⁻¹ (Zlotkin *et al.* 2001). Based on these assumptions, at least 17 infants were required per group to detect a 10 g L⁻¹ difference in Hb concentration at the 8-week follow-up.

Data processing

Data were double-entered in Visual Fox Pro 6.0 (Microsoft, Redmond, Washington, USA) using a customized data entry program. Data were electronically sent to the study centre at the Hospital for Sick Children in Toronto, Canada where they were checked for numeric and logical consistency before analyses. We dichotomized CRP concentrations using a cut-off level of >8.5 mg L⁻¹ (Ramco Laboratories,

Stafford, TX, USA). In addition, we calculated adherence as the percentage of empty sachets (or bottles in the FS group) out of the total assigned for each child; we subtracted the number of lost sachets (or bottles) from the total. Similarly, we computed the values for ease of use and darkening of the stools by taking the percentage of 'yes' responses out of the total responses of each caregiver; for the former, 'yes' indicated that the intervention was easy to use, and for the latter, it indicated that the stools were dark. For diarrhoea, we calculated the number of episodes (reported number of times caregivers reported loose stools over the course of the study) per child. We dichotomized the data on staining of the teeth; thus a 'yes' response indicated that the caregiver reported staining of the child's teeth at least once during the weekly visits.

Analyses

Preliminary analyses included descriptive statistics, histograms and box plots. Univariate analyses examining differences in iron status from baseline to 8 weeks in each of the five treatment groups were conducted using a paired *t*-test for continuous data (Hb concentration) and McNemar's test for categorical data (proportion of children with IDA). Multivariate analyses comparing Hb concentrations over the study period across the five treatment groups were performed using mixed linear models for repeated-measures analysis. Model development included treatment group as the independent variable of interest; housing compound and child as random effects; and age, sex, timing of introduction of complementary foods, and malaria status as covariates potentially related to Hb concentration (Brotanek *et al.* 2005; Le Hung *et al.* 2005; Mamiro *et al.* 2005; Spinelli *et al.* 2005). Models were based on an unstructured covariance structure, which assumes correlation between children residing in the same housing compound and between Hb concentrations taken from the same child at baseline, week 3 and week 8. All models were examined for convergence criteria and normally distributed residuals. Tukey's method was used to adjust for multiple comparisons. In addition, baseline iron status ($\text{sTfR} > 8.5 \text{ mg L}^{-1}$

vs. $\leq 8.5 \text{ mg L}^{-1}$) and an interaction between treatment group and iron status were entered in the final model to examine whether Hb concentration was independently associated with treatment group and iron status, or whether baseline iron status modifies the association between Hb concentration and treatment group. Multivariate analyses examining serum ferritin (log-transformed) measured at 8 weeks as the dependent variable were also performed using the random effects linear model. Independent variables included treatment group, baseline serum ferritin and CRP ($> 8.5 \text{ mg L}^{-1}$) in order to adjust for falsely elevated serum ferritin concentrations due to infection. Differences in adherence, ease of use and side effects between children in the Sprinkles versus iron drop groups were conducted using Kruskal-Wallis test for nominal or interval data, and the chi-squared test for categorical data. Analyses were based on an intention-to-treat principle. A two-sided *P*-value of 0.05 indicated statistical significance. Statistical analyses were conducted using SAS (version 9.1; SAS Institute Inc., Cary, NC, USA).

Ethics approval

Ethics approval was obtained from the Research Ethics Committees at the Hospital for Sick Children, Toronto Canada and the Kintampo Health Research Center, Kintampo, Ghana. Verbal consent to conduct the study in the Kintampo district was obtained from the District Assembly of Elected Representatives and from elders in each village. Informed and signed consent was individually obtained from mothers of the participating children.

Results

Recruitment

A total of 284 children from 265 housing compounds were assessed for eligibility, and out of them, 133 children (127 housing compounds) entered the study. A total of 118 (89%) children completed the study; we had complete data including biochemical indices for 114 (86%) children. Fifteen children were lost to follow-up because their families migrated out of the study area, and four children had missing data for

Table 1. Baseline characteristics

	Ferrous fumarate (encapsulated)			Ferric pyrophosphate (micronized)	Ferrous sulphate
	12.5 mg	20 mg	30 mg	20 mg	12.5 mg
<i>n</i>	22	25	25	26	18
Age months*	11.2 (3.3)	13.1 (3.4)	12.0 (3.5)	12.0 (3.3)	10.7 (4.3)
Boys [†]	10 (45.5)	17 (68.0)	15 (60.0)	16 (61.5)	10 (55.6)
Weight kg*	8.1 (1.2)	7.9 (1.2)	8.1 (1.2)	7.9 (1.5)	7.6 (1.4)
Length cm*	69.2 (4.1)	70.6 (4.2)	70.4 (4.2)	70.4 (5.4)	68.7 (5.6)
Breastfeeding ≥ 6 months [‡]	4 (18.2)	3 (12.0)	3 (12.0)	3 (11.5)	4 (22.2)
Malarial parasite positive [§]	14 (73.7)	16 (69.6)	16 (66.7)	15 (60.0)	11 (64.7)
CRP > 8.5 mg L ⁻¹ [#]	8 (36.4)	6 (24.0)	8 (32.0)	6 (23.1)	8 (44.4)

*Mean (SD). [†]No. (%). [‡]Measured using rapid antigen test. [§]CRP, C-reactive protein.

biochemical indices because of difficulties in collecting or processing blood samples.

Baseline characteristics

Baseline characteristics of the children on whom complete data were available are shown in Table 1. The mean age of children was in the interval of 10.7–13.1 months. The distribution of other baseline characteristics was similar across groups.

Effect on haemoglobin

Table 2 shows haematologic indices in the five groups. At baseline, mean Hb varied from 87 to 91 g L⁻¹; at week 3, mean Hb varied from 100 to 107 g L⁻¹; and at week 8, mean Hb varied from 102 to 110 g L⁻¹, across the five groups. Box plots of Hb concentrations at these three time points for the five groups are presented in Fig. 2. The change in Hb concentrations from baseline to week 8 was significant in all groups (Table 2). However, the overall difference in mean Hb concentrations across groups was not significant [$t(145) = 0.71$, $P = 0.78$]. In addition, no trend was observed from lower to higher doses. Simpler methods yielded similar results, indicating robustness of findings. Also, the differences between any two groups were not significant – the 95% confidence intervals for the pair-wise differences between each Sprinkles group and the FS are shown in Fig. 3. Subgroup analysis by iron status revealed that: (1) the

effect on Hb concentrations did not significantly depend upon the group allocation ($P = 0.38$); (2) the effect on Hb concentrations significantly depended upon the baseline iron status ($P = 0.0002$); and (3) the magnitude of effect on Hb concentrations for a group significantly differed by baseline iron status ($P = 0.03$).

Effect on iron deficiency anaemia

The prevalence of IDA varied from 50% to 70% across groups at baseline (Table 2). At week 8, this prevalence was in the interval of 22% to 36%. Thus, there was a significant reduction in the absolute risk of IDA, which varied from 28% to 46%; however, the overall difference between groups was not significant [$t(54) = 0.58$, $P = 0.68$].

Effect on serum ferritin

Geometric means of serum ferritin varied from 18.6 to 44.0 $\mu\text{g L}^{-1}$ at baseline (Table 2). At week 8, these means were in the interval of 48.0–78.3 $\mu\text{g L}^{-1}$. The overall difference across groups was not significant [$t(43) = 1.55$, $P = 0.24$]; also the differences between any two groups were not significant.

Adherence, ease of use and side effects

Table 3 shows data on adherence, ease of use and side effects. Among groups that received Sprinkles,

Table 2. Haematologic indices at baseline, 3 weeks and 8 weeks

	Ferrous fumarate (encapsulated)			Ferric pyrophosphate	Ferrous sulphate	<i>P</i> overall
	12.5 mg	20 mg	30 mg	20 mg	12.5 mg	
Haemoglobin g L ⁻¹ *						
<i>n</i>	22	25	25	26	18	
Baseline	87 (7)	89 (8)	88 (9)	91 (8)	89 (8)	
3 weeks	100 (13)	101 (14)	107 (13)	102 (12)	106 (14)	
8 weeks	103 (13)	102 (16)	104 (17)	103 (16)	110 (16)	0.78†
<i>P</i> for change (Baseline–8 weeks)‡	0.0001	0.0006	0.0001	0.0007	0.0001	
Serum ferritin µg L ⁻¹ §						
<i>n</i>	22	24	23	25	18	
Baseline	24.9 (4.9–182.5)	18.6 (5.0–162.0)	24.1 (3.9–189.0)	23.8 (0–150.9)	44.0 (8.6–268.5)	
8 weeks	78.3 (16.4–468.2)	59.4 (9.1–226.1)	48.0 (5.4–346.9)	50.4 (8.3–46.4)	62.1 (12.6–83.2)	0.24¶
Iron deficiency anaemia††,‡‡						
<i>n</i>	20	25	25	26	18	
Baseline	14 (70)	16 (64)	15 (60)	18 (69)	9 (50)	
8 weeks	7 (35)	9 (36)	6 (24)	6 (23)	4 (22)	0.68§§
<i>P</i> for change¶¶	0.03	0.03	0.006	0.0005	0.05	

*Mean (SD). †Based on a linear mixed effects model which had haemoglobin (Hb) concentrations (baseline, 3 weeks and 8 weeks) as the dependent variables, group allocation and dichotomized soluble transferrin receptor (sTfR) (>8.5 mg L⁻¹) as independent variables, and child and housing compound as random effects. We also adjusted for malaria status at baseline as it was significantly associated with Hb in the model. ‡Based on paired *t*-tests. §Geometric Mean (Range). ¶Based on a random effects model which had serum ferritin at 8 weeks (log-transformed) as the dependent variable, group allocation, baseline serum ferritin and dichotomized CRP (>8 mg L⁻¹) as independent variables, and housing compound as a random effect. ††No. (%). ‡‡Iron deficiency anaemia defined as Hb < 100 g L⁻¹ and sTfR > 8.5 mg L⁻¹. §§Based on a logistic regression model which had iron deficiency anaemia at 8 weeks as the dependent variable, and group allocation and iron deficiency anaemia at baseline as independent variables. ¶¶Based on McNemar test. CRP, C-reactive protein.

Table 3. Adherence and side effects

	Ferrous fumarate (encapsulated)			Ferric pyrophosphate (micronized)	Ferrous sulphate	<i>P</i> overall
	12.5 mg	20 mg	30 mg	20 mg	12.5 mg	
<i>n</i>	26	28	27	27	25	
Adherence*,†	85 (8)	84 (9)	84 (12)	83 (8)	69 (7)	0.001
Ease of use*,‡	95 (8)	99 (3)	98 (6)	99 (3)	82 (22)	0.001
Diarrhoea episodes/child§	1.3 (1.2)	1.0 (1.2)	0.9 (1.2)	0.9 (1.1)	1.2 (1.4)	0.66
Darkening of stools*,††	50 (33)	60 (31)	54 (37)	51 (34)	59 (36)	0.8
Staining of teeth*,‡‡	2 (8)	6 (21)	8 (30)	1 (4)	12 (46)	0.02

*Mean per cent (SD). †Adherence was determined by calculating the percentage of empty sachets or bottles out of the total assigned. ‡Ease of use was determined by calculating the percentage of positive responses to questions about the ease of use. §Mean (SD). Episode defined as the number of reported loose stools over the course of the study. ¶Diarrhoea episodes were determined by calculating the mean number of reported episodes of loose stools per child. ††Darkening of the stool was determined by calculating the percentage of positive responses to questions about the colour of the stool. ‡‡Staining of the teeth was determined by calculating the percentage of responses reporting staining of the teeth with the intervention.

Fig. 2. Box plots of haemoglobin concentrations for the five groups over time. Lower edge, line and upper edge of the box represent the 25th, 50th and 75th percentiles, respectively. Endpoints of lower and upper whiskers represent the minimum and maximum values for haemoglobin concentrations, respectively. FF12.5, FF20 and FF30 denote 12.5, 20 and 30 mg of ferrous fumarate groups, and FP and FS denote ferric pyrophosphate and ferrous sulphate groups, respectively.

Fig. 3. The 95% confidence intervals for the difference in mean haemoglobin concentrations between groups. Confidence intervals from linear mixed effect models with group allocation as main effect, baseline iron status as a covariate, compound and person as random effects and haemoglobin concentrations at 0, 3 and 8 weeks as outcome. Left: without adjusting for multiple comparisons; right: after adjusting for multiple comparisons. FF12.5, FF20 and FF30 denote 12.5, 20 and 30 mg of ferrous fumarate groups, and FP and FS denote ferric pyrophosphate and ferrous sulphate groups, respectively.

adherence was 85% (SD = 8%) in FF12.5, 84% (SD = 9%) in FF20, 84% (SD = 12%) in FF30 and 83% (SD = 8%) in MFP. Adherence was 69% (SD = 7%) in the FS group, which was significantly lower compared with Sprinkles groups ($P = 0.001$). In

Sprinkles groups, on average 95–99% of the caregivers reported that the sachets were easy to use compared with 82% in the FS group ($P = 0.001$). All groups reported similar rates for darkening of stools (50–59%) per child ($P = 0.8$). For other side effects,

the group means were 0.9–1.3 for episodes of diarrhoea per child in the Sprinkles group compared with 1.2 episodes per child in the FS group ($P = 0.66$); 1–8 of the caregivers reported staining of the teeth in the Sprinkles group compared with 12 in the FS group ($P = 0.006$).

Discussion

Sprinkles is a home-fortification approach for providing iron and other micronutrients to young children at risk of IDA (Zlotkin *et al.* 2005). Because in Ghana, the molar ratio of phytate:iron in complementary foods is very high, 9.8 (Zlotkin *et al.* 2006), lower amounts of iron may be absorbed from multi-micronutrient Sprinkles, which are mixed into foods as compared to the 'traditional' FS drops which are administered on an empty stomach. This potential shortcoming may be circumvented by using higher doses of iron. However, in iron-deficient states, higher amounts of iron are absorbed (and vice versa) (Hallberg *et al.* 1997), thus, the most appropriate dose and form of iron to use in multi-micronutrient Sprinkles for community interventions remains uncertain. Although various doses and forms of iron have been investigated, to the best of our knowledge, no study has simultaneously evaluated the haematologic response from multiple doses and forms of iron. Results from the current study demonstrated that there was a significant increase in mean Hb concentration from baseline to week 8 in all groups, with no significant differences between groups. Adherence and side effects were similar among multi-micronutrient Sprinkles groups; however, adherence and reported ease of use were lower and reporting of staining of the teeth was higher in the FS group.

Previous studies that have examined the absorption of iron from FS, encapsulated ferrous fumarate and micronized ferric pyrophosphate have found that iron-deficient children absorb more iron than non-deficient children even from a food-based intervention. Of these studies, two have directly measured the absorption of labelled ferrous fumarate from cereal in infants: Davidsson *et al.* (2000) added labelled ferrous fumarate to low phytate-containing wheat and soy-based cereals provided to 6–12-month-old non-

anaemic infants – the mean iron bioavailability at fortification levels (10 mg iron per 100 g dry cereal) was 4.1% (range: 1.7–14.7%); Tondeur *et al.* (2004) used intrinsically labelled lipid-microencapsulated ferrous fumarate in multi-micronutrient Sprinkles, which was added to high phytate-containing maize-based cereals – the geometric mean absorption of iron was 4.6% in non-anaemic children (range 1.5–12.3%), and 8.3% (range 2.9–17.8%) in children with IDA. Although the bioavailability of ferric pyrophosphate is lower than that of ferrous fumarate (Le Hung *et al.* 2005), newer micronized forms with smaller particle size have recently been developed to increase its bioavailability. Of these, SunActive Fe™ has recently been shown to be absorbed at a rate of 3.4% when added to infant cereal. This rate of absorption is comparable with that of FS (4.1%; $P = 0.24$) (Fidler *et al.* 2004).

The results of this study are comparable to those of previous studies on multi-micronutrient Sprinkles (Zlotkin *et al.* 2001, 2003b). They are also comparable to the results of studies that have used traditional forms of iron supplements. For example, in a study in Indonesia, a cohort of 67 anaemic children received a daily dose of 30 mg of iron as FS for 8 weeks; mean Hb increased from 98 (SD = 14) to 116 g L⁻¹ (SD = 7) (Schultink *et al.* 1997). Similarly, in a recent study in Zanzibar, a cohort of 614 children (6–59 months) received 20 mg of iron as FS for 12 months; geometric mean of serum ferritin was 29.2 µg L⁻¹ at baseline and 57.5 µg L⁻¹ at the end of intervention (Stoltzfus *et al.* 2001).

Our findings of high rates for adherence and 'ease of use' in the multi-micronutrient Sprinkles groups are also consistent with those of our previous research (Zlotkin *et al.* 2005). The higher adherence rates in Sprinkles groups compared with the FS group may in part be explained by more positive responses for 'ease of use' in these groups. Field workers were unaware of the study hypotheses and encouraged adherence in all groups during weekly monitoring visits. We believe that the better adherence and 'ease of use' is a direct result of the simple and passive nature of the use of multi-micronutrient Sprinkles. They are tasteless, mix well with any semi-solid food, do not necessitate any measurement, and do not

appreciably change the taste or colour of the food to which they are added. In contrast, FS drops have to be measured carefully before use, have a metallic taste, and generally are not easy to use. Thus, our results suggest that multi-micronutrient Sprinkles may have better acceptability and compliance in a community setting.

Previous studies have shown that staining of the teeth and gastrointestinal discomfort are common among children who are supplemented with iron drops (Mora 2002), which appear to be dose dependent. Thus, lower doses of iron within multi-micronutrient Sprinkles may have better acceptability in terms of side effects. In the current study, caregivers reported fewer incidences of staining of teeth in the multi-micronutrient Sprinkles groups compared with the FS group, although diarrhoea episodes were similar. These results do not support a dose-dependent effect on side effects. Nevertheless, the side effects were benign, and no child required extra medical care or hospitalization.

In the present study, a significant association was found between baseline measures of low Hb concentration and malaria positivity. At baseline, approximately 69% (48/70) of children were positive for malaria. Two recently large-scale, well-designed studies suggest that provision of iron plus folic acid supplementation to pre-school children in malaria endemic regions is associated with increased risk of adverse events and hospitalizations (Sazawal *et al.* 2006; Tielsch *et al.* 2006). Given the slower absorption of iron with fortified foods compared with supplements, the results of these two studies, however, cannot be applied to fortification or food-based approaches (WHO Statement 2006). As recommended by the World Health Organization (WHO Statement 2006), greater caution needs to be exercised when providing iron plus folic acid supplements to young children in high malaria transmission areas. Furthermore, adequately powered studies investigating the impact of fortified foods (commercial or home-based) on morbidity and mortality in young children in regions of high malaria transmission are needed.

Limitations of this study include sample size and generalizeability. A *post hoc* power analysis was

conducted to examine whether an inadequate sample size could account for the unobserved differences between treatment groups in the present study. It was calculated that to reach significant differences in Hb concentrations with the variance and differences observed between groups in the present investigation, we would have needed to include at least 54 subjects per group. Therefore, with an adequate number of subjects, Hb concentrations may differ by dose and method of treatment. While comparing Hb concentrations at baseline to 8 weeks of follow-up within individual treatment groups was not the primary objective of this study, the *post hoc* power analysis indicates that the present study was adequately powered (86%) to detect a clinically significant difference of 10 g L^{-1} at a significance level of 0.05 and standard deviation of 15 g L^{-1} . In terms of the generalizeability of the study findings, we studied children with Hb concentrations between 70 and 99 g L^{-1} and supplemented these children for 8 weeks. In programme settings, children would likely have Hb concentrations in a broader range. In most developing countries, children with anaemia comprise 60–90% of the total population of young children. Therefore, our results are still generalizable to settings where the prevalence of anaemia is high. A dose of iron within multi-micronutrient Sprinkles that could achieve a therapeutic response in children with mild to moderate anaemia ($\text{Hb } 70\text{--}100 \text{ g L}^{-1}$), would also be beneficial to children with $\text{Hb} > 100 \text{ g L}^{-1}$, provided the duration of intervention is short (approximately 8 weeks). Thus, our results are not generalizable to iron fortification programmes that are meant for the entire population (including adult males) and for indefinite periods of time. The current trial should only be seen as a dose-finding study.

Because individualized screening for IDA would not be possible in large-scale programmes (because of high costs), a single dose of iron that is effective in treating those who have IDA yet not excessive in those who have sufficient iron stores is required. Our results suggest that an iron dose as low as 12.5 mg in the form of encapsulated ferrous fumarate may be effective in treating IDA and hence suitable for such programmes. With the advent of a new micronized

form of ferric pyrophosphate (used in the current study) that has shown promising results in terms of bioavailability and organoleptic properties (that allows it to be miscible in liquids in addition to solid foods) (S.H. Zlotkin, personal communication). Sprinkles with micronized ferric pyrophosphate can be added to liquids as well. This would allow greater flexibility for programme applications but at a higher cost, and thus requires careful consideration in terms of cost-effectiveness.

In conclusion, the significant effects on haematologic indices observed in the current study with different doses and forms of iron suggest that iron is well absorbed in iron-deficient children even when the iron is mixed with foods and when the dose of iron is as low as 12.5 mg. Further research is needed to determine the effectiveness of this dose of iron in programme settings.

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S. Zlotkin owns the intellectual property rights to micronutrient Sprinkles™. All profit net of expenses from the licensing of Sprinkles is donated to the Hospital for Sick Children Foundation. There are no other 'competing interests'.

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