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**THE HOSPITAL
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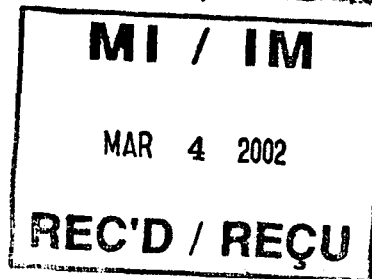**University of Toronto
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9/0313-B8

February 22, 2002

The Micronutrient Initiative
P.O. Box 56127
Ottawa, Ontario
K1R 7Z1

To whom it may concern:

Re: Zinc Supplementation to Prevent Zinc Deficiency in Anemic Infants and Young Children Receiving Treatment with Iron: A Follow-up Randomized Controlled Trial (Ghana) Centre/MI File: 5600-0007-08-300

A manuscript emanating from the project listed above has been submitted to the American Journal of Clinical Nutrition. I provide, for your review, a copy of the manuscript which was recently submitted. If you have any questions, please feel free to contact me at the address listed on this letterhead.

Sincerely,

Stanley H. Zlotkin, MD, PhD, FRCPC

SHZ:ls

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2 5

1   Zinc supplementation did not improve linear growth decline in anemic

2   Ghanaian infants treated with microencapsulated ferrous fumarate

3

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6

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13   **Health Research Unit, Ministry of Health, Ghana** (P Arthur MD, MPH, MSc, Kojo

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16

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20   Running head: Length decline with zinc and iron supplements

1   **Abstract**

2   **Background:** High rates of iron deficiency anemia and early linear growth faltering

3   possibly due to zinc deficiency are observed in infants in the developing world. We

4   recently demonstrated that microencapsulated ferrous fumarate sprinkles were efficacious

5   in the treatment of anemia, yet infants manifested a rapid decline in linear growth. Since

6   iron supplementation can depress zinc absorption we speculated that the growth faltering

7   might be due to zinc deficiency.

8   **Objective:** To determine the effect of zinc supplementation on linear growth in anemic

9   infants treated with microencapsulated ferrous fumarate.

10   **Design:** In a prospective randomized double-masked clinical trial, we studied 304

11   anemic infants (mean age 10.3 ± 2.5 months; hemoglobin 87.4 ± 8.4 g/L) in rural Ghana.

12   The intervention group (n= 160) received a daily sachet of microencapsulated ferrous

13   fumarate (80 mg iron) and zinc gluconate (10 mg) in powder form to be sprinkled on to

14   any complementary food; the control group (n=144) received an identical sachet without

15   added zinc. Both groups received the sachets once daily for 2 months. Anthropometric

16   measurements, plasma zinc, hemoglobin and ferritin were measured at baseline and end.

17   78.6% of infants completed the study.

18   **Results** At baseline, 80.7% of infants had normal plasma zinc concentrations but were

19   stunted. Stunting significantly worsened after zinc supplementation (z-score, -1.70 start,

20   -1.81 end, p=0.001) when compared to the control group (z-score, -1.81 start, -1.86,

21   p=0.0985). Mean plasma zinc concentration decreased significantly in both groups

22   (p<0.05). The rate of recovery from anemia was higher in the control group than in the

23   zinc supplemented group (74.8% (86/115) vs 62.9% (78/124); p = 0.048).

1   **Conclusion** Short-term use of zinc supplements in anemic children did not prevent

2   growth faltering and was associated with a lower rate of successful treatment of anemia.

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14   <u>Key Words</u>: zinc, iron; infants and children; anemia, microencapsulated iron; ferrous

15   fumarate; linear growth faltering, malnutrition.

1 **Introduction**

2 High prevalence rates for anemia and early linear growth faltering are common

3 features of malnourished infants in the developing world (1, 2). Both are associated with

4 diminished cognitive and physical development that may not be reversible (3). Anemia

5 and stunting are also common consequences of the plant- and cereal-based

6 complementary diet typically fed to infants and children in developing countries (4).

7 These complementary foods are low in energy and poor sources of bioavailable iron and

8 zinc. They are high in phytic acid, which further reduces the absorption of both

9 micronutrients from the diet. To improve the nutritional status of these infants, it has been

10 suggested that supplementation with micronutrients may be the most appropriate strategy

11 (5)

12

13 In an effort to improve the iron status of infants, we recently developed a novel

14 supplementation approach, which provides microencapsulated ferrous fumarate and

15 ascorbic acid in powder form. Packaged in individual sachets, the supplement is

16 designed to be sprinkled on to complementary foods after the food is cooked. In a

17 randomized controlled trial we demonstrated that microencapsulated ferrous fumarate

18 sprinkles were as efficacious as ferrous sulfate drops in the treatment of anemia in infants

19 6-18 months of age (6). Despite the positive effect on anemia status, we observed a rapid

20 and significant decline in linear growth over the two-month study period. Height-for-age

21 z-scores in both groups (drops and sprinkles) decreased from a mean of -1.36 ± 1.12 at

22 the beginning, to -1.53 ± 1.16 at the end of the two-month intervention ($p<0.0001$) without

23 concurrent changes in either weight-for-age or weight-for-height z-scores in either group.

1   We speculated that this rapid decline in linear growth might have been due to a

2   combination of *de novo* and iatrogenic zinc deficiency, since linear growth failure in

3   infants is a principle clinical feature of zinc deficiency (7-10).

4

5   The interaction between iron and zinc has been well-described (11-13). At an

6   estimated molar ratio (Fe:Zn) of >2:1 in adults, iron can depress zinc absorption when

7   iron is given as a supplement (14). Moreover, iron supplementation has also been

8   associated with impaired linear growth in Honduran infants (15). To explain the linear

9   growth faltering observed among infants in our original study, we speculated that iron

10   supplementation provided to the infants may have depressed the absorption of

11   endogenous zinc from primarily cereal-based weaning diet, thereby, exacerbating an

12   already precarious zinc status. This may have led to the observed rapid decline in linear

13   growth.

14

15   In the prospective double-masked randomized controlled trial reported here, we

16   tested the hypothesis that anemic infants receiving daily zinc supplementation combined

17   with microencapsulated ferrous fumarate sprinkles plus ascorbic acid would manifest

18   improved height-for-age z-scores and plasma zinc concentrations when compared to

19   anemic infants receiving microencapsulated ferrous fumarate sprinkles with ascorbic acid

20   alone. Our objective therefore was to determine the effect of zinc supplementation on

21   linear growth in anemic infants treated with microencapsulated ferrous fumarate.

1   Methods

2   *Study area, subjects and recruitment*

3   The research took place in the field study area for the Kintampo Health Research

4   Centre (KHRC), located in the Brong Ahafo Region of Ghana. This is a malaria-endemic

5   area where the principle complementary food is a maize-based porridge, low in

6   bioavailable iron and zinc. The prevalence of anemia in young children is estimated at

7   about 70%, a significant proportion of which is due to iron deficiency (16).

8   Eligible infants were identified from an existing surveillance database of births in

9   the district. To be included in the study, infants had to be 6 to 24 months old at the time

10   of recruitment; ingesting a weaning food in addition to breast milk; with a hemoglobin

11   concentration between 70 and 99 g/L, as measured during a baseline assessment.

12   Children who were severely anemic (hemoglobin <70 g/L) were excluded from the trial

13   and treated.

14

15   *Study Design*

16   Since it was unethical to provide a placebo to a child with anemia at the start of the

17   trial, we did not include a placebo control. Randomization to one of the two treatment

18   groups was done with sealed opaque envelopes containing group designations, which

19   were generated randomly by computer with Microsoft Access 97 (Microsoft Corporation,

20   Seattle, WA). All individuals involved in the study (including parents and field workers)

21   were blinded to group assignments until the code was broken at the completion of the

22   data analysis.

1   The intervention group received microencapsulated ferrous fumarate (80 mg of

2 elemental iron) and zinc gluconate (10 mg of zinc) packaged in a sachet with ascorbic

3 acid (50 mg), added to the child's meal serving (after it was cooked) once daily. The

4 control group received iron sprinkles (80 mg of encapsulated ferrous fumarate plus 50 mg

5 of ascorbic acid) administered similarly, once daily. The dose of iron was identical to that

6 which had been shown to be efficacious in our earlier study (6). Because the ferrous

7 fumarate was lipid-coated, we documented minimal intestinal irritation from this

8 relatively high dose of iron.

9

10   During the baseline assessment, a written questionnaire was administered to collect

11 demographic, nutritional, and health data for each infant. Field workers visited infants at 2-

12 week intervals after the baseline visit, for a total of 5 visits. At each visit, a questionnaire

13 regarding side effects (diarrhea, constipation, and general discomfort), ease of use and

14 adherence over the preceding 7 days was completed. Questions related to ease of use

15 included whether children objected to taking the iron and whether microencapsulated

16 ferrous fumarate changed the colour, taste or texture of the infants' food. To evaluate

17 adherence, during each visit, the number of used (empty) sachets was counted.. At each

18 visit, fieldworkers provided parents with verbal educational reinforcement to maximize

19 adherence to the intervention.

20   Anthropometric measurements, including weight and height were completed

21 during baseline and final visits as previously described (6). Capillary blood samples at

22 baseline and final visits were obtained from a finger prick using aseptic techniques, and

23 hemoglobin concentration was determined on the spot using a portable HEMOCUE B-

1   hemoglobin photometer (Hemocue Inc, Angelholm, Sweden) by trained technicians using

2   standardized techniques (17). Malaria parasite smears were taken (at the final visit only),

3   and 500 μL blood samples were collected and preserved in ice-lined cold boxes. Blood

4   samples were returned to the base station within 6 hours of collection, where the plasma

5   was separated by centrifugation (10 minutes at 1300 RPM) before storage at -40^0C.

6   Serum ferritin was assayed in duplicate by a commercial enzyme-linked immunosorbent

7   assay (ELISA), using a Spectro Ferritin Kit (Ramco Laboratories, Houston, TX) (18).

8   Baseline and final ferritin samples from an individual subject were assayed on the same

9   day (in a single batch) on one 96-well microtitre plate to minimize inter-assay variation.

10   An external reference standard (Lyphochek Anaemia Control, Bio-Rad, Anaheim, CA)

11   was assayed in duplicate on each microtitre plate for the ferritin assay. Plasma zinc

12   concentration was determined by inductively coupled plasma mass spectrophotometry

13   (ICP-MS) (19).

14

15   *Sample size and power*

16   Based on a literature review and data from our previous study in Ghana, in which

17   initial mean height-for-age z-score was -1.36 ± 1.12, we expected that zinc

18   supplementation would improve the z-score by 0.49 standard deviation units. Using an α

19   = 0.05 and power = 0.80 the estimated sample size was 112 subjects per group (20).

20   Assuming a 20 % dropout rate, we planned to recruit 135 infants per group.

21

1   *Data processing and analysis*

2   Data were entered in Visual Fox Pro 6.0 (Microsoft Corporation), verified, and

3   checked for range and consistency with customized data-entry and processing programs

4   (Microsoft Access 97, Microsoft Corporation) as previously described (6). Data were

5   analyzed with Statistical Analysis Software, version 8.0 (SAS Institute, Inc, Carey, NC).

6   Paired *t* tests were used to analyze the change in plasma zinc and anthropometric

7   measurements, as well as hemoglobin and ferritin, over time. Differences between groups

8   in anthropometric measurements, plasma zinc, hemoglobin and ferritin, at the beginning

9   and the end of the study were assessed by ANOVA (with proc GLM). Analysis of

10   ferritin values was conducted on log-transformed data because of their skewed frequency

11   distribution. The proportion of children who went from an anemic to a non-anemic state

12   (hemoglobin >100 g/L) and from iron depleted to an iron replete state (ferritin >12 µg/L)

13   was compared between the groups with chi-square analysis. McNemar's test was run to

14   compare change in anemia and ferritin status at the beginning and end of the study. The

15   acceptable level of statistical significance for all tests was $p<0.05$.

16

17   *Ethics approval and consent*

18   Ethics approval was obtained from The Hospital for Sick Children (Toronto,

19   Canada), the London School of Hygiene and Tropical Medicine (London, UK), and

20   Ghana's Ministry of (Kintampo, Ghana).

21   Oral consent to conduct the study in the Kintampo district was obtained from the

22   District Assembly of Elected Representatives; in each village from village elders; and

23   individual signed consent was obtained from the mothers of infants in the study.

1   **Results**

2   After the screening survey, a total of 529 infants were found to be eligible for the

3   study. 57.5% (304 infants) had hemoglobin concentrations between 70.0 g/L and 99.9

4   g/L. Their mean age was 10.3 ± 2.5 months. Infants were randomized into 2 groups

5   (Figure). 65 (21.4%) of the 304 infants did not attend the final assessment visit.

6   Consequently, a total of 239 infants completed the final assessment, including

7   anthropometric measurements and blood sampling.

8   At baseline there were no significant differences in mean [SD] plasma zinc

9   (p=0.58), age (p=0.78), hemoglobin (p=0.95) or ferritin (p=0.44) values between the

10   treatment groups.

11

12   *Anthropometric measurements*

13   There was no effect of group, gender or their interaction on initial and final z-

14   score values. The mean weight-for-age, height-for-age and weight-for-height z-scores at

15   baseline and final were all below zero (table 1). There were no differences between

16   groups at baseline and end. Both groups had a significant decrease in mean weight-for-

17   age and weight-for-height z-scores between baseline and final. Infants in the 'Iron + zinc'

18   group had a significant decrease in their mean height-for-age z-score (p=0.001), whereas

19   there was no significant decrease in the 'Fe- alone' group (p=0.099). There was a

20   significant negative association between initial age and final mean weight-for-age

21   (p=0.02) and weight-for-height z-scores (p=0.02) among the entire sample population.

22

23   *Plasma Zinc Response*

1   At baseline, the mean plasma zinc concentrations were similar between groups

2   (Table 2). From baseline to the end of the study the mean plasma zinc concentration

3   decreased significantly in both groups although there was a trend toward higher mean

4   zinc concentrations in the 'Fe + zinc' group at the end.

5

6   At baseline there was no difference between the groups in the proportion of

7   infants with low plasma zinc values (p=0.4601). Overall, 43/223 (19.3%) had plasma

8   zinc values below 10.7 μmol/L (normal range 11.5 -22.2 μmol/L for infants under the age

9   of 1 year and 10.7 - 20.0 μmol/L for infants >1 year; HSC reference values). The

10   proportion of infants with zinc values below 10.7μmol/L increased significantly in the

11   'Iron' only group from 23/108 (21.3%) at baseline to 39/108 (36.1%) at the end

12   (p=0.016).

13

14   *Hemoglobin response*

15   There was no effect of initial hemoglobin, group, gender or age on final

16   hemoglobin. In both groups, there was a significant increase in hemoglobin concentration

17   from baseline to the end of the study (p< 0.0001; table 3). Overall, 164/239 (68.6%) of

18   infants advanced from an anemic to a non-anemic state (hemoglobin values >100 g/L).

19   The hemoglobin concentration in the 'Iron' group was significantly higher than the "Iron

20   + zinc' group at the end of the study (p=0.024). The rate of recovery was higher in the

21   'Iron' group 86/115 (74.8%) than in the 'Iron + zinc' group 78/124 (62.9%) (p = 0.048).

22   Data were also analyzed to determine the percentage of infants who positively

23   responded to iron treatment (a positive response was defined as an increase in

1   hemoglobin of 10 g/L or greater at the final blood sample). In the 'Iron group, 89/115

2   (77.4%) of the infants responded; in the 'Iron + zinc' group, 84/124 (67.7%) responded

3   (p=0.028). The relative risk of remaining anemic after two months of treatment was 0.74

4   times lower for the 'Iron' group (95% CI 0.54 - 1.02; p=0.049).

5

6   *Ferritin response*

7   At baseline and at the end, geometric mean ferritin values were similar between the two

8   groups (p= 0.44; table 4). There was a significant increase in both after the 2-month

9   intervention (p<0.0001). The variance for ferritin values was wide at both baseline and at

10   the end of the study, as is commonly found in malaria endemic regions. (21). At baseline

11   there was no difference in the proportion of infants with iron depletion (defined as ferritin

12   <12μg/L) between treatment groups (p=0.49). McNemar's analysis showed that there

13   was a significant decrease in the number of infants with iron depletion after two months

14   of treatment within both groups. In the 'Iron' group the rate of iron depletion decreased

15   from 36/92 (39.13%) at baseline to 22/92 (23.91%) at the end (p=0.0043) and from

16   49/110 (44.5%) to 17/110 (15.45%) in the 'Iron + Zn' group (p<0.0001). The rate of

17   decrease observed in the zinc supplemented group was significantly greater (p<0.0001).

18

19

20   *Malaria status*

21   178/286 (62.24%) infants tested positive for malaria parasites. Infants who tested

22   positive for malaria were more likely to be anemic in both groups (p< 0.0001). There was

23   no difference in malaria status different between groups.

1 *Compliance*

2   Over the two-month intervention, 82.1% of the infants received sprinkles at least

3 5 times a week. Only 3.4% of parents reported having any problems using sprinkles. Of

4 those who reported problems, only 1.8% reported that they had an unpleasant odor while

5 80.5% reported that the sprinkles changed the colour of their infant's food (much like the

6 effect of adding a condiment such as pepper to food). Fewer than 3% of all caregivers

7 gave the sprinkles to a 'non-study' child and 69.7% reported using the full contents of the

8 sachet all of the time. All infants were breast-feeding at the start of the study and

9 continued breast-feeding during the two-month period, although not exclusively. None of

10 the children received commercial infant formulas.

11

1 **Discussion**

2   In the current study, we proposed that zinc was the limiting nutrient for the

3 promotion and maintenance of linear growth and that supplementation with iron would

4 further predispose to zinc deficiency and growth faltering, while zinc supplementation

5 would sustain growth. Continued growth faltering was observed in both groups, thus

6 zinc-supplementation did not improve growth. It is likely, therefore, that growth faltering

7 is due to multiple factors in addition to marginal zinc status.

8   With the single exception of the height-for-age z-score in the unsupplemented

9 group, weight-for-age, weight-for-height and height-for-age z-scores decreased

10 significantly in both groups over the study period. Linear growth faltering was, however,

11 greater in the zinc supplemented group. This suggests that zinc was not the limiting factor

12 for linear growth. The majority of infants had adequate zinc status at baseline despite

13 their food supply that was limited in zinc and high in zinc-binding phytate. Others have

14 made similar observations (22). We believe that there are four possible explanations for

15 this observation. Firstly, zinc is likely preserved when growth is limited. A rapidly

16 growing infant needs more nutrients than a slowly growing one. Thus if growth is limited

17 because of inadequate energy, for example, zinc needs may be concomitantly decreased.

18 Secondly, increased stool losses of zinc from diarrhea is often a predisposing cause of

19 zinc deficiency (7). The frequency of diarrhea in infants in the current study was not

20 high, possibly because the study was conducted during the 'dry season'. Thirdly, zinc

21 status as assessed by plasma zinc concentration is of limited value because of its poor

22 sensitivity and specificity to changes in dietary zinc and the inability to adequately

23 control for postprandial variation and infection (23). Finally, it has been suggested that as

1   dietary intake becomes limited, endogenous zinc losses are homeostatically decreased

2   (24). One or more of these reasons may explain the preservation of zinc status at baseline.

3   It is notable that infants in the unsupplemented group were able to maintain their

4   initial height-for-age z-scores without further significant growth faltering, while the zinc-

5   supplemented group did not. This refutes our original hypothesis that iron supplementation

6   alone was the major contributing factor to linear growth faltering. Alternatively, it suggests

7   that the iron may have had a protective effect on further faltering, possibly through the

8   greater improvement of iron status in the unsupplemented group. The impact of iron

9   supplementation on growth could not be directly assessed in this study because, for ethical

10   reasons, a placebo group of anemic infants was not included. The effect of iron

11   supplementation on linear growth has been equivocal with some reports describing

12   enhanced growth and others the opposite (25).

13   Like others, we observed a decrease in mean weight-for-age and weight-for-

14   height z-scores in infants between 6 and 24 months of age (25,26). Factors that could

15   have affected growth included infant and maternal stores of nutrients at birth, multiple

16   deficiencies of macro- and micronutrients, and the impact of infectious diseases (25). Our

17   results showed a significant negative association between initial age and final

18   underweight z-scores. These results imply that with increasing age, infants may not have

19   met their dietary energy and nutrient requirements. Similar results were recently

20   described in Ghana, where weight-for-length z-scores significantly decreased between

21   ages 2 to 12 months (22). These observations are consistent with Brown and Dewey's

22   conclusions that unfortified cereal-based complementary foods are inadequate total

23   sources of nutrition for breast-feeding infants in the first years of life (27).

1   A significant decrease in mean plasma zinc concentrations was observed in both

2   groups over the two-month study. However, the decrease was smaller and only

3   marginally significant in the zinc-supplemented group (p=0.046). This would suggest

4   that either the amount of zinc provided in the sachet or the bioavailability of the zinc

5   compound was insufficient to maintain zinc status during the two-month study period or

6   that the intervention period was too short. Dirren et al recently documented a significant

7   increase in plasma zinc concentrations in children supplemented with 10 mg of zinc/day

8   compared to a placebo, but over a 15-month period. Thus duration of supplementation

9   may be a contributing factor (28). Lartey et al in Ghana, observed an inverse relationship

10   between dietary available zinc and plasma zinc concentrations in a similar group of

11   infants (29). When the estimate was adjusted for calcium, phytate and animal protein, the

12   inverse relationship was relinquished. Thus, the bioavailability of zinc, when added to

13   food as a powdered sprinkle, is likely strongly influenced by the content of other

14   nutrients in the food to which it is added.

15   We had originally hypothesized that iron supplementation alone depressed zinc

16   absorption leading to linear growth faltering. The mechanism by which iron and zinc

17   compete for absorption is not fully understood. Results of past research on the effect of

18   dietary iron on zinc absorption are conflicting. Studies have shown that iron-fortified

19   infant foods did not interfere with zinc absorption at Fe:Zn molar ratios of as high as 57:1

20   (30,31). However, there is evidence that iron provided at supplementation levels may

21   have an adverse effect on zinc absorption when Fe:Zn ratios exceed 2:1 (12,32).

22   Furthermore, studies on the effect of prenatal iron supplements have found a decrease in

23   fractional zinc absorption when iron was provided at amounts as small as 18 mg/d (32-

1   35). In the current study, the Fe:Zn molar ratio was relatively high at 9:1. There is no way

2   of determining whether iron affected zinc absorption.

3   Although the primary purpose of this study was to examine the effect of

4   supplementary zinc on linear growth, a secondary objective was to confirm the positive

5   effect of microencapsulated ferrous fumarate sprinkles on the treatment of anemia, as had

6   been previously shown (6). In the current study we confirmed our earlier observations

7   that iron sprinkles are an efficacious alternative to treating anemia in infants. In fact, the

8   overall rate of successful treatment in the current study was even higher than in our

9   original report. However, the rate of successful treatment of anemia in the iron-alone

10   group was higher. The difference remained after adjusting for age, initial hemoglobin

11   and plasma zinc levels and malaria status. Dijkhuizen et al reported a similar antagonistic

12   interaction on combined supplementation when compared to iron alone, which was also

13   more effective in reducing the prevalence of anemia in Indonesian infants (36). These

14   results imply that iron absorption was greater in infants receiving iron supplements

15   without zinc. Inhibition of iron absorption in the zinc-supplemented group may have been

16   a result of zinc competing with iron for the same receptor sites on intestinal mucosal cells

17   (37). Although a few studies have demonstrated an effect of zinc on iron absorption when

18   Zn:Fe molar proportions were equal (38,39), there is no data on the effect of zinc on iron

19   absorption when iron molar proportions exceed those of zinc.

20   Results of this study indicate that in a controlled setting, micronutrient sprinkles

21   with iron and zinc do not prevent linear growth faltering in anemic infants, although

22   sprinkles are very successful in treating anemia. Early growth faltering in this population

23   is likely of multifactorial origin. Sprinkles with iron alone did not contribute to an

1   increased risk of linear growth faltering and although the addition of zinc had a

2   marginally negative effect on linear growth, one must be careful not to over-interpret

3   these results. Had the study lasted for longer than 2 months, we may have seen a positive

4   effect of zinc supplementation on growth as has been previously reported (40-42).

5   Further research is in progress to directly examine the interaction between iron and zinc

6   in sprinkles using stable isotope methodology.

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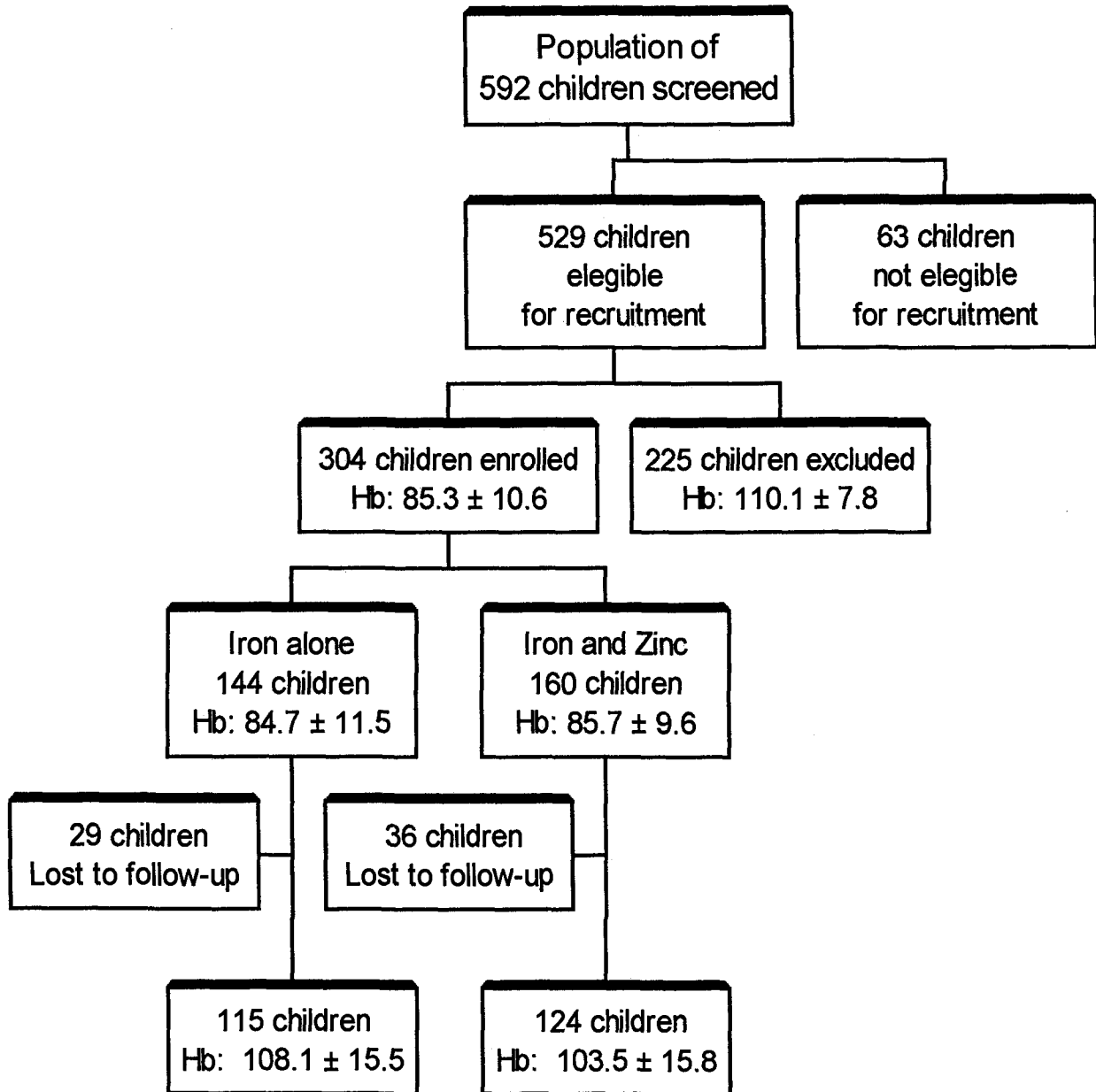
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21

22

23

Population of
592 children screened

529 children
elegible
for recruitment

63 children
not elegible
for recruitment

304 children enrolled
Hb: 85.3 ± 10.6

225 children excluded
Hb: 110.1 ± 7.8

Iron alone
144 children
Hb: 84.7 ± 11.5

Iron and Zinc
160 children
Hb: 85.7 ± 9.6

29 children
Lost to follow-up

36 children
Lost to follow-up

115 children
Hb: 108.1 ± 15.5

124 children
Hb: 103.5 ± 15.8

1

2   Figure 1. Trial Profile

1   Table 1: Anthropometry as determined by z-score values for weight- and height-for-age

2   and weight for height at baseline and at the end of the two-month intervention.

3   (i) Weight-for-Age Z-score

Group	Iron	Iron + Zinc	P-value
Baseline	-1.80 ± 1.14	-1.69 ±1.01	0.4243
Final	-1.95 ± 1.09	-1.89 ± 0.93	0.6898
Differences	-0.14 ± 0.47	-0.20 ± 0.42	0.3033
	p = 0.0022	p < 0.0001	

4   N= 230 paired samples

5

6   (ii) Height-for-Age Z-scores

Group	Iron	Iron + Zinc	P-value
Baseline	-1.81 ± 1.12	-1.70 ± 1.14	0.4890
Final	-1.86 ± 1.11	-1.81 ± 1.10	0.7321
Differences	-0.05 ± 0.32	-0.10 ± 0.34	0.2244
	p = 0.0985	p = 0.0011	

7

1   (iii) Weight-for-Height Z-scores

Group	Fe	Fe + zinc	P-value
Baseline	-0.65 ± 0.93	-0.60 ± 0.86	0.5863
Final	-0.92 ± 0.93	-0.88 ± 0.71	0.6773
Differences	-0.27 ± 0.54	-0.29 ± 0.50	0.7801
	$p < 0.0001$	$p < 0.0001$	

2

3

1   Table 2. Mean plasma zinc concentration by treatment group at baseline

2   and two months later *

3

Group	Iron (n=108)	Iron + Zinc (n=115)	p-value
		Plasma Zinc (µmol/L)	
Baseline	14.04 ± 4.42	14.36 ± 4.40	0.585
Final	12.44 ± 3.29	13.36 ± 3.81	0.056
Differences	-1.60 ± 4.90	-1.00 ± 5.33	0.3877
	p = 0.0010	p = 0.0461	

4

5   *Values are means ± SD

1   Table 3. Mean hemoglobin values at baseline and after the two-month intervention *.

2

Group	Iron (n=115)	Iron + Zinc (n=124)	p-value
		Hemoglobin (g/L)	
Baseline	87.4 ± 8.2	87.4 ± 8.5	0.9527
Final	108.1 ± 15.5	103.5 ± 15.8	0.0235
Differences	20.7 ± 15.3	16.1 ± 16.4	0.0282
	$p \leq 0.0001$	$p \leq 0.0001$	

3

4   * Values are means $\pm$ SD.

5

1   Table 4: Geometric mean ferritin values and range, by treatment group, at baseline and

2   after the 2 months intervention *

3

Group	Iron (n=92)	Iron + Zinc (n=110)	p-value
		Ferritin (µg/L)	
Baseline	16.3 (0.2-316.8)	13.7 (0.03-365.2)	0.4416
Final	37.4 (1.2 -390.1)	51.0 (1.4-386.1)	0.2926
Differences †	21.1 p<.0001	37.3 p<.0001	0.1140

4

5   *Data are geometric means and range; analysis was done with log-transformed values

6   since ferritin values are not normally distributed. † Mean ferritin increased significantly

7   from baseline to the final visit in both groups (p<0.001). Normal values are 12-400 µg/L

8   (48). Cut off values used: 400 (µg/L)

9