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

This report is presented as received by IDRC from project recipient(s). It has not been subjected to peer review or other review processes.

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Zinc supplementation did not improve linear growth decline in anemic Ghanaian infants treated with microencapsulated ferrous fumarate.

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Nutrition Institute of the ILSI Research Foundation. Mead Johnson Canada generously provided material support.

Running head: Length decline with zinc and iron supplements
Abstract

Background: High rates of iron deficiency anemia and early linear growth faltering possibly due to zinc deficiency are observed in infants in the developing world. We recently demonstrated that microencapsulated ferrous fumarate sprinkles were efficacious in the treatment of anemia, yet infants manifested a rapid decline in linear growth. Since iron supplementation can depress zinc absorption we speculated that the growth faltering might be due to zinc deficiency.

Objective: To determine the effect of zinc supplementation on linear growth in anemic infants treated with microencapsulated ferrous fumarate.

Design: In a prospective randomized double-masked clinical trial, we studied 304 anemic infants (mean age 10.3 ± 2.5 months; hemoglobin 87.4 ± 8.4 g/L) in rural Ghana. The intervention group (n= 160) received a daily sachet of microencapsulated ferrous fumarate (80 mg iron) and zinc gluconate (10 mg) in powder form to be sprinkled on to any complementary food; the control group (n=144) received an identical sachet without added zinc. Both groups received the sachets once daily for 2 months. Anthropometric measurements, plasma zinc, hemoglobin and ferritin were measured at baseline and end. 78.6% of infants completed the study.

Results At baseline, 80.7% of infants had normal plasma zinc concentrations but were stunted. Stunting significantly worsened after zinc supplementation (z-score, -1.70 start, -1.81 end, p=0.001) when compared to the control group (z-score, -1.81 start, -1.86, p=0.0985). Mean plasma zinc concentration decreased significantly in both groups (p<0.05). The rate of recovery from anemia was higher in the control group than in the zinc supplemented group (74.8% (86/115) vs 62.9% (78/124); p = 0.048).
Conclusion: Short-term use of zinc supplements in anemic children did not prevent growth faltering and was associated with a lower rate of successful treatment of anemia.

Key Words: zinc, iron; infants and children; anemia, microencapsulated iron; ferrous fumarate; linear growth faltering, malnutrition.
Introduction

High prevalence rates for anemia and early linear growth faltering are common features of malnourished infants in the developing world (1, 2). Both are associated with diminished cognitive and physical development that may not be reversible (3). Anemia and stunting are also common consequences of the plant- and cereal-based complementary diet typically fed to infants and children in developing countries (4). These complementary foods are low in energy and poor sources of bioavailable iron and zinc. They are high in phytic acid, which further reduces the absorption of both micronutrients from the diet. To improve the nutritional status of these infants, it has been suggested that supplementation with micronutrients may be the most appropriate strategy (5).

In an effort to improve the iron status of infants, we recently developed a novel supplementation approach, which provides microencapsulated ferrous fumarate and ascorbic acid in powder form. Packaged in individual sachets, the supplement is designed to be sprinkled on to complementary foods after the food is cooked. In a randomized controlled trial we demonstrated that microencapsulated ferrous fumarate sprinkles were as efficacious as ferrous sulfate drops in the treatment of anemia in infants 6-18 months of age (6). Despite the positive effect on anemia status, we observed a rapid and significant decline in linear growth over the two-month study period. Height-for-age z-scores in both groups (drops and sprinkles) decreased from a mean of -1.36 ± 1.12 at the beginning, to -1.53±1.16 at the end of the two-month intervention (p<0.0001) without concurrent changes in either weight-for-age or weight-for-height z-scores in either group.
We speculated that this rapid decline in linear growth might have been due to a combination of \textit{de novo} and iatrogenic zinc deficiency, since linear growth failure in infants is a principle clinical feature of zinc deficiency (7-10).

The interaction between iron and zinc has been well-described (11-13). At an estimated molar ratio (Fe:Zn) of >2:1 in adults, iron can depress zinc absorption when iron is given as a supplement (14). Moreover, iron supplementation has also been associated with impaired linear growth in Honduran infants (15). To explain the linear growth faltering observed among infants in our original study, we speculated that iron supplementation provided to the infants may have depressed the absorption of endogenous zinc from primarily cereal-based weaning diet, thereby, exacerbating an already precarious zinc status. This may have led to the observed rapid decline in linear growth.

In the prospective double-masked randomized controlled trial reported here, we tested the hypothesis that anemic infants receiving daily zinc supplementation combined with microencapsulated ferrous fumarate sprinkles plus ascorbic acid would manifest improved height-for-age z-scores and plasma zinc concentrations when compared to anemic infants receiving microencapsulated ferrous fumarate sprinkles with ascorbic acid alone. Our objective therefore was to determine the effect of zinc supplementation on linear growth in anemic infants treated with microencapsulated ferrous fumarate.
Methods

Study area, subjects and recruitment

The research took place in the field study area for the Kintampo Health Research Centre (KHRC), located in the Brong Ahafo Region of Ghana. This is a malaria-endemic area where the principle complementary food is a maize-based porridge, low in bioavailable iron and zinc. The prevalence of anemia in young children is estimated at about 70%, a significant proportion of which is due to iron deficiency (16).

Eligible infants were identified from an existing surveillance database of births in the district. To be included in the study, infants had to be 6 to 24 months old at the time of recruitment; ingesting a weaning food in addition to breast milk; with a hemoglobin concentration between 70 and 99 g/L, as measured during a baseline assessment. Children who were severely anemic (hemoglobin <70 g/L) were excluded from the trial and treated.

Study Design

Since it was unethical to provide a placebo to a child with anemia at the start of the trial, we did not include a placebo control. Randomization to one of the two treatment groups was done with sealed opaque envelopes containing group designations, which were generated randomly by computer with Microsoft Access 97 (Microsoft Corporation, Seattle, WA). All individuals involved in the study (including parents and field workers) were blinded to group assignments until the code was broken at the completion of the data analysis.
The intervention group received microencapsulated ferrous fumarate (80 mg of elemental iron) and zinc gluconate (10 mg of zinc) packaged in a sachet with ascorbic acid (50 mg), added to the child’s meal serving (after it was cooked) once daily. The control group received iron sprinkles (80 mg of encapsulated ferrous fumarate plus 50 mg of ascorbic acid) administered similarly, once daily. The dose of iron was identical to that which had been shown to be efficacious in our earlier study (6). Because the ferrous fumarate was lipid-coated, we documented minimal intestinal irritation from this relatively high dose of iron.

During the baseline assessment, a written questionnaire was administered to collect demographic, nutritional, and health data for each infant. Field workers visited infants at 2-week intervals after the baseline visit, for a total of 5 visits. At each visit, a questionnaire regarding side effects (diarrhea, constipation, and general discomfort), ease of use and adherence over the preceding 7 days was completed. Questions related to ease of use included whether children objected to taking the iron and whether microencapsulated ferrous fumarate changed the colour, taste or texture of the infants’ food. To evaluate adherence, during each visit, the number of used (empty) sachets was counted. At each visit, fieldworkers provided parents with verbal educational reinforcement to maximize adherence to the intervention.

Anthropometric measurements, including weight and height were completed during baseline and final visits as previously described (6). Capillary blood samples at baseline and final visits were obtained from a finger prick using aseptic techniques, and hemoglobin concentration was determined on the spot using a portable HEMOCUE B-
hemoglobin photometer (Hemocue Inc, Angelholm, Sweden) by trained technicians using standardized techniques (17). Malaria parasite smears were taken (at the final visit only), and 500 µL blood samples were collected and preserved in ice-lined cold boxes. Blood samples were returned to the base station within 6 hours of collection, where the plasma was separated by centrifugation (10 minutes at 1300 RPM) before storage at −40°C. Serum ferritin was assayed in duplicate by a commercial enzyme-linked immunosorbent assay (ELISA), using a Spectro Ferritin Kit (Ramco Laboratories, Houston, TX) (18). Baseline and final ferritin samples from an individual subject were assayed on the same day (in a single batch) on one 96-well microtitre plate to minimize inter-assay variation. An external reference standard (Lyphochek Anaemia Control, Bio-Rad, Anaheim, CA) was assayed in duplicate on each microtitre plate for the ferritin assay. Plasma zinc concentration was determined by inductively coupled plasma mass spectrophotometry (ICP-MS) (19).

Sample size and power

Based on a literature review and data from our previous study in Ghana, in which initial mean height-for-age z-score was −1.36 ± 1.12, we expected that zinc supplementation would improve the z-score by 0.49 standard deviation units. Using an α = 0.05 and power = 0.80 the estimated sample size was 112 subjects per group (20). Assuming a 20 % dropout rate, we planned to recruit 135 infants per group.
Data processing and analysis

Data were entered in Visual Fox Pro 6.0 (Microsoft Corporation), verified, and checked for range and consistency with customized data-entry and processing programs (Microsoft Access 97, Microsoft Corporation) as previously described (6). Data were analyzed with Statistical Analysis Software, version 8.0 (SAS Institute, Inc, Carey, NC). Paired t tests were used to analyze the change in plasma zinc and anthropometric measurements, as well as hemoglobin and ferritin, over time. Differences between groups in anthropometric measurements, plasma zinc, hemoglobin and ferritin, at the beginning and the end of the study were assessed by ANOVA (with proc GLM). Analysis of ferritin values was conducted on log-transformed data because of their skewed frequency distribution. The proportion of children who went from an anemic to a non-anemic state (hemoglobin >100 g/L) and from iron depleted to an iron replete state (ferritin >12 µg/L) was compared between the groups with chi-square analysis. McNemar's test was run to compare change in anemia and ferritin status at the beginning and end of the study. The acceptable level of statistical significance for all tests was p<0.05.

Ethics approval and consent

Ethics approval was obtained from The Hospital for Sick Children (Toronto, Canada), the London School of Hygiene and Tropical Medicine (London, UK), and Ghana’s Ministry of (Kintampo, Ghana).

Oral consent to conduct the study in the Kintampo district was obtained from the District Assembly of Elected Representatives; in each village from village elders; and individual signed consent was obtained from the mothers of infants in the study.
Results

After the screening survey, a total of 529 infants were found to be eligible for the study. 57.5% (304 infants) had hemoglobin concentrations between 70.0 g/L and 99.9 g/L. Their mean age was 10.3 ± 2.5 months. Infants were randomized into 2 groups (Figure). 65 (21.4%) of the 304 infants did not attend the final assessment visit. Consequently, a total of 239 infants completed the final assessment, including anthropometric measurements and blood sampling.

At baseline there were no significant differences in mean [SD] plasma zinc (p=0.58), age (p=0.78), hemoglobin (p=0.95) or ferritin (p=0.44) values between the treatment groups.

Anthropometric measurements

There was no effect of group, gender or their interaction on initial and final z-score values. The mean weight-for-age, height-for-age and weight-for-height z-scores at baseline and final were all below zero (table 1). There were no differences between groups at baseline and end. Both groups had a significant decrease in mean weight-for-age and weight-for-height z-scores between baseline and final. Infants in the ‘Iron + zinc’ group had a significant decrease in their mean height-for-age z-score (p=0.001), whereas there was no significant decrease in the ‘Fe- alone’ group (p=0.099). There was a significant negative association between initial age and final mean weight-for-age (p=0.02) and weight-for-height z-scores (p=0.02) among the entire sample population.

Plasma Zinc Response
At baseline, the mean plasma zinc concentrations were similar between groups (Table 2). From baseline to the end of the study the mean plasma zinc concentration decreased significantly in both groups although there was a trend toward higher mean zinc concentrations in the ‘Fe + zinc’ group at the end.

At baseline there was no difference between the groups in the proportion of infants with low plasma zinc values (p=0.4601). Overall, 43/223 (19.3%) had plasma zinc values below 10.7 \(\mu\)mol/L (normal range 11.5 -22.2 \(\mu\)mol/L for infants under the age of 1 year and 10.7 - 20.0 \(\mu\)mol/L for infants >1 year; HSC reference values). The proportion of infants with zinc values below 10.7\(\mu\)mol/L increased significantly in the 'Iron' only group from 23/108 (21.3%) at baseline to 39/108 (36.1%) at the end (p=0.016).

**Hemoglobin response**

There was no effect of initial hemoglobin, group, gender or age on final hemoglobin. In both groups, there was a significant increase in hemoglobin concentration from baseline to the end of the study (p< 0.0001; table 3). Overall, 164/239 (68.6%) of infants advanced from an anemic to a non-anemic state (hemoglobin values >100 g/L). The hemoglobin concentration in the ‘Iron’ group was significantly higher than the “Iron + zinc” group at the end of the study (p=0.024). The rate of recovery was higher in the ‘Iron’ group 86/115 (74.8%) than in the ‘Iron + zinc’ group 78/124 (62.9%) (p = 0.048).

Data were also analyzed to determine the percentage of infants who positively responded to iron treatment (a positive response was defined as an increase in
hemoglobin of 10 g/L or greater at the final blood sample). In the 'Iron group, 89/115 (77.4%) of the infants responded; in the 'Iron + zinc' group, 84/124 (67.7%) responded (p=0.028). The relative risk of remaining anemic after two months of treatment was 0.74 times lower for the 'Iron' group (95% CI 0.54 - 1.02; p=0.049).

Ferritin response

At baseline and at the end, geometric mean ferritin values were similar between the two groups (p= 0.44; table 4). There was a significant increase in both after the 2-month intervention (p<0.0001). The variance for ferritin values was wide at both baseline and at the end of the study, as is commonly found in malaria endemic regions. (21). At baseline there was no difference in the proportion of infants with iron depletion (defined as ferritin <12µg/L) between treatment groups (p=0.49). McNemar's analysis showed that there was a significant decrease in the number of infants with iron depletion after two months of treatment within both groups. In the 'Iron' group the rate of iron depletion decreased from 36/92 (39.13%) at baseline to 22/92 (23.91%) at the end (p=0.0043) and from 49/110 (44.5%) to 17/110 (15.45%) in the 'Iron + Zn' group (p<0.0001). The rate of decrease observed in the zinc supplemented group was significantly greater (p<0.0001).

Malaria status

178/286 (62.24%) infants tested positive for malaria parasites. Infants who tested positive for malaria were more likely to be anemic in both groups (p< 0.0001). There was no difference in malaria status different between groups.
Compliance

Over the two-month intervention, 82.1% of the infants received sprinkles at least 5 times a week. Only 3.4% of parents reported having any problems using sprinkles. Of those who reported problems, only 1.8% reported that they had an unpleasant odor while 80.5% reported that the sprinkles changed the colour of their infant’s food (much like the effect of adding a condiment such as pepper to food). Fewer than 3% of all caregivers gave the sprinkles to a ‘non-study’ child and 69.7% reported using the full contents of the sachet all of the time. All infants were breast-feeding at the start of the study and continued breast-feeding during the two-month period, although not exclusively. None of the children received commercial infant formulas.
Discussion

In the current study, we proposed that zinc was the limiting nutrient for the promotion and maintenance of linear growth and that supplementation with iron would further predispose to zinc deficiency and growth faltering, while zinc supplementation would sustain growth. Continued growth faltering was observed in both groups, thus zinc-supplementation did not improve growth. It is likely, therefore, that growth faltering is due to multiple factors in addition to marginal zinc status.

With the single exception of the height-for-age z-score in the unsupplemented group, weight-for-age, weight-for-height and height-for-age z-scores decreased significantly in both groups over the study period. Linear growth faltering was, however, greater in the zinc supplemented group. This suggests that zinc was not the limiting factor for linear growth. The majority of infants had adequate zinc status at baseline despite their food supply that was limited in zinc and high in zinc-binding phytate. Others have made similar observations (22). We believe that there are four possible explanations for this observation. Firstly, zinc is likely preserved when growth is limited. A rapidly growing infant needs more nutrients than a slowly growing one. Thus if growth is limited because of inadequate energy, for example, zinc needs may be concomitantly decreased. Secondly, increased stool losses of zinc from diarrhea is often a predisposing cause of zinc deficiency (7). The frequency of diarrhea in infants in the current study was not high, possibly because the study was conducted during the ‘dry season’. Thirdly, zinc status as assessed by plasma zinc concentration is of limited value because of its poor sensitivity and specificity to changes in dietary zinc and the inability to adequately control for postprandial variation and infection (23). Finally, it has been suggested that as
dietary intake becomes limited, endogenous zinc losses are homeostatically decreased (24). One or more of these reasons may explain the preservation of zinc status at baseline.

It is notable that infants in the unsupplemented group were able to maintain their initial height-for-age z-scores without further significant growth faltering, while the zinc-supplemented group did not. This refutes our original hypothesis that iron supplementation alone was the major contributing factor to linear growth faltering. Alternatively, it suggests that the iron may have had a protective effect on further faltering, possibly through the greater improvement of iron status in the unsupplemented group. The impact of iron supplementation on growth could not be directly assessed in this study because, for ethical reasons, a placebo group of anemic infants was not included. The effect of iron supplementation on linear growth has been equivocal with some reports describing enhanced growth and others the opposite (25).

Like others, we observed a decrease in mean weight-for-age and weight-for-height z-scores in infants between 6 and 24 months of age (25,26). Factors that could have affected growth included infant and maternal stores of nutrients at birth, multiple deficiencies of macro- and micronutrients, and the impact of infectious diseases (25). Our results showed a significant negative association between initial age and final underweight z-scores. These results imply that with increasing age, infants may not have met their dietary energy and nutrient requirements. Similar results were recently described in Ghana, where weight-for-length z-scores significantly decreased between ages 2 to 12 months (22). These observations are consistent with Brown and Dewey's conclusions that unfortified cereal-based complementary foods are inadequate total sources of nutrition for breast-feeding infants in the first years of life (27).
A significant decrease in mean plasma zinc concentrations was observed in both groups over the two-month study. However, the decrease was smaller and only marginally significant in the zinc-supplemented group (p=0.046). This would suggest that either the amount of zinc provided in the sachet or the bioavailability of the zinc compound was insufficient to maintain zinc status during the two-month study period or that the intervention period was too short. Dirren et al recently documented a significant increase in plasma zinc concentrations in children supplemented with 10 mg of zinc/day compared to a placebo, but over a 15-month period. Thus duration of supplementation may be a contributing factor (28). Lartey et al in Ghana, observed an inverse relationship between dietary available zinc and plasma zinc concentrations in a similar group of infants (29). When the estimate was adjusted for calcium, phytate and animal protein, the inverse relationship was relinquished. Thus, the bioavailability of zinc, when added to food as a powdered sprinkle, is likely strongly influenced by the content of other nutrients in the food to which it is added.

We had originally hypothesized that iron supplementation alone depressed zinc absorption leading to linear growth faltering. The mechanism by which iron and zinc compete for absorption is not fully understood. Results of past research on the effect of dietary iron on zinc absorption are conflicting. Studies have shown that iron-fortified infant foods did not interfere with zinc absorption at Fe:Zn molar ratios of as high as 57:1 (30,31). However, there is evidence that iron provided at supplementation levels may have an adverse effect on zinc absorption when Fe:Zn ratios exceed 2:1 (12,32). Furthermore, studies on the effect of prenatal iron supplements have found a decrease in fractional zinc absorption when iron was provided at amounts as small as 18 mg/d (32-
In the current study, the Fe:Zn molar ratio was relatively high at 9:1. There is no way of determining whether iron affected zinc absorption.

Although the primary purpose of this study was to examine the effect of supplementary zinc on linear growth, a secondary objective was to confirm the positive effect of microencapsulated ferrous fumarate sprinkles on the treatment of anemia, as had been previously shown (6). In the current study we confirmed our earlier observations that iron sprinkles are an efficacious alternative to treating anemia in infants. In fact, the overall rate of successful treatment in the current study was even higher than in our original report. However, the rate of successful treatment of anemia in the iron-alone group was higher. The difference remained after adjusting for age, initial hemoglobin and plasma zinc levels and malaria status. Dijkhuizen et al reported a similar antagonistic interaction on combined supplementation when compared to iron alone, which was also more effective in reducing the prevalence of anemia in Indonesian infants (36). These results imply that iron absorption was greater in infants receiving iron supplements without zinc. Inhibition of iron absorption in the zinc-supplemented group may have been a result of zinc competing with iron for the same receptor sites on intestinal mucosal cells (37). Although a few studies have demonstrated an effect of zinc on iron absorption when Zn:Fe molar proportions were equal (38,39), there is no data on the effect of zinc on iron absorption when iron molar proportions exceed those of zinc.

Results of this study indicate that in a controlled setting, micronutrient sprinkles with iron and zinc do not prevent linear growth faltering in anemic infants, although sprinkles are very successful in treating anemia. Early growth faltering in this population is likely of multifactorial origin. Sprinkles with iron alone did not contribute to an
increased risk of linear growth faltering and although the addition of zinc had a marginally negative effect on linear growth, one must be careful not to over-interpret these results. Had the study lasted for longer than 2 months, we may have seen a positive effect of zinc supplementation on growth as has been previously reported (40-42). Further research is in progress to directly examine the interaction between iron and zinc in sprinkles using stable isotope methodology.
References


Figure 1. Trial Profile

Population of 592 children screened

529 children eligible for recruitment

63 children not eligible for recruitment

304 children enrolled

225 children excluded

Hb: 85.3 ± 10.6

Hb: 110.1 ± 7.8

Iron alone

144 children

Hb: 84.7 ± 11.5

29 children

Lost to follow-up

Iron and Zinc

160 children

Hb: 85.7 ± 9.6

36 children

Lost to follow-up

115 children

Hb: 108.1 ± 15.5

124 children

Hb: 103.5 ± 15.8
Table 1: Anthropometry as determined by z-score values for weight- and height-for-age and weight for height at baseline and at the end of the two-month intervention.

(i) Weight-for-Age Z-score

<table>
<thead>
<tr>
<th>Group</th>
<th>Iron</th>
<th>Iron + Zinc</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>-1.80 ± 1.14</td>
<td>-1.69 ± 1.01</td>
<td>0.4243</td>
</tr>
<tr>
<td>Final</td>
<td>-1.95 ± 1.09</td>
<td>-1.89 ± 0.93</td>
<td>0.6898</td>
</tr>
<tr>
<td>Differences</td>
<td>-0.14 ± 0.47</td>
<td>-0.20 ± 0.42</td>
<td>0.3033</td>
</tr>
<tr>
<td>p = 0.0022</td>
<td>p &lt; 0.0001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N= 230 paired samples

(ii) Height-for-Age Z-scores

<table>
<thead>
<tr>
<th>Group</th>
<th>Iron</th>
<th>Iron + Zinc</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>-1.81 ± 1.12</td>
<td>-1.70 ± 1.14</td>
<td>0.4890</td>
</tr>
<tr>
<td>Final</td>
<td>-1.86 ± 1.11</td>
<td>-1.81 ± 1.10</td>
<td>0.7321</td>
</tr>
<tr>
<td>Differences</td>
<td>-0.05 ± 0.32</td>
<td>-0.10 ± 0.34</td>
<td>0.2244</td>
</tr>
<tr>
<td>p = 0.0985</td>
<td>p = 0.0011</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
(iii) Weight-for-Height Z-scores

<table>
<thead>
<tr>
<th>Group</th>
<th>Fe</th>
<th>Fe + zinc</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>-0.65 ± 0.93</td>
<td>-0.60 ± 0.86</td>
<td>0.5863</td>
</tr>
<tr>
<td>Final</td>
<td>-0.92 ± 0.93</td>
<td>-0.88 ± 0.71</td>
<td>0.6773</td>
</tr>
<tr>
<td>Differences</td>
<td>-0.27 ± 0.54</td>
<td>-0.29 ± 0.50</td>
<td>0.7801</td>
</tr>
</tbody>
</table>

p < 0.0001  p < 0.0001
Table 2. Mean plasma zinc concentration by treatment group at baseline and two months later *

<table>
<thead>
<tr>
<th>Group</th>
<th>Iron (n=108)</th>
<th>Iron + Zinc (n=115)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma Zinc (μmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>14.04 ± 4.42</td>
<td>14.36 ± 4.40</td>
<td>0.585</td>
</tr>
<tr>
<td>Final</td>
<td>12.44 ± 3.29</td>
<td>13.36 ± 3.81</td>
<td>0.056</td>
</tr>
<tr>
<td>Differences</td>
<td>-1.60 ± 4.90</td>
<td>-1.00 ± 5.33</td>
<td>0.3877</td>
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</table>

p = 0.0010  p = 0.0461

*Values are means ± SD
Table 3. Mean hemoglobin values at baseline and after the two-month intervention *.

<table>
<thead>
<tr>
<th>Group</th>
<th>Iron</th>
<th>Iron + Zinc</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n=115)</td>
<td>(n=124)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Hemoglobin</strong> (g/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>87.4 ± 8.2</td>
<td>87.4 ± 8.5</td>
<td>0.9527</td>
</tr>
<tr>
<td>Final</td>
<td>108.1 ± 15.5</td>
<td>103.5 ± 15.8</td>
<td>0.0235</td>
</tr>
<tr>
<td>Differences</td>
<td>20.7 ± 15.3</td>
<td>16.1 ± 16.4</td>
<td>0.0282</td>
</tr>
</tbody>
</table>

* Values are means ± SD.

p ≤ 0.0001
Table 4: Geometric mean ferritin values and range, by treatment group, at baseline and after the 2 months intervention *

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

*Data are geometric means and range; analysis was done with log-transformed values since ferritin values are not normally distributed. † Mean ferritin increased significantly from baseline to the final visit in both groups (p<0.001). Normal values are 12-400 μg/L (48). Cut off values used: 400 (μg/L)