Original Article

Zinc-iron, but not zinc-alone supplementation, increased linear growth of stunted infants with low haemoglobin

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Zinc supplementation has been shown to benefit linear growth. However the effect may depend on whether zinc is the most limiting nutrient. This study aims to investigate the effect of supplementation with zinc-given alone or with iron and vitamin-A in improving infants' micronutrient status and linear growth. The study was a double-blind-community-intervention study involving 800 infants aged 3-6months in rural East Lombok, West Nusa Tenggara. Syrup consisting of zinc-alone, Zn (10 mg/d), zinc+iron, Zn+Fe (10 mg/d of each), zinc+iron+vitamin-A, Zn+Fe+vit.A (10 mg/d of each zinc and iron *plus* 1,000 IU vitamin-A), or placebo were given daily for six months. Outcomes measured were length, weight, and micronutrient status (haemoglobin, serum zinc, ferritin and retinol). Zn+Fe and Zn+Fe+vit.A supplementations benefit zinc and iron status of the subjects, while Zn-alone supplementation disadvantaged haemoglobin and iron status. The highest increment in vitamin A and haemoglobin status was shown in Zn+Fe+vit.A group. An effect on linear growth was observed among initially-stunted subjects in Zn+Fe and Zn+Fe+vit.A groups who grew 1.1-1.5cm longer than placebo. On the other hand, in the Zn-alone group, mean height-for-age Z-score decreased to a greater extent than placebo. The between-group difference in HAZ among initially-stunted subjects was significant after four months supplementation. While the difference was not significant in follow-up after 6 months, the pattern remained the same where means height-for-age Z-score in Zn+Fe+vit.A and Zn+Fe groups were higher than placebo and Zn-alone groups. Given the low haemoglobin/iron status of the subjects, zinc supplementation would have positive effect on growth if the low haemoglobin/iron status is also addressed and corrected.

Key Words: optimal growth, infant, iron, micronutrient, zinc

Introduction

It has been well established, both by studies in experimental animals and by human intervention trials, that zinc deficiency is growth-limiting. National prevalence of stunting or underweight among children under 5 years of age has been suggested as indirect indicators of a population's risk of zinc deficiency. A currently developed map which was reproduced from the WHO data indicates that those countries where the prevalence of childhood stunting exceeds 20%, such as Indonesia, are likely to have zinc deficiency. In addition estimated percentage of population at risk of having inadequate zinc intake also suggested that risk of zinc deficiency is high in Indonesia.[1]

While there is still lack of data to confirm zinc deficiency in Indonesia, data on iron deficiency anemia (IDA) showed that it is one of the most common nutrition problems in the country. In 1995, 40% of underfive children suffered from IDA and the figure is particularly high in the younger children <24 months.[2] The condition is exacerbated by the rich phytate content in the cereal-based complementary foods which inhibits the absorption of both iron and zinc.[3,4]

In area where stunting prevalence is high zinc supplementation is expected to improve linear growth; however the effect may be variable depending on whether zinc is the limiting or problem nutrient and on whether deficiencies in other nutrients which may limit a growth response to zinc supplementations exist.[3] While zinc and iron share common food sources, high dose of zinc can potentially induce antagonistic interactions with iron.[4] Vitamin A has also been shown to have specific benefit on linear growth i.e. on children with vitamin A deficiency.[5] Studies with zinc supplementation often only include parameter on zinc status and hence miss the information on how other nutrients status affected growth.

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The aim of this study is to investigate the effect of six-month zinc supplementations given in different regimes (zinc-alone, zinc+iron, zinc+iron+vit.A) on infants' micronutrient (zinc, iron, vitamin-A) and haemoglobin status as well as on linear growth of Indonesian infants. To assess if growth outcome is sustained after supplementation was ended, linear growth was also assessed six months after the end of supplementation.

Materials and methods

Subjects

The study was carried out in East Lombok district, West Nusa Tenggara province, Indonesia from July 1998 to March 1999, and a follow-up anthropometric measurement was conducted 6-month after the end of supplementation (September 1999). East Lombok was selected because preliminary data suggested that infant feeding practices in the area would most probably lead to iron and zinc deficiency.

The subjects were selected on the basis of the following criteria: aged 3-6 months old at enrollment, predominantly breast-fed, and free from apparent congenital abnormalities. Eligible infants were identified through data obtained from Integrated Health Service Posts *(Posyandu)* and by the village health volunteers in every sub-village. Mothers of eligible infants were informed of the procedures and purposes of the study and signed an informed consent form if they agreed their children participated in the study.

The study was approved by the Committee on Health Research Ethics, Faculty of Medicine, University of Indonesia, Jakarta.

Study Design and Sample Size

The study was a randomized, double-blind, placebo-controlled supplementation trial and was part of UNICEF's Multi-center Study for a Trial of Iron and Zinc Supplementation during Infancy.

Two hundred infants per groups (total 800 infants) were recruited in this study based on protocol of the multi-center study.[6] This sample size was based on the ability to determine with an α=0.05, 1-β=0.95 a difference in mean height-for-age Z-score of 0.45 SD and standard deviation of 1.15 and account for 20% drop-out rate. Subsample for biochemical assessment (n=53/group) was based on the ability to determine with an α=0.05, 1-β=0.95 a difference in serum zinc of 1.4 µmol/L and standard deviation of 2.0 µmol/L.[7]

Supplements

There were four supplementation groups in the study: zinc alone (10 mg/d), iron and zinc (10 mg of each/day), iron, zinc, and vitamin A (10 mg/day of each iron and zinc and 1,000 IU/d vitamin-A), and placebo. Zinc and iron were given as *zinc sulfate* and *ferrous sulfate,* while vitamin-A as *retinol-acetate.* The supplements were made by a local pharmaceutical company (PT. Kenrose, Indonesia) in cooperation with UNICEF-Jakarta. To avoid vitamin A deficiency becoming a limiting factor to child health, and to iron metabolism during the study period, vitamin A at a dose of 100,000 IU was given at the start of the study to all children**.** No de-worming was given since worm infestation was expected to be low among the infants.

Allocation to supplementation groups was conducted using systematic random sampling in each sex group This was done since gender might interfere with the growth rate i.e. effects are more pronounced in boys, perhaps because boys have higher estimated zinc requirements than do girls during infancy.[3]

The supplements were coded with a letter and the code was safe-kept at the SEAMEO laboratory by a laboratory assistant. Neither the investigator nor field assistants knew the codes until all subjects had completed the trial. Every subject received a personal bottle with a dosing syringe, labeled with the subject's name and code (the code indicated the location and the responsible cadre as well). The bottles were kept safe by the health volunteer who gave the supplements (2 mL syrup/day) for 30 days per month by door-to-door visits to the subjects' houses. The cadres recorded subjects' compliance in compliance forms i.e. whether subjects took the syrup/not and if not why, also on any side effects reported by the mothers. Every month the field coordinators distributed new bottles to cadres according to the list of subjects' allocation to the supplements' codes. The bottle was labeled with the subject's name and weighed to estimate bottle+syrup weight. After the 30th day the bottles were collected and weighed. The difference between bottle+syrup before and after distribution for each subject was used as an estimate of syrup consumed by the subject and was converted to days of compliance i.e. days of compliance = (syrup weight : weight per ml): 2 mL/day. Compliance was calculated as days of compliance divided by 180 days of intervention times 100%. Compliance based on bottle weighing was used as reference whereas compliance based on the cadres' record was used to cross-check the compliance data from bottle weighing.

Data collection

Weight and length were measured at baseline and monthly during six month supplementation by trained nutritionist using standard methods.[8] Weight was measured to the nearest 0.1kg with the child minimally clothed using an electronic weighing scale (SECA 770). Recumbent length was recorded to the nearest 0.1cm using a wooden length board (SEAMEO TROPMED). In addition, anthropometric measurements were also performed six months after the end of supplementation. Eight field workers (nutritionists and nurse) were trained and standardized by one of the authors (UF) to take all of the anthropometric measurements. Four persons with the highest precision and accuracy on length measurement which met the required standard were assigned as the nutritionist whereas the other four persons helped in holding the subjects for length measurements.[9]

Non-fasting blood samples were collected between 0700 and 1200. About 3-5 ml whole blood was withdrawn and put into acid-wash vacutainers, immediately stored in cool box prior to separation. Serum was separated by centrifugation at 2500 rpm for 10 min at room temperature. Serum samples were put into separate vials for assessment of serum zinc (in acid-washed vial), serum ferritin and serum retinol (non acid-washed vials). The

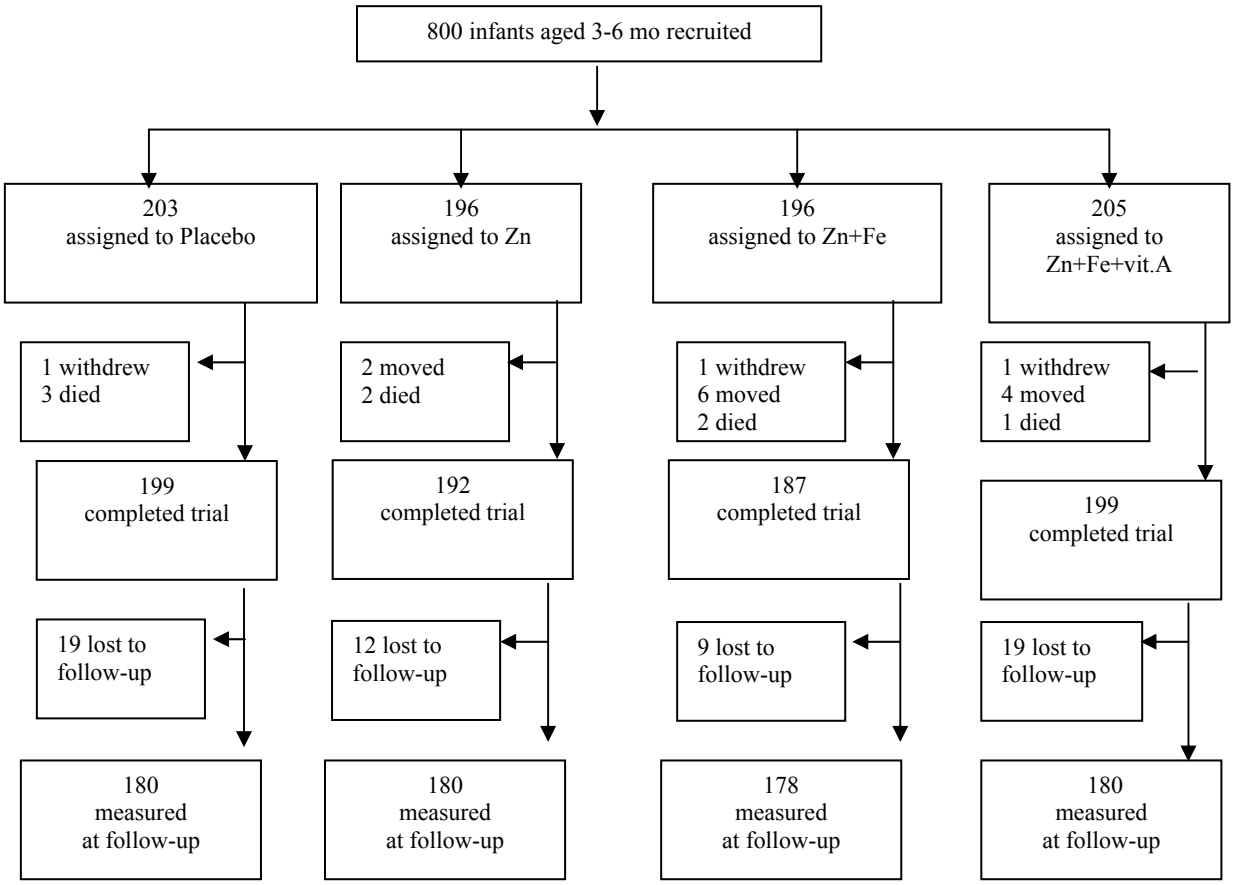

Figure 1. Trial profile of subjects

vials were stored at -30^{0}C until analysis.

Serum zinc was analyzed with flame atomic absorption spectrophotometry at Nutrition Research Development Center laboratory. Serum ferritin was determined with enzyme-linked immunoassay (ELISA) and serum retinol with high pressure liquid spectroscopy (HPLC) at the SEAMEO-TROPMED University of Indonesia laboratory. Between-duplicate variability were <5% for all biochemical analyses (serum zinc, ferritin, and retinol).

Haemoglobin samples were collected using filter paper methods of blood samples obtained from heel-prick and were analyzed by standard cyanmethemoglobin method.[10]

Information on complementary feeding and breastfeeding was obtained at baseline and every month using single 24hour-recall with mothers (or main caregivers). Since subjects started the study at different ages (3-6 months) dietary analyses was performed for intakes at 6-9 month of age in which all infants had the data. World Food 2 program (University of California, Davis) was used to calculate nutrient intake from complementary foods.

Data processing and statistical analysis

Z-scores for weight and length (weight-for-age *WAZ*; length-for-age *HAZ*; and weight-for-length, *WHZ*) were calculated with EPI-Info 6 using WHO recommended growth reference i.e. NCHS 1997.

Data were analyzed using SPSS 10.0 (SPSS Inc, Chicago, IL) software package. Each dependent variable was tested for homogeneity of its variance (normal distribution) using the Kolmogorov-Smirnov test. Data were reported as means ± SD for the normally distributed variables and as median (inter-quartile range) for non-normally distributed variables. Serum ferritin concentrations were transformed to logarithms before statistical analysis. Differences in prevalence were tested with Pearson's Chi-square test and differences among groups were examined using ANOVA for parametric variables and Kruskall-Wallis test for nonparametric variables. Since energy intake (as percentage of RDA) slightly differed among groups (p<0.05), this variable was included as covariate in the analyses of growth variables (ANCOVA test), however it was not shown to be significant covariate. When the overall F-test was significant, differences between the groups were further investigated with post-hoc multiple comparison for ANOVA. A paired *t*-test was used to evaluate the changes over time within each group for parametric variables. The McNemar test was applied to measure differences in prevalence within each group over time.

Differences between the supplementation groups for anthropometry at recruitment and subsequent 2-monthy intervals (2nd, 4th, 6th months) of supplementation were analyzed with repeated measurements multivariate ANOVA statistics. Sex and supplementation groups were included as between-subject factors. In addition, analyses were repeated including supplementation with/without zinc (placebo vs the remaining three groups), with/without iron (placebo and Zn-alone group vs Zn+Fe and Zn+Fe+vit.A group), with/without vitamin A (Zn+Fe+vit.A vs the remaining three groups). Infants with HAZ<-5.00 or >3.00 in the 2-monthly interval data were excluded from growth analysis.[11] Analyses of biochemical indicators included only subjects with available samples both before and after supplementation.

For interpretation of the statistical analyses, significance was set at *p* values less than 0.05.

Results

In total, 800 infants were recruited from 12 villages in the four sub-districts. Of the recruited infants, 777 (97%) completed the intervention and 718 (90%) could be measured at follow-up. Twenty-three infants (3%) dropped out during the intervention study for the following reasons: moving house (12 cases), withdrawal (three cases) and death (eight cases). The infants who dropped out did not differ from the infants who completed the trial for age, sex, nutritional status and parental characteristics (Fig 1).

Subjects in the four study groups were comparable in most of the parental and socio-economic background. There was no significant difference in education and occupation of mothers or fathers among the four supplementation groups, where 48% of the fathers and 27% of the mothers worked as farmers in their own land or as farm laborers. Most of the households were of low socio-economic as identified by non-permanent houses and low income (i.e. about one third had income below poverty cut-off for West Nusa Tenggara in 1998 which was Rp 25,000/capita.month).

Mean±SD age of the subjects at start of the study was 5.1±1.1 month and 51.5% were boys. All (100%) of the subjects were breastfed at the start of the study. There was no difference across the groups at age 6-9 months in terms of zinc, iron, and vitamin A intakes from both complementary food and breastmilk. Intakes of iron, vitamin A, and zinc were low: respectively 6%, 15%, and 50% of the estimated needs from complementary food (assuming low bioavailability of iron and zinc). Although there was difference between the groups in energy ($p<0.05$) and slightly for protein ($p<0.10$) –the highest being in Zn-alone, the lowest in Zn+Fe-- in all of the four groups the energy intake from complementary foods was still below the required energy of 269 kcal (6-8 months of age) from complementary food.[12] In general, prior to the supplementation, the four supplementation groups were comparable in their nutritional status and child characteristics (Table 1).

Median [range] of the overall compliance was 97% [range: 52-100%] and did not differ between the four groups. Table 2 showed changes on the biochemical indicators. There was an increase in serum zinc in the three groups which received zinc; the increase being highest in

Table 1. Characteristics of subjects in each treatment group at baseline[1]

Characteristics	Placebo (n=199)	Zn (n=192)	Zn+Fe (n=187)	Zn+Fe+vit.A (n=199)
Child age, month[2, †]	5.1±1.1	5.0±1.1	5.0±1.1	5.0±1.1
Child sex: boys, % [‡]	45.7	54.2	54.5	51.8
Mothers' education, %[‡]				
<3 y	20.6	22.4	28.9	26.1
3y – primary school	47.2	46.9	38.5	42.7
Secondary school	27.8	28.2	29.4	30.1
University or higher	1.5	2.6	1.6	0.5
No answer	2.5	-	1.6	0.6
Fathers' education, %[‡]				
<3 y	16.5	18.7	16.0	23.6
3y – primary school	42.7	46.9	44.4	35.7
Secondary school	31.1	27.6	35.3	33.2
University or higher	6.5	6.8	2.6	7.0
No answer	3.2	-	1.7	0.5
Mothers' occupation, %[‡]				
Housewife	52.8	57.3	57.8	58.3
Working in the farm	26.6	26.6	26.2	26.1
Others	9.0	8.3	10.2	9.5
No answer	11.6	7.8	5.8	6.1
Father's occupation, %[‡]				
Farmer	47.7	47.4	46.0	48.2
Fisherman	3.5	4.7	7.5	6.0
Monthly worker (teacher, civil servant)	11.1	6.8	4.3	8.0
Others (sellers, craftsman, working abroad)	35.1	38.6	38.5	35.1
Not working in previous 3-mo	0	0.5	1.1	0.5
No answer	2.7	2.0	2.6	2.2
Non-permanent house	49.7	57.3	61.0	53.8
Prevalence of micronutrient deficiencies, n (%)[‡]				
Anemia	119 (79.3)	127 (83.0)	128 (82.6)	128 (84.2)
Iron deficiency	0 (0)	4 (13.8)	1 (3.4)	4 (10.8)

[1] There were no significant differences among treatment groups for any value at baseline. [2] Mean ± SD. [†]ANOVA test. [‡]Chi-square test.

Table 2. Indicators of micronutrient status among infants before and after 6-month supplementation[1,2]

Indicators	Placebo	Zn	Zn+Fe	Zn+Fe+vit.A
Serum zinc(µmol/L)	n=34	n=25	n=32	n=35
• Start	15.3 [13.8 to 17.2]	15.3 [12.2 to 18.4]	15.3 [13.8 to 16.8]	15.3 [13.8 to 16.8]
• 6th mo [2]	15.3 [13.8 to 16.8]	18.4 [16.8 to 23.0][2, ***]	16.8 [14.2 to 19.9][2, **]	16.8 [13.8 to 19.9][2, *]
• Changes	0 [-1.9 to 1.5]	3.1 [0.8 to 8.4][2, ***]	1.5 [0 to 4.6][2, **]	1.5 [0 to 3.1][2, **]
Haemoglobin (g/dL) [3]	n=150	n=153	n=155	n=152
• Start	9.58 ± 1.59	9.65 ± 1.54	9.66 ± 1.45	9.54 ± 1.49
• 6th mo	9.08 ± 1.52[4,a, †]	9.02 ± 1.52[4,a, †]	9.63 ± 1.60[4,b]	9.69 ± 1.75[4,b]
• Changes	-0.50 ± 2.08[4,a]	-0.64 ± 2.01[4,a]	-0.03 ± 2.04[4,ab]	0.16 ± 2.15[4,b]
Serum ferritin (µg/L)	n=29	n=29	n=29	n=37
• Start	37.7 ± 2.2	29.9 ± 2.8	45.2 ± 2.8	29.6 ± 3.2
• 6th mo	14.1 ± 2.7[4,a, †]	9.4 ± 3.5[4,a, †]	31.1 ± 2.2[4,b]	22.9 ± 2.3[4,b]
• Changes	-22.2 [-49.7 to -7.7]	-18.0 [-36.8 to -10.3]	-10.4 [-70.6 to 12.9]	-2.9 [-58.9 to 14.1]
Serum retinol (µmol/L)	n=37	n=26	n=34	n=42
• Start	0.92 ± 0.19	0.90 ± 0.17	0.92 ± 0.21	0.93 ± 0.22
• 6th mo	0.97 ± 0.21[4,ab]	0.93 ± 0.19 [4,a]	0.91 ± 0.20[4,a]	1.04 ± 0.26[4,b, ‡]
• Changes	0.05 ± 0.22	0.03 ± 0.22	-0.01 ± 0.24	0.11 ± 0.35

[1]Values are mean ± SD, except for serum zinc and ferritin changes where values are median [25th to 75th percentile]. [2] Value is significantly different than placebo value, Mann-Whitney *U* test (* $p<0.05$, ** $p<0.01$, *** $p<0.001$). [3] Excluding Hb<6.00 g/L at start and final. [4] Values within one row with different letters as supercripts indicate significant differences among the groups (Anova & Bonferroni tests, $p<0.05$). [†] Within-group difference: significantly *lower* than values at start, paired-T test (* $p<0.01$). [‡] Within-group difference: significantly *higher* than values at start, paired-T test (* $p<0.05$)

Table 3. Prevalence (%) of under-nutrition by supplementation groups, before and 6 months after supplementation, and at follow-up[1]

Indicators	Placebo	Zn	Zn+Fe	Zn+Fe+vit.A
Stunting (HAZ<-2.00)	n=197	n=191	n=186	n=199
- start	16.8	13.1	16.1	16.6
- 6th mo [2]	28.1[†,**]	24.3[†,***]	27.6[†,***]	28.4[†,***]
- follow-up [3]	61.43[†,***]	57.4[†,***]	63.6[†,***]	65.1[†,***]
Wasting (WHZ<-2.00)	n=197	n=191	N=186	n=199
- start	2.5	1.6	0	1.0
- 6th mo [2]	13.5[†,***]	10.1[†,***]	8.1[†,***]	12.9[†,***]
- follow-up [3]	9.8	9.7	9.7	8.0 [†]
Underweight (WAZ<-2.00)	n=197	n=191	N=186	N=199
- start	5.6	6.3	2.2	4.5
- 6th mo [2]	41.7[†,***]	39.2[†,***]	33.5[†,***]	45.4[†,***]
- follow-up [3]	41.6	40.9	38.6	43.4
Anemia (hb < 10.0 g/dL)	n=150	n=153	n=155	n=152
- start	79.3	83.0	82.6	84.2
- 6th mo	92.0[†,**]	91.5[†,*]	83.9	80.3

[1] Number of samples (n) is number of samples at start and end unless otherwise specified. [2] Number of samples at 6th mo: placebo=192, Zn=189, Zn+Fe=185, Zn+Fe+vit.A=194. [3] Number of samples at follow-up: placebo=173, Zn=176, Zn+Fe=176, Zn+Fe+vit.A=175. [†] Within-group difference: Significantly different than prevalence at start (for 6th mo value) or than prevalence at 6th mo (for follow-up value); *$p<0.05$, **$p<0.01$, ***$p<0.001$, McNemar test

Zn group. Significant increase in serum retinol was only observed in Zn+Fe+vit.A group. On the other hand there was a decrease in mean haemoglobin and serum ferritin levels after six months; however the decrease was only significant in placebo and Zn groups for haemoglobin and in all except Zn+Fe+vit.A group for serum ferritin. Anaemia prevalence was already high (>80%) at the start of the study, when the infants were 3-6 months old. After six months, anemia prevalence increased significantly in placebo and Zn-alone groups, but not significantly in Zn+Fe and Zn+Fe+vit.A groups.

There was no significant difference in length, weight, and Z-scores across the groups after the supplementation. In all of the four groups, there was an increase in stunting, wasting and underweight ($p<0.001$). Furthermore, stunting prevalence increased to more than twice between end of supplementation and six months later i.e. follow-up ($p<0.001$ compared to prevalence at 6th mo), Table 3.

Table 4. Nutritional status indicators before and after supplementation, and at follow-up by supplementation group[1]

	Placebo (n=192)	Zn (n=189)	Zn+Fe (n=185)	Zn+Fe+vit.A (n=194)
HAZ				
- Initial	-1.04 ± 1.05	-0.93 ± 1.00	-1.00 ± 1.02	-1.04 ± 1.00
- 6th mo	-1.47 ± 0.94 †	-1.39 ± 0.96 †	-1.43 ± 0.92 †	-1.48 ± 0.94 †
- follow-up[2]	-2.26 ± 0.88 †	-2.19 ± 0.92 †	-2.28 ± 0.89 †	-2.26 ± 0.87 †
WAZ				
- Initial	-0.60 ± 0.98	-0.48 ± 1.00	-0.38 ± 0.94	-0.57 ± 0.92
- 6th mo	-1.86 ± 0.93[3,a, †]	-1.71 ± 0.89[3,c, †]	-1.63 ± 0.92[3,bc, †]	-1.86 ± 0.85[3,ab, †]
- *follow-up*[2]	-1.64 ± 1.02	-1.67 ± 1.02	-1.59 ± 1.13	-1.65 ± 1.07
WHZ				
- Initial	0.26 ± 1.07	0.29 ± 1.00	0.50 ± 0.90	0.29 ± 1.00
- 6th mo	-1.00 ± 0.93[3,a, †]	-0.88 ± 0.97[3,ab, †]	-0.73 ± 1.03[3,b, †]	-0.98 ± 0.93[3,a, †]
- *follow-up*[2]	-0.47 ± 1.31†	-0.58 ± 1.31†	-0.39 ± 1.43†	-0.49 ± 1.28†

[1] Values are means ± SD. [2] Number of samples at follow-up: placebo=176, Zn=177, Zn+Fe=176, Zn+Fe+vit.A=177. [3] Values with different alphabets were significantly different at $p< 0.05$ (LSD, post-hoc ANOVA). † Within-group difference: values were significantly lower than values at start (for 6th mo value) or than values at 6th mo (for follow-up value); $p<0.001$, Paired-*t* test

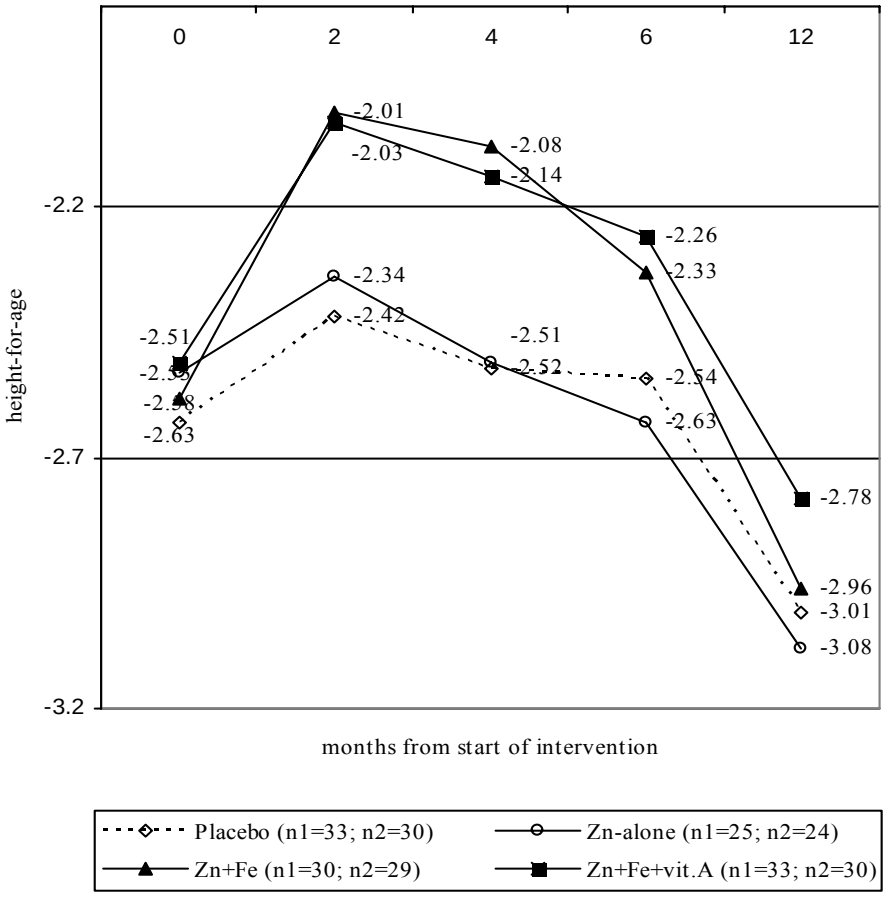

Figure 2. HAZ of the initially stunted subjects during the 6-month supplementation and at follow-up. Note: n1=number of subjects at start to 6th month after start of supplementation (the supplementation period); n2= number of samples at 12th month (follow up i.e. 6-month after end of supplementation)

Despite the supplementation Z-scores decreased during 6-month supplementation (HAZ, WHZ, WAZ) and 6-month after the end of supplementation (HAZ and WAZ). In average mean HAZ decreased by 0.4SD during the 6-month supplementation and by 0.8SD six months after end of supplementation; by this time mean HAZ was already <-2.0SD (Table 4). On the other hand, among initially stunted infants there was an *increase* in mean HAZ during the 6-month supplementation. Among initially stunted, HAZ increment was largest in the first 2-month of supplementation and became significantly larger in Zn+Fe and Zn+Fe+vit.A groups after 4-month supplementation than in both placebo and Zn-alone groups (Fig 2). After four months supplementation initially stunted subjects in these groups grew 1.1-1.5 cm longer than placebo and until follow-up the difference remained at 1 cm (Fig 3). Mean length change in Zn-alone group was similar with placebo and their HAZ even decreased larger than the placebo.

When supplementation groups were re-categorized by

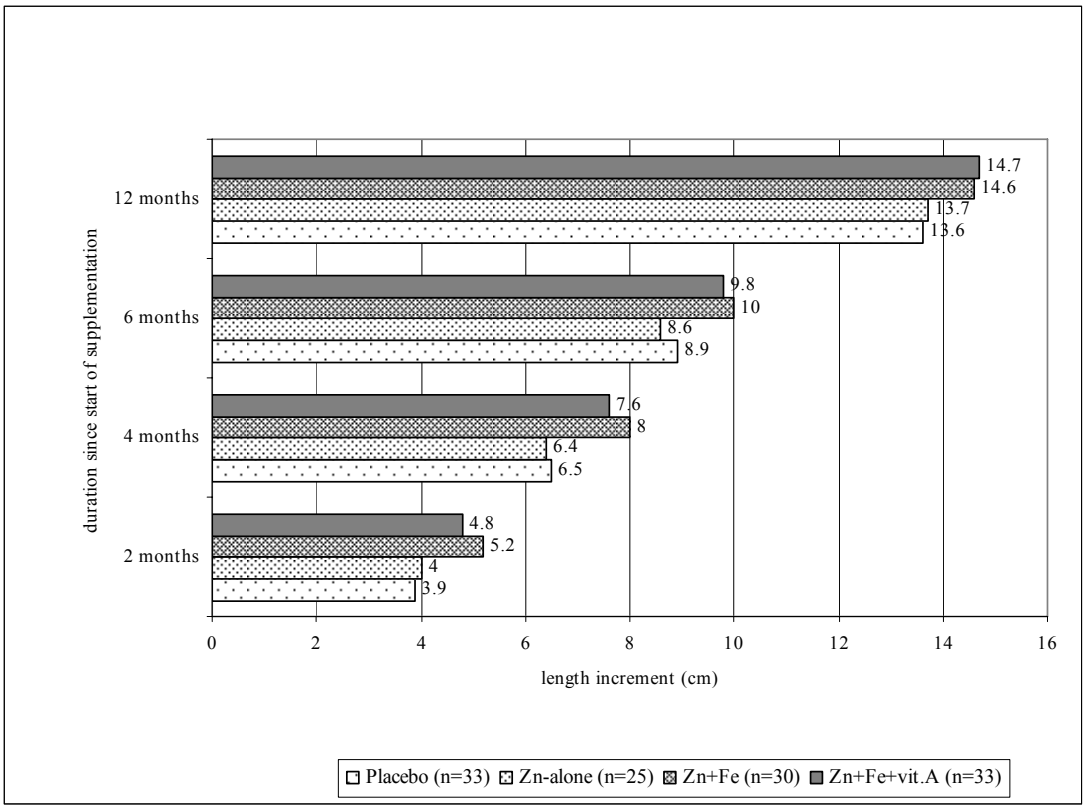

Figure 3. Length increment from start of the 6-mo supplementation among initially stunted subjects

nutrient (zinc, iron, vitamin A i.e. with/without each), changes of HAZ over time were significantly determined by stunting, sex, iron-supplementation*stunting interaction, and stunting*sex interaction. Boys and supplementation with iron were also significant between-subject factors among the initially stunted subjects i.e. mean HAZ after 6-mo supplementation was 0.4SD higher in boys and 0.2SD higher in groups receiving iron. Interaction of supplementation with iron and boys was not significant suggesting that stunted girls also benefit from iron in the supplement although to a lesser extend than boys.

Discussion

Result from this study showed that zinc status was improved by zinc supplementations whereas iron and vitamin A status also improved where the respective nutrient was in the supplements. We showed that, among infants with poor haemoglobin and iron status, zinc supplementations would lead to the improvements in linear growth only when iron was included in the supplement. In particular, we showed that benefit of the zinc+iron supplementations were significantly higher among initially stunted subjects and among boys. More importantly our findings suggested that zinc supplementation without iron did not benefit haemoglobin, iron status and linear growth of these infants.

Serum zinc levels of the subjects did not suggest that zinc deficiency was as significant public health problem as anemia or iron deficiency in the study area. Although resulted in hardly any changes in haemoglobin, iron in the supplement prevented ferritin and haemoglobin values from dropping in an important way. On the contrary, Zn-alone group experienced a large drop in ferritin and haemoglobin levels. Increase in prevalence of iron deficiency which was of the same pattern with increase in prevalence of anaemia indicated an IDA. This data was in line with low iron intake of the subjects with very low proportion (±10%) of heme iron *(data not shown)*. The observed low haemoglobin increment in this study could be attributable to low iron intake and low mean HAZ, as suggested by earlier study.[13] During the supplementation period, most subjects received 1-2 high dose vitamin A capsule from government which had been blanket national supplementation policy in every August and February; this may explain absence of vitamin A deficiency among the subjects both before and after supplementation. Despite the non-deficient status, additional vitamin A in the daily supplement was related with the highest change in haemoglobin level.

The improved micronutrient status, however, is not sufficient to allow for optimal growth in these infants, and is supported by an earlier study.[14] Positive effect on growth was found only among initially stunted subjects in Zn+Fe and Zn+Fe+vit.A groups. Despite positive effect on serum zinc among subjects supplemented with zinc-alone, their growth was not improved. This finding was also observed among the initially stunted subjects. This fact suggests that zinc is not the most limiting nutrient among this study subjects[4]; iron and other macronutrients i.e. energy and protein[15,16] were more limiting than zinc. Subjects in this study clearly had inadequate energy intake since the median energy intake (excluding breastmilk) between 6-8 months of age was between 82-109 kcal below the recommended level of 269 kcal[12]. Many studies suggested that fulfilment of macronutrient requirement is prerequisite for good absorption of micronutrient supple-

ment. The high correlation between haemoglobin and HAZ and the negative effect on HAZ of the Zn-alone group as compared to placebo (*data not shown*) further supported that iron was more limiting than zinc. In this regard, the 2RDA zinc in the supplement (ratio Zn:Fe of 2:1), which was initially thought would result in greater response in linear growth, would further dilute lack of iron in the overall diet i.e. food intake plus supplement. Perhaps Zn:Fe ratio of 1:1 or slightly less would better correct the anaemia and poor iron status and eventually growth.

This study support previous meta-analysis of 33 Zn-studies[17] which found greater response among initially stunted subjects and more earlier studies which found more pronounced response among boys.[18,19] Our finding was somewhat different with that found by Umeta et al[20] -- where zinc-alone supplementation (10 mg/d for six months) gave positive benefit for both boys and girls-- but supports previous findings that beneficial effect of zinc supplementation on growth is related to the degree of stunting and of zinc deficiency.[4] While baseline serum zinc level was not assessed in that study, the level among placebo group at the end of the supplementation suggested that zinc status was lower than our study. In addition to zinc status, it is possible that subjects in that study also had better iron and haemoglobin status than this current study, although these data were not available. It is possible that beneficial effect of zinc-alone supplementation on growth is more pronounced in severe zinc deficiency and/or mild iron deficiency. Comparing serum zinc levels and linear growth response in our study and others suggested that positive growth response in both boys and girls is related to more severe zinc deficiency while response among boys may only indicate the mild zinc deficiency.

Despite no positive response among the Zn-alone supplemented group, we found among initially stunted subjects receiving Zn+Fe and Zn+Fe+vit A increase in HAZ of 0.25±0.80 and 0.26±0.94, respectively. These changes were of greater magnitude than that reported among stunted infants in Ethiopia (0.14±0.46) given 10 mg Zn/d over 6-month period.[20] Similarly, length increment was 10.0±2.7 cm and 9.8±2.6 cm in these two groups, which was higher than length increment found in that study (7.0±1.0 cm). However, six months after supplements were no longer given HAZ decreased sharply and difference between Zn-alone and Zn+Fe or Zn+Fe+vit.A supplements became smaller, although means HAZ of the previously zinc-and-iron supplemented groups were higher than Zn-alone and placebo groups.

Our results provide evidence that growth is more determined by iron rather than zinc status among subjects with low haemoglobin and iron status. In order to have a positive benefit on linear and optimal growth in these subjects, zinc supplementation must be given together with iron with consideration on optimal iron:zinc ratio. The micronutrient supplementation must be accompanied by efforts to secure energy and protein requirements and therefore improving quality of home feeding and caregiving should be considered to sustain the effect of the micronutrient supplementations.

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References

1. International Zinc Nutrition Consultative Group (IZiNCG) Technical Document #1 Assessment of the Risk of Zinc Deficiency in Populations and Options for Its Control. Food Nutr Bull 2004; 25: s2.
2. Atmarita (2005). Nutrition Problems in Indonesia. www.gizi.net/download/nutrition%20problem%20in%20Indonesia.pdf (Accessed May 2006).
3. Gibson RS. Zinc nutrition in developing countries. Nutr Res Rev 1994; 7: 151-73.
4. Gibson RS. Zinc supplementation for infants. Lancet 2000; 355: 2008-9.
5. Hadi H, Stoltzfus RJ, Dibley MJ, Moulton LH, West KP Jr, Kjolhede CL, Sadjimin T. Vitamin A supplementation selectively improves the linear growth of indonesian preschool children: results from a randomized controlled trial. Am J Clin Nutr 2000; 71: 507-13.
6. Krause V, Yip R. Improving Iron and Zinc Nutrition in Infancy and Early Childhood: Proceedings of the Bali Consultation Meeting for the Planning of Multi-country Iron and Zinc Intervention Trials. Jakarta, UNICEF, 1997.
7. Cavan KR, Gibson RS, Grazioso CF, Isalgue AM, Ruz M, Solomons NW. Growth and body composition of periurban Guatemalan children in relation to zinc status: a longitudinal zinc intervention trial. Am J Clin Nutr 1993; 57: 344-52.
8. Gibson RS. Principles of Nutritional Assessment. New York: Oxford University Press, 1990.
9. WHO. Measuring Change in Nutritional Status. Geneva: WHO, 1983.
10. International Nutrition Anemia Consultative Group Iron Deficiency in Women. Nutrition Foundation. Washington DC: INACG Institute of Life Sciences, 1984.
11. WHO. Physical Status: The Use and Interpretation of Anthropometry. Geneva: WHO, 1995.
12. WHO. Complementary Feeding of Young Children in Developing Countries: A Review of Current Scientific Knowledge. Geneva: WHO, 1998.
13. Allen LH, Rosado JL, Casterline JE, Lopex P, Munoz E, Garcia OP, Martinez H. Lack of haemoglobin response to iron supplementation in anemic Mexican preschoolers with multiple micronutrient deficiencies. Am J Clin Nutr 2000; 71: 1485-94.
14. Dijkhuizen MA, Wieringa FT, West CE, Martuti S, Muhilal. Effects of iron and zinc supplementation in Indonesian infants on micronutrient status and growth. J Nutr 2001; 131: 2860-5.
15. Golden MHN. Specific deficiencies versus growth failure: Type I and Type II nutrients. SCN News 1995; 12:10-4.
16. Rivera JA, González-Cossió T, Flores M, Romero M, Rivera M, Tellez-Rojo MM, Rosado JL, Brown KH. Multiple micronutrient supplementation increases the growth of Mexican infants. Am J Clin Nutr 2001; 74: 657-63.

17. Brown KH, Peerson JM, Rivera J, Allen LH. Effect of supplemental zinc on the growth and serum zinc concentrations of prepubertal children: a meta-analysis of randomized controlled trials. Am Clin Nutr 2002; 75: 1062-71.
18. Walravens PA, Chakar A, Mokni R, Denise J, Lemonnier D. Zinc supplements in breastfed infants. Lancet 1992; 340: 683-5.
19. Schlesinger L, Arevalo M, Arredondo S, Diaz M, Lonnerdal B, Stekel A. Effect of a zinc-fortified formula on immunocompetence and growth of malnourished infants. Am J Clin Nutr 1992; 56: 491-8.
20. Umeta M, West CE, Haidar J, Deurenberg P, Hautvast JGAJ. Zinc supplementation and stunted infants in Ethiopia: a randomized controlled trial. Lancet 2000; 355: 2021-6.

Original Article

Zinc-iron, but not zinc-alone supplementation, increased linear growth of stunted infants with low haemoglobin

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補充鋅-鐵，而非單獨補充鋅，可促進發育不良併有低血紅素的嬰兒線性生長

補充鋅指出有益於線性生長，然而其效應可能取決於鋅是否為最限制的營養素。本研究目的為調查單獨給予鋅或是和鐵及維生素 A 一起補充，對於改善嬰兒微量營養素狀況與線性生長的效應。本研究為雙盲社區介入研究，800 名居住在東 Lombok 鄉村、西 Nusa Tenggara 的 3-6 個月嬰兒參與研究。每天給予研究對象含鋅(鋅 10mg/d)、鋅+鐵(鋅跟鐵各 10mg/d)、鋅+鐵+維生素 A(鋅跟鐵各 10mg/d 加上 1,000IU 的維生素 A)，或是安慰劑的糖漿為期六個月。結果變項為身長、體重及微量營養素狀況(血紅素、血清鋅、鐵蛋白與視網醇)。補充鋅+鐵與鋅+鐵+維生素 A 對於研究對象的鋅及鐵狀況有益，然而單獨給予鋅時則不利於血紅素及鐵的狀況。維生素 A 與血紅素增加最多的為鋅+鐵+維生素 A 組。原本為發育不良的研究對象，在補充鋅+鐵及鋅+鐵+維生素 A 後比給予安慰劑組成長了 1.1-1.5 公分。另一方面，在單獨補充鋅組，平均年齡身高別 Z 分數下降程度較安慰劑組大。原本為發育不良的研究對象在經過四個月的補充之後，HAZ 在組別間有顯著的差異。然而在追蹤六個月後，儘管平均年齡身高別 Z 分數仍維持相同的模式，即鋅+鐵+維生素 A 及鋅+鐵的組較安慰劑組或單獨補充鋅組高，但其間的差異不再顯著。對血紅素/鐵狀況較差的研究對象而言，在低血紅素/鐵狀況已被改善的條件下，補充鋅將對生長狀況具有正面的影響。

關鍵字：理想生長、嬰兒、鐵、微量營養素、鋅。