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Long-term effects of iron and zinc supplementation during infancy on cognitive performance and growth 8 years later: a follow-up study

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Doctor of Philosophy

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An Abstract of
a dissertation submitted to the Faculty of the Graduate
School of Emory University in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy

in

Division of Biological and Biomedical Sciences
Nutrition and Health Sciences

2010

ABSTRACT

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Iron and zinc are important micronutrients for child cognitive development and growth, particularly during infancy when brain development and physical growth are rapid. Many studies have investigated the effects of iron and zinc interventions in infancy, but the benefits of these interventions have been assessed only in terms of outcomes in infancy. None of the intervention studies during the critical phase of infancy have reported effects measured in school-aged children and beyond.

A randomized, placebo controlled trial of iron and zinc supplementation was conducted in 4-6 month-old breastfed infants in Khon Kaen, Thailand from 1998-1999. Infants were randomly divided into 4 groups receiving 1) 10 mg iron, 2) 10 mg zinc, 3) 10 mg iron+10 mg zinc, or 4) a placebo for 6 months. Improvements in iron and zinc status and weight were found; no measures of cognitive development in infancy were assessed in this study. These children were followed-up at 9 years of age in order to assess long-term effects of iron and/or zinc supplementation on intellectual functioning and physical growth (n=560). Results showed no differences in intelligence quotient (IQ), Raven's CPM scores, or school performance among the 4 groups. There were no differences in physical growth among these 4 groups. We also investigated the influence of prenatal and postnatal growth on IQ and Raven's CPM scores at 9 years of age. Results showed that early infancy growth that included a gain in weight, length, and head circumference was correlated positively with measured IQ at 9 years of age. In addition, solely a gain in length at late infancy was also positively correlated with measured IQ.

In summary, we found no long-term benefits of iron and/or zinc supplementation during infancy on cognitive performance and growth at 9 years of age. However, we found a significant relationship between early to late infancy growth and intellectual functioning at 9 years of age. These findings suggest that nutrition intervention programs should emphasize the importance of early childhood nutrition.

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ACKNOWLEDGEMENTS

I would like to thank the many people who have helped me complete this dissertation. I am especially grateful to my advisor, Dr. Reynaldo Martorell for his generous and intellectual comments and advice. His comments and advice were invaluable in the completion of my research and dissertation. I would like to extend my gratitude to my committee members, Dr. Usha Ramakrishnan, Dr. Ann M. DiGirolamo, and Dr. Rafael Flores who gave me insightful guidance. My deep appreciation also goes to my advisor in Thailand, Dr. Pattanee Winichagoon who has been an excellent teacher and mentor.

This dissertation would not have been possible without the participation of children and parents, local health workers, and school teachers in Khon Kaen province. I thank the investigators and fieldworkers at the Institute of Nutrition, Mahidol University (INMU) and clinical psychologists at Khon Kaen University for their data collection efforts and expertise. My gratitude also goes to Dr. Emorn Wasantwisut and her colleagues who allowed me to use the data from the intervention trial. I would like to thank for the generous financial assistance I received from the Ellison Medical Foundation Foundation /International Nutrition Foundation and Mahidol University. I also thank to my colleagues at Emory, Meng Wang, Cheng Huang, and my NHS classmates Phuong Nguyen, Jenifer Johnson, Maggie Phan, Beth Imhoff-Kunsch, Camila Corvalan, Reena Oza, Smita Iyer, and Vinh Bui for their help, encouragement, and friendship. My gratitude also goes to Nicholas Welch who helped me with editing assistance on this dissertation. Finally, I am deeply grateful to my parents, sister, and brother for their love and constant encouragement that inspired me to reach the goal.

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CHAPTER 1
INTRODUCTION

Infancy is a period of rapid brain growth (1). Any perturbation during early childhood such as undernutrition, environmental toxin, stress, and poor stimulation and social interaction can cause long-term effects on brain structure and function (2). Physical growth is also rapid during early childhood (3). Limiting nutrients as well as prolonged infection can contribute to growth failure (4).

Iron deficiency is the primary cause of anemia, which remains a significant public health problem. About 25% of the world's population suffers from anemia (5). In Thailand, the prevalence of anemia in 2003 was 56% in children 6-11 months and 26% in children 1-5 years old (6). Prevalence data for zinc deficiency are lacking. Because sources of iron and zinc in the diet are similar and iron and zinc have the similar problem of bioavailability, zinc deficiency is likely to be a public health problem in many countries with high rates of iron deficiency anemia (7). In most developing countries, poor dietary quality is a major determinant of inadequate micronutrient intakes (8). Cereal grains, which are usually low in iron and zinc content, are the major constituents of complementary food for infants in most settings (6, 9). Therefore, it is difficult for infants to meet iron and zinc requirements in these settings.

Iron and zinc are important micronutrients for growth and development in children. Iron is essential for normal neurological function. Iron deficiency affects myelination, neurotransmitter metabolism, and iron-containing enzymes (10). Children with iron deficiency anemia have poor psychomotor development. Moreover, moderately severe iron deficiency in early childhood causes long-lasting impaired development (11-

13). Iron supplementation during infancy has potential long-term benefits (i.e., at least 4 years after supplementation) (11). Children with treated iron deficiency during the infancy period had the same level of development test scores at 5 years of age as children who had maintained normal iron status. Iron may be involved in growth through its effect on immunity, appetite, thermogenesis, and thyroid hormone metabolism (14). However, iron supplementation alone failed to show a positive effect on both weight and height of children less than 18 years old in a meta-analysis (15). Zinc is important for the development of the central nervous system (16). Early infant zinc deficiencies may affect infant neurological development but the evidence is inconclusive (17). Zinc supplementation in infants and preschool children (18, 19) resulted in higher vigorous and functional activity level, but there was no effect on motor development. Zinc is involved in deoxyribonucleic acid (DNA) synthesis, ribonucleic acid (RNA) synthesis, and cell division (16). Therefore, zinc deficiency is associated with growth retardation and failure to thrive. Effects of zinc supplementation on growth were examined in a meta-analysis of 33 randomized intervention trials that were conducted in children less than 12 years old (20). Positive effects on height and weight increments were reported. Responses to zinc supplementation were significantly greater in those studies that enrolled subjects with preexisting stunting or underweight. By contrast, there were no significant effects of zinc supplementation in those studies that enrolled mostly children who were non-stunted and/or had adequate weight-for-age. However, a recent meta-analysis did not show significant effects of zinc interventions on weight and height gain (21). This meta-analysis included only studies among children $\leq$ 5 years of age.

Because iron and zinc deficiencies likely coexist in infants due to high requirements and poor intakes of common food sources, combined supplementation would be an appropriate strategy to prevent the deficiencies. However, there has been concern that iron and zinc might interact when they are given together. High iron intake reduces zinc absorption (22) and high level of zinc supplementation also reduces iron absorption (23). It was reported previously that iron and zinc may compete for transportation sites in the small intestine. However, later reports revealed that iron and zinc may interact during post-absorption because they are transported by different proteins (24). Many studies assessed the effect of iron and zinc supplementation in infancy on their iron and zinc status, growth, development, and morbidity (25-30). These studies report inconsistent short-term effects (i.e., concurrent effects). A study in West Java, Indonesia (27) and a study in Thailand (29) did not show effects of iron and zinc combined on growth, but a study in Vietnam suggested a negative effect of iron on weight when iron was added to zinc supplement (28). A study in Bangladesh showed a beneficial effect of combined iron and zinc supplementation on motor development (30). In contrast, no significant effect of combined iron and zinc supplementation on development was found in a study in Central Java (26). Severity of iron and zinc deficiencies may explain these inconsistent results. Nevertheless, most studies showed an effect of supplementation on biochemical status. None of these studies have investigated the long-term effects.

In a study in Thailand (29), iron and zinc supplementation in infancy improved iron status, zinc status, and weight gain. However, cognitive performance was not evaluated in this study. Since this supplementation trial was performed during the first

two years of life, a critical period of child growth and development (1, 3), we expect that the benefits of the supplementation will be long-lasting. Therefore, this study was conducted to test the following hypotheses:

1) Iron and/or zinc supplementation during infancy benefits cognitive performance at 9 years of age.
2) Iron and/or zinc supplementation during infancy leads to improved physical growth at 9 years of age.
3) Prenatal and early postnatal growth has a greater influence on intellectual functioning at late childhood than late postnatal growth.

This dissertation includes (a) a detailed review of the literature regarding iron and zinc deficiencies; benefits of iron and zinc supplementation on cognitive development and growth; Thailand: country overview; and description of the intervention trial in infancy (Chapter 2); (b) a description of the study objectives, study design, and statistical methods (Chapter 3); (c) a report describing long-term effects of iron and zinc supplementation on cognitive performance in children at 9 years of age (Chapter 4); (d) a report describing long-term effects of iron and zinc supplementation on physical growth in children at 9 years of age (Chapter 5); (e) a report describing the influence of prenatal and postnatal growth on intellectual functioning at late childhood (Chapter 6); and (f) a summary of the overall findings, conclusions, and implications of this research project (Chapter 7).

Findings from the proposed study will contribute new knowledge about long-term effects of iron and zinc supplementation during early childhood on cognitive performance and growth in mid-childhood. This knowledge will be important for developing

appropriate timing, duration, and combination of iron and zinc supplementation to overcome iron and zinc deficiencies. Furthermore, the knowledge from the study will also be relevant to other developing countries where iron and zinc deficiencies are prevalent.

CHAPTER 2
LITERATURE REVIEW

2.1 Overview of iron and zinc deficiencies

2.1.1 Iron and zinc deficiencies situation and causes

Iron deficiency is the primary cause of anemia which remains a significant public health problem. About 25% of the world's population suffers from anemia and the estimated prevalence for preschool-age children is nearly 50% (5). Individuals with zinc deficiency have been rarely reported because zinc status is difficult to assess. However, the sources of iron and zinc in the diet are similar and iron and zinc have the similar problem of bioavailability. Thus, iron and zinc deficiencies may coexist and may be prevalent in the populations that have limited intake of meat and high intake of phytate (31).

Iron deficiency and anemia can result from poor dietary intake, increased requirement during rapid growth, and excessive iron losses due to menstruation or parasitic infection (32, 33). Low consumption of iron-rich foods such as meat, consumption of food with poorly bioavailable iron, and high consumption of foods that contain inhibitors of iron absorption such as phytate and polyphenols are important causes of iron deficiency. An increased requirement for iron during rapid growth in infants, young children, adolescence, and pregnant women is another important cause of iron deficiency if iron consumption is inadequate to meet the requirement. Iron deficiency also results from abnormal iron loss as seen in hookworm infections or cow's milk sensitivity in infants and children. Anemia is also found as a co-morbidity with infectious diseases such as malaria or human immunodeficiency virus (HIV) infection.

Preterm and low birth weight infants have less iron storage than full-term infants. Thus, they are at higher risk of iron depletion at 2-3 months of age.

Risk factors for zinc deficiency include: inadequate intake, poor absorption, and low bioavailability of dietary zinc. In many developing countries, the consumption of animal products is low. In addition, food taboos in some Asian countries prevent pregnant women, lactating women, and young children from consuming adequate zinc and protein-rich foods. Moreover, plant foods, containing phytate, fiber, oxalate, tannin, and lignin known to inhibit the absorption of zinc, are the main diet among these populations (7). Increased losses and impaired utilization in hot and humid climates during diarrhea or parasitic infection, as well as increased requirements during pregnancy, lactation, young childhood, and adolescence also contribute to zinc deficiency (7).

In most developing countries, cereal grains are the major constituents of complementary food. In Malawi, cereals are the most prevalent sources of energy intake, iron, and zinc (9). In Thailand, about 72% of infants receive instant rice porridge or mashed banana but only 10% receive mashed rice with egg yolk or liver (6). The quality of the diet among older children is also poor, especially in the Northeast region of Thailand. Thus, not only iron and zinc content but also bioavailability are low in these food sources and would contribute significantly to iron and zinc deficiency in early childhood.

2.1.2 Control of iron and zinc deficiencies

Strategies to prevent and control micronutrient deficiencies include dietary modification, fortification, and supplementation (8). Control of helminth and malaria

infection is also an important strategy for iron deficiency control and prevention. Dietary diversification is considered sustainable, economically feasible, and culturally acceptable. It is focused on promoting the availability of, and access to, iron and zinc rich foods. It is also focused on promoting consumption of foods which enhance the absorption or utilization of zinc and iron. Appropriate methods of food preparation and processing can reduce absorption inhibitors. Several methods such as plant breeding and genetic modification can be employed to increase bioavailability (34, 35).

There is a consensus that fortification of food is an effective long-term approach to improve the micronutrient status of populations. Fortification of wheat flour, soy sauce, and fish sauce with iron has been implemented in many countries (36). Infant formula and complementary foods for children are fortified with zinc and other micronutrients in some countries (37). Sprinkles are an innovation in home fortification (38). They are micronutrients in powder form, which are easily mixed and incorporated into home-made foods. Delivery micronutrients with a sprinkles sachet have been studied in both developed and developing countries (38-41). The overall results showed good efficacy, bioavailability, safety, and acceptability. Complementary food supplements are also available in the form of crushable or water soluble tablets (foodlets) and fortified spread (37).

Supplementation has focused on single micronutrients, mainly iron, iodine, zinc, and vitamin A. Iron supplementation is regularly provided to pregnant women as universal supplementation in order to reduce iron deficiency. However, no universal iron supplementation has been provided for iron deficiency anemia prevention in infants, except for those who have low birth weight (34). Zinc deficiency can be prevented by

zinc supplementation. Doses of supplementation for prevention of zinc deficiency are suggested by IZiNCG (35). Nonetheless, there is no universal zinc deficiency control program.

During rapid growth in infancy, a relatively high iron intake is needed. However, after 6 months of age, iron content of breast milk is not sufficient to meet infants' requirement. Therefore, in the setting where iron-fortified complementary foods are not widely and regularly consumed by young children and complementary foods are usually low in iron contents, routine iron supplementation is recommended as an appropriate strategy to prevent iron deficiency anemia in children 6-24 months of age (36).

2.2 Roles of iron and zinc in cognitive development and growth during childhood

2.2.1 Roles of iron and zinc in cognitive development

Human brain construction and development occur shortly after conception and continue until adolescence (42). Postnatal growth of the brain is characterized by its rapid growth during infancy and early childhood, a much more gradual increment during mid-childhood, and very small terminal increase throughout adolescence. Nearly half of the postnatal growth of the brain has been accomplished by the end of the first year, three fourths by the third year, and nine tenths by the seventh year (1).

Herschkowitz (43) divided brain development into four periods. The first period of brain development occurs between the 8^{th} and 32^{nd} week of gestation. Neuroblast proliferation and migration and aggregation of neurons occur in this period. In the second period, between the 32^{nd} week of gestation and birth, cell migration, cell differentiation, and proliferation of glial cells, appear. Myelination begins in this period. The third period

extends between birth and 2 years of age. There is intensive brain growth and differentiation during this period. Brain weight at 2 years is the same as adult brain weight. It is a period of intensive neuronal differentiation with formation of dendrites and synapses and myelogenesis in the central nervous system. After 2 years of age, it is considered as the fourth period. Brain growth and myelination slow down, but differentiation still exists. The differences of brain growth in each period suggest that the critical periods in human brain development are fetal and growth before 2 years of age (1).

Iron is essential for normal neurological function. It has an important role in myelination and white matter of cerebellar folds (10). Iron is a cofactor for enzymes including tyrosine hydroxylase and tryptophan hydroxylase. These enzymes are involved in the synthesis of neurotransmitters such as dopamine, serotonin, and norepinephrine (44). Brain iron is also associated with γ-aminobutyric acid (GABA) in which GABA is altered by iron deficiency (10). Iron deficiency has both direct and indirect effects on cognitive function. Iron deficiency affects myelination, neurotransmitter metabolism, and iron containing-enzymes. In iron-deficient animals, learning ability decreased when non-heme iron concentrations in the brain and number of dopamine D2 receptors in the cortex were decreased by iron deficiency (45, 46). Anemia can alter behavior patterns which leads to functional isolation, an indirect mechanism linking anemia to poor cognitive development (47, 48). Several studies have shown that anemic children ambulate and explore less in their environment as compared to nonanemic children. In addition, anemic children tend to demonstrate hesitancy, wariness, withdrawal, fatigue, and less reactivity to usual stimuli than children with adequate iron status (46, 47). Because of these

behaviors, their caregivers offer less stimulation which may alter the acquisition of new skills and finally delay the development of the children.

Many studies have reported a poor level of development, cognitive function, and behavioral development in iron deficient children (49). In a cross-sectional study, children aged 6 to 12 years in Mexico were administered psychological tests including Wechsler Intelligence Scale for Children-Revised and Dynamic evaluation of Learning (50). The results showed that iron deficient, but not anemic children scored lower than iron replete children. In Thailand, the scores of Thai language in 9 to 11 years old iron deficient children before iron supplementation were lower than those of normal iron status children (51).

Zinc is important for the development of the central nervous system (16). The human brain contains a significant amount of zinc. Zinc concentrations are highest in the telencephalon and grey matter of the cerebral cortex (45). It is important for both structure and function of the brain (52, 53). Zinc has important functions in biochemical processes like myelination and it is involved in the precursor production of neurotransmitters and the release of neurotransmitters such as GABA and glutamate (52). It is also important for neuronal migration and synaptogenesis and is also involved in metabolism, function, and transport of thyroid hormones as well as other hormones that may influence the central nervous system. Moreover, zinc dependent enzymes are involved in brain growth (16). Results from animal studies suggest that development during the periods of brain growth and the pre-adolescent growth spurt are most affected by zinc deficiency (52). Zinc deficiency during the rapid period of brain growth can alter

emotional behavior, decreased spontaneous activity, and impaired memory, attention, and learning ability (16).

2.2.2 Roles of iron and zinc in growth

Physical growth occurs rapidly during the early childhood, especially the first year of life (3). Daily weight gain is approximately 20-30 g during the first 6 months and decreases to 12-15 g during 6 to12 months (54). After the first year, daily weight gain decreases to 8 g during 1 to 3 years and 6 g during 4-6 years. Length increases by 2.0-3.5 cm/month in the first 6 months. Then, it reduces to 1.2-1.5 cm/month during 6 to 12 months and 1 cm/month during 1 to 3 years. Tremendous reduction on growth of length happens during 4 to 6 years in which length increases only 3 cm/year.

Anemia, especially iron deficiency anemia is associated with poor physical growth and development (14). Iron does not have a direct role in growth, but it may be involved through its effect on immunity, appetite, thermogenesis, and thyroid hormone metabolism (14). A study in Bangladesh suggested iron deficiency may contribute to growth retardation (55). Children who drank low iron content water were shorter than those who drank water with normal iron content.

Zinc deficiency is associated with growth retardation. The role of zinc in deoxyribonucleic acid (DNA) synthesis, ribonucleic acid (RNA) synthesis, and cell division is likely to be crucial (16, 56). Zinc also stimulates the DNA synthesis in bone cells, thus enhances vitamin D effects on bone metabolism. Zinc interacts with hormones involved in bone growth such as somatomedin-c, osteocalcin, testosterone, thyroid hormones, and insulin. Zinc also participates in growth hormone synthesis and secretion (16). In addition, zinc participates in metabolism of macronutrients (16). Zinc may

indirectly affect linear growth by altering smell and taste leading to decreased appetite and consequently decreased food consumption (16). Zinc deficiency also reduces resistance to infection. In addition, infections decrease zinc stores in the body and thus further adversely affect growth (57).

Many studies have shown that zinc depletion limits growth in children. A study in school age children aged 9 to 12 years showed an increased prevalence of low hair and plasma zinc values in children with short stature (58). In another study of 2-10 years old Papua New Guinean children, where 38% of males and 20% of females had height-for-age Z score (HAZ) < -2 SD, the results showed that hair zinc associated with HAZ. The authors concluded that stunting in this population was related to chronic deficits in energy and protein and was exacerbated in older boys with suboptimal zinc deficiency (59).

2.3 Benefits of iron supplementation on cognitive development and growth during childhood

2.3.1 Benefits of iron supplementation on cognitive development

2.3.1.1 Iron supplementation in infants

Long-term (i.e., 2-6 months) iron intervention studies in infants suggested some beneficial effect on cognitive development (Table 2.1). A study in Indonesian infants showed that Bayley scales of mental and motor development of iron deficient infants aged 12 to18 months improved to the same level as iron-sufficient infants, after receiving iron supplementation for 4 months (60). In a study in Costa Rica, iron supplementation during infancy showed the potential of long-term effects (11). Children whose iron

deficiency had been fully corrected by 6 months iron supplementation in infancy had the same level of developmental test scores at 5 years of age as the children who had maintained normal hemoglobin status.

Some studies showed that moderately severe iron deficiency in early childhood may cause irreversible impaired development and this impairment cannot be corrected by iron supplementation. Anemic infants in Chile who were treated with oral iron at 3 months did not improve in cognitive test scores (61). Cognitive function of these children was assessed again at about 5 to 6 years old (13). Children who had iron deficiency anemia (IDA) and received iron supplementation during infancy still had lower cognitive test scores compared to normal children. Another study was conducted in Costa Rica (62) where infants with hemoglobin (Hb) less than 120 g/L randomly received intramuscular iron injection or oral iron, or oral placebo for 1 week and non anemic infants randomly received oral iron or oral placebo for 1 week. After 1 week, infants who received iron injection and iron-sufficient infants were given placebo drops for 12 weeks, while all other infants were given oral iron. After intervention, mental and motor test scores of IDA infants whose anemia and iron deficiency were corrected were improved. However, initially anemic infants who had more severe or chronic iron deficiency still had significant lower mental and motor test scores compared to those who were iron-sufficient. The authors suggested that iron treatment may be inadequate to improve cognitive function or it might be that the negative effects on cognitive function was long-lasting, depending on the timing, severity, or chronicity of iron deficiency anemia in infancy (62). Some of these children were assessed again at 5 years old (11). Although, they had normal iron status, those who had Hb $\leq$ 110 g/L still had lower mental and

motor test scores compared to other children. However, those whose iron deficiency had been fully corrected performed as well as those who had maintained good iron status during infancy. These children were also assessed at 10 years after the intervention (12). Children who had severe, chronic iron deficiency in infancy still had lower scores in arithmetic, reading, directed writing task, and test of motor proficiency, compared to those who maintained good iron status in infancy.

2.3.1.2 Iron supplementation in preschool children

Several iron supplementation studies have been conducted to assess the effect of iron on cognitive function in preschool children (Table 2.2). These results show significant benefits of iron treatment, especially in anemic children. A study in Indonesia was conducted in children with mean age of 54 months (63). Children were randomly assigned to receive iron or placebo. Treated anemic children had more improvement on two tests of oddity learning and one test of discrimination learning compared to placebo anemic children. Seshadri (64) conducted four iron supplementation studies in children 5 to15 years. The first study was conducted among children aged 5-8 years to investigate the effect of 20 mg iron and 0.1 mg folic acid supplementation on cognition. The result showed significant increases in verbal, performance, and total intelligence quotient (IQ) in iron supplemented group, while the increase was not found in the placebo group. Among supplemented children, improvement in total scores of anemic children was significantly higher than those of nonanemic children only in the 7-8 y age group.

Table 2.1 Studies of the effect of iron supplementation on cognitive development in infants

Study	Sample size, age	Supplementation	Outcome measures	Results
Idjradinata, 1993 (60) Indonesia	N=126 12-18 mo	3 mg Fe/kg/d (ferrous sulfate) Placebo 4 mo	Bayley MDI and PDI	Significant change in mean MDI and PDI scores in IDA children Developmental delay reversed
Walter, 1989 (61) De Andraca, 1990 (13) Chile	N= 196 12 mo	15 mg Fe/d x 3 (ferrous sulfate) or placebo at 12 mo for 10 d Followed by 15 mg Fe/d x 3 (all subjects) for 3 mo	Bayley MDI and PDI 5 y: Standford-Binet Intelligence Test, Illinois Psycholinguistic Abilities Test, Psychoeducational Abilities Test, Bruininks-Oseretsky Test of Motor Proficiency, VMI	*After supplementation* - IDA: lower MDI and PDI *At 5-6 y of age* - IDA: lower cognition test scores
Lozoff, 1987 (62) Lozoff, 1991 (11) Lozoff, 2000 (12) Costa Rica	N = 191 12-23 mo	- Hb < 120 g/L: IM /oral 5 mg Fe/kg/d x 2 (ferrous sulfate) or placebo for 1 wk - Non anemic: oral Fe or placebo Followed by - IM and Fe-sufficient: placebo - Others: oral 3 mg Fe/kg/d x 2 for 12 wk	Bayley MDI and PDI 5 y: WPPSI, Goodenough-Harris Draw-a-Man Test, Beery Developmental Test of Visual-Motor Integration, Spanish version of the Woodcock-Johnson Psycho-Educational Battery, Bruininks-Oseretsky Test of Motor Proficiency 10 y: WISC-R, Wide Range Achievement Test-Revised, Directed Writing Task, Bender Visual-Motor Gestalt Test, Bruininks-Oseretsky Test of Motor Proficiency, Central/Incidental Serial Recall Test, Attentional Capacity Test, Underlining Test, K-ABC Spatial Memory, Tactual Performance Task, Cognitive Abilities Tests	*After supplementation* IDA initially and remained ID: lower MDI and PDI Fully corrected: improved MDI and PDI *At 5 y of age* - IDA initially and remained ID : lower cognitive and motor test scores - Fully corrected: performed similar to normal group *At 11-14 y of age* - IDA: lower cognitive and motor test scores

IDA, iron deficiency anemia; MDI, Mental Development Index; PDI, Psychomotor Development Index; VMI, Development Test of Visual Motor Integration; WPPSI, Wechsler Preschool and Primary Scale of Intelligence; WISC-R, Wechsler Intelligence Scale for Children-Revised

Table 2.2 Studies of the effect of iron supplementation on cognitive development in preschool children

Study	Sample size, age	Supplementation	Outcome measures	Results
Soewondo, 1989 (63) Indonesia	N=127 < 5 y	50 Fe/d placebo 2 mo	Two discrimination learning, three oddity learning tasks PPVT	Scores in two tests of oddity learning and one test of discrimination learning: Treated IDA > placebo
Seshadri, 1989 (64) study 1 India	N = 94 5-8 y	20 mg Fe/d + 0.1 mg Folic acid/d Placebo 2 mo	WISC	Significantly improved verbal, performance, and total scores in supplemented group 7-8 y: Higher mean improvement of total IQ in supplemented anemic children compared to nonanemic children
Seshadri, 1989 (64) study 2 India	N = 28 5-6 y	40 mg Fe/d + 0.2 mg Folic acid/d Placebo 2 tablets x 3 d of mebendazole in both groups 2 mo	WISC	Significant increase in verbal, performance, and total scores in supplemented group compared to placebo
Stoltzfus, 2001(65) Zanzibar	N=614 6-59 mo	10 mg Fe/d (ferrous sulfate) 500 mg mebendazole/3 mo Fe + mebendazole Placebo 12 mo	Gross motor and language milestones	Fe improved language development Fe improved motor development in children with Hb < 90 g/L No benefits of mebendazole on motor and language development

IDA, iron deficiency anemia; Hb, hemoglobin; IQ, Intelligence Quotient; PPVT, Peabody Picture Vocabulary Test; WISC, Wechsler Intelligence Scale for Children

The second study conducted by Seshadri (64) assessed the effect of 40 mg iron and 0.2 mg folic supplementation in 5-6 years old children. All children were dewormed with mebendazole in this study. After supplementation, children in the supplemented group had significantly higher verbal and performance scores compared to the placebo group. The third and the fourth studies were performed in 8-15 years old children (64). A study in Zanzibar (65) where children aged 6-59 months received iron, mebendazole, iron

and mebendazole, or a placebo reported a significant improvement on language development in children who received iron supplementation. The effect size compared iron vs. no iron was 0.8 (0.2-14) and the effect size comparing mebendazole vs. no mebendazole was 0.3 (-0.3-0.9). Motor development was improved in iron supplemented children who had Hb < 90 g/L. There were no significant additional benefits of mebendazole treatment on both language and motor development.

2.3.1.3 Iron supplementation in school age children and adolescents

Many iron supplementation studies were conducted in school age children and adolescents to assess the effect of supplementation on cognitive development (Table 2.3). A positive effect has been shown mostly in supplemented anemic children. A study in Indonesian children showed that IDA children who received 10 mg of iron daily had more significant improvement on a school achievement test and the Bourden-Wisconsin test of concentration compared to IDA children who received a placebo (66). Two of four studies conducted by Seshadri assessed the benefits of iron supplementation in children aged 8-15 years in India (64). In one study, boys were supplemented with daily 30 mg iron, 40 mg iron, or a placebo for 2 months. A significant improvement on visual recall, digit span, and clerical tasks was shown in both iron supplemented groups. When anemic and nonanemic boys were analyzed separately, significant treatment effect was restricted only to anemic boys (Hb < 105 g/L). Another study was conducted in girls and 60 mg of iron were administered daily. The results showed an improvement of cognitive test scores in the iron supplemented group, especially in anemic girls who received iron supplementation. However, a significant supplemented effect was found on the maze task in nonanemic girls.

Two studies in Thailand which administered both iron supplementation and anthelminthic treatment, however, did not show significant effects of the intervention. A study in children aged 9-11 years who received anthelminthic treatment at baseline and at 3-month after intervention started did not show a significant effect of 16 weeks iron intervention on either IQ or school achievement (51). In another study in Thailand (67), primary school children with mean age of 9.6 years were treated with albendazole and then received 60 mg daily or weekly iron supplementation or a placebo. The results revealed that those who received daily supplementation, although had increases in serum ferritin (SF), had lower changes in IQ compared to those who received weekly supplementation or a placebo (3 vs. 6 IQ point).

Table 2.3 Studies of the effect of iron supplementation on cognitive development in school age children and adolescents

Study	Sample size, age	Supplementation	Outcome measures	Results
Soemantri, 1985 (66) Indonesia	N=119 10-11 y	2 mg Fe/kg/d (ferrous sulfate), placebo 3 mo	RCPM (baseline only), educational achievement test, Bourden-Wisconsin test for concentration	Higher educational achievement test Higher Bourden-Wisconsin (IDA children only)
Seshadri, 1989 (64) study 3 India	N=48 8-15 y boys	Each triplet: 30 mg Fe/d (ferrous sulfate) or 40 mg Fe/d or placebo 2 mo	Visual recall, digit span (WISC), maze (WISC) and clerical tasks	Significant higher scores (both 30 mg and 40 mg groups) in all tests except maze Higher overall score in anemic children only
Seshadri, 1989 (64) study 4 India	N=130 8-15 y girls	60 mg Fe/d (ferrous sulfate) Placebo 2 mo x 2 in one school year	Visual recall, digit span (WISC), maze (WISC) and clerical tasks	Higher scores in clerical task, mazes, overall scores in anemic children only Higher scores in mazes in nonanemic children
Pollitt, 1989 (51) Thailand	N=1358 9-11 y	10 mg/d Fe (ferrous sulfate) 2 wk followed by 20 mg/d 14 wk, Placebo 200 mg albendazole for all children 16 wk	RCPM, Thai language and Math tests	NS RCPM, Thai language, Math Poorer performance in anemic children on IQ and Language tests
Sungthong, 2004 (67) Thailand	N=397 6-13 y	60 mg Fe/d (ferrous sulfate) 60 mg Fe/wk Placebo 400 mg albendazole at baseline and 11wk for all children 16 wk	Test of Nonverbal Intelligence-II Thai language and Math test	Daily Fe group had lower IQ NS Thai language and Math

IDA, iron deficiency anemia; RCPM, Raven's Colored Progressive Matrices; NS, non significant difference between groups; WISC, Wechsler Intelligence Scale for Children

2.3.2 Benefits of iron supplementation on growth

Regarding the effects of iron supplementation on growth, results from randomized controlled trials showed a tendency of improving linear and ponderal growth in children with IDA at baseline. Twenty one studies were analyzed in a meta-analysis of iron intervention and the effect on growth among infants and children less than 18 years old (15). Results failed to show a significant effect of iron supplementation on either height or weight. The overall effect size was 0.09 (95% CI: -0.07, 0.24) for height and 0.13 (95% CI: -0.05, 0.30) for weight. When the studies were stratified based on baseline hemoglobin status, lack of significant differences still existed. Even though effect sizes for height and weight gain were greater among subjects who were anemic initially, the differences were not significant. A recent meta-analysis which included only the intervention trial in children ≤ 5 years of age also showed no significant effect of iron intervention on weight and height gain (21).

2.3.2.1 Iron supplementation in infants

Among studies in infants, most of them revealed no significant benefit and some studies showed an adverse effect of iron supplementation on growth (Table 2.4). A study conducted in Sweden and Honduras examined the effect of iron supplementation on growth of breast-fed infants (68). Infants aged 4 months were randomly assigned to three groups to receive a placebo from 4 to 9 months, a placebo from 4 to 6 months and then 1 mg iron/kg daily from 6 to 9 months, or iron supplementation from 4 to 9 months. The results indicated that daily low dose iron supplementation had a negative effect on linear growth and head circumference. Among the Swedish infants, gains in length and head circumference were significantly lower in those who received iron than in those who

received a placebo from 4 to 9 months. The same effect on length was seen in Honduras where gains in length were lower in the iron groups compared to the placebo group, but only at the 4–6 month-interval among those with initial Hb ≥ 110 g/L. There was no significant effect on weight gain. A study in Indonesian infants showed that 3 mg of daily iron supplementation for 4 months did not affect length and arm circumference (69). Weight gain was higher in the placebo group compared to the iron supplemented group. In a study in India (70), iron-sufficient infants were randomly assigned to receive 2 mg iron/kg daily or a placebo and iron deficient infants received 6 mg iron/kg daily. Weight and height gain were measured every 2 weeks. In the iron deficient group, iron supplemented infants had a significant improvement of mean monthly weight gain and linear growth compared to the placebo group. However, iron significantly decreased weight gain and linear growth of iron-replete children.

Table 2.4 Studies of the effect of iron supplementation on growth in infants

Study	Sample size, age	Supplementation	Outcome measures	Results
Dewey, 2002 (68) Sweden and Honduras	N=131 4-9 mo	1 mg Fe/kg/d (ferrous sulfate) from 4 to 9 mo Placebo from 4 to 6 mo then 1 mg Fe/kg/d from 7 to 9 mo Placebo from 4-9 mo	Weight, length, head circumference (HC)	Sweden: gains in length and HC were lower in iron groups compared to placebo Honduras: gains in length were lower in iron groups compared to placebo in children 4-6 mo with Hb ≥ 110 g/L Weight gain NS
Idjradinata, 1994 (69) Indonesia	N=47 12-18 mo	3 mg Fe/kg/d (ferrous sulfate) Placebo 4 mo	Length, weight, arm circumference	Length and arm circumference NS Weight gain was lower in iron group
Majumdar, 2003 (70) India	N=150 6-24 mo	Iron sufficient: 2 mg Fe/kg/d or placebo Iron-deficient: 6 mg Fe/kg/d 4 mo	Weight, length, head circumference (HC)	Iron-sufficient: less weight gain and linear growth in iron group Iron-deficient: greater mean weight gain and linear growth in iron group

NS, non significant difference between groups; Hb, hemoglobin

2.3.2.2 Iron supplementation in preschool children

Studies among preschool children showed inconsistent effects of iron supplementation and/or deworming on growth (Table 2.5). In a study in Indonesia, anemic preschool children who had low weight-for-age Z score (WAZ) were randomly given 20 mg iron plus vitamin C or vitamin C only (control) (71). Children with parasites were given anthelminthic tablets before starting supplementation. After supplementation, iron supplementation showed to improve growth of these anemic and low weight-for-height Z score (WHZ) preschool children in which iron group had higher increases in height and HAZ compared to control group. In another study in Indonesia (72), 2-5 years old children received iron supplementation and deworming or iron alone or a placebo. There were no effect of supplementation in changes in both weight and height. A study in Benin was conducted to assess the effect of iron and deworming on growth (73). Preschool children aged 3- 5 years were randomly allocated into 4 groups to received 60 mg iron/d and 200 mg albendazole for 3 consecutive days and repeated 1 month later, iron and albendazole-placebo, albendazole and iron-placebo, or placebos for 3 months. There was no significant difference in changes in weight, height, MUAC, triceps skinfold, WAZ, and HAZ over 3 and 10 months between study groups. In addition, sub-sample analyses of stunted and anemic subjects also showed no significant effect.

The last two studies evaluated 1 year iron supplementation with and without deworming. In Bangladesh study, children 0.5-6 years old received either daily iron supplementation with multivitamins or multivitamins alone (74). Results from this study failed to show a significant benefit of iron supplementation on weight and height gain. A study in Zanzibar was conducted in children 6-71 months old (75). Children 6-59 months

old were randomly to received iron, mebendazole, iron and mebendazole, or a placebo for 12 months. There was no effect of iron on mild wasting (WHZ < -1) or stunting (HAZ < -2) after supplementation. Mebendazole significantly decreased mild wasting and small arm circumference prevalence (< 5^{th} percentile) in group with age < 30 months. However, adverse effects of mebendazole were found on mild wasting in children ≥ 48 months old.

Table 2.5 Studies of the effect of iron supplementation on growth in preschool children

Study	Sample size, age	Supplementation	Outcome measures	Results
Angeles, 1993 (71) Indonesia	N=76 2-5 y anemic with low WAZ children	30 mg Fe/ d (ferrous sulfate) + 20 mg vitamin C 20 mg vitamin C Antihelminthic treatment in children with parasites 2 mo	Weight and height	Increases in height and HAZ were larger in iron group compared to control
Palupi, 1997 (72) Indonesia	N=194 2-5 y	30 mg Fe/wk (ferrous sulfate) + 400 mg albendazole 30 mg Fe/wk Placebo 2 mo	Weight and height	No effect on weight and height
Dossa, 2001 (73) Benin	N=140 3-5 y	60 mg Fe/d (ferrous sulfate) + 200 mg albendazole/d x 3 d and repeated at 1 mo later Fe + albendazole-placebo Albendazole + Fe-placebo Placebo 3 mo	Weight, height, MUAC, triceps skinfold	No effect on growth in all subjects and in stunted and anemic sub-groups
Rahman, 1999 (74) Bangladesh	N=317 0.5-6 y	15 mg Fe/d (ferrous sulfate) + vitamin A, D, and C Vitamins only 12 mo	Weight and height	No effect on weight and height increment
Stoltzfus, 2004 (75) Zanzibar	N=459 6-71 mo	10 mg Fe/d (ferrous sulfate) 500 mg mebendazole/3 mo Fe+ mebendazole placebo 12 mo	Weight, height, MUAC	No effect of iron on mild wasting (WHZ <-1) or stunting (HAZ < -2) Mebendazole decreased mild wasting and small arm circumference prevalence (<5th percentile) in group with age <30 mo Adverse effect of mebendazole on mild wasting in children ≥ 48 mo

WHZ, weight-for-height Z scores; HAZ, height-for-age Z scores; MUAC, mid-upper arm circumference

2.3.2.3 Iron supplementation in school age children and adolescents

Studies among school age and adolescent children revealed significant effect of iron supplementation only in children with IDA at baseline (Table 2.6). A study conducted in Indonesia (76) where school age children received supplementation for 12 weeks found a significant treatment effect on height, weight, and MUAC in anemic children treated with iron supplementation compared to anemic children in the placebo group. Mean changes for height, weight, and mid-upper arm circumference (MUAC) between treated and placebo groups were -2.42 vs. -3.98 cm, 0.41 vs.-1.21 kg, and 1.12 vs. -0.74 cm, respectively. There was no difference between the iron-treated and the placebo groups for nonanemic children (76). A study in Kenya showed a significant benefits of iron supplementation on weight, height, MUAC, triceps skinfold, and subscapular skinfold (77). Mean baseline Hb of children was 111.4 g/L. Mean changes of weight and height in the iron treated group were 1.6 ± 0.6 kg and 1.4 ± 0.5 cm, while mean changes of weight and height in the placebo group were 0.7 ± 0.4 kg and 1.1 ± 0.5 cm. This study also indicated that iron improved appetite and the authors mentioned that the improvement of growth may result from the improvement of appetite and total intake. A study of iron and folic acid supplementation in adolescent girls aged 10-18 years with mean baseline Hb of 109.1 g/L showed a significantly improvement in weight gain in the supplemented group compared to the placebo group (mean changes: 0.83 vs. 0.04). The growth increment was greater in the 10-14 years old group than in the 15-18 years old group (78). In a study in Thailand (79), 16 weeks of iron supplementation and deworming showed that weekly iron supplementation can lead to an increase in linear growth compared to those receiving a daily supplement. Mean changes in height were 2.3 ± 0.8

cm and 2.6 ± 0.9 cm, respectively. However, the treatment groups did not differ from the placebo groups, and none of supplementation groups showed a treatment effect on weight, WAZ, or HAZ. That the beneficial effect of iron supplementation was not observed in this study might be due to the low prevalence of IDA (4.2%) or to the existence of thalassemia in the population.

Table 2.6 Studies of the effect of iron supplementation on growth school age children and adolescents

Study	Sample size, age	Supplementation	Outcome measures	Results
Chwang, 1988 (76) Indonesia	N=119 8.2-13.5 y	2 mg Fe/kg/d (ferrous sulfate) Placebo 12 wk	Weight, height, MUAC	Fe increased weight, height, and MUAC in anemic children only
Lawless, 1994 (77) Kenya	N=87 6-11 y	150 mg Fe/d (sustained-release ferrous sulfate), Placebo 14 wk	Weight, height, MUAC, TS, SSS	Fe group increased in height, weight, WAZ, HAZ, WHZ, MUAC, triceps skinfold, and subscapular skinfold
Kanani, 2000 (78) India	N=203 10-18 y girls	60 mg Fe + 0.5 mg folic acid/d Placebo 12 wk	Weight and height	Significant weight gain No change in BMI in Fe group, BMI decreased in placebo group
Sungthong, 2002 (79) Thailand	N=397 6-13y	60 mg Fe/d (ferrous sulfate) 60 mg Fe/wk Placebo 400 mg Albendazole at baseline and 11wk for all children 16 wk	Weight, height	Higher height gain in weekly group compared to daily group NS weight, WAZ, HAZ

WAZ, weight-for-age Z scores; WHZ, weight-for-height Z scores; HAZ, height-for-age Z scores; MUAC, mid-upper arm circumference; TS, triceps skinfold; SSS, supscapular skinfold; NS, non significant difference between groups

2.3.3 Summary of iron supplementation on cognitive development and growth

Studies of iron supplementation during infancy on cognitive function have shown inconsistent results. Some studies report irreversible effects on cognitive function in anemic children who received iron supplementation but some studies have shown that the negative effect of IDA can be corrected. Lozoff (62) mentioned that the long-lasting negative effects on cognitive function might be directly related to timing, severity, or chronicity of iron deficiency anemia in infancy. Iron supplementation in preschool children, school age children, and adolescents suggest more beneficial effects in initially anemic children. Regarding growth, a meta-analysis showed no positive effect of iron supplementation on both weight and height. When considering the studies by age group, some studies in infants reveal no significant benefit and some studies show an adverse effect. Studies in preschool children, school age children, and adolescents have shown beneficial effects mostly in children who were anemic at baseline.

Helminth control has shown to improve iron status, growth, and cognition, although the benefits have not been consistent (75). The studies reviewed here rarely assessed the effects of deworming, although several studies administered anthelminthic tablets. However, among those 2 studies that compared the effect between the deworming group to the placebo group, one study showed a positive effect on growth. The effect sizes between study that included the deworming and the studies that did not employ the deworming cannot be compared here, since supplementation regimens were not the same.

2.4. Benefits of zinc supplementation on cognitive development and growth during childhood

2.4.1 Benefits of zinc supplementation on cognitive development

2.4.1.1 Zinc supplementation in infants

Zinc supplementation during infancy showed a potential beneficial effect on activity pattern (Table 2.7). A study in 12-23 months old Indian infants indicated that supplemental zinc and multivitamins (vitamin A, B1, B2, B6, D3, E, and niacinamide) for 7 months resulted in higher activity level than when multivitamins alone were given (18). In a zinc supplementation trial among infants in rural Guatemala, attainment of motor milestones was not affected, but activity patterns were improved with zinc supplementation (19). Infants in the zinc supplemented group were more frequently observed in sitting, playing, and lying down less as compared to non supplemented infants. However, another study among low birth weight infants in Brazil showed no effects on mental development of infants after 8 weeks of zinc supplementation (80). Only infants who received 5 mg of zinc, but not those who received a placebo or 1 mg of zinc, were observed to be more responsive to developmental test administrators. A study in neonates in Chile found that those who received zinc supplementation had higher motor quality factors than those in the placebo group at 12 months after supplementation (81). There was no significant difference in mean Mental Development Index (MDI) and Psychomotor Development Index (PDI) between 2 groups. However, fewer infants in the zinc supplemented group had an MDI score <100 compared to those in the placebo group. A randomized trial in 12-18 months old in India reported no significant effect of 10 mg zinc/d supplementation for 4 months on MDI and PDI (82).

Table 2.7 Studies of the effect of zinc supplementation on cognitive development in infants

Study	Sample size, age	Supplementation	Outcome measures	Results
Sazawal, 1996 (18) India	N=93 12-23 mo	10 mg Zn/d (zinc gluconate) + MM MM 7 mo	Behavior observation 5 h/d for 2 d	Spent more time in high movement activities
Bentley, 1997 (19) Guatemala	N = 85 6-9 mo	10 mg Zn(zinc sulfate) Placebo 7 mo	Behavior observation for one 12 h at baseline, 2 mo, and 7 mo later	NS motor milestones Zn group spent more time in sitting, playing than lying down
Ashworth, 1998 (80) Brazil	N=205 6-12 mo	1 mg Zn/d (zinc sulfate) 5 mg Zn/d Placebo 8 wk	Bayley MDI and PDI	NS MDI, PDI High responsiveness in 5 mg Zn group
Castillo-Duran, 2001 (81) Chile	N=112 Neonate	5 mg Zn/d (zinc sulfate) Placebo 1 y	Bayley MDI and PDI Behavior rating scale (BRS; orientation/engagement, emotional regulation factor, and motor quality factor)	NS MDI, PDI Fewer infants in Zn group had MDI < 100 compared to placebo NS BRS Zn group had higher motor quality factor than placebo group at 12 mo
Taneja, 2005 (82) India	N=571 12-18 mo	10 mg Zn/d (zinc gluconate) Placebo 4 mo	Bayley MDI and PDI	NS MDI, PDI

IDA, iron deficiency anemia; MDI, Mental Development Index; PDI, Psychomotor Development Index; NS, non significant different between groups; MM, multivitamins (vitamin A, B1, B2, B6, D3, E, and niacinamide)

2.4.1.2 Zinc supplementation in preschool and school age children

Effects of zinc supplementation on cognitive function in preschool and school age have been inconsistent (Table 2.8). In a study in 5-7 years old boys in Canada, cognitive function was measured using 4 subtests of Detroit Tests of Learning Abilities including sentence imitation, word sequences, oral directions, and design reproduction which emphasize mental concentration and short-term memory, and thus provide a measure of attention. The result revealed that attention was unaffected by zinc supplementation (83). A study conducted in Guatemala where children 7-8 years old received either zinc supplementation or a placebo for 6 months showed no treatment effect on cognitive function (84). This study also used the Detroit Tests of Learning Aptitudes (letter sequences, oral directions, design reproduction).

A positive effect of zinc supplementation in school age children was shown in a study in China. Children aged 6-9 years received either 20 mg zinc alone, 20 mg zinc with selected micronutrients, or micronutrients alone for 10 weeks. Cognitive function was assessed by the computerized Cognition-Psychomotor Assessment System-Revised which aims to measure fine and gross motor skill, eye-hand coordination, sustained attention and dyscontrol, visual perception, short-term visual memory, and formation and abstract reasoning. Zinc supplemented children demonstrated improved cognitive test performance and the greatest improvements were observed when other micronutrients were also given together with zinc as compared to zinc and micronutrient alone (85).

Table 2.8 Studies of the effect of zinc supplementation on cognitive development in preschool and school age children

Study	Sample size, age	Supplementation	Outcome measures	Results
Gibson, 1989 (83) Canada	N = 60 5-7 y	10 mg Zn/d (zinc sulfate) Placebo 12 mo	Detroit Tests of Learning Abilities (sentence imitation, word sequences, oral directions, design reproduction)	NS cognition
Cavan, 1993 (84) Guatemala	N=162 7-8 y	10 mg/d (amino acid chelate) Placebo 6 mo	Detroit Tests of Learning Aptitudes (letter sequences, oral directions, design reproduction)	NS cognition
Sandstead, 1998 (85) China	N = 760 6-9 y	20 mg Zn/d 20 mg Zn/d + MN MN 10 wk	CPAS-R: Finger tapping, visual motor tracking, continuous performance, design matching, delayed design matching, oddity task	Improved fine and gross motor skills Sustained attention and capacity for concept formation and abstract reasoning

MN, micronutrients (copper, selenium, iodine, fluoride, manganese, molubdenum, chromium, retinol, vitamin D, phytonadione, vitamin E, thamine, pyridoxine, riboflavin, niacin, folic acid, B12); CPAS-R, Cognition-Psychomotor Assessment System-Revised; NS, non-significant difference between groups

2.4.2 Benefits of zinc supplementation on growth

The effects of zinc supplementation on growth were examined in a meta-analysis of 33 randomized intervention trials that were conducted in children less than 12 years old (20). Results confirmed that zinc supplementation had a positive effect on height and weight increments. Zinc supplementation produced an overall effect size for linear growth of 0.35, which increased to 0.46 in the stunted children. Similarly, the overall weighted mean effect size for the change in body weight was 0.31 and 0.56 in response to zinc supplementation in all children and underweight children, respectively. However, zinc supplements did not have any significant effect on WHZ.

In a recent meta-analysis, there was no positive benefit of zinc supplementation on growth among children ≤ 5 years of age. Results of this study were not consistent with the earlier meta-analysis studies (20, 86) and the authors mentioned that only studies among ≤ 5 year old children were included in the recent meta-analysis and better baseline nutritional status in the recent trials included in the analysis may attenuate the concurrent benefit of zinc supplementation on growth.

2.4.2.1 Zinc supplementation in infants

In studies among infants, some studies showed no benefit of zinc supplementation on growth but some studies found a positive effect (Table 2.9). A study in Gambia was conducted in infants aged 0.6-2.3 years old (87). There was a small (2%) but significant effect of zinc supplementation on arm circumference increment, even though no benefits of supplementation on weight gain or height were shown. A study in Chilean infants showed no benefit of zinc supplementation on growth (81). In Guatemala, an increase in MUAC was greater in the zinc supplemented group compared to the placebo group.

However, benefits of zinc on gains in length were shown only in children who were stunted (88). Significant effects of zinc supplementation on growth were found in a study in Vietnam. Infants aged 4 to 36 months were randomly to received zinc supplementation or a placebo for 5 months (89). Weight gain and height gain at the end of the study were higher in zinc supplemented group as compared to the placebo group. Effects of zinc supplementation on weight were shown at 2 months after supplementation and effects of zinc on height were shown at 1 month after supplementation.

Two studies of zinc supplementation during early childhood in developing countries (90, 91) which were not examined in the meta-analysis by Brown (20) showed inconsistent results. In a study conducted in Bangladesh (90), infants were enrolled at 4 weeks to receive 5 mg of zinc or a placebo until 24 weeks of age. Infants were assessed after supplementation. Among infants who had low serum zinc at baseline (< 9.18 μmol/L), the results showed significantly greater weight gains and head circumference increases in those who received zinc compared to those who received a placebo. However, no significant difference in weight gain was found among infants with normal serum zinc at baseline. There was no significant difference in length, chest circumference, and MUAC between the supplemented and the placebo groups. Another study was conducted in Burkina Faso (91). Children aged 6-31 months received zinc or placebo for 6 months. There were no significant differences between the supplemented and the placebo groups on length/height, weight, MUAC, HAZ, WAZ, and WHZ. In addition, sub-group analysis showed no significant effect of zinc on growth among stunted infants.

Table 2.9 Studies of the effect of zinc supplementation on growth in infants

Study	Sample size, age	Supplementation	Outcome measures	Results
Bates, 1993 (87) Gambia	N=170 0.6-2.3 y	70 mg Zn (zinc acetate, and then zinc gluconate) Placebo 2 times/wk as a drink 1.25 y	Weight, length, MUAC	No effect on weight and length Significant effect on MUAC
Ninh, 1996 (89) Vietnam	N=146 4-36 mo	10 mg Zn/d (zinc sulfate) placebo 5mo	Weight, height	Zn group had higher weight gain, height gain, WAZ, and HAZ
Rivera, 1998 (88) Guatemala	N=89 6-9 mo	10 mg Zn/d (zinc sulfate) Placebo As a drink 6.9 mo	Weight, length, MUAC,HC, triceps skinfold	Zn increased MMA Greater length gain in Zn group compared to placebo in stunted children
Castillo-Duran, 2001(81) Chile	N=112 neonate	5 mg Zn/d (zinc sulfate) Placebo 1 y	Weight, length, HC	No effect on weight, length, WLZ
Osendarp, 2002 (90) Bangladesh	N=301 3-5 wk	5 mg Zn/d (zinc acetate) Placebo 30 wk	Weight, height, HC, MUAC, chest circumference	Greater weight gain and change in HC only in zinc deficient
Muller, 2003 (91) Burkina Faso	N=685 6-31 mo	12.5 mg Zn/d (zinc sulfate) Placebo 6 mo	Weight, height, MUAC	NS weight, height, WAZ, HAZ, WHZ, MUAC

MN, multivitamins and minerals; WAZ, weight-for-age Z scores; WHZ, weight-for-height Z scores; HAZ, height-for-age Z scores; MUAC, mid-upper arm circumference; MMA, mid-upper arm muscle area; HC, head circumference; NS, non significant difference between groups

2.4.2.2 Zinc supplementation in preschool and school age children

Studies in preschool children were shown in Table 2.10. A study in Chile revealed only significant effects of zinc on height gain, especially in boys (92). There was no different in mid upper arm muscle area and mid upper arm fat area. However, there was a trend toward increased changes in mid upper muscle area in boys who received supplementation. A study in preschool children in Uganda (93) where children 33-89 months received 10 mg of zinc supplementation daily or a placebo showed no effect of

supplementation on either weight and height gain. Zinc supplementation increased MUAC at the end of the study while a placebo did not. Another study in preschool children which was conducted in children 22-66 months in Belize (94) did not show a significant effect on weight and height gain after 6 months of weekly zinc supplementation.

Studies in school age children seem to show more promising benefits. In Thailand, a study (95) was conducted in 133 children aged 6 to 13 years. These children were randomly assigned to receive daily zinc, vitamin A, combination of zinc and vitamin A, or a placebo for 6 months. At baseline, 28 percent of the children were stunted. Although serum zinc concentrations increased significantly at the end of the study, the investigators did not observe any significant difference in linear or ponderal growth of children supplemented with zinc compared to those receiving vitamin A or a placebo. The second study was conducted in Japan (96). Twenty one pre-pubertal, stunted children were given 5 mg zinc sulfate/kg or placebo daily for 6 months. The results showed a significant improvement in linear growth velocity in the supplemented group compared to the placebo group. A ten week double-blind trial in 6-9 years old Chinese children (85) also had relevant findings, but was not included in the meta-analysis by Brown (20) because of the absence of a placebo group. In this study, the children were given either 20 mg zinc alone, 20 mg zinc with micronutrients, or micronutrients alone for 10 weeks. A measurement of knee height was used to assess linear growth velocity in this study. All treatments resulted in increased growth, but zinc alone had the least effect on growth whereas the combination of zinc and other micronutrients had the largest effect. The authors concluded that it was evident that the

children were deficient in several micronutrients essential for growth. A study in Chile also found a significant effects on height and HAZ, although only among boys (97). In addition, zinc supplementation studies in Guatemalan (84) and Zimbabwean children (98) found treatment effects on body composition measures including MUAC and arm muscle area-for-age, respectively.

2.4.3 Summary of zinc supplementation on cognitive development and growth

Zinc supplementation in infants showed some positive effect on activity patterns. However, significant effect on MDI was not reported. Studies in preschool and school age children revealed inconsistent results. In the studies in Canada and Guatemala, a limited range of cognitive functions was assessed (99). In addition, there might be possible that children in Canada were not zinc deficient, thus they did not respond to supplementation (99).

A meta-analysis of zinc supplementation and growth in children suggests a beneficial effect on both weight and height gain (20). When considering studies in infants, preschool, and school age children separately, the results were inconsistent. This might be partly due to the fact that children in these studies were deficient in several micronutrients as demonstrated in the study in China where the combination of zinc and micronutrients supplementation was the most effective (85). However, a more recent meta-analysis did not find any beneficial effect of zinc supplementation on growth (21). The differences between these meta-analyses may be due to the differences in severity of deficiency and the differences in age of participants included in the analyses.

Table 2.10 Studies of the effect of zinc supplementation on growth in preschool and school age children

Study	Sample size, age	Supplementation	Outcome measures	Results
Ruz, 1997 (92) Chile	N=96 27-50 mo	10 mg Zn/d (zinc sulfate) Placebo 14 mo	Weight, Height, MUAC, TS, MMA, MAFA	Higher height gain in Zn group in boys NS weight gain NS MMA, MAFA
Kikafunda, 1998 (93) Uganda	N=155 33-89 mo	10 mg Zn/d (zinc sulfate) Placebo	Weight, height, MUAC	NS weight and height gain Zn improved MUAC
Smith, 1999 (94) Belize	N=43 22-66 mo	70 mg Zn/wk (zinc gluconate) 3030 RE vitamin A/wk (retinyl palmitate) Zn + vitamin A Placebo As a drink 6 mo	Weight, height	No effect of zinc on weight and height gain
Udomkesmalee, 1992 (95) Thailand	N=133 6-13 y	25 mg Zn/d (zinc gluconate), 1500 RE vitamin A/d (retinyl palmitate) Zn +Vitamin A Placebo. 6 mo	Weight , height	NS height and weight in Zn group compared with VA and placebo
Nakamura, 1993 (96) Japan	N=21 5.9 y	5 mg Zn/kg/d (zinc sulfate), control 6 mo	Height	Improved height and height velocity
Sandstead, 1998 (85) China	N = 760 6-9 y	20 mg Zn/d 20 mg Zn/d + MN MN 10 wk	Knee height	Improved knee height in Zn + MN, followed by MN
Castillo-Duran, 1994 (97) Chile	N=42 8.7 y	10 mg Zn/d (zinc sulfate), placebo 12 mo	Height, armspan, length of lower segment, weight	Improved height and HAZ in males only
Cavan, 1993 (84) Guatemala	N=162 6.8 y	10 mg Zn/d (amino acid chelate), placebo 6 mo	Weight, height, MUAC, TS	TS, MUAC
Friis, 1997 (98) Zimbabwe	N=191 10.1 y	15 mg Zn/d (zinc sulfate) 3 mo	Height, weight, MUAC, TS	Improved weight, WAZ, arm-muscle area for age

HAZ, height-for-age Z scores; WAZ, weight-for-age Z scores; WHZ, weight-for-height Z scores; ; MUAC, mid-upper arm circumference; MMA, mid-upper arm muscle area; MAFA, midarm fat area ;TS, triceps skinfold; MN, micronutrients (copper, selenium, iodine, fluoride, manganese, molybdenum, chromium, retinol, vitamin D, phytonadione, vitamin E, thiamine, pyridoxine, riboflavin, niacin, folic acid, B12); NS, non significant difference between group

2.5 Iron and zinc interaction

There has been a concern of iron and zinc interaction when they are given together. High iron intake (as ferrous sulfate) reduced zinc absorption (as zinc sulfate) when iron and zinc were given in water solution (22). However, the impaired zinc absorption was not observed when iron and zinc were given in a meal. A high level of zinc supplementation has also been suggested to reduce iron absorption (23). Apart from the influence on intestinal absorption, the following mechanism was postulated in 1980 by Davis (100). High levels of zinc may affect iron metabolism by impairing the incorporation of iron into or release from ferritin. Moreover, high levels of zinc lead to a faster turnover of iron due to shortened red blood cell life spans (100).

It was reported previously that an interaction between iron and zinc is due to the competition for transportation by the divalent metal transporter-1 (DMT1) in the small intestine. However, it is found later that iron and zinc are transported by different transporters (24). Iron is transported across the epical membrane of the small intestine by the DMT1 and it is exported across the basolateral membrane by ferroportin (FPN). On the other hand, zinc is transported across apical membrane by human intestinal zinc transporters (hZIP) and transported across basolateral membrane by another zinc transporter (ZnT1) (24, 101). Zinc uptake is independent of the DMT1 mechanism and zinc transporters do not appear to involve in iron uptake, thus it is suggested that the iron and zinc interaction is not due to competitive absorption by DMT1 and zinc does not interfere with iron absorption. In animal models, zinc carbonate absorption was not affected by elemental iron, but the retention of zinc was lower when it was given with ferrous sulfate, due to increased zinc excretion. Although these results may be difficult to

interpret because the iron was administered in different forms , they suggest that iron and zinc competition may occur during post-absorption (24). To date, the possible post-absorption mechanism remains unclear.

Another possible mechanism is that the competitive absorption of iron and zinc may be due to physiochemical interaction rather than physiological competition (24). This is supported by the evidence that iron and zinc can form complexes with other food constituents in the intestinal lumen such as carboxylic acids, which could affect the absorptive ability.

Kordas (24) revealed that the interaction between iron and zinc may occur in tissues other than the intestinal tissue such as brain tissue. However, information on iron and zinc interaction in neural tissue is limited. A study in rats found that iron alone or zinc alone was not effective in increasing the number of ferritin-containing mossy fiber (MF) cells, the cell which is thought to involve in long-term potentiation (LTP-an electrophysical model of memory and learning). When rats were administered iron and zinc together, the MF cells were higher than in the rat administered with other treatments. This study provides evidence of possible biological interdependence of iron and zinc in the brain. Nonetheless, further investigation is needed to understand the possible mechanisms.

2.6 Effects of combined iron and zinc supplementation

2.6.1 Multi-country studies of iron and zinc supplementation in Southeast Asia

Multi-country iron and zinc supplementation studies were conducted with support from the United Nations Children's Fund (UNICEF). These studies were performed in Indonesia, Vietnam, and Thailand (27-29). In all studies, 10 mg iron, 10 mg zinc, 10 mg iron and 10 mg zinc combined, and a placebo were administered daily to infants 4 to 6 months for the duration of 6 months. The effects of supplementation and interaction after supplementation on iron and zinc status, growth and morbidity were assessed. These results were inconsistent (Table 2.11). The studies in West Java, Indonesia and Thailand revealed significant improvements of iron and zinc status when iron and zinc were given alone. Negative effects of iron supplementation on zinc status and the negative effects of zinc supplementation on iron status were not found in the Indonesia study. However, the interaction of iron and zinc was noted on serum ferritin and serum zinc status in the study in Thailand. With regards to growth, no benefit of iron and zinc supplementation was shown among Indonesian infants. However, iron but not zinc, improved ponderal growth in Thai infants. In the Vietnam study, iron alone and iron and zinc combined provided similar effect on iron status. In contrast, zinc alone resulted in better zinc status than iron and zinc combined. The zinc supplementation group had higher weight gains. There was no significant effect on morbidity. Different findings among these studies may be due to severity status of iron and zinc deficiencies. In addition, these children may differ in underlying deficiencies in other nutrients.

Table 2.11 Multi-country studies of iron and zinc supplementation in infants in Southeast Asia

Study	Sample size, age	Supplementation	Outcome measures	Results
Dijkhuizen, 2001 (27) Indonesia	N=360 4 mo	10 mg Fe/d 10 mg Zn/d Fe+Zn Placebo 6 mo	Hb, SF, Zinc Weight, length	Biochemical indicator at 6 mo Hb (g/L): P=106, Fe=115, Zn=106, Fe+Zn=110 SF (µg/L): P=14 , Fe=37, Zn=12L, FeZn=27 Zinc (µmol/L): P=13 , Fe=14, Zn=16, Fe+Zn=15 Interaction: NS Growth: NS
Berger, 2006 (28) Vietnam	N=784 4-7 mo	10 mg Fe/d (ferrous sulfate) 10 mg Zn/d (zinc sulfate) Fe+Zn Placebo 6 mo	Hb, SF, Zinc Weight, length Morbidity	Biochemical indicator at 6 mo Hb (g/L): P=118, Fe=132, Zn=118, Fe+Zn=129 SF (µg/L): P=17, Fe=58, Zn=17, Fe+Zn=52 Zinc (µmol/L): P=16, Fe=15, Zn=23, Fe+Zn=22 Interaction: NS Zinc increased weight Length: NS Morbidity: NS
Wasantwisut, 2006 (29) Thailand	N=609 4-6 mo	10 mg Fe/d (ferrous sulfate) 10 mg Zn/d (zinc sulfate) Fe+Zn Placebo 6 mo	Hb, SF, Zinc Weight, length	Biochemical indicator at 6 mo Hb (g/L): P=106, Fe=118, Zn=102, Fe+Zn=113 SF (µg/L): P=24, Fe=70, Zn=24, Fe+Zn=51 Zinc (µmol/L): P=10, Fe=12, Zn=17, Fe+Zn=12 Interaction on SF Interaction on serum zinc Length: NS Fe increased weight

Fe, iron supplementation; Zn, zinc supplementation; Hb, hemoglobin; SF, serum ferritin; Zinc, plasma/serum zinc; NS, non significant difference between groups

2.6.2 Studies of iron and zinc supplementation in other areas

Other iron and zinc supplementation studies in infants and toddlers are presented in Table 2.12. A study similar to the multi-country studies was performed in Central Java, Indonesia (26, 102). The study showed that the infants in the iron group had higher Hb and SF, while infants in the zinc group had higher serum zinc. The improvement of iron and zinc status was less when iron and zinc were combined. Results also indicate benefits on growth from zinc supplementation as well as benefits on growth and psychomotor development from iron supplementation. However, there was no significant effect on growth or development when iron and zinc were combined. A study in Bangladeshi infants has shown a beneficial effect of weekly combined iron and zinc supplementation and micronutrient mix on motor development (30). The effect sizes were 0.35-0.39, respectively. In contrast, the effect sizes were smaller among infants receiving iron or zinc alone. Iron alone and the groups receiving combined iron and zinc had higher orientation-engagement scores as compared to the control group. The effect sizes were 0.30 to 0.41. Olney (103) conducted iron-folic acid and zinc supplementation in infant 5-11 months in Zanzibar. The results showed that iron-folic acid reduced time to walking unassisted. Iron-folic acid alone and zinc alone improved Hb and zinc protoporphyrin compared to the placebo. There were no significant treatment effects on changes in HAZ or WAZ. In a study in Mexico, children aged 18-36 months were randomly assigned to receive daily iron, zinc, iron and zinc combined, or a placebo for 1 year (104). Biochemical, anthropometry, and morbidity status were assessed at 12 months after supplementation. Children in both iron supplemented groups had higher SF levels after supplementation compared to those in the placebo or zinc supplemented groups. In

addition, children in both zinc supplemented groups had higher serum zinc compared to those in the placebo or iron supplemented groups. Children who received iron and zinc combined or zinc alone had fewer episodes of diseases. There was no effect of treatment on growth or body composition.

2.6.3 Summary of iron and zinc supplementation

Most studies of iron and zinc supplementation during infancy revealed concurrent positive effect of supplementation on biochemical status. Negative interactions between iron and zinc were found on iron and zinc status in some studies. Benefits of supplementation on growth were seen in a few studies. Cognitive development was rarely investigated in these studies. Positive effects from either iron alone or iron and zinc combined was found in some studies and one study suggested stronger effect in iron deficient anemic infants. Few studies investigated morbidity status and one study showed a positive effect of iron and zinc combined and zinc alone in reducing disease episodes.

Table 2.12 Iron and zinc supplementation studies

Study	Sample size, age	Supplementation	Outcome measures	Results
Lind, 2003 (25) Lind, 2004 (26) Indonesia	N=549 ≤ 6 mo	10 mg Fe/d (ferrous sulfate) 10 mg Zn/d (zinc sulfate) Fe+Zn Placebo 6 mo	Hb, SF, sTfR, Zinc, Copper Weight, height, MUAC, knee height Bayley MDI and PDI BRS Morbidity	Hb (g/L): P=114, Fe=119, Zn=116, Fe+Zn=115 SF (µg/L): P=13, Fe=47, Zn=13, Fe+Zn=32 Zinc (µmol/L): P=9, Fe=9, Zn=12, Fe+Zn=11 sTfR (mg/L): P=9, Fe=8, Zn=10, Fe+Zn=8 Interaction on Hb and SF WAZ: Highest in Zn Zn effect on WHZ Interaction on WAZ, knee height, MUAC Interaction on PDI MDI and BRS: NS Morbidity: NS
Black, 2004 (30) Bangladesh	N=221 6-12 mo	20 mg Fe (ferrous sulfate) + 1 mg B2 20 mg Zn (zinc acetate) +1 mg B2 Fe + Zn MM B2 (control)Weekly for 6 mo	Bayley MDI and PDI Behavior Rating scale (BRS; Orientation/engagement, Emotional regulation Motor quality factor) Weight, height Hb	MDI: NS PDI: Fe+Zn, MM > control Orientation/engagement: Fe+Zn, Fe > control Hb: NS Growth: NS
Olney, 2006 (103) Zanzibar	N=354 5-11 mo	12.5 mg Fe + 50 µg folic acid (FeFA) 10 mg Zn FeFA + Zn Placebo Daily for 1 y (children <12 mo = ½ dose)	Hb, ZPP Weight, length, MUAC Motor milestone	Hb (g/L): P=-8, FeFA=4, Zn=3, Zn+FeFA=1 FeFA walked unassisted sooner Compared to no FeFA and effect was stronger in IDA at baseline FeFA and Zn improved Hb, ZPP HAZ, WAZ: NS
Rosado, 1997 (104) Mexico	N=219 18-36 mo	20 mg Fe/d (ferrous sulfate) 20 mg Zn/d (zinc methionine) Fe+Zn Placebo12 mo	Hb, SF, Zinc Height, weight, MUAC, TS Morbidity	Hb (g/L): P=118, Fe=118, Zn=118, Fe+Zn=118 SF (µg/L): P=23, Fe=47, Zn=20, Fe+Zn=44 Zinc (µmol/L): P=14, Fe=15, Zn=17, Fe+Zn=18 Growth: NS Fe+Zn, Zn reduced episode of diseases

Fe, iron supplementation; Zn, zinc supplementation; Hb, hemoglobin; SF, plasma/serum ferritin; Zinc, plasma/serum zinc; sTfR, serum transferrin receptor; ZPP, zinc protoporphyrin; RBP, Retinol binding protein; NS, non significant difference between groups; MDI, Mental Development Index; PDI, Psychomotor Development Index; MUAC, mid-upper arm circumference; TS, triceps skinfold; WAZ, weight-for-age Z scores; WHZ, weight-for-height Z scores; MM, micronutrient mix (B1, niacin, folic acid, pantothenic acid, iodine, copper, manganese, selenium, C, D, E, B6, and B12, 20 mg Fe, 20 mg Zn, 1 mg B2)

2.7 Long-term effects of nutrition intervention during early childhood on cognitive development and growth

Some studies have investigated the long-term effect of nutrition interventions on growth and cognitive development. One study was conducted among Indonesian children who received high energy dietary supplement for 3 months when they were 6 to 60 months old and the benefits of which were studied 8 years later. The results revealed that among those who received supplementation before 18 months of age, the cognitive test scores on working memory were higher than those in the control group (105). Another study was a longitudinal study conducted in Guatemala (106). Results showed significantly higher scores on tests of knowledge, numeracy, reading, and vocabulary at 11 to 24 years of age among populations who received high energy and protein drink supplementation prenatally and during at least the first two years of life compared to those who received low energy drink. A study conducted among stunted Jamaican children who received milk-based formula supplementation with or without psychosocial stimulation at 9 to 24 months of age for 2 years showed no remaining benefit of intervention on growth at 4 years after the end of intervention (107, 108). However, a small benefit of cognitive function from both intervention groups compared to the control group was found. There was no additive effect from combined intervention. These children were assessed again at 8 years after the end of intervention. There were no significant benefits from supplementation on growth and cognition; however, children who received stimulation still had higher cognitive test scores than control children.

Few studies have investigated the long-term effect of iron supplementation on growth and cognitive development. Lozoff (11) assessed the long-term cognitive

outcomes of iron deficiency during infancy and the effect of iron intervention when children were 5 years old. Iron deficient children who had initial Hb above 100 g/L and iron deficiency was fully corrected after 3 months of iron treatment at infancy, had similar cognitive test scores as those who had normal iron status. However, those whose iron deficiency was not fully corrected had lower scores on developmental tests as compared to those whose iron deficiency had been fully corrected and children with normal iron status. This study also followed children 10 years after the intervention (12). Combined moderately anemic children and iron deficient children before and after treatment in infancy were compared to children with good iron status in infancy. Children who had moderate anemia or were iron deficient during infancy had lower scores on mental and motor functioning compared to those who had good iron status during infancy.

Despite some evidence of concurrent positive effects of zinc supplementation on growth and development, there is no study that investigates the long-term effects. In addition, there is no study that has investigated the long-term effects of iron and zinc combined.

2.8 Thailand: country overview and description of intervention trial in infancy

2.8.1 Thailand: country overview

2.8.1.1 General information

Thailand is situated in Southeast Asia and shares a border with Cambodia to the east, Laos to the northeast, Myanmar to the west, and Malaysia to the south. It borders two bodies of water, namely the Gulf of Thailand to the south and the Indian Ocean to the west. The population of Thailand is approximately 64.6 million. The total area of the country is 513,000 square kilometers, and it is divided into four natural regions: the mountains and forests of the North; the vast rice fields of the Central Plains; the semi-arid farm lands of the Northeast plateau; and the tropical islands and long coastline of the peninsula South. Thailand is divided into 75 provinces and 2 special governed districts: the capital Bangkok and Pattaya. Each province is divided into smaller districts, sub-districts, and villages. Thailand has a warm, tropical climate affected by a seasonal monsoon. Three distinct seasons are hot and dry from February to May (average temperature 34 degrees Celsius and 75% humidity), rainy with plenty of sunshine from June to October (average day temperature 29 degrees Celsius and 87% humidity), and cool from November to January (temperatures range from 32 degrees Celsius to below 20 degrees Celsius with a drop in humidity). The southern region is always hot and humid. The Thai economy is export-dependent, with exports accounting for 60% of GDP. Thailand is a lower middle income country with per capita income of $2,750 in 2005 (109). Roughly 60% of Thailand's labor force is employed in agriculture. Rice is the country's most important crop and Thailand is a major exporter in the world rice market.

2.8.1.2 Iron and zinc deficiencies in Thailand

A high prevalence of anemia has been reported in Thailand. In 1995, the prevalence of anemia using hemoglobin concentrations among children aged 0-5 years was 25.2% and prevalence among children aged 6-14 years was 20.5%. Pregnant women with hematocrit < 33% was 16.3% (110). In 2003, prevalence of anemia among 32 infants aged 6-11 months was 56.3% (6). Prevalence of anemia in children 1-5 years old was 25.9%. Prevalence of anemia in school age children was reported separately by age group. The prevalence was 46.7 % in children aged 6-8 years, 25.4% in children aged 9-11 years, and 15.7% in children aged 12-14 years. Despite the high prevalence of anemia, IDA prevalence among school age children was low as reported in several studies. Only 4.2% of school age children in southern region were IDA (Serum ferrittin (SF) ≤2 0 µg/L and Hb < 115 g/L for 5-11 y; Hb < 120 g/L for 12-13 y), while 22% of children were anemia (111). In the northeastern region, a study in school age children found that only 4.4% in children with heterozygous for hemoglobin type E (Hb AE) and only 4.3% of children with normal hemoglobin (Hb AA) were IDA (serum transferrin receptor (TfR) > 8.5 mg/L and Hb < 115 g/L for 6-11 y; Hb < 120 g/L for those aged ≥ 12 y), although the prevalence of anemia among all children was 31% (112). In a study of iron and zinc supplementation during infancy, the original study (29), the prevalence of IDA among infants at baseline was only 1.7-6.2% in each group (SF < 20 µg/L and Hb < 110 g/L), while prevalence of anemia was 23.1-38.8%. The authors suggested that a high prevalence of hemoglobinopathy or vitamin A deficiency may be associated with the high prevalence of anemia. However, the benefit of iron supplementation in these infants suggests that they were affected by some degrees of iron deficiency regardless of high

serum ferritin level, Zinc deficiency was not assessed in the National Nutrition Surveys. Results from two studies conducted in the northeastern region suggest a high prevalence of low serum zinc in school age children. The prevalence from a study conducted in 1990 was 70% (serum zinc < 10.7 μmol/L) (113), and the prevalence from a study conducted in 2002 was 57% (serum zinc: < 9.9 μmol/L in children < 10 y; < 10.7 μmol/L in boy ≥ 10 y; < 10.5 μmol/L in girl ≥ 10 y) (112).

There has been a surveillance system in place to assist with prevention and control of anemia among pregnant women and school age children in Thailand. Pregnant women have been routinely given iron supplementation through ante-natal care service (114). For school age children, curative iron supplementation is provided and a weekly school supplementation program was launched as a pilot program in schools in 13 provinces in 2000. In addition to iron supplementation, instant noodle condiments have been voluntary fortified with multiple micronutrients including iron, vitamin A, and iodine. The fortified instant noodle products have been widely distributed throughout the country. Fortification of fish sauce with iron is another potential strategy. It has been studied and is ready to be launched in the market. There are no national prevention and control programs for iron deficiency in infants or zinc deficiency in any age group.

2.8.2 Description of the intervention trial in infancy

A randomized, double blind, placebo controlled trial of iron and zinc supplementation in infants in Khon Kaen province, northeast, Thailand was conducted during 1998 to 1999 by Institute of Nutrition, Mahidol University (29). A total of 675 infants aged 4-6 months were recruited and 609 infants completed the study. Infants were randomly divided into 4 groups receiving 10 mg iron alone, 10 mg zinc alone, 10 mg iron

and 10 mg zinc, or a placebo. Infants received supplements on a daily basis for 6 months. All children received one dose of 1500 µg retinol equivalent (RE) vitamin A at the beginning of the study. Socio-demographic information was collected at baseline. Anthropometric and 24-hr recall data were collected at baseline, 2nd month, 4th month, and at the end of the intervention. Iron and zinc status were measured in the subset of infants at baseline and at the end of the intervention.

At baseline, mean Hb, serum zinc, and median SF did not differ among groups. Overall, 3% of infants were stunted at baseline and none were underweight. Using the WHO cutoff of Hb < 110 g/L, the overall prevalence of anemia was 28.8% at baseline. After 6 months supplementation, iron alone and iron and zinc combined supplementation resulted in higher Hb than placebo and zinc supplementation. Iron significantly increased Hb by 10.8 g/L, but zinc decreased Hb by 3.4 g/L. Interaction of iron and zinc supplementation on Hb status was not significant. SF concentration in iron and iron+zinc groups were higher than that in placebo and zinc groups. SF was the highest in the iron group. Interaction of iron and zinc supplementation on SF was significant. Iron significantly increased SF by 31.8 µg/L, but zinc decreased SF by 8.5 µg/L. Serum zinc was the highest in zinc group. Iron+zinc and iron groups did not differ in serum zinc, but serum zinc concentrations in these groups were higher than that in placebo. Interaction of iron and zinc supplements on serum zinc was significant. Iron decreased zinc concentration by 0.8 µmol/L, whereas zinc increased 8.5 µmol/L with zinc supplementation. Supplements did not affect linear growth. With regard to the effect on ponderal growth, only iron supplementation resulted in a significant improvement on infants' weight with an estimated effect size of 80 g and significant improvement of

weight-for-length Z scores. In contrast, there were no significant effects on growth due to zinc supplementation.

2.9 Window of vulnerability during infancy

Infancy is a period of rapid brain and physical growth. Postnatal growth of the brain is characterized by its rapid growing during infancy and early childhood. Nearly half of the postnatal growth of the brain has been accomplished by the end of the first year, three fourths by the third year, and nine tenths by the seventh year (1). Physical growth also occurs rapidly during early childhood, especially the first year of life (3). Daily weight gain is approximately 20-30 g during the first 6 months and decreases to 12-15 g during 6 to12 months (54). After the first year, daily weight gain decreases to 6-8 g during 1 to 6 years. Length increases by 2.0-3.5 cm/month in the first 6 months. Then, it reduces to 1.2-1.5 cm/month during 6 to 12 months and 1 cm/month during 1 to 3 years. Tremendous reduction on growth of length happens during 4 to 6 years in which length increases only 3 cm/year. Due to rapid brain and physical growth, any perturbations during early childhood such as undernutrition, stress, poor stimulation and social interaction, and poor health can have long-term effects on brain structure and function as well as contribute to growth failure (2, 4).

2.10 Influence of early growth on later cognitive development

Evidence from previous studies suggests that child malnutrition is associated with poor health and development later in life (115). Studies that focused on prenatal growth reported mixed results. Some studies reported an association of size at birth and cognitive

abilities (116-119). For example, intrauterine growth restriction (116, 120) has been associated with lower IQ scores at 9-10 years or poorer academic achievement at 9-11 years of age. A study conducted in the United States also reported a significant positive association of birth weight and IQ at 7 years of age (117). In addition, a study in Scotland indicated an association of birth weight and cognitive functioning at 11 years of age (121). Among children who were born at term in a longitudinal study in Finland, lower birth weight and length as well as smaller head circumference (HC) were associated with a lower level of cognitive abilities at 56 months (118). However, a British birth cohort that assessed the influence of HC at birth on IQ at 4 and 8 years of age among term-born children (122) found a significant association of HC at birth with only IQ at 4 years of age, but not at 8 years. A study in Brazil also showed no significant association of low birth weight and IQ at 8 years, after controlling for socio-demographic variables (123). In addition, studies in Guatemala (124) and United Kingdom (125) did not show a significant relationship among birth weight, length, or HC and cognitive abilities later in life. A study in Australia showed that association of birth weight and cognitive functioning declined as age increased (126).

Studies that investigated the association of postnatal growth and later intellectual function tended to report the importance of early postnatal growth, rather than late postnatal growth. A positive association of early postnatal growth, but not late postnatal growth was also found in a study that investigated the importance of head growth on IQ at 4 and 8 years of age (122). In a study in Guatemala (124), only early postnatal growth was associated with cognitive performance at adulthood with no association between late postnatal growth and cognitive abilities. It is also important to mention a study among

Filipino children that showed a decline in the degree of association between growth from birth to 2 years of age and cognitive abilities at 8 years of age (127); however, this trend was not further demonstrated when cognitive abilities were measured at 11 years of age.

2.11 Chapter summary

Anemia and zinc deficiency are prevalent in Thailand, and other developing countries. Infants in developing countries are at risk of iron and zinc deficiency due to several reasons such as high requirements for rapid growth, low bioavailability and low quantity of iron and zinc in complementary foods, as well as untimely introduction of complementary foods. Literature has demonstrated that infancy is a critical period of growth and development. However, there is no universal control program of iron and zinc deficiency of infants in vulnerable areas. Literature also suggests critical roles of iron and zinc deficiency on growth and development and potential concurrent benefits of iron or zinc supplementation. In addition, study findings suggest a potential long-term benefit of iron supplementation on cognitive function in IDA children, although this is dependent on dose and timing of supplementation as well as severity and chronicity of iron deficiency. Furthermore, iron, zinc, or combined iron and zinc supplementation in early childhood has shown to improve iron and zinc status, although a negative interaction was found in some studies. Concurrent benefit of the combination on growth was not observed. Although there are limited data on the effect of iron and zinc supplementation on cognition and morbidity, beneficial effects were revealed. However, clear positive or negative interactions are not clear. Data of long-term effects of combined iron and supplementation are not available.

Most studies have investigated short-term effects of iron, zinc, or iron and zinc supplementation, but long-term effects have rarely been assessed. Since the period of infancy is a critical period, there is a possibility that the long-term effects of supplementation during infancy may be greater than the effects of the supplementation during school age. Thus, comparison of the long-term effects of supplementation during infancy and the concurrent effects of school age supplementation would be of interest. However, current data do not allow for such a comparison. With regards to interventions during school age period, conditions such as better status in early childhood, better current status, or participation in other inventions may interact with the intervention itself. These possible interactions may lessen or enhance the effect size.

In addition to the importance of micronutrient supplementation on cognitive abilities and growth, literature reports mixed evidence of the association between growth and cognitive abilities. Thus, longitudinal data on growth from birth to late childhood would allow us to confirm the findings of the influence of growth in different phases during childhood and intellectual functioning at 9 years of age. These findings would be useful for nutrition intervention program planning to achieve optimal childhood development.

CHAPTER 3
METHODS

This chapter describes the research objectives and hypotheses, study design, study setting, study participants, data collection methods, and statistical analysis.

3.1 Objectives and hypotheses

Primary objectives

1. To investigate effects of daily iron and zinc supplementation during infancy on cognitive performance in children at 9 years of age.
2. To investigate effects of daily iron and zinc supplementation during infancy on physical growth in children at 9 years of age.

Secondary objective

1. To examine the influence of prenatal and postnatal growth on intellectual functioning in late childhood.

Specific hypotheses

1. Compared to children who received a placebo, children who received iron or zinc or iron and zinc combined will perform better on cognitive tests and will have better growth status at 9 years of age.
2. Compared to children who received iron or zinc alone, children who received iron and zinc combined will perform differently on cognitive tests and will have different growth status at 9 years of age.
3. Prenatal and early postnatal growth will have a greater influence on intellectual functioning at 9 years of age than late postnatal growth.

3.2 Study design

This study was a cross-sectional, follow-up study of a randomized controlled iron and zinc supplementation trial in infants. Data collection was performed from August 2007 to January 2008 when the children were approximately 9 years of age. Village Health Volunteers and school teachers assisted in locating the children in the original villages or in nearby areas. Written informed consent was obtained from parents or caregivers and verbal assent was obtained from children. Children were excluded from the study if they have been diagnosed with neurological disorders, physical disabilities, or chronic illnesses.

The follow-up protocol was approved by the Institution Review Boards of Emory University, Atlanta, Georgia and the Human Ethics Committee of Mahidol University, Thailand. This study was registered at clinicaltrials.gov as NCT 00824304.

3.3. Study setting

The study was conducted in 106 villages that were included in the intervention trial. These villages are located in 3 rural districts, namely Ubonrat, Ban Fang, and Nampong, in Khon Kaen province. We also followed-up children who moved to nearby districts, including Muang and Khao Suankwang. Khon Kaen province is situated in northeast, Thailand. It is about 450 kilometers far from Bangkok, the capital city. The five districts included in this study are similar to other rural northeast districts with regards to socio-economic profiles for population, village, and household size (29).

The northeast region is the poorest region of Thailand. The main income for the people in this area is from agriculture, especially the cultivation of rice. However, crop

yields lag behind the other parts of country due to the hot and dry climate as well as poor quality of soil. The main language of this region is Thai-Isan (which is similar to Laotian).

3.4 Study participants

Availability of children to participate was checked during February to July 2006 by village health volunteers (VHVs) and school teachers. We were able to locate 562 of 609 children who completed the intervention trial. The main reason for loss to follow-up was migration (n=47). One parent refused to participate and one child was diagnosed with a neurological disorder. This resulted in a final sample size of 560 children, with 139, 147, 139, and 135 in the placebo, iron, zinc, and iron plus zinc groups, respectively. The sample size was 92% of the original sample size. Comparisons of selected characteristics between non-participating (n=49) and participating children in the follow-up were performed. We found no differences in the distribution of supplementation type ($P = 0.122$), sex ($P = 0.579$), birth weight ($P = 0.511$), age at recruitment into the trial ($P = 0.940$), and biochemical and anthropometric status at recruitment into the trial [hemoglobin ($P = 0.676$), serum zinc ($P = 0.818$), weight ($P = 0.668$), and height ($P = 0.922$)].

We calculated the effect size using Power Analysis of Sample Size (PASS) (128) based on the sample size of 130 children per group. With 80% power and alpha of 0.05, we had an ability to detect a difference of Intelligence Quotients (IQ) as small as 1.4 points based on a standard deviation (SD) of 12 from a previous study (67) or the ability

to detect the difference of HAZ as small as 0.1 based on a SD of 0.9 (79). The detectable differences reflected small effects (0.1 SD for both IQ and HAZ).

3.5 Data collection

Socio-economic, demographic, morbidity history, schooling status, and school characteristics

Children and parents were interviewed by trained research assistants using pre-tested questionnaires, including demographic and socioeconomic variables such as sex, age, family composition, housing characteristics, household assets, access to services, parental and caregiver education, parental and caregiver occupation, child's morbidity history, eating habits, and participation in nutrition and health interventions such as deworming, school lunch, supplementation, and instant noodle fortification. Schooling information including age at school entry and grade attended was collected. Information about school characteristics, including school type (public or private), school size, location of school (urban or rural), and the availability of nutrition interventions at school was obtained from the school principals.

Early life status

Data were abstracted from files from the intervention trial during infancy, including iron and zinc status at baseline and after supplementation, anthropometric data at baseline and after supplementation, birth weight, gestational age and socio-economic status during infancy. Maternal height (cm) and supplement compliance (%) were also obtained from the original trial.

Cognitive performance

Cognitive performance was assessed using the a) Wechsler Intelligence Scale for Children-Third edition (WISC-III), Thai version (129, 130) and b) Raven's *Colored Progressive Matrices* (Raven's CPM) (131). WISC-III is an intelligence test designed to measure a variety of skills in children aged 6 years to 16 years. The test can reflect both verbal and nonverbal abilities and does not require reading or writing.

The WISC-III consists of 6 verbal subtests (information, similarities, arithmetic, vocabulary, comprehension, and digit span) and 6 performance subtests (picture completion, coding, picture arrangement, block design, object assembly, and symbol search). Maze, a supplemental subtest, was not performed in our study because it is not used in the computation of the IQ and the index scores. Arithmetic, digit span, coding, and symbol search subtests of WISC-III measured the performance associated with short-term memory, attention, and reasoning which are known to be affected by iron and zinc deficiencies (46, 53, 132). In addition, information, similarities, vocabulary, and comprehension subtests measured verbal ability, which reflects experiences and educational environments (133). Picture completion, picture arrangement, block design, and object assembly measured visual-motor coordination, abstract-visual problem solving, planning, and logical thinking. In the Thai version of WISC-III, the items in two subtests including information and vocabulary were rearranged and the answers in four subtests including information, similarities, vocabulary, and comprehension were adjusted to be appropriate for Thai children. Reliability of the test was performed and the Thai norm was developed based on the data from 3,300 Thai children aged 6-16 years (130). These children were selected from both rural and urban areas in 13 provinces.

Reliability of WISC-III, Thai version was assessed using Cronbach's alpha. Reliability was fair to good and ranged from 0.63-0.80 for all subtests. Reliability for full scale, verbal, and performance IQ ranged from 0.88-0.93 and reliability for index scores ranged from 0.88-0.91.

Raven's CPM is a nonverbal test designed for young children and the elderly. It measures a person's ability to form perceptual relations and to reason by analogy independent of language and formal schooling. The test consists of 3 sets (A, Ab, B) with 12 items in each set. Children were asked to find the missing pattern in each item. Each set of items gets progressively harder and requires greater cognitive capacity. Most items are presented on a color background which makes them easily recognized by the participants with minimal verbal explanation. Reliability of Raven's CPM was assessed in 467 northeast Thai children aged 6-11 years (134). The Kuder-Richardson Formula 20 (KR-20), a derivative of the Cronbach's alpha, indicated high reliability of the test in these children (KR-20 = 0.92).

Both WISC-III and Raven's CPM tests were administered by 12 qualified clinical psychologists using a standardized protocol. The psychologists were unaware of the child's intervention group. The administration of the tests was carried out on weekends or holidays due to the availability of unattended school rooms, which provided a quiet environment for testing. The administration of the tests was performed at a school or health center near the homes of children. All children received a package of wheat flour based snack and a carton of milk (180 ml) and were encouraged to consume these foods before the test was administered. Each child completed the WISC-III subtests and the Raven's CPM individually in about 1.5-2.0 hours. Raw scores from each subtest of

WISC-III were transformed to scaled scores and the age-adjusted intelligence quotient (IQ) including full scale IQ, verbal IQ, and performance IQ were calculated based on the Thai Norm. The WISC-III index scores were also generated. These index scores include Verbal Comprehension Index (derived from information, similarities, vocabulary, and comprehension subtests), Perceptual Organization Index (derived from picture completion, picture arrangement, block design, and object assembly subtests), Freedom from Distractibility Index (derived from arithmetic and digit span subtests), and Processing Speed Index (derived from coding and symbol search subtests) (135). The raw scores for all items in all three sets of the Raven's CPM test were summed and the total score was used in the analysis.

Inter-rater reliability test for the WISC-III was performed to ensure the reliability of subtest scoring and IQ and index score calculation among these psychologists. The test was performed in 30 primary school children. Each psychologist performed the test in 2-3 children. The administration of the test was video recorded. After that, each psychologist reviewed all 30 video records and assigned the scores on the record forms. Three hundred and sixty record forms were included in the inter-rater reliability analysis. The mean intra-class correlation coefficient (ICC) for each subtest ranged from 0.86 to 1.00 and the mean ICC for IQ and index score calculation ranged from 0.91 to 1.00.

We performed correlation tests between Raven's CPM and WISC-III IQ. Correlation coefficients between Raven's CPM and full scale IQ and performance IQ were quite high (0.59 and 0.64, respectively) (Table 3.1). However, a correlation coefficient between Raven's CPM and verbal IQ was moderate (0.38).

Data on school performance for each child, including Thai, English, Mathematics, and Science scores, were abstracted from school records. The records for the 1st semester of the 2007 academic year were used. The scores were derived from tests developed by each school and were recorded as percentage of total points achieved.

Anthropometry

Weight and length at birth were obtained from the trial during infancy (29). Weight and length at birth was measured by health personnel and was recorded in the child's health booklet. Weight, length, mid-upper arm circumference (MUAC), and head circumference (HC) at baseline and endline of the intervention trial were also obtained from the original trial. Methods of body size measurements during infancy was described previously (29). Weight-for-age Z score (WAZ), length-for-age Z score (LAZ) and body mass index-for-age Z score (BMIZ) were calculated based on the 2006 World Health Organization (WHO) growth standards for children 0-5 years old. Weight, height, MUAC, and HC at 9 years of age were carried out in triplicate according to the standard techniques (136). The child was instructed to wear light clothes and weight was measured to the nearest of 0.1 kg using a digital weighing scale (Seca digital scale model 841, Seca Corporation, Hamburg, Germany). Standing height was measured to the nearest of 0.1 cm using a wooden height board. MUAC was measured at the midpoint of the upper right arm using a non-stretching tape and was read to the nearest 0.1 cm. Triceps and subscaptular skinfolds thickness were measured using a Holtain skinfold caliper (Holtain Ltd., Crosswell, UK). Body mass index (BMI) was calculated (BMI = weight (kg)/height^2 (m)). WAZ, height-for-age Z score (HAZ), and BMIZ were calculated using the 2007 WHO growth reference for school-aged children and

adolescents.

Prior to anthropometric measurement, the precision of the anthropometric measurement was determined by calculating the coefficients of reliability (CRs) for each measurement on 20 children (136). The CRs for weight and height measurements were 0.9999. The CRs for HC, MUAC, triceps skinfold thickness, and subscaptular skinfold thickness were 0.9958, 0.9989, 0.9947, and 0.9997, respectively.

Digit preference of anthropometric measures was assessed. Birth weight was reported in grams. About 67% of the measurements ended with 100 g and almost 25% of the measurements ended with 50 g. Birth length was reported to the nearest 1 cm in 91% of the measurements. Only 1% of the measurements were reported to the nearest 0.1 cm. Almost 80% of head circumference at birth (n=331) ended with 0 and 2% ended with 5. The distribution of the ending digits of weight, height, HC, and MUAC at 4 mo, 1 y and 9 y are presented in Table 3.2. There was no digit preference in all measurements.

3.6 Statistical analysis

Data were entered and checked for accuracy using Epi-Info 2004. Incorrect entries and missing data were checked and verified on a systematic basis using range checks for values beyond permissible values and missing values. Extremely low and high values [less than (1^{st} quartile - (3*inter-quartile range)) or greater than (3^{rd} quartile + (3*inter-quartile range))] were set to missing. Missing values were also set to missing. Data that were available for analyses were summarized in Table 3.3.

Statistical analyses were performed using SAS for Windows 9.2 (SAS Institute Inc., Cary, NC, USA). All continuous variables were tested for normality using the

Kolmogorov-Smirnov test. Mean and standard deviation or median and interquartile ranges were calculated as appropriate. Differences among supplementation groups were analyzed using Analysis of Variance (ANOVA) with Tukey's post-hoc test for continuous variables and using chi-square test for categorical variables. The Kruskal-Wallis test was used to compare differences among groups for non-normally distributed data. Since children were clustered by school, the General Linear Mixed Model (MIXED) was used for analyses of cognitive performance including WISC-III IQ and index scores, Raven's CPM score, and school performance. The MIXED model was also used to test the significance of the interactions between intervention group and baseline status. Generalized Linear Model (GLM) was used to assess the effects of iron and/or zinc supplementation on the growth and the significance of the interactions between intervention group and baseline status, after adjusting for covariates. Multiple Stage Least Squares (SLS) analyses were used to assess association of prenatal growth (at birth), early postnatal growth (birth to age 1 year), and late postnatal growth with IQ (age 1 year to age 9 years). Detailed statistical analyses for each objective were described as follows:

Long-term effects of iron and zinc supplementation on cognitive performance

Statistical analyses were based on intention-to-treat. Main outcomes were full scale, verbal, and performance IQ, WISC-III index scores, Raven's CPM score, and school performance at 9 years of age. Other school variables, including nursery school attendance, kindergarten attendance, and current grade attendance were secondary outcomes. Differences among supplementation groups were analyzed using Analysis of Variance (ANOVA) with Tukey's post-hoc test for continuous variables including age

and characteristics at infancy and using chi-square test for categorical variables such as distribution of sex, parental education and occupation, SES, and schooling outcomes. The Kruskal-Wallis test was used to compare differences among groups for non-normally distributed data. The MIXED Model was used for analyses of WISC-III IQ and index scores, Raven's CPM score, and school performance to take into account the fact that children were clustering within school.

Sub-analyses were performed to assess whether the effect of iron and/or zinc supplementation on full scale IQ and Raven's CPM score differed by micronutrient status, anthropometric status, socio-economic status, and supplement compliance at infancy. Each variable, namely hemoglobin at entry, serum zinc at entry, birth weight, LAZ at entry, household income at infancy, and supplement compliance was tested separately, together with its interaction term using the MIXED model. Each model also controlled for child's age and sex, maternal education, availability of mother, school location, and household SES. Household SES was not included in the model that assessed the interaction between intervention group and household income at baseline.

Long-term effects of iron and zinc supplementation on physical growth

The primary analyses were based on intention-to-treat. Differences of anthropometric indices among supplementation groups were analyzed using Analysis of Variance (ANOVA) with Tukey's post-hoc test for continuous variables. The Chi-square test was used to assess differences among groups for categorical variables. The Kruskal-Wallis analysis was used to compare differences among groups for non-normally distributed data.

Generalized Linear Models (GLM) were performed to assess effects of iron and/or zinc supplementation on the main outcomes, including weight, height, BMI, MUAC, WAZ, HAZ, and BMIZ at 9 years of age, adjusting for size at 1 year, child age, sex, maternal height, maternal education, and household SES. The analyses that included interactions between intervention group and supplement compliance, WAZ, LAZ, and BMIZ at baseline were also performed to test whether supplement compliance and anthropometric status at baseline modified the intervention effect. Statistical significance was set at $P < 0.05$.

The influence of prenatal and postnatal growth on intellectual functioning at 9 years of age

The main outcomes were WISC-III full scale IQ, verbal IQ, performance IQ, and Raven's CPM score at 9 years of age. Independence T-test or Analysis of Variance (ANOVA) with Tukey's post-hoc test was used to assess differences in the main outcomes by socio-demographic variables. Ordinary Least Squares (OLS) regression analyses were performed to assess the associations between body size at birth, 4 months, 1 year, and 9 years and the main outcomes, adjusting for socio-demographic variables and maternal height. The regression coefficients for body size were presented as standard deviation scores (SDSs), which were calculated by subtracting the individual measurement value by the mean of that measurement and then dividing by its SD.

Multiple Stage Least Squares (SLS) analyses were used to assess associations between prenatal growth (at birth), early infancy growth (birth to 4 months), late infancy growth (4 months to 1 year), and late postnatal growth (1 year to 9 years) and IQ or Raven's CPM. The SLS analyses were used in an earlier study that assessed the relative

importance of prenatal and postnatal growth on women's education achievement (124). The 2-SLS analysis was used to estimate the effect of prenatal growth and the effect of early infancy growth, independent of prenatal growth. In the first stage, the influence of birth size on size at 4 months was estimated for each measurement (weight and height). We modeled size at 4 months on birth size to estimate predicted size at 4 months. After that, the residual, which was interpreted to represent the early infancy effect (R1), was calculated by subtracting the predicted size from the observed size at 4 months. In the last stage, we modeled birth size and the R1 residual on IQ in order to assess the relative importance of prenatal and early infancy effects. Size at birth and the residual were used as SDSs in this stage to facilitate comparisons of the relative influence of growth on IQ at different ages. Other covariates including child sex, maternal height and education, availability of mother, SES, and location of school were included in the analysis.

The 3-SLS analysis was used to estimate the effect of late infancy growth independent of previous growth. We first obtained the R1 as in the first stage of the 2-SLS analysis. In the second stage, size at 1 year was modeled on size at birth and the R1 to get the predicted size at 1 year of age. Then, the late infancy residual (R2) was calculated by subtracting the predicted size at 1 year from the observed size. After that, we performed the third stage regression by modeling birth size, R1, and R2 on IQ.

In the 4-SLS analysis, we obtained the R1 and R2 as described in the 3-SLS. Then, we modeled size at 9 years on birth size, R1 and R2 in order to get the predicted size at 9 years. The third residual, R3, was calculated by subtracting the predicted size at 9 years from the observed size. Finally, the fourth stage regression was performed by

modeling birth size, R1, R2, and R3 on IQ to assess prenatal, early infancy, late infancy, and late postnatal effects without the influences of the initial growth.

HC at birth was obtained from health record; the distribution was extremely skewed, suggesting poor data quality. Therefore, we included only the measurements at 4 months, 1 year, and 9 years in the SLS analysis. In the 2-SLS analysis, we first modeled HC at 4 months on HC at 1 year to estimate predicted size at 1 year. The late infancy growth (R1) was calculated by subtracting the predicted HC at 1 year from the observed HC. Then, we modeled HC at 4 months and the R1 residual on IQ to assess the effects of early infancy growth and late infancy growth separately. The 3-SLS was performed to assess the effects of growth in HC at early infancy, late infancy, and late postnatal. In addition to R1 estimation, we modeled on HC at 9 years on HC at 1 year to get the predicted HC. Then, we subtracted the predicted HC from the observed HC to get the late postnatal growth in HC (R2). In the last stage, we used HC at 4 months, R1, and R2 as dependent variables to assess the effects of growth in HC at early and late infancy and late postnatal on IQ at 9 years of age.

Examples of SLS

Two Stage Least Square regression (2-SLS):

- Stage 1: Length at 4 mo (S_1) = $\beta_0 + \beta_1 X_1$ (Birth length)

  Predicted length at 4 mo: $\hat{S}_1 = \hat{\beta}_0 + \hat{\beta}_1 X_1$ (Birth length)

  Early infancy effect (R_1) = Length at 4 mo - predicted length at 4 mo
- Stage 2: Full scale IQ (S_2) = $\alpha_0 + \alpha_1 X_1 + \alpha_2 R_1$ + covariates

Three Stage Least Square regression (3-SLS):

- Stage 1: Length at 4 mo (S_1) = $\beta_0 + \beta_1 X_1$ (Birth length)

  Predicted length at 4 mo: $\hat{S}_1 = \hat{\beta}_0 + \hat{\beta}_1 X_1$

  Early infancy effect (R_1) = Length at 4 mo - predicted length at 4 mo

- Stage 2: Length at 1 year (S2)

  Predicted length at 1 y: $\hat{S}_2 = \hat{\beta}_0 + \hat{\beta}_1 X_1 + \hat{\beta}_2 R_1$

  Late infancy effect (R_2) = Length at 1 y - predicted length at 1 y

- Step 3: Full scale IQ (S_2) = $\alpha_0 + \alpha_1 X_1 + \alpha_2 R_1 + \alpha_2 R_2$ + covariates

Table 3.1 Correlation coefficient between Raven's CPM score and WISC-III IQ and index scores

Variable	r	*P*
WISC-III IQ		
Full scale IQ	0.59	<0.001
Verbal IQ	0.38	<0.001
Performance IQ	0.64	<0.001

Table 3.2 Distribution of ending digits of weight, height, head circumference, and mid-upper arm circumference

Digit	Weight (%)	Height (%)	HC (%)	MUAC (%)
		At 4 mo		
0	7.5	17.8	16.8	12.0
1	8.4	10.0	8.8	8.6
2	9.6	10.9	9.3	10.0
3	9.8	7.7	7.3	8.8
4	12.3	8.8	7.3	7.3
5	9.5	15.4	17.7	16.6
6	13.0	8.0	9.3	8.4
7	9.5	8.2	6.8	9.1
8	11.6	8.4	10.7	10.4
9	8.8	4.8	6.1	8.9
		At 1 y		
0	7.7	17.8	16.6	14.5
1	10.2	10.2	10.0	8.8
2	8.8	11.6	9.5	11.4
3	11.8	6.6	9.0	8.4
4	12.0	7.0	7.3	7.3
5	9.6	15.7	15.2	14.7
6	10.5	6.8	8.8	9.8
7	9.3	7.9	10.0	9.3
8	10.7	8.4	9.7	8.9
9	9.5	8.0	3.8	6.8
		At 9 y		
0	10.9	19.1	10.2	10.9
1	9.5	10.0	9.8	10.7
2	10.4	5.9	8.6	10.9
3	9.6	6.3	9.6	10.7
4	11.2	13.0	12.0	8.4
5	13.9	13.0	15.9	10.2
6	11.3	7.7	7.9	8.9
7	7.0	5.0	8.6	9.1
8	7.9	8.9	8.7	8.2
9	8.4	11.1	8.7	12.0

HC, head circumference; MUAC, mid-upper arm circumference

Table 3.3 Data availability of selected variables (n=560)

Variable	Available data, n (%)
Cognitive test	
WISC-III IQ	560 (100.0)
Raven's CPM score	560 (100.0)
Anthropometry	
Weight at birth	560 (100.0)
Length at birth	462 (82.5)
HC at birth	331 (59.1)
Weight at 4 mo	560 (100.0)
Length at 4 mo	560 (100.0)
HC at 4 mo	560 (100.0)
MUAC at 4 mo	560 (100.0)
Weight at 1 y	560 (100.0)
Length at 1 y	560 (100.0)
HC at 1y	559 (99.8)
MUAC at 1 y	559 (99.8)
Weight at 9 y	560 (100.0)
Height at 9 y	560 (100.0)
HC at 9 y	560 (100.0)
MUAC at 9 y	560 (100.0)
Socio-demographic variables	
Maternal education	557 (99.5)
Paternal education	551 (98.4)
Maternal occupation	555 (99.1)
Paternal occupation	534 (95.4)
Availability of mother	560 (100.0)
School location	560 (100.0)
SES score	560 (100.0)
Household income	549 (98.0)
Other variables	
Maternal height	554 (98.9)
Gestational age	465 (83.0)

CHAPTER 4

LONG-TERM EFFECTS OF IRON AND ZINC SUPPLEMENTATION DURING INFANCY ON COGNITIVE FUNCTION AT 9 YEARS OF AGE AMONG NORTHEAST THAI CHILDREN: A FOLLOW-UP STUDY

Long-term effects of iron and zinc supplementation during infancy on cognitive function at 9 years of age among northeast Thai children: A follow-up study

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Running title: Long-term effects of iron and zinc supplementation on cognitive function

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Reprints will not be available from the author.

Sources of support: This study was supported by grants from International Nutrition Foundation/ Ellison Medical Foundation and Mahidol University Research fund.

ABSTRACT

Background: Iron and zinc are important micronutrients for child growth and development, particularly early in life. One would expect that iron and zinc supplementation in infancy would be an appropriate strategy to promote long-term cognitive development and school achievement, but this has not been evaluated.

Objective: To investigate the impact of iron and/or zinc supplementation during infancy on cognitive performance 8 years after supplementation.

Design: A follow-up study was performed among 560 9-year old children in rural districts of Khon Kaen, Thailand. These children had participated in a randomized controlled trial involving 4 groups receiving daily iron, zinc, iron and zinc, or a placebo at 4-6 months of age for a duration of 6 months. The children examined at 9 years represent 92% of those who participated in the earlier trial. Cognitive performance was assessed using the Wechsler Intelligence Scale for Children-Third Edition (WISC-III), Thai version, the Raven's Colored Progressive Matrices (Raven's CPM), and school performance tests. General Linear Mixed models were performed to assess the long term impact of supplementation and to assess whether the effects of supplementation differed by biochemical status, anthropometric status, socio-economic status, and supplement compliance at infancy.

Results: There were no significant differences at follow-up among the 4 supplementation groups for the full scale, verbal, and performance IQ, and Raven's CPM score at 9 years of age. The IQs of children ranged from 92.9 to 93.7 for full scale IQ, 93.9 to 95.4 for verbal IQ, and 93.1 to 94.0 for performance IQ. There were differential effects of

supplementation on Full scale IQ and Raven's CPM score by per capita household income at infancy

Conclusions: Supplementation with iron and/or zinc during infancy does not lead to long term cognitive improvement in 9 year old children.

INTRODUCTION

Iron and zinc are essential for normal neurological function. Iron deficiency affects myelination, neurotransmitter metabolism, and iron containing-enzymes (1, 2). Functional isolation, i.e. reduced exploration of the environment and reduced activity, is an indirect mechanism linking anemia to poor cognitive development (3). Zinc is important for both structure and function of the brain (4, 5). Zinc deficiency during rapid periods of brain growth can alter emotional behavior, decrease spontaneous activity, and impair memory, attention, and learning ability (6). Infancy is a period of rapid brain growth (7). Any perturbations during early childhood, such as malnutrition and poor stimulation, can lead to long-term impairment of brain structure and function (8). Infants in developing countries are at risk of iron and zinc deficiency due to low intakes, poor bioavailability in complementary foods, as well as because of untimely introduction of these foods (9, 10). Thus, combined supplementation of iron and zinc appears to be an appropriate strategy to prevent deficiencies and promote optimal brain development.

Many studies have assessed the effect of iron and zinc supplementation in infants on their iron and zinc status, growth, development, and morbidity (11-16). Most of the studies show inconsistent short-term impact on biochemical status and development. Coordinated, multi-country iron and zinc supplementation studies were conducted in Indonesia, Vietnam, and Thailand (13-15). In these studies, iron, zinc, iron plus zinc, or a placebo was administered daily to infants aged 4 to 6 months for 6 months. These studies showed improvement in iron and zinc status; however, none of these multi-country studies assessed cognitive performance. A similar but independent study was performed in Central Java, Indonesia (12, 17). This study demonstrated benefits on psychomotor

development from iron but not zinc supplementation. However, there was no effect on development when both iron and zinc were provided. On the other hand, a study in Bangladesh showed a beneficial effect of weekly combined iron and zinc supplementation and micronutrient mix on motor development in infancy (16). Effect sizes were smaller among infants receiving iron or zinc alone. Olney (18) conducted a trial of iron-folic acid and zinc supplementation in Zanzibar. Infants aged 5-11 months were randomly assigned to receive iron+folic acid, zinc, iron+folic acid+zinc, or a placebo. Infants who received iron+folic acid or iron+folic acid+zinc walked unassisted sooner than those who received zinc alone or a placebo. Overall these studies suggest a possible benefit of iron supplementation but are unclear as to the effects of combining iron and zinc on infant development.

There are no studies that have investigated the long-term effects of iron and zinc supplementation. Only one study reported a long-term effect of iron supplementation during infancy on developmental outcome at five years of age (19). Iron deficient children who had baseline Hb above 100 g/L and whose iron deficiency was fully corrected after 3 months of iron treatment at infancy had similar cognitive test scores as those who had normal iron status at baseline. Those whose iron deficiency was not fully corrected had lower scores on developmental tests compared to those whose iron deficiency had been fully corrected and children with normal iron status.

There is no study that has investigated whether micronutrient supplementation during infancy has long-term effects on cognitive development. Therefore, the aim of this study is to assess the impact of iron and zinc supplementation on cognitive performance approximately 8 years after an intervention during infancy in Thailand (15). Since the

supplementation was performed during infancy, a critical period of child growth and development (7, 20), we hypothesized that the benefits of the supplementation would be long-lasting.

METHODS

Brief description of the trial in infancy

A randomized controlled trial of iron and zinc supplementation in infants was conducted in Khon Kaen province, northeast Thailand from 1998 to 1999. A detailed description of the location and methodologies used in the original supplemental trial has been published elsewhere (15). Briefly, 675 infants aged 4-6 months were recruited of which 609 infants completed the study. Infants were randomly assigned to one of 4 groups: 10 mg iron in the form of ferrous sulfate, 10 mg zinc in the form of zinc sulfate, 10 mg iron and 10 mg zinc, or a placebo. Infants received supplements on a daily basis for 6 months. Socio-demographic information was collected at baseline. Anthropometric data were collected from all infants and blood samples were collected for iron and zinc status determinations in a subset of infants (42%) at baseline and at the end of the intervention.

At baseline, mean hemoglobin (Hb), mean serum zinc, and median serum ferritin (SF) did not differ among groups. Overall, 3% of infants were stunted at baseline and none were underweight. The overall prevalence of anemia was 28.8%. After 6 months of supplementation, iron alone and iron plus zinc supplementation resulted in higher Hb than zinc supplementation and placebo groups. Iron significantly increased Hb by 10.8 g/L, but zinc decreased Hb by 3.4 g/L. There was no interaction between iron and zinc

supplementation on Hb status. SF concentration in iron and iron plus zinc groups was higher than that in the placebo and zinc groups. SF was highest in the iron group. There was a significant interaction between iron and zinc supplementation on SF. Iron significantly increased SF by 31.8 µg/L but zinc decreased SF by 8.5 µg/L. Serum zinc was highest in the zinc group. The iron plus zinc and iron groups did not differ in terms of serum zinc, but serum zinc in these groups was higher than that in the placebo group. The interaction between iron and zinc supplementation on serum zinc was significant. Iron decreased zinc concentration by 0.8 µmol/L, whereas zinc increased by 8.5 µmol/L with zinc supplement. Supplementation did not affect linear growth. However, iron supplementation increased weight by 80 g and increased weight-for-length Z scores. Zinc supplementation had no impact on weight. Cognitive development was not assessed in this study.

Study design and participants

A follow-up cross-sectional study was conducted from August 2007 to January 2008 when the children were approximately 9 years of age. Village Health Volunteers and school teachers assisted in locating the children in the original villages or in nearby areas. Written informed consent was obtained from parents or caregivers of 560 children (92 % of the original sample) and verbal assent was obtained from all children. The follow-up protocol was approved by the Institution Review Boards of Emory University, Atlanta, Georgia and the Human Ethics Committee of Mahidol University, Thailand. This study was registered at clinicaltrials.gov as NCT 00824304.

Assessments

Cognitive performance

Cognitive performance was assessed using the a) Wechsler Intelligence Scale for Children-Third edition (WISC-III), Thai version (21, 22) and b) Raven's *Colored Progressive Matrices* (Raven's CPM) (23). The WISC-III is an intelligence test designed to measure a variety of skills in children aged 6 years to 16 years. The test can reflect both verbal and nonverbal abilities and does not require reading or writing. The Thai version of WISC-III and the Thai norm were developed to be appropriate for Thai children. The WISC-III consists of 6 verbal subtests (information, similarities, arithmetic, vocabulary, comprehension, and digit span) and 6 performance subtests (picture completion, coding, picture arrangement, block design, object assembly, and symbol search). Maze, a supplemental subtest, was not performed in our study because it is not used in the computation of the IQ and the index scores. The Raven's CPM is a nonverbal test designed for young children and the elderly. It measures a person's ability to form perceptual relations and to reason by analogy independent of language and formal schooling. The test consists of 3 sets (A, Ab, B) with 12 items in each set. Children were asked to find the missing pattern in each item. Each set of items gets progressively harder and requires greater cognitive capacity. Most items are presented on a color background which makes them easily recognized by the participants with minimal verbal explanation.

Both WISC-III and Raven's CPM tests were administered by clinical psychologists using a standardized protocol. The psychologists were unaware of the child's intervention group. The administration of the tests was carried out on weekends or

holidays due to the availability of unattended school rooms, which provided a quiet environment for testing. The administration of the tests was performed at a school or health center near the homes of children. All children received a package of wheat flour based snack and a carton of milk (180 ml) and were encouraged to consume these foods before the test was administered. Each child completed the WISC-III subtests and the Raven's CPM individually in about 1.5-2.0 hours. Raw scores from each subtest of WISC-III were transformed to scaled scores and the age-adjusted intelligence quotient (IQ) including full scale, verbal, and performance IQ were calculated based on the Thai Norm. The WISC-III index scores were also generated as described below. Verbal Comprehension Index was calculated based on information, similarities, vocabulary, and comprehension. Perceptual Organization Index was calculated based on picture completion, picture arrangement, block design, and object assembly. Freedom from Distractibility Index was calculated based on arithmetic and digit span. Processing Speed Index was calculated based on coding and symbol search. The scores for all items in all three sets of the Raven's CPM test were summed and the total score was used in the analysis.

Data on school performance for each child, including Thai, English, Mathematics, and Science scores, were abstracted from school records. The records for the 1st semester of the 2007 academic year were used. The scores were derived from tests developed by each school and were recorded as percentage of total points achieved.

Socio-demographic and schooling variables

Children and their parents or main caregivers were interviewed by trained research assistants using a pretested questionnaire. Information regarding child age and sex, parental education and occupation, availability of mother at home, nursery school attendance, age at entry to first grade, and the location of the school were obtained from the questionnaire. Parental education was categorized into 2 groups, 1) lower than or equal to grade 6 and 2) higher than grade 6. Parental occupation was categorized into 4 groups, 1) housewife or unemployed, 2) agriculture, 3) wage laborer, and 4) others (including clerical duties, social service, skilled trader, merchant, and professional). Data on the availability of mother at home was coded as present or absent/occasionally present. The location of the school was coded as urban or rural. Nursery school and kindergarten attendance data were coded as never attended or attended.

Standardized household socio-economic scores (SES) were calculated using principal component analysis (24, 25). The variables included in the analysis were housing characteristics, household assets, and access to services. SES scores were then categorized into tertiles.

Health and socio-demographic status at infancy

Anthropometric and biochemical (hemoglobin and serum zinc) data at entry and endline were taken from the original study. Weight-for-age Z scores (WAZ) and length-for-age Z scores (LAZ) were calculated using the 2006 World Health Organization (WHO) growth standards for children 0-5 years old. Biochemical measures were assessed only in a sub-sample of infants in the original study. Details of anthropometric measurement and blood collection and analyses were described earlier (15). Data on age

at enrollment in the intervention trial and household income were also retrieved from the original study.

Statistical analysis

Statistical analyses were performed using SAS for Windows 9.2 (SAS Institute Inc., Cary, NC, USA). The analyses were based on intention-to-treat. Main outcomes were full scale, verbal, and performance IQ, WISC-III index scores, Raven's CPM total score, and school performance at 9 years of age. Other school variables, including nursery school attendance, kindergarten attendance, and current grade attendance were secondary outcomes. Mean and standard deviation or median and interquartile ranges were calculated. Differences among supplementation groups were analyzed using Analysis of Variance (ANOVA) with Tukey's post-hoc test for continuous variables and using chi-square test for categorical variables. The Kruskal-Wallis test was used to compare differences among groups for non-normally distributed data. The General Linear Mixed Model (MIXED) was used for analyses of WISC-III IQ and index scores, Raven's CPM score, and school performance to take into account the fact that children were clustering within school.

Sub-analyses were performed using the MIXED models to assess whether the effect of iron and/or zinc supplementation on full scale IQ and Raven's CPM score differed by micronutrient status, anthropometric status, socio-economic status, and supplement compliance at infancy. Each variable, namely hemoglobin at entry, serum zinc at entry, birth weight, LAZ at entry, household income at infancy, and supplement compliance was tested separately, together with its interaction term, e.g., hemoglobin at entry x intervention groups or serum zinc at entry x intervention groups. Each model also

controlled for child's age and sex, maternal education, availability of mother, school location, and household SES. Household SES was not included in the model that assessed the interaction between intervention group and household income at baseline.

RESULTS

Characteristics of the participants

It was possible to locate 562 of 609 children who completed the earlier trial. The main reason for loss to follow-up (n=47) was migration. One parent refused to participate and one child was diagnosed with a neurological disorder. This resulted in a final sample size of 560 children, with 139, 147, 139, and 135 in the placebo, iron, zinc, and iron plus zinc groups, respectively (Figure 4.1). The sample size was 92% of the original sample size, and ranged between 89% to 100% in each supplementation group. Most children continued to live in the original villages or nearby communities because parents generally leave their children with grandparents or extended family when they move to work in other areas. We compared non-participating (n=49) and participating children in the follow-up and found no differences in the distribution of supplementation type (P = 0.122), sex (P = 0.579), birth weight (P = 0.511), age at recruitment into the trial (P = 0.940), and biochemical and anthropometric status at recruitment into the trial [hemoglobin (P = 0.676), serum zinc (P = 0.818), weight (P = 0.668), and height (P = 0.922)].

Mean age of children at follow-up was 9 years. Children in the iron groups were slightly older than those in other groups (Table 4.1). Distribution of sex, SES tertiles, parental education and occupation, availability of mother, and location of school were

similar across supplementation groups. About a third of parents had primary education or less and their main occupation was agriculture (45-57%). More than 80% of the children in each group studied at the school located in the village.

Infant characteristics of children recruited for the follow-up study are presented in Table 4.2. Children in the iron and zinc group were slightly older than the rest (4.4 vs. 4.5 mo). There were no differences among supplementation groups in biochemical measures or in anthropometric characteristics at enrollment. There were no significant differences among groups in median per capita annual household income. Children who received iron supplementation had higher hemoglobin levels than the others at the end of supplementation. Similarly, children who received zinc supplementation had higher serum zinc levels compared to others.

Effects of supplementation on schooling outcomes and cognitive performance

The percentages of children who attended nursery school and kindergarten were similar across groups (Table 4.3). Mean age at first grade entry was 7 years old. There were no significant differences among groups. The percentage of children attending grade 4 was highest in the iron group. This was not related to greater grade repetition of children in other groups; rather, it was due to the older age of the iron group.

There were no significant differences among groups in IQ scores. IQs ranged from 92.9 to 93.7 points for full scale IQ, 93.9 to 95.4 points for verbal IQ, and 93.1 to 94.0 points for performance IQ (Table 4.4). The WISC-III index scores of the children in the placebo group, including verbal comprehension, perceptual organization, freedom from distractibility and processing speed did not differ from those of children in iron and/or zinc groups. There were no differences among the 4 groups in mean scaled scores

of selected subtests, including arithmetic, digit span, coding, and symbol search (data not shown). These subtests measure the performance associated with short-term memory, attention, and reasoning which are known to be affected by iron and zinc deficiencies (4, 26, 27). Similarly, the Raven's CPM score, adjusted for age, were not different among the 4 groups and ranged from 21.5 to 22.4. The school performance scores were not different among groups. The scores ranged from 70.7 to 72.3 for Thai, 67.2 to 69.8 for English, 69.2 to 70.7 for Mathematics, and 69.9 to 72.0 for Science.

Differential effects of supplementation on full scale IQ and Raven's CPM score by characteristics at infancy

Sub-analysis to assess whether the effects of supplementation differed by micronutrient status, anthropometric status, or supplement compliance at infancy did not identify any significant interaction term. Significance interaction terms were found only in the case of intervention group x per capita household income at infancy. These are illustrated by the plot of full scale IQ by intervention group and per capita household income (P=0.003) (Figure 4.2) and the plot of Raven's CPM score by intervention group and per capita household income (P=0.028) (Figure 4.3). The plot suggested that children in the iron group with higher per capita household income tended to have lower full scale IQ compared to other groups. In addition, children in the iron and zinc groups with higher per capita household income tended to have lower Raven's CPM score compared to the placebo and iron+zinc groups.

DISCUSSION

Many studies have investigated the effects of micronutrient interventions in infancy on developmental outcomes in infancy. What are missing are studies of micronutrient interventions during the critical phase of infancy that report effects measured in school children and beyond. This study followed the children who received daily iron and/or zinc supplementation during infancy to 9 years of age. We evaluated the long-term effects of the intervention on childhood cognitive performance and found no long-term benefits of daily iron and/or zinc supplementation during infancy on IQ, Raven's CPM score, and school performance at 9 years of age.

Lack of long-term effects cannot be easily attributed to lack of deficiency. Prevalence of anemia at baseline of the intervention trial was quite high (28.8%) and prevalence of zinc deficiency was also high, ranging from 44.8 to 63.1%. High prevalence of anemia and zinc deficiency indicated severe micronutrient malnutrition in our children.

The intervention period may have been too short. Although iron and zinc supplementation at 4-6 months of age improved hemoglobin, iron status, and zinc status at 1 year of age, these children could have become deficient again in the second year of life or beyond. Therefore, providing iron and zinc supplementation only during the early childhood may not be sufficient to promote better cognitive development. Providing prenatal and extended postnatal supplementation may have been required. Further studies are needed to investigate whether longer intervention periods are needed to maintain micronutrient status or to influence later cognitive development.

Nine years of age, while children had not completed primary school, may have been too early to detect long-term effect on cognitive performance. A follow-up study of high protein-energy supplementation during pregnancy and the first 2 years of life (28) found inconsistent but moderately positive effects of supplementation on cognitive abilities when children were assessed during infancy and preschool periods; benefits of supplementation on intellectual functions were larger when the subjects were tested in adolescence and adulthood (29-31). One of these studies showed that the effect of supplementation on educational achievement in women was significant only among those who completed primary school (30). Thus, it may be possible to find long-term effect of supplementation on cognitive performance if we test when children completed primary school.

The benefit of iron and zinc supplementation during infancy on later cognitive function may be influenced by other factors, such as other nutrients, health status, and social and economic factors (32, 33). It is possible that our children were deficient in other nutrients, such as protein and energy, iodine, folate, vitamin B-12, and omega-3 unsaturated fatty acids, which have been known to be associated with cognitive performance (32, 34).

As presented in our findings, children in this study were from rural areas with some social and economic disadvantages. Most participants study in the school located in the villages, have parents with only elementary education, and mostly engage in agricultural and manual labor as occupations. These factors may dilute the effect of iron and zinc supplementation. However, our study was a randomized controlled trial and we also adjusted for these factors (maternal education, availability of mother, school

location, and SES) in the sub-analyses and the results were still insignificant. Thus, lack of significant finding in our study may not be related to the influence of social and economic factors.

We performed sub-analyses to assess whether the effect of iron and/or zinc supplementation on full scale IQ and Raven's CPM scores differ by supplement compliance, and micronutrient, anthropometric, and socio-economic status at infancy by including the interaction terms between supplementation and hemoglobin at entry, serum zinc at entry, birth weight, LAZ at entry, household income at infancy, and supplement compliance in each GLM model. The interaction term between supplementation and household income was significant on Full scale IQ and Raven's CPM score. This indicated that household income during infancy modified the effect of supplementation. We do not have an explanation for these significant findings. Since we tested 12 interaction terms in our analysis (12 models; 6 interaction terms and 2 outcomes), these findings may occur by chance. Thus, the findings should be interpreted with caution.

The major strength in our study is that we obtained a high participatory rate, 92% of the intervention trial in infancy. Since this follow-up study did not plan during the intervention trial, the sample size we had was what existed. However, we performed the power calculation to ensure that the study did not lack of power. From the power calculation based on the sample size of 130 children per group, 80% power, and alpha of 0.05, we had an ability to detect a difference as small as 1.4 IQ points (effect size = 0.1). Therefore, lack of significant finding was not due to insufficient power. Another strength is that we assessed a variety of cognition skills including both verbal and nonverbal cognitive abilities. We found that the correlation coefficients between 2 cognition tests

used in our study, the WISC-III IQ and Raven's CPM score, were quite high (0.4 to 0.6). Consistent findings from both tests strengthened our findings.

This study also has several limitations. One limitation in this study is that we did not have the opportunity to measure behavioral and physical activity of the children which have been known to be indirect consequences of nutritional deficiency (3, 35). Although these measurements are not related to insignificant findings in the current study, they may help to explain the benefits of iron and zinc supplementation. Another limitation is that school performance, including Thai, English, Mathematics, and Science scores were derived from tests that were developed by each school. However, we addressed this in our analysis by using the MIXED model to account for the clustering within school. Gestational age data were retrieved from available records and were usually estimated from recall of the last menstrual period. We had high missing values for the gestational age variable (17%). Prevalence of preterm births (gestational age < 37 weeks) and means of gestational age were similar in all 4 intervention groups. Thus, we did not include this variable in our analysis.

In conclusion, although iron and zinc are important for optimal brain development and early childhood is a critical of brain growth, this long-term follow-up study did not show any benefits of iron and/or zinc supplementation during six months in infancy on cognitive performance at 9 years of age. Since this is a first study to investigate long-term effect of iron and zinc supplementation on cognitive performance at 9 years of age, more evidence is needed to support our null findings. In addition, further research may be needed to investigate whether the continuation of iron and zinc supplementation from early childhood through late childhood and adolescence will provide any long-term

benefits, and whether benefits will occur when the assessment is performed during adolescence and adulthood.

We would like to thank project staff, participants, local health workers, and school teachers for their participation and cooperation. We also thank to Emorn Wasantwisut and her colleagues for sharing the data of the original study and Jintana Singkhornard and her colleagues for performing the Wechsler Intelligence Scale for Children and Raven's Color Progressive Matrices tests.

TP, AD, PW, UR, RF, and RM contributed to the study design. TP collected and analyzed the data under the supervision of RM and PW. TP wrote the manuscript with the contributions from all coauthors. None of the authors had a conflict of interest.

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Table 4.1 Comparisons of socio-demographic characteristics by supplementation group

Characteristic	Placebo (n=139)	Iron (n=147)	Zinc (n=139)	Iron+Zinc (n=135)	P^2
Age (y)	9.3 ± 0.3[1,b]	9.4 ± 0.4[a]	9.2 ± 0.3[b]	9.2 ± 0.3[b]	<0.001
Boys:Girls (%)	53.2:46.8	50.3: 49.7	50.4: 49.6	48.9: 51.1	0.908
SES tertiles (%)					
Low	38.9	32.0	32.4	29.6	0.639
Medium	27.3	36.0	34.5	36.3	
High	33.8	32.0	33.1	34.1	
Caretaker, %					
Dad/Mom	77.0	78.2	79.1	74.8	0.389
Grandparents	19.4	20.4	15.1	22.2	
Others	3.6	1.4	5.8	3.0	
Maternal education (%), n=557					
≤ Grade 6	69.3	61.9	65.2	55.6	0.114
> Grade 6	30.7	38.1	34.8	44.4	
Paternal education (%), n=551					
≤ Grade 6	65.9	66.9	69.9	59.9	0.370
> Grade 6	34.1	33.1	30.2	40.2	
Maternal occupation (%), n=555					
Housewife	3.6	6.9	2.9	6.7	0.355
Agriculture	56.5	55.2	57.3	44.8	
Wage laborer	32.6	31.7	29.7	39.6	
Others	7.3	6.2	10.1	9.0	
Paternal occupation (%), n=534					
Unemployed	0.7	1.4	0.7	0.8	0.795
Agriculture	55.0	48.6	50.4	48.4	
Wage laborer	34.1	42.0	39.3	45.2	
Others	8.2	8.0	9.6	5.6	
Availability of mother (%)					
Present	87.8	86.4	87.0	85.9	0.972
Absent/Occasionally present	12.2	13.6	13.0	14.1	
School location (%)					
Rural	84.2	85.7	83.4	86.7	0.876
Urban	15.8	14.3	16.6	13.3	

[1] Mean ± SD (all such values).

[2] Chi square test for categorical variables; ANOVA with Tukey post-hoc test for continuous variables, values in a row without a common letter differ, P <0.05.

Table 4.2 Characteristics at infancy by supplementation group for participants in the follow-up at 9 years of age

Variable	Placebo (n=139)	Iron (n=147)	Zinc (n=139)	Iron+Zinc (n=135)	P^3
Age at enrollment (y)	4.5 ± 0.5[1]	4.5 ± 0.5	4.5 ± 0.6	4.4 ± 0.5	0.037
Birth weight (kg)	3.1 ± 0.4	3.0 ± 0.4	3.1 ± 0.4	3.1 ± 0.4	0.733
Hemoglobin at entry (g/L), n=239	116.7 ± 12.0	118.0 ± 11.3	113.7 ± 11.1	114.1 ± 11.6	0.119
Hemoglobin at exit (g/L), n=239	106.5 ± 13.3[b]	117.9 ± 12.3[a]	101.5 ± 13.4[b]	112.5 ± 11.2[a]	<0.001
Serum zinc at entry (μmol/L), n=239	11.1 ± 2.7	10.4 ± 2.5	11.6 ± 3.0	11.3 ± 2.2	0.080
Serum zinc at exit (μmol/L), n=239	9.8 ± 1.9[c]	11.5 ± 2.5[b]	16.4 ± 5.1[a]	11.8 ± 3.3[b]	<0.001
Weight-for-age Z score at entry	-0.6 ± 0.9	-0.5 ± 0.9	-0.6 ± 0.9	-0.6 ± 0.8	0.882
Weight-for-age Z score at exit	-0.9 ± 0.9	-0.8 ± 0.9	-0.9 ± 0.9	-0.9 ± 0.9	0.587
Length-for-age Z score at entry	-0.8 ± 0.9	-0.9 ± 0.9	-0.9 ± 0.9	-0.8 ± 0.9	0.897
Length-for-age Z score at exit	-1.0 ± 1.0	-1.0 ± 0.9	-1.0 ± 1.0	-1.0 ± 0.9	0.913
Supplement compliance (%)	95.4 (4.7)[2a]	94.9 (5.1)[a,b]	93.4 (6.7)[b]	93.2 (6.5)[b]	0.004
Per capita annual household income (THB), n=549	7700 (3899, 13500)[2]	8200 (4100, 14700)	8900 (4100, 17000)	8200 (3900, 15300)	0.648[4]

[1] Mean ± SD (all such values).

[2] Median (IQR) (all such values).

[3] ANOVA with Tukey post-hoc test, values in a row without a common letter differ, $P < 0.05$.

[4] Kruskal-Wallis test

[5] THB, Thai baht

Table 4.3 Schooling outcomes by supplementation group

Variable	Placebo (n=139)	Iron (n=147)	Zinc (n=139)	Iron+Zinc (n=135)	P^2
Nursery school attendance (%)					
Never attended	49.6	46.3	48.9	38.5	0.234
Attended	50.4	53.7	51.1	61.5	
Kindergarten attendance (%)					
Never attended	0.0	0.7	1.4	1.5	0.508
Attended	100.0	99.3	98.6	98.5	
Age at 1st grade entry (y)	6.9 ± 0.2[1]	6.9 ± 0.3	6.9 ± 0.3	6.9 ± 0.3	0.858
Current grade (%)					
Grade 2	2.9	4.1	5.0	7.4	<0.001
Grade 3	84.9	58.5	83.5	85.9	
Grade 4	12.2	37.4	11.5	6.7	

[1] Mean ± SD (all such values).

[2] Chi square test for categorical variables; ANOVA for continuous variables.

Table 4.4 Average intelligence quotient (IQ), WISC-III index scores, Raven's CPM score, and school performance scores[1]

Variable	Placebo (n=139)	Iron (n=147)	Zinc (n=139)	Iron+Zinc (n=135)	*P*[4]
WISC-III IQ					
Full scale IQ	93.5 ± 11.7[2]	93.3 ± 9.5	92.9 ± 12.3	93.7 ± 11.3	0.855
Verbal IQ	94.1 ± 10.8	93.9 ± 8.6	94.0 ± 11.1	95.4 ± 10.6	0.202
Performance IQ	94.0 ± 12.7	93.9 ± 11.4	93.1 ± 13.2	93.1 ± 12.5	0.911
WISC-III index scores					
Verbal Comprehension	92.5 ± 11.2	92.7 ± 8.9	92.9 ± 11.6	93.9 ± 10.6	0.393
Perceptual Organization	94.7 ± 13.7	94.9 ± 12.0	93.8 ± 13.6	94.2 ± 13.0	0.919
Freedom from Distractibility	103.0 ± 10.8	101.1 ± 11.6	100.2 ± 11.9	101.4 ± 12.1	0.053
Processing Speed	97.3 ± 10.4	96.0 ± 10.4	96.3 ± 9.1	96.0 ± 11.2	0.574
Raven's CPM score[3]	21.5 ± 5.2	21.7 ± 6.1	21.4 ± 5.4	22.4 ± 6.0	0.398
School performance scores					
Thai	72.3 ± 15.3	70.7 ± 13.3	71.5 ± 14.1	70.8 ± 13.9	0.517
English	69.8 ± 14.1	67.2 ± 14.5	69.1 ± 16.5	68.0 ± 12.8	0.062
Mathematics	70.7 ± 15.3	69.2 ± 15.8	69.5 ± 15.3	69.8 ± 15.1	0.430
Science	72.0 ± 14.1	70.7 ± 13.3	70.7 ± 16.5	69.9 ± 15.1	0.100

[1] WISC-III, Wechsler Intelligence Scale for Children-Third edition; Raven's CPM, Raven's *Colored Progressive Matrices.*

[2] Mean ± SD (all such values).

[3] Adjusted for age.

[4] MIXED procedure

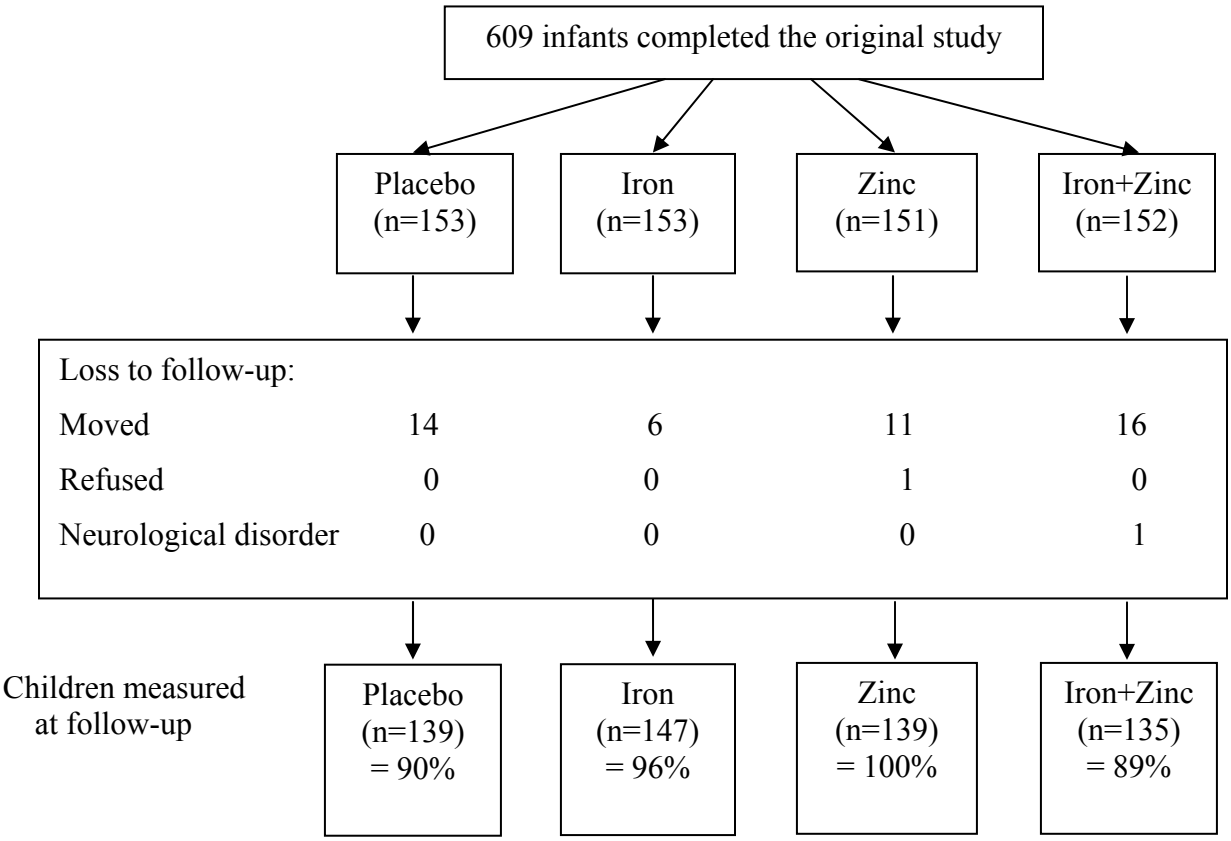

Figure 4.1 Participant profile

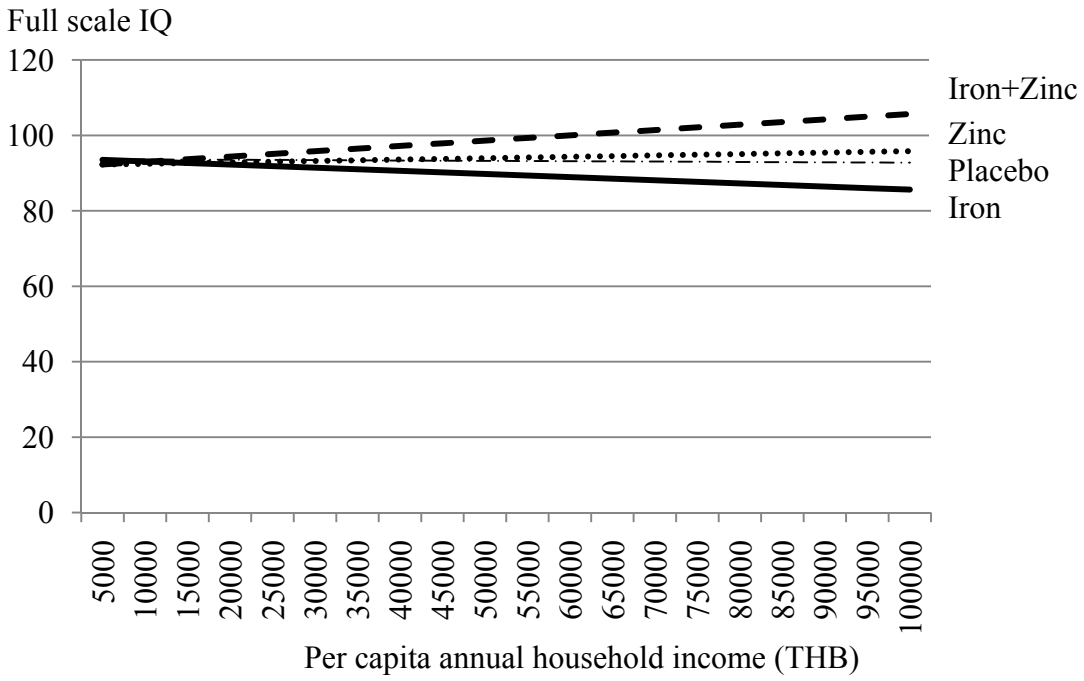

Figure 4.2 Full scale IQ by intervention group and household income at baseline of the intervention trial. The interaction between intervention group and household income was significant ($P = 0.003$) in MIXED model that adjusted for child age and sex, maternal education, availability of mother, and school location. THB, Thai baht

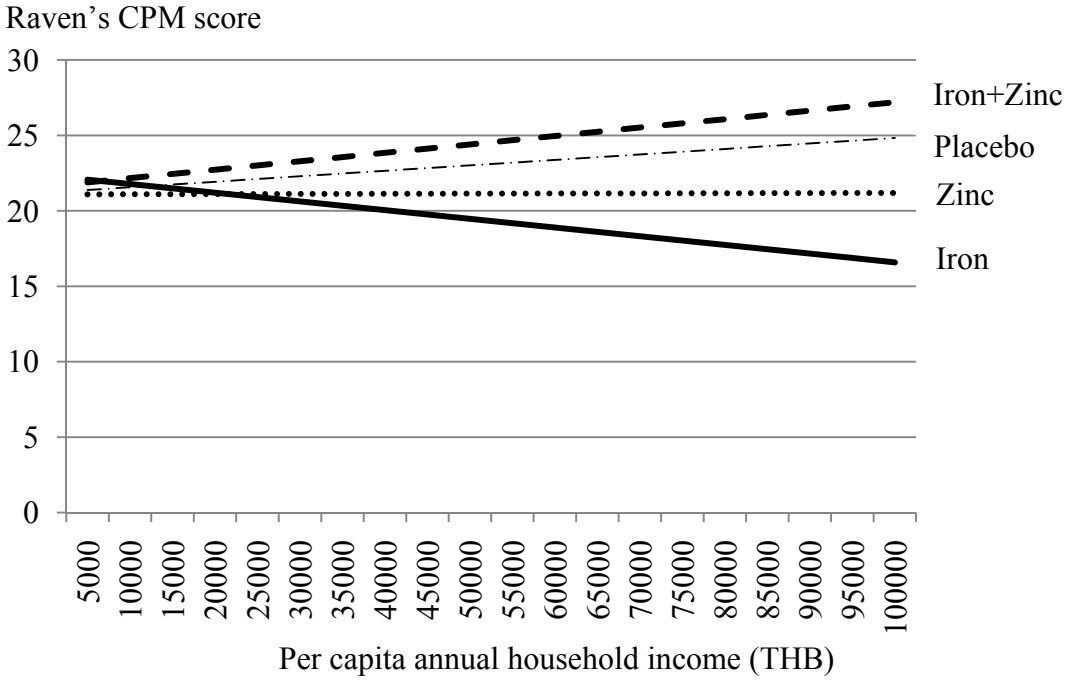

Figure 4.3 Raven's CPM score by intervention group and household income at baseline of the intervention trial. The interaction between intervention group and household income was significant ($P = 0.028$) in MIXED model that adjusted for child age and sex, maternal education, availability of mother, and school location. THB, Thai baht

CHAPTER 5

LONG-TERM EFFECTS OF IRON AND ZINC SUPPLEMENTATION DURING INFANCY ON GROWTH AMONG 9 YEAR-OLD NORTHEAST THAI CHILDREN: A FOLLOW-UP STUDY

Long-term effects of iron and zinc supplementation during infancy on growth among 9 year-old northeast Thai children: A follow-up study

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Short title: Long-term effects of iron and zinc supplementation on growth

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Reprints will not be available from the author.

Sources of support: This study was supported by grants from International Nutrition Foundation/ Ellison Medical Foundation and Mahidol University Research fund.

ABSTRACT

Background: Deficiencies of iron and zinc are prevalent in young children, especially among those in the areas where complementary foods are mainly plant-based. Studies of iron and/or zinc supplementation during infancy, the period of rapid growth, have shown inconsistent concurrent benefits on physical growth; however, none of these studies have investigated long-term effects of infant supplementation.

Objective: We aimed to evaluate long-term effect of iron and/or zinc supplementation during infancy on physical growth at 9 years of age.

Design: Weight, height, and mid-upper arm circumference (MUAC) measurements were performed among 9 year old northeast Thai children (n=560). These children had participated in a randomized trial of daily iron and/or zinc supplementation at 4-6 months of age for 6 months. Anthropometric status and socio-economic data during infancy were obtained from the original trial. General Linear Model was used to assess the effect of supplementation on anthropometric measures and indices 9 years, adjusted for size at the endline of supplementation. Interactions between supplementation and anthropometric indices at baseline were tested.

Results: There were no significant effects of iron and/or zinc supplementation on weight, height, body mass index (BMI), MUAC, and Z scores for weight (WAZ), height, and BMI at 9 years of age. WAZ at baseline modified the effect of supplementation on WAZ at 9 years of age in which positive effect was shown among children with lower baseline WAZ while negative effect was shown among those with higher baseline WAZ.

Conclusions: There was no long-term effect of iron and zinc supplementation during infancy on growth at 9 years of age. WAZ at baseline modified the effect of

supplementation on WAZ at 9 years of age. Iron and zinc supplementation in infants should be restricted to those who are at risk of undernutrition in order to ensure optimal benefit and minimize resulting detrimental effects on growth in late childhood seen in this study.

INTRODUCTION

Iron deficiency is the primary cause of anemia, which remains a significant public health problem. About 25% of the world's population suffers from anemia and the estimated prevalence for preschool-age children is nearly 50%. In Thailand, the prevalence of anemia in 2003 was 56% children 6-11 months and 26% in children 1-5 years old (1). Large scale data for zinc deficiency have been rarely reported because zinc status is difficult to assess. However, the sources of iron and zinc in the diet are similar and iron and zinc have similar problem of bioavailability, thus zinc deficiency is likely to be a public health problem in many countries (2). In most developing countries, cereal grains, which are usually low in iron and zinc content, are the major constituents of complementary food for infants in most settings (1, 3). Therefore, attaining dietary requirements for iron and zinc in infants is difficult.

Anemia, especially iron deficiency anemia is associated with poor physical growth and development (4). Iron does not have a direct role in growth, but it may be involved through its effect on immunity, appetite, thermogenesis, and thyroid hormone metabolism (4). A study in Bangladesh suggested iron deficiency may contribute to growth retardation (5). However, iron supplementation alone failed to show an improvement on both weight and height in a meta-analysis of studies among children less than 18 years old (6) and a meta-analysis of studies among children less than 5 years old (7) .

Zinc deficiency is associated with growth retardation. It is involved in deoxyribonucleic acid (DNA) synthesis, ribonucleic acid (RNA) synthesis, and cell division, and also interacts with hormones involved in bone growth and participates in

metabolism of macronutrients (8). Zinc may also indirectly affect linear growth by altering perception of smell and taste thus leading to decreased appetite and consequently decreased food consumption (8). Previous studies have shown that zinc depletion limits growth in children (9, 10). In a supplementation trial in preschool children, zinc supplemented children had better changes in body composition after 25 weeks supplementation (11). In addition, positive effects of zinc supplementation on height and weight were reported in a meta-analysis of 33 randomized intervention trials that were conducted in children less than 12 years old (12). Responses to zinc supplementation were significantly greater in those studies that enrolled subjects with preexisting stunting or that were underweight. However, a recent meta-analysis did not show significant effects of zinc interventions on weight and height gain (7). This meta-analysis included only studies among children ≤ 5 years of age.

Among studies included in a recent meta-analysis (7), studies conducted in Thailand and the nearby countries showed an impact of iron and zinc supplementation in infancy on biochemical status; however, effects on growth were inconsistent (13-17). A study in West Java, Indonesia (15) did not show effects of iron, zinc, or iron plus zinc on growth, while a study in Vietnam suggested a negative effect of iron supplementation on weight when iron was added to zinc supplementation (16). A study performed in Central Java, Indonesia indicated benefits of iron or zinc supplementation on weight from zinc supplementation (14). However, there was no significant effect on growth when iron and zinc were combined.

A study in Thailand showed a small positive effect of iron supplementation on weight, but not on length (17). None of these studies has investigated long-term effects. Since it has been suggested that physical growth occurs rapidly during early childhood, especially the first year of life (18), we therefore conducted a follow-up study to determine long-term effects of iron and zinc supplementation during infancy on physical growth of supplemented children at 9 years of age.

METHODS

Study design and participants

A follow-up cross-sectional study was conducted from August 2007 to January 2008 among children who had previously participated in an intervention trial of iron and zinc supplementation during infancy in Khon Kaen, Thailand. These children were enrolled in an intervention trial that took place during 1998-1999. They were randomly divided into 4 groups receiving 10 mg iron alone, 10 mg zinc alone, 10 mg iron plus 10 mg zinc, or a placebo on a daily basis from 4-6 months of ages for the duration of 6 months. A detailed description of the study and results were reported earlier (17). At follow-up, all children from the original trial who were available in the study area and the areas nearby were recruited. Of 690 children who completed the original trial, 560 children were included in the current study (92 % of the original sample) (Figure 5.1). Written informed consent was obtained from parents or caregivers of these children and verbal assent was obtained from all children. The study protocol was approved by the Institution Review Boards of Emory University, Atlanta, Georgia and the Human Ethics

Committee of Mahidol University, Thailand. This study was registered at www.clinicaltrials.gov as NCT 00824304.

Anthropometric variables

Weight and length at birth and weight, length, and mid-upper arm circumference (MUAC) at baseline and endline of the intervention trial were obtained from the original trial (17). Weight and length at birth was measured by health personnel and was recorded in the child's health booklet. Methods of body size measurements during infancy were described elsewhere (17). Z scores for weight-for-age (WAZ), length-for-age (LAZ), and BMI-for-age (BMIZ) were calculated based on the 2006 World Health Organization (WHO) growth standards for children 0-5 years old. Weight, height, and MUAC at 9 years of age were carried out in triplicate according to standard technique (19). The child was instructed to wear the light clothes and the weight was measured to the nearest 0.1 kg using a digital weighing scale (Seca digital scale model 841, Seca Corporation, Hamburg, Germany). The standing height was measured to the nearest 0.1 cm using a wooden height board. MUAC was measured at the midpoint of the upper left arm using a non-stretching tape and was read to the nearest 0.1 cm. Each measurement was taken by the same investigator. WAZ, height-for-age Z score (HAZ), and BMIZ were calculated using the 2007 WHO growth reference for school-aged children and adolescents. The precision of the anthropometric measurement was determined by calculating the coefficients of reliability (CRs) for each measurement on 20 children prior to the data collection. The CRs were 0.9999, 0.9999, and 0.9989 for weight, height, and MUAC measurements, respectively.

Other variables

Socio-demographic data were obtained from the trial in infancy (17). These data were collected by questionnaire. Variables included in this analysis were child age and sex, maternal education, and household income. Household income data were presented as per capita annual household income. Maternal height (cm) and supplement compliance (%) used in this analysis were also obtained from the trial infancy. The supplement compliance was daily recorded in the monthly record form by mothers or village health workers and was reviewed by field workers. Research investigators reviewed the record again and measured the leftover syrup during the monthly field visit.

Maternal education and SES score were retrieved from the follow-up dataset. Maternal education was coded as less than or equal to grade 6 or higher than grade 6. Standardized household socio-economic scores (SES) were calculated using principal component analysis (20, 21). The variables included in the analysis were housing characteristics, household assets, and access to services. SES scores were then categorized into tertiles. However, SES scores were included as continuous data in the model that assessed the effect of supplementation on growth.

Statistical analysis

All variables were tested for normality by the Kolmogorov-Smirnov test. The primary analyses were based on intention to treat. Mean and standard deviation or median and interquartile ranges were calculated. Differences of anthropometric indices among supplementation groups were analyzed using Analysis of Variance (ANOVA) with Tukey's post-hoc test for continuous variables. The Chi-square test was used to assess differences among groups for categorical variables. The Kruskal-Wallis analysis was

used to compare differences among groups for non-normally distributed data. The main outcomes were weight, height, BMI, MUAC, WAZ, HAZ, and BMIZ.

Additional analyses were performed using Generalized Linear Models (GLM) to assess long-term effects of iron and/or zinc supplementation on growth including weight, height, BMI, MUAC, WAZ, HAZ, and BMIZ at 9 years of age. The models were adjusted for size at 1 year, child sex, supplement compliance, maternal height, maternal education, and SES score. Change in age from 1 to 9 years of age was also included as a covariate in the model that included weight, height, MUAC, or BMI as an outcome. The analyses that included interaction terms between intervention group and supplement compliance and baseline anthropometric status (WAZ, LAZ, and BMIZ) were also performed to test whether supplement compliance and anthropometric status at baseline modified the intervention effect. We performed 6 GLM models to test these 4 interaction terms. The interaction terms and outcomes were as follows: intervention * compliance on WAZ, HAZ, and BMIZ at 9 y; intervention * baseline WAZ on WAZ at 9 y; intervention * baseline LAZ on HAZ at 9 y; intervention * baseline BMIZ on BMIZ at 9 y. All statistical analyses were performed using SAS for Windows 9.2 (SAS Institute Inc., Cary, NC, USA). Statistically significant level was set at $P < 0.05$.

RESULTS

Selected socio-demographic data at infancy including, child sex, maternal education, and household income of 560 children who participated in this follow-up study were presented in Table 5.1. All 4 groups were similar by sex, maternal education, SES score, and household income. The majority of mothers had educational levels of less

than or equal to grade 6. Per capital annual household income was approximately 8,000 baht. Maternal height was also similar across all 4 groups. Supplement compliance was generally high in all groups (93-95%). However, the compliance was slightly higher in the placebo group compared to the compliance in the zinc and iron plus zinc groups (P = 0.004).

The anthropometric characteristics at birth, at baseline and endline of the intervention trial, as well as at 9 years of age of the 560 children who completed the follow-up study are presented in Table 5.2. Although the mean birth length of children who received iron plus zinc supplementation was lower than that of children who received a placebo (49.8 cm vs 50.8 cm, $P < 0.05$), length at baseline of the intervention trial was similar across the 4 groups. There were no significant differences when comparing 4 groups for other anthropometric indices at other time points.

We further assessed the effect of iron and/or zinc supplementation on weight, height, BMI, and MUAC by first adjusting for these anthropometric measures at 1 year of age, child sex and age change. Age change was included in the model in which weight, height, BMI, or MUAC was the outcome in order to correct for the difference in age at the time of measurement. There were no significant effects of supplementation on these anthropometric measurements (Table 5.3 and 5.4). We then added the supplement compliance variable and found that the effects of supplementation and the compliance variable were not significant in models (data not shown). Therefore, we did not include the compliance variable in the last models to which we further added maternal height, maternal education, and SES score as the covariates. Effects of iron and zinc supplementation on weight, height, BMI, and MUAC were not significant in these

models. Among covariates, being a boy was associated with lower weight, height, and MUAC (P <0.05). Effects of maternal height and SES on these anthropometric measures were not significant. We also further assessed the effect of iron and/or zinc supplementation on WAZ, HAZ, and BMIZ at 9 years of age, by adjusting for the same covariates, except for age change. The effects of supplementation on WAZ, HAZ, and BMIZ were not significant (Table 5.5). The effect of maternal height on WAZ and HAZ was significant (P <0.05). A one centimeter increase in maternal height was associated with a 0.02 and 0.03 SD increase in WAZ and HAZ, respectively.

We did not find that supplement compliance modified the effect of the intervention on WAZ, HAZ, and BMIZ at 9 years of age when we assessed the interaction between intervention group and supplement compliance. We also did not find that LAZ and BMIZ at baseline modified the effect of supplementation on HAZ, and BMIZ at 9 years of age. However, WAZ at baseline modified the effect of supplementation on WAZ at 9 years of age (P=0.038 for interaction; Figure 5.2). Children who received iron and/or zinc supplementation and had lower WAZ at baseline tended to have higher WAZ at 9 years of age compared to those who received a placebo. In contrast, those who received iron and/or zinc supplementation and had higher WAZ at baseline tended to have lower WAZ at 9 years of age compared to those who received a placebo.

DISCUSSION

We assessed the long-term effect of iron alone, zinc alone, or combined iron and zinc supplementation during infancy on physical growth at 9 years of age among children

in rural communities of northeast Thailand. Results from the present study indicate no long-term effects of iron and/or zinc supplementation on anthropometric measures (weight, height, BMI, and MUAC) and anthropometric indices (WAZ, HAZ, and BMIZ) at 9 years of age. Additional analysis showed that WAZ at baseline modified the effect of supplementation on WAZ at 9 years of age. Children with lower baseline WAZ tended to benefit from iron and zinc supplementation. However, results suggest that supplementation may be harmful to those with higher baseline WAZ.

Many studies have investigated the effect of iron and/or zinc supplementation during infancy, but none of these studies have investigated the effects on growth beyond infancy. Most iron intervention studies during infancy have shown no significant concurrent benefit or adverse effects of iron supplementation on growth (22-24). In addition, a systematic review (25) and meta-analyses (6, 7) of studies in infants and older children did not suggest a concurrent benefit of iron supplementation on physical growth. A recent meta-analysis also documented no concurrent effect of zinc supplementation on growth among children ≤ 5 years of age. Results of this study were not consistent with earlier meta-analysis studies (12, 26). The authors mentioned that only studies among ≤ 5 year old children were included in the recent meta-analysis and better baseline nutritional status in children in the recent trials may attenuate the concurrent benefit of zinc supplementation on growth. In addition, this analysis included both supplementation and fortification trials.

To our knowledge, this is a first study that investigated the long-term effects of iron supplementation during infancy on physical growth at 9 years of age. A non positive long-term effect of iron and/or supplementation during infancy in our study seems to be

in agreement with previous studies, albeit said studies investigated only the concurrent benefit. Although data from the intervention trial during infancy suggests a high prevalence of zinc deficiency (45-63%) and anemia (23-39%) at baseline among these children (17), prevalence of underweight and prevalence of stunting were low (3.9% and 8.2%, respectively). Thus, apparently normal anthropometric status at baseline may explain a non beneficial long-term effect of iron and zinc supplementation on growth. Lack of long-term benefit maybe due to the fact that the benefit was diluted by the presence of other nutrients related to physical growth such as energy, protein, and iodine (10, 27).

Interestingly, our results were in partial agreement with an earlier meta-analysis that showed a greater effect of zinc supplementation on weight among children who were underweight at baseline (12). We found an adverse long-term effect of iron and/or zinc supplementation on change in WAZ among children who were better in WAZ at baseline. Publications regarding the long-term effects of iron and zinc supplementation on growth are not currently available, thus we cannot provide a definite conclusion of long-term positive or adverse effects of iron and/or zinc supplementation at infancy on growth in weight at late childhood until we have more supportive evidence.

As indicated by our results, anthropometric status during infancy progressed toward a malnourished status with increased age (Figure 5.3 and 5.4). WAZ at birth were slightly lower than the 2006 WHO growth standards, while WAZ at 4 months and 1 year of age were a lot lower than the standard. LAZ at birth were slightly higher than the 2006 WHO growth standards, whereas LAZ at 4 months and 1 year of age were a lot lower than the standard. However, the anthropometric status was slightly better when children

were at 9 years of age. Our results were similar to the results from the Fourth National Nutrition Survey (NNS) in Thailand in 1995 (28) in which the 1987 Thai growth reference was used. The NNS showed that prevalence of underweight was lower in children less than one year old (29.6%) while the prevalence of underweight became higher among 1 year, 2 years, and 3 years old children (34.5%, 35.1%, and 35.7%, respectively). Then, the prevalence of underweight became lower in 4 years old children (31.4%). However, the prevalence of underweight became higher again in 6-14 year old children (33.7%). A similar pattern was reported for the prevalence of stunting. This evidence may warrant changes in complementary food practices and health care among young children.

Our study has several strengths. First, we had a low percentage of loss to follow-up and thus decrease the selection bias due to loss to follow-up. Second, supplement compliance was high in our study. This is important because we can eliminate the factor that might be related to the null findings. Finally, we conducted a community-based study and the children participated in our study were apparently healthy. Thus, findings from this study could be generalized to similar populations in rural communities of developing countries when more evidence is available. This study also has a limitation. Since we did not plan to assess body composition in the intervention trial, data on anthropometric measures were not available and we cannot calculate body composition such as arm fat area and arm muscle area. Therefore, we were not able to demonstrate whether there was any long-term benefit of iron and/or zinc supplementation on body composition.

Although more studies are needed to confirm our findings, a preliminary implication that can be obtained from the current findings is that iron and/or zinc

intervention during infancy may not provide the same long-term benefit on growth in all children. Thus, investment in iron and/or zinc interventions should consider the baseline nutritional status of targeted infants and interventions should be restricted to infants who are at risk of undernutrition in order to ensure the benefit and minimize the adverse effect on growth during late childhood.

We are grateful to all of the participants, project staff, local health personnel, and school teachers for their continue involvement in this study. We also wish to thank to Emorn Wasantwisut and her colleagues for sharing the data of the original study.

TP, UR, PW, RF, and RM contributed to the study design. TP collected and analyzed the data under the supervision of RM and PW. TP wrote the manuscript with the contributions from all coauthors. None of the authors had a conflict of interest

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Table 5.1 Selected characteristics for participants in the follow-up study

	Placebo (n=139)	Iron (n=147)	Zinc (n=139)	Iron+Zinc (n=135)	P^3
Boys:Girls (%)	53.2:46.8	50.3: 49.7	50.4: 49.6	48.9: 51.1	0.908
Maternal education (%), n=557					
≤ Grade 6	69.3	61.9	65.2	55.6	0.114
> Grade 6	30.7	38.1	34.8	44.4	
SES tertiles (%)					
Low	38.9	32.0	32.4	29.6	0.639
Medium	27.3	36.0	34.5	36.3	
High	33.8	32.0	33.1	34.1	
Per capita annual household income (THB), n=549	7700 (3899, 13500)[1]	8200 (4100, 14700)	8900 (4100, 17000)	8200 (3900, 15300)	0.648
Maternal height (cm), n=554	153.2 (5.0) [2]	153.5 (5.3)	153.4 (5.8)	152.8 (4.9)	0.685
Supplement compliance (%)	95.4 (4.7) [a]	94.9 (5.1)[a,b]	93.4 (6.7)[b]	93.2 (6.5)[b]	0.004

[1] Median (25th percentile, 75th percentile) (all such values).
[2] Mean (SD) (all such values).
[3] ANOVA with Tukey post-hoc, Kruskal-Wallis test, or Chi-square test, values in row with superscripts without a common letter differ, $P < 0.05$.
THB, Thai baht

Table 5.2 Selected anthropometric characteristics by supplementation groups

	Placebo (n=139)	Iron (n=147)	Zinc (n=139)	Iron+Zinc (n=135)	P^3
At birth					
Birth weight (kg)	3.1 (0.4)[1]	3.0 (0.4)	3.1 (0.4)	3.1 (0.4)	0.733
Birth length (cm), n=462	50.8 (2.6)[a]	50.5 (2.8)[a,b]	50.0 (2.5)[a,b]	49.8 (2.7)[b]	0.021
Weight-for-age Z score	-0.4 (0.9)	-0.5 (0.9)	-0.4 (0.9)	-0.4 (0.8)	0.748
Length-for-age Z score	0.6 (1.4)	0.4 (1.4)	0.2 (1.4)	0.1 (0.9)	0.897
At 4 mo (baseline)					
Age at measurement (mo)	4.5(0.5)	4.5 (0.5)	4.5 (0.6)	4.4 (0.5)	0.037
Weight (kg)	6.6 (0.8)	6.6 (0.8)	6.5 (0.8)	6.5 (0.7)	0.535
Length (cm)	62.2 (2.2)	62.1 (2.5)	62.0 (2.3)	61.9 (2.1)	0.622
BMI (kg/m^2)	16.9 (1.4)	17.0 (1.4)	16.8 (1.4)	16.8 (1.4)	0.710
MUAC(cm)	13.6 (0.9)	13.7 (1.0)	13.5 (1.1)	13.5 (0.9)	0.516
Weight-for-age Z score	-0.6 (0.9)	-0.5 (0.9)	-0.6 (0.9)	-0.6 (0.9)	0.882
Length-for-age Z score	-0.8 (0.9)	-0.9 (0.9)	-0.9 (0.9)	-0.8 (0.9)	0.897
BMI-for-age Z score	-0.1 (1.0)	-0.1 (0.9)	-0.2 (1.0)	-0.1 (0.9)	0.770
At 1 year (endline)					
Age at measurement (mo)	10.5 (0.5)	10.5 (0.5)	10.5 (0.6)	10.4 (0.6)	0.173
Weight (kg)	8.1 (0.9)	8.2 (0.9)	8.1 (0.9)	8.1 (0.9)	0.689
Length (cm)	70.7 (2.4)	70.6 (2.5)	70. 6 (2.5)	70.4 (2.2)	0.701
BMI (kg/m^2)	16.2 (1.1)	16.4 (1.2)	16.1 (1.2)	16.3 (1.4)	0.173
MUAC (cm)	13.9 (1.0)	14.0 (1.0)	13.8 (1.0)	13.9 (1.0)	0.461
Weight-for-age Z score	-0.9 (0.9)	-0.8 (0.9)	-0.9 (0.9)	-0.9 (0.9)	0.587
Length-for-age Z score	-1.0 (0.9)	-1.0 (0.9)	-1.0 (1.0)	-1.0 (0.9)	0.913
BMI-for-age Z score	-0.5 (0.8)	-0.3 (0.8)	-0.5 (0.9)	-0.4 (0.9)	0.136
At 9 years					
Age at measurement (y)	9.4 (0.3)[b]	9.6 (0.4)[a]	9.3 (0.3)[b,c]	9.3 (0.3)[c]	<0.001
Weight (kg)	26.2 (5.0)	26.2 (5.7)	25.9 (5.2)	26.3 (5.9)	0.563
Height (cm)	129.1 (6.0)	129.8 (6.1)	129.0 (5.6)	129.1 (5.8)	0.685
BMI (kg/m^2)	15.6 (2.1)	15.8 (2.4)	15.5 (2.2)	15.7 (2.4)	0.804
MUAC (cm)	18.5 (17.3, 19.7)[2]	18.7 (17.7, 19.9)	18.7 (17.1, 19.6)	18.5 (17.4, 19.6)	0.113
Weight-for-age Z score	-0.9 (1.1)	-0.9 (1.1)	-0.9 (1.2)	-0.8 (1.2)	0.916
Length-for-age Z score	-0.9 (0.9)	-0.9 (0.9)	-0.8 (0.9)	-0.8 (0.9)	0.676
BMI-for-age Z score	-0.6 (1.1)	-0.5 (1.1)	-0.6 (1.1)	-0.5 (1.1)	0.627

[1] Mean (SD) (all such values); [2] Median (25th percentile, 75th percentile) (all such values); [3] ANOVA with Tukey post-hoc or Kruskal-Wallis test, values in row with superscripts without a common letter differ, $P < 0.05$.
BMI, body mass index; MUAC, mid-upper arm circumference.

Table 5.3 Effects of iron and zinc supplementation during infancy on weight and height at 9 years of age

	Adjusted effect: model 1			Adjusted effect: model 2		
	Coefficient (95% CI)		*P*	Coefficient (95% CI)		*P*
	Weight at 9 y (kg), n=551					
Supplementation						
Iron	0.06	(-1.08, 1.20)	0.915	0.09	(-1.05, 1.23)	0.872
Zinc	0.01	(-1.13, 1.16)	0.985	0.03	(-1.11, 1.28)	0.955
Iron + Zinc	0.16	(-1.00, 1.32)	0.789	0.27	(-0.90, 1.43)	0.655
Weight at endline (kg)	3.05	(2.56, 3.54)	<0.001	2.94	(2.45, 3.45)	<0.001
Sex, male	-1.91	(-2.79, -1.04)	<0.001	-1.91	(-2.78, -1.04)	<0.001
Age change (y)	1.89	(0.61, 3.1)	0.004	1.98	(0.70, 3.26)	0.003
Maternal height (cm)				0.06	(-0.02, 0.14)	0.169
Maternal education, ≤ grade 6				0.46	(-0.44, 1.35)	0.316
SES score				0.22	(-0.06, 0.81)	0.088
	Height at 9 y (cm), n=551					
Supplementation						
Iron	0.19	(-0.84, 1.21)	0.721	0.16	(-0.84, 1.17)	0.752
Zinc	0.34	(-0.69, 1.37)	0.520	0.34	(-0.67, 1.35)	0.511
Iron + Zinc	0.70	(-0.34, 1.75)	0.187	0.80	(-0.24, 1.83)	0.130
Height at endline (cm)	1.74	(1.57, 1.91)	<0.001	1.60	(1.43, 1.78)	<0.001
Sex, male	-3.06	(-3.86, -2.27)	<0.001	-2.90	(-3.68, -2.11)	<0.001
Age change (y)	4.18	(3.03, 5.33)	<0.001	4.24	(3.10, 5.37)	<0.001
Maternal height (cm)				0.16	(0.09, 0.24)	<0.001
Maternal education, ≤ grade 6				0.33	(-0.47, 1.12)	0.420
SES score				0.24	(-0.15, 0.62)	0.227

SES, socio-economic status.
Model 1, adjusted for sex and age change; Model 2; adjusted for sex, age change, maternal height, maternal education, and socio-economic status.

Table 5.4 Effects of iron and zinc supplementation during infancy on body mass index and mid-upper arm circumference at 9 years of age

	Adjusted effect: model 1			Adjusted effect: model 2		
	Coefficient (95% CI)		*P*	Coefficient (95% CI)		*P*
	BMI at 9 y (kg/m^2), n=551					
Supplementation						
Iron	0.03	(-0.48, 0.53)	0.914	0.04	(-0.46, 0.55)	0.873
Zinc	-0.09	(-0.60, 0.41)	0.724	-0.08	(-0.59, 0.43)	0.756
Iron + Zinc	-0.07	(-0.58, 0.45)	0.797	-0.04	(-0.56, 0.48)	0.877
BMI at endline (kg/m^2)	0.70	(0.55, 0.85)	<0.001	0.70	(0.55, 0.85)	<0.001
Sex, male	-0.27	(-0.63, 0.09)	0.141	-0.28	(-0.65, 0.08)	0.124
Age change (y)	0.14	(-0.42, 0.71)	0.620	0.18	(-0.39, 0.74)	0.543
Maternal height (cm)				-0.01	(-0.04, 0.03)	0.895
Maternal education, ≤ grade 6				0.16	(-0.23, 0.56)	0.417
SES score				0.16	(-0.03, 0.35)	0.094
	MUAC at 9 y (cm), n=550					
Supplementation						
Iron	0.24	(-0.26, 0.75)	0.343	0.26	(-0.25, 0.76)	0.321
Zinc	0.05	(-0.46, 0.56)	0.850	0.06	(-0.45, 0.56)	0.824
Iron + Zinc	0.23	(-0.29, 0.74)	0.383	0.25	(-0.26, 0.77)	0.339
MUAC at endline (cm)	0.81	(0.62, 0.99)	<0.001	0.80	(0.61, 0.98)	<0.001
Sex, male	-0.42	(-0.78, -0.05)	0.026	-0.43	(-0.79, -0.06)	0.022
Age change (y)	0.45	(-0.11, 1.02)	0.115	0.49	(-0.07, 1.06)	0.089
Maternal height (cm)				0.01	(-0.03, 0.04)	0.938
Maternal education, ≤ grade 6				0.12	(-0.27, 0.52)	0.539
SES score				0.17	(-0.02, 0.37)	0.075

BMI, body mass index; MUAC, mid-upper arm circumference; SES, socio-economic status.
Model 1, adjusted for sex and age change; Model 2; adjusted for sex, age change, maternal height, maternal education, and socio-economic status.

Table 5.5 Effects of iron and zinc supplementation during infancy on WAZ, HAZ, and BMIZ at 9 years of age

	Adjusted effect: model 1			Adjusted effect: model 2		
	Coefficient (95% CI)		*P*	Coefficient (95% CI)		*P*
	WAZ at 9 y, n=549					
Supplementation						
Iron	-0.04	(-0.28, 0.19)	0.704	-0.04	(-0.27, 0.19)	0.750
Zinc	0.01	(-0.22, 0.24)	0.928	0.01	(-0.22, 0.24)	0.918
Iron + Zinc	0.03	(-0.21, 0.26)	0.824	0.05	(-0.18, 0.29)	0.670
WAZ at endline	0.67	(0.58, 0.76)	<0.001	0.64	(0.54, 0.73)	<0.001
Sex, male	0.08	(-0.08, 0.25)	0.327	0.06	(-0.10, 0.23)	0.437
Maternal height (cm)				0.02	(0.01, 0.03)	0.031
Maternal education, ≤ grade 6				0.10	(-0.08, 0.29)	0.263
SES score				0.09	(-0.01, 0.18)	0.051
	HAZ at 9 y, n=551					
Supplementation						
Iron	0.01	(-0.16, 0.17)	0.944	0.01	(-0.16, 0.16)	0.956
Zinc	0.07	(-0.10, 0.23)	0.417	0.07	(-0.09, 0.23)	0.403
Iron + Zinc	0.12	(-0.05, 0.29)	0.158	0.14	(-0.03, 0.31)	0.097
HAZ at endline	0.67	(0.61, 0.74)	<0.001	0.62	(0.55, 0.68)	<0.001
Sex, male	0.08	(-0.03, 0.20)	0.165	0.07	(-0.05, 0.18)	0.266
Maternal height (cm)				0.03	(0.02, 0.04)	<0.001
Maternal education, ≤ grade 6				0.07	(-0.06, 0.20)	0.275
SES score				0.04	(-0.02, 0.10)	0.202
	BMIZ at 9 y, n=551					
Supplementation						
Iron	-0.05	(-0.50, -0.10)	0.692	-0.04	(-0.28, 0.20)	0.743
Zinc	-0.05	(-0.29, 0.19)	0.707	-0.04	(-0.28, 0.20)	0.729
Iron + Zinc	-0.04	(-0.29, 0.19)	0.761	-0.03	(-0.27, 0.22)	0.838
BMIZ at endline	0.57	(-0.29, 0.21)	<0.001	0.56	(0.47, 0.66)	<0.001
Sex, male	0.03	(0.50, 0.66)	0.696	0.03	(-0.14, 0.20)	0.764
Maternal height (cm)				0.01	(-0.02, 0.02)	0.901
Maternal education, ≤ grade 6				0.08	(-0.11, 0.27)	0.424
SES score				0.08	(-0.01, 0.17)	0.079

WAZ, weight-for-age Z score; HAZ, height-for-age Z score; BMIZ, BMI-for-age Z score; SES, socio-economic status.
Model 1, adjusted for sex; Model 2; adjusted for sex, maternal height, maternal education, and socio-economic status.

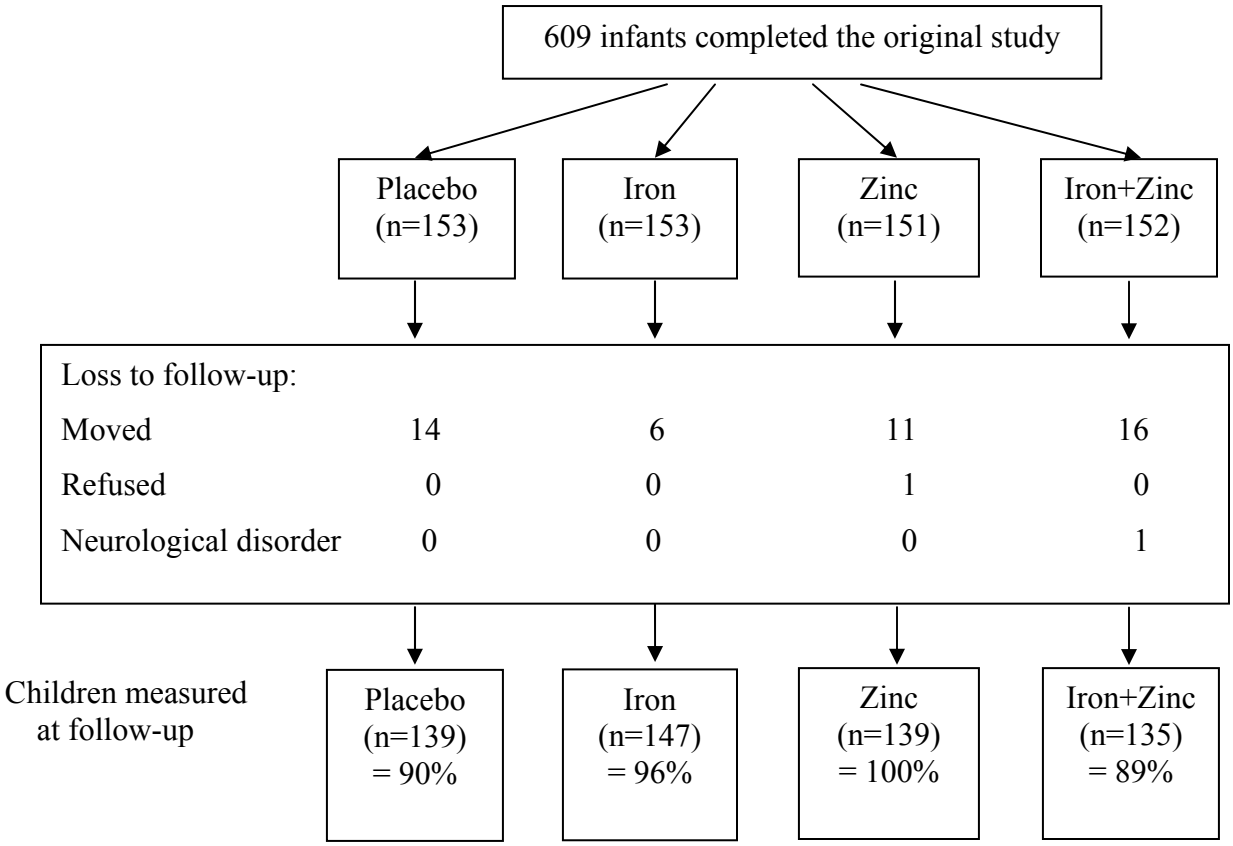

Figure 5.1 Participant profile

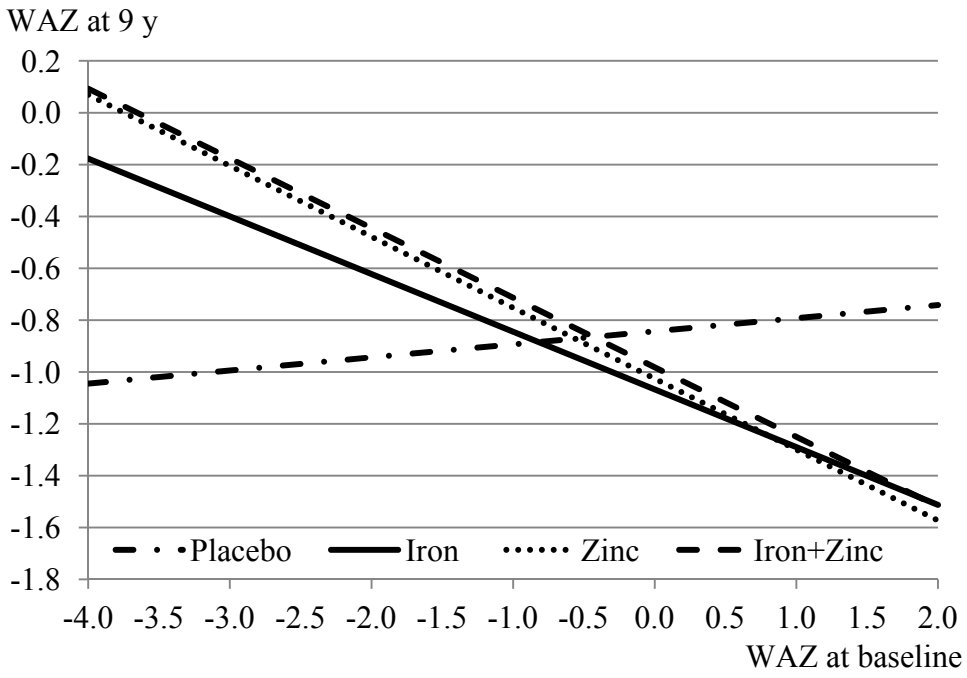

Figure 5.2 WAZ at 9 y by intervention group and WAZ at baseline of the intervention trial. The interaction between intervention group and WAZ at baseline was significant ($P = 0.038$) in general linear model that adjusted for child sex, maternal height, maternal education, and socio-economic status.

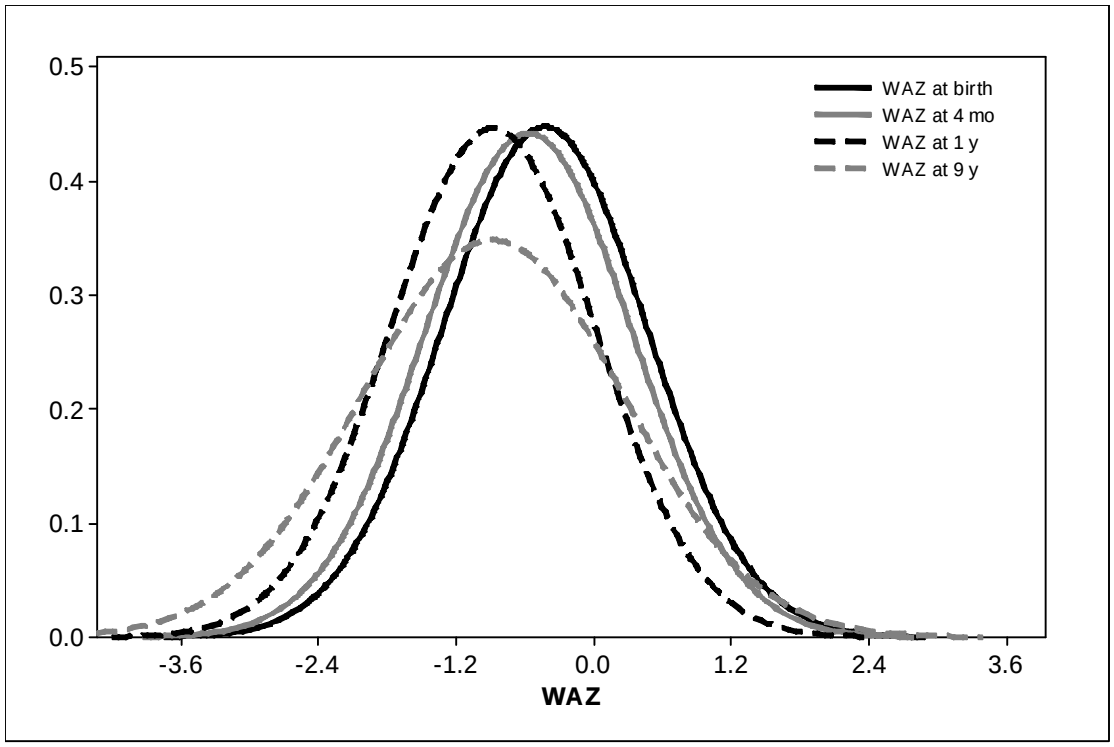

Figure 5.3 Distribution of weight-for-age Z scores at birth, 4 mo, 1 y, and 9 y of age

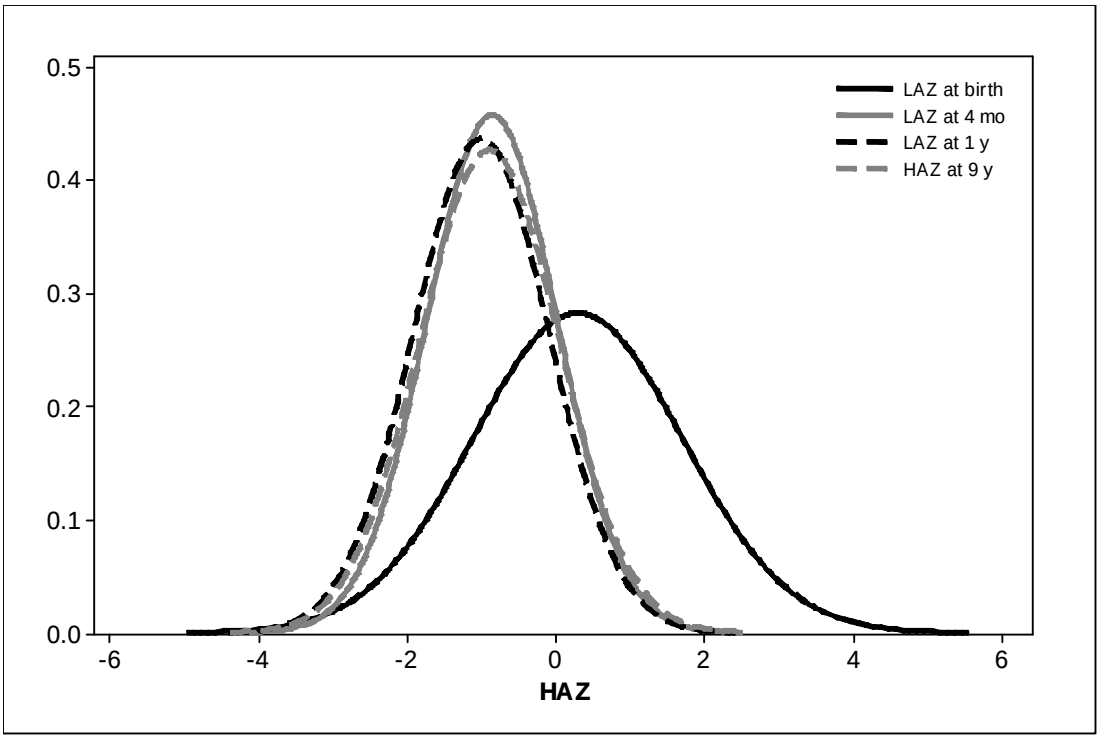

Figure 5.4 Distribution of length/height-for-age Z scores at birth, 4 mo, 1 y, and 9 y of age

CHAPTER 6

THE INFLUENCE OF PRENATAL AND POSTNATAL GROWTH ON INTELLECTUAL FUNCTIONING AT 9 YEARS OF AGE AMONG RURAL THAI CHILDREN

The influence of prenatal and postnatal growth on intellectual functioning at 9 years of age among rural Thai children

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Short title: Influence of prenatal and postnatal growth on intellectual functioning

Abbreviations: HC--Head circumference

WISC-III-- Wechsler Intelligence Scale for Children-Third edition

Raven's CPM -- Raven's Colored Progressive Matrices

SLS--Multiple Stage Least Squares analyses

SDS--Standard deviation score

ABSTRACT

Background: Poor growth during childhood is associated with cognitive impairment in many studies, but less is known about the long-term effects of growth during specific periods from birth to late childhood. In addition, the significance of growth between early and late infancy has rarely been distinguished.

Objective: This study aimed to assess the relative influence of size at birth, early postnatal growth (early and late infancy), and late postnatal growth on intellectual functioning at 9 years of age.

Methods: Intellectual functioning of 560 participants in a trial of iron/zinc supplementation during infancy in Thailand was measured using the Wechsler Intelligence Scale for Children-Third edition (WISC-III), Thai version and the Raven's Colored Progressive Matrices (Raven's CPM). Data of weight, length, and head circumference (HC) were collected at birth, 4 months, 1 year, and 9 years. Multiple Stage Least Squares analyses that generate conditional variables accounting for the correlation among size measures across age were performed to assess the relative importance of prenatal (size at birth), early infancy (birth to 4 months), late infancy (4 months to 1 year), and late postnatal (1 year to 9 years) growth, adjusting for socio-demographic variables.

Results: Weight gain, length gain, and HC at early infancy period were positively associated with IQ at 9 years of age. Length gain at early infancy period was associated with Raven's CPM score. Length gain at late infancy period was also associated with IQ. Effects of size at birth and growth from 4 months to 1 year were smaller and inconsistent.

There was no association between growth from 1 year to 9 years and IQ or Raven's CPM.

Conclusion: Early infancy growth in weight and HC as well as early to late infancy growth in length are the strong and consistent influences on intellectual functioning at 9 years of age in this cohort of children in Thailand.

INTRODUCTION

Child malnutrition is associated with poor health and developmental consequences. Many studies have investigated the influence of prenatal and postnatal growth on cognitive performance later in life. Studies that focused on prenatal growth reported mixed results. Some studies reported the association of size at birth and cognitive abilities (1-4). For example, intrauterine growth restriction (1) has been associated with lower IQ scores at 9-10 years. A study conducted in the United States also reported the significant positive association of birth weight and IQ at 7 years of age (2). Among children who born at term in a longitudinal study in Finland, lower birth weight and length, and smaller head circumference (HC) were associated with lower level of cognitive abilities at 56 months (3). However, a British birth cohort that assessed the influence of HC at birth on IQ at 4 and 8 years of age among term-born children (5) found a significant relationship of HC at birth and IQ at 4 years of age, but not at 8 years. A study in Brazil also showed no significant association of low birth weight and IQ at 8 years, after controlling for socio-demographic variables (6). In addition, studies in Guatemala (7) and United Kingdom (8) did not show a significant relationship among birth weight, length, or HC and cognitive abilities later in life. In a study in Guatemala, only early postnatal growth was associated with cognitive performance at adulthood. A non significant association of late postnatal growth and cognitive abilities appeared in this study. A positive association of early postnatal growth, but not late postnatal growth was also found in a study that investigated the importance of head growth on IQ at 4 and 8 years of age (5).

Although previous studies tend to show the importance of growth during prenatal and early postnatal periods on cognitive performance rather than that of growth at later ages, the results are still controversial. In addition, growth during infancy has been rarely studied separately as in early and late infancy. We therefore followed 560 9-year old children who had previously participated in a trial of iron and zinc supplementation during infancy. In the present study, we evaluated the relative influence of growth at prenatal (at birth), early infancy (birth to 4 months), late infancy (4 months to 1 year), and late postnatal (1 year to 9 years) periods on intellectual functioning of children at 9 years of age.

METHODS

Study Design and Participants

A follow-up, cross-sectional study was carried out among children who had participated in a randomized controlled trial of iron and zinc supplementation during infancy in Khon Kaen province, northeast Thailand during 1998 to 1999 (9) In the trial, breast-fed infants aged 4-6 months were randomly assigned to receive daily oral supplementation of iron, zinc, iron plus zinc, or a placebo for the duration of 6 months. Of the 609 infants that completed the original study, 562 children participated in the present study. However, one child was diagnosed with a neurological disorder and one parent refused to participate in the study. These children were not included and the final sample size of this study resulted as 560 children. Written informed consent was obtained from parents/caregivers and verbal assent was obtained from all children. This study was

approved by the Institution Review Boards of Emory University, Atlanta, Georgia and the Human Ethics Committee of Mahidol University, Thailand.

Measurements

Intellectual functioning

Intelligence quotient (IQ) was assessed at 9 years of age by clinical psychologists, using the Wechsler Intelligence Scale for Children-Third edition (WISC-III), Thai version (10, 11). The WISC-III administered in this study included six verbal subtests (Information, Similarities, Arithmetic, Vocabulary, Comprehension, and Digit Span) and six performance subtests (Picture Completion, Coding, Picture Arrangement, Block Design, Object Assembly, and Symbol Search). Raw scores from each subtest were transformed to the scaled scores and then to the age-adjusted full scale, verbal, and performance IQ according to the Thai Norm. We also performed the Raven's *Colored Progressive Matrices* (Raven's CPM) (12). The raw scores for all items in all three sets of the Raven's CPM test were summed and the total score was used in the analysis. The administration of the tests was carried out at a school or health center near the homes of children on weekends or holidays due to the availability of unattended rooms, which provided a quiet environment for testing. All children received a snack and milk before test administration and then individually performed the test for 1.5-2.0 hours.

Anthropometry Measurements

Weight, length, and head circumference (HC) at birth, at the beginning of the trial (age range: 3.5-6.5 months; referred to as at 4 months) and at the end of the trial (age range: 9.3-12.7 months; referred to as at 1 year) were obtained from the intervention trial infancy. Methods of weight and height measurement performed during infancy were

explained in the previous publication (9). *Z* scores for age were generated for weight, height, and HC, based on the 2006 World Health Organization (WHO) growth standard for children 0-5 years old. Weight at birth was measured as a routine practice by health personnel and was recorded in the child's health booklet. Length at birth was measured in 462 children and HC at birth was measured in 254 children. Weight, height, and HC at 9 years of age (age range: 8.8-10.1 years) were assessed using a standard procedure (13). Weight was measured to the nearest of 0.1 kg using a digital weighing scale (Seca digital scale model 841, Seca Corporation, Hamburg, Germany). The standing height was measured to the nearest of 0.1 cm using a wooden height board. HC was measured to the nearest 0.1 cm using non-stretching tape. Weight-for-age and height-for-age *Z* scores were generated using the 2007 WHO Growth Reference for school-aged children and adolescents.

Covariates

Socio-demographic data including child age and sex, maternal education, availability of a mother at home, household socioeconomic status (SES), and the location of school being attended by the child were obtained using a pretested questionnaire. Maternal education was categorized into 2 groups, 1) lower than or equal to grade 6 and 2) higher than grade 6. Maternal occupation was categorized into 4 groups, 1) housewife or unemployed, 2) agriculture, 3) wage laborer, and 4) others (including clerical duties, social service, service provider, merchant, and professional). Availability of mother at home was coded as present or absent/occasionally present. Location of schools was categorized as in urban or rural area. Standardized household SES scores were generated using principal component analysis (14, 15). Housing characteristics, household assets,

and access to services were included in this computation. Maternal height was measured during the recruitment at infancy.

Statistical Analysis

Statistical analyses were performed using SAS for Windows 9.2 (SAS Institute Inc., Cary, NC). The main outcomes were WISC-III full scale IQ, verbal IQ, performance IQ, and Raven's CPM score at 9 years of age. Mean and standard deviation or median and interquartile range were calculated. Independence T-test or Analysis of Variance (ANOVA) with Tukey's post-hoc test was used to assess the differences of the main outcomes by socio-demographic variables. Ordinary Least Squares (OLS) regression analyses were performed to assess the associations between body size at birth, 4 months, 1 year, and 9 years and the main outcomes, adjusting for socio-demographic variables and maternal height. The regression coefficients for body size were presented as standard deviation scores (SDSs) which were calculated by subtracting the individual measurement value by the mean of that measurement and then dividing by its SD.

We defined prenatal growth as size at birth. Postnatal growth included early and late postnatal growth. Early postnatal growth included early infancy which was defined as growth from birth to 4 months of age and late infancy growth which was defined as growth from 4 months to 1 years of age. Late postnatal growth was defined as growth from 1 year to 9 years of age. Multiple Stage Least Squares (SLS) analyses were used to assess associations between prenatal growth, early infancy growth, late infancy growth, and late postnatal growth and intellectual functioning at 9 years of age. The SLS method was used in an earlier study that assessed the relative importance of prenatal and postnatal growth on women's education achievement (7). The 2-SLS analysis was used to

estimate the effect of prenatal growth and the effect of early infancy growth, independent of prenatal growth. In the first stage, the influence of birth size on size at 4 months was estimated for each measurement (weight and height). We modeled size at 4 months on birth size to estimate predicted size at 4 months. After that, the residual, which was interpreted to represent the early infancy effect (R1), was calculated by subtracting the predicted size from the observed size at 4 months. In the last stage, we modeled birth size and the R1 residual on IQ in order to assess the relative importance of prenatal and early infancy effects. Size at birth and the residual were used as SDSs in this stage to facilitate comparisons of the relative influence of growth on IQ at different ages. Other covariates including child sex, maternal height and education, availability of mother, SES, and location of school were included in the analysis.

The 3-SLS analysis was used to estimate the effect of late infancy growth independent of previous growth. We first obtained the R1 as in the first stage of the 2-SLS analysis. In the second stage, size at 1 year was modeled on size at birth and the R1 to get the predicted size at 1 year of age. Then, the late infancy residual (R2) was calculated by subtracting the predicted size at 1 year from the observed size. After that, we performed the third stage regression by modeling birth size, R1, and R2 on IQ.

In the 4-SLS analysis, we obtained the R1 and R2 as described in the 3-SLS. Then, we modeled size at 9 years on birth size, R1 and R2 in order to get the predicted size at 9 years. The third residual, R3, was calculated by subtracting the predicted size at 9 years from the observed size. Finally, the fourth stage regression was performed by modeling birth size, R1, R2, and R3 on IQ to assess prenatal, early infancy, late infancy, and late postnatal effects without the influences of the initial growth.

HC at birth was obtained from health records. However, the distribution was extremely skewed, suggesting poor data quality (Figure 6.1). Therefore, we included only the measurements at 4 months, 1 year, and 9 years in the SLS analysis. In the 2-SLS analysis, we first modeled HC at 4 months on HC at 1 year to estimate predicted size at 1 year. The late infancy growth (R1) was calculated by subtracting the observed HC at 1 year by the predicted HC. Then, we modeled HC at 4 months and the R1 residual on IQ to assess the effects of early infancy growth and late infancy growth separately. The 3-SLS was performed to assess the effects of growth in HC at early infancy, late infancy, and late postnatal. In addition to R1 estimation, we modeled on HC at 9 years on HC at 1 year to get the predicted HC. Then, we subtracted the predicted HC from the observed HC to get the late postnatal growth in HC (R2). In the last stage, we used HC at 4 months, R1, and R2 as dependent variables to assess the effects of growth in HC at early and late infancy and late postnatal on IQ at 9 years of age.

RESULTS

Description of the Participants

Children were 9.3 years of age when the cognition tests were administered (Table 6.1). The proportion of boys and girls are similar (boys = 284; girls = 276). The mean (SD) birth weight was 3.1 (0.4) kg. The mean (SD) weight-for-age *Z* scores were -0.4 (0.9) at birth, -0.6 (0.9) at 4 months, -0.9 (0.9) at 1 year, and -0.9 (1.2) at 9 years. The mean (SD) length/height-for-age Z scores were 0.3 (1.4) at birth, -0.8 (0.9) at 4 months, -1.0 (0.9) at 1 year, and -0.9 (0.9) at 9 years. The mean (SD) HC-for-age *Z* scores at 4 months and 1 year were -0.8 (0.9) and -0.9 (0.9).

Bivariate Analysis

Intellectual functioning data, including full scale, verbal, performance IQ, and Raven's CPM score were presented by socio-demographic variables in Table 6.2. Performance IQ and Raven's CPM score of the boys was significantly higher than that of the girls. Full scale and verbal IQ were higher for children whose mothers had higher education. Full scale IQ was also higher in children whose mothers were in the other occupational group as compared to those whose mothers were in the agricultural occupational group. Verbal IQ was also higher in children whose mothers were available at home. All IQs and Raven's CPM score were significantly higher among children in the higher SES household and those whose schools were located in urban area. The mean (SD) full scale IQs were 96.0 (9.1), 92.8 (9.6), and 90.5 (9.9) points for children in high, medium, and low SES tertiles, respectively and the full scale IQs of children who attended schools located in urban and rural area were 98.3 (9.8) vs. 92.2 (9.5) points.

Multivariate Analysis

In OLS multiple linear regression models using the IQ scores and Raven's CPM score measured at 9 years as the dependent variables, the significance of weight, length/height, and HC at each time point was analyzed after adjusting for sex, maternal height and education, availability of mother at home, SES, and the location of the school being attended by children (Table 6.3). Weight and length at birth were not associated with full scale, verbal IQ, and Raven's CPM score at 9 years of age. However, length at birth was associated with performance IQ. Length/height and head circumference at 4 months, 1 year, and 9 years were associated with all IQs ($p < 0.05$). Association of weight at later ages and IQ was inconsistent. Only length at 4 months and 1 year was associated

with Raven's CPM score. There was no association of weight and head circumference and Raven's CPM score at 9 years of age.

Table 6.4 shows the relationship between body size measures at various ages. Weight at birth, 4 months, 1 year, and 9 years were positively correlated ($p < 0.05$). Positively significant relationships were also shown for length or height and head circumference. Since body size measures at later ages were significantly associated with the measures at earlier ages, we performed the SLS analyses to take into account the correlation among the measures at different time points. The residuals of different time points estimated from the SLS models were mutually uncorrelated with r = 0.0 and *p*-value =1.0, we therefore can included them in the model simultaneously.

Results from the SLS analyses were presented in Table 6.5-6.10. In the 4-SLS, gain in weight from birth to 4 months was strongly associated with all IQs at 9 years in which 1 SD gain in weight increased 1.3, 0.8, and 1.6 points in full scale, verbal, and performance IQ, respectively (Table 6.5). Weight at birth was positively associated with only performance IQ. There was no significant association of IQ and gain in weight from 4 months to 1 year and from 1 to 9 years. A similar pattern was shown in length/height in which gain in length from birth to 4 months was positively associated with all IQs at 9 years (Table 6.6). Specifically, 1 SD gain in length from birth to 4 months increased 1.8, 1.3, and 2.2 points in full scale, verbal, and performance IQ, respectively. Length at birth was significant associated with full scale and performance IQ. Gain in length from 4 months to 1 year was also associated with all measures of IQ. However, the associations of length at birth and gain in length from 4 months to 1 year and IQ were smaller in extent as compared to the associations between gain in length from birth to 4 months and

IQ. Gain in length/height from 1 year to 9 years was not associated with any IQ scores at 9 years. HC at 4 months was significantly associated with IQ at 9 years. In the 3-SLS, a 1 SD increase in HC was associated with a corresponding 1.8 point, 1.5 point, and 2.0 point increase in full scale, verbal, and performance IQ (Table 6.7). Change in HC from 4 months to 1 year and from 1 year to 9 years was not significantly associated with IQ at 9 years. HC at birth was not included in this analysis.

Weight and head circumference at birth, 4 months, 1 year, and 9 years of age were not associated with Raven's CPM score at 9 years of age (Table 6.8 and 6.10). Only gain in length from birth to 4 months was associated with Raven's CPM score (Table 6.9). However, there was no association between length at birth or gain in length at later ages and Raven's CPM score.

Among covariates included in the regression models, school location and SES were the strongest and most consistent predictors of IQ and Raven's CPM score at 9 years of age. Children who were attending a school in urban area had higher full scale IQ, verbal IQ, and Raven's CPM score compared to those attending a school in rural area. SES score was positively associated with all IQs and Raven's CPM score. Child sex was only consistently associated with verbal IQ in which being male was associated with lower verbal IQ as compared to being female. Availability of mother was also associated with IQ, but the associations were less consistent. Maternal height was associated with full scale IQ in the 2-SLS model that included birth weight and growth in weight from birth to 4 months as main predictors. Maternal education was not associated with IQ scores in this study.

DISCUSSION

Our study used the data on growth from birth to late childhood in an apparently healthy population in order to investigate the influences of growth at birth, early postnatal, and late postnatal periods on intellectual functioning in late childhood. We separated prenatal growth into early infancy (from birth to 4 months) and late infancy (from 4 months to 1 year). Our findings suggest that early postnatal growth, particularly during early infancy period is the most important period of child growth in terms of weight, length, and HC. This period was consistently associated with intellectual functioning at 9 years when compared to other periods. It is important to note that length gain during late infancy was also associated with intellectual functioning at 9 years of age, although in smaller extent.

One would expect that growth during the prenatal period is important for cognitive abilities. However, our findings showed inconsistent and weaker associations of prenatal growth or size at birth on IQ as compared to other periods. These findings contrast with a study by Heinonen et al (3) which showed a slightly stronger influence of weight, length, and HC at birth on cognitive abilities at 56 months compared to growth at 5, 20, and 56 months. For example, children with 1 SD lower in birth weight performed 1.9 points lower in general reasoning test, while children with 1 SD slower in weight gain performed 1.1 points lower. Similar patterns occurred among the majority of the cognitive tests and also appeared for length and HC. The difference in time of cognitive measurement between this study and our study (56 vs. 108 months old) may result in different conclusion. In addition, findings in our study may not imply that prenatal growth is less important for cognitive abilities at late childhood because this weak

association might be due to low variation of body size at birth in our sample. Only 5.5% of children were low birth weight (<2500 g), thus this may attenuate the influence of size at birth on cognitive abilities.

Although our results were not in accordance with the above study, our findings confirm previous longitudinal studies in both developed and developing countries that early postnatal growth was the most important for cognitive abilities later in life as compared to growth in other periods (5, 7, 8, 16). Even though some of these studies investigated the influence of growth on cognitive abilities in adulthood while our study assessed cognitive abilities at 9 years of age, the same trend was shown. These studies demonstrated that birth weight was not associated with cognitive abilities at 11 years of age (8) or at adulthood (7). Gain in length at early postnatal period was more influential on educational achievement (EA) in adult women than birth length or gain in height at late postnatal (7). Change in HC at early postnatal was more important than HC at birth and change in HC at the late postnatal period on IQ at 8 years of age (5), 9 years of age (16), or EA at adulthood (7).

Apart from the influence of growth on intellectual functioning, growth which reflects nutritional status of the child may indirectly influence intellectual functioning through the interaction between caregiver and child. Although malnourished children may not receive more or less attention from mothers because their mothers may not notice that their children are malnourished (17), these children may explore and interact less socially and with their environment (18, 19) and thus respond less to their mothers. In addition, malnourished children tend to receive less food which may result in less stimulation during feeding (20).

We investigated the relationship between growth and intellectual functioning by taking the socio-demographic variables and maternal height into account. Among these variables, having attended school in an urban area was strongly influential on IQ and Raven's CPM score. In general Thai rural settings, children usually attend a school located near their residence. Only parents who can afford for higher expenses or can easily find modest transportation may arrange for their children to attend school in the city. Thus, this finding suggests that educational system needs to be explored and improved in order to make the quality of schools comparable for both rural and urban areas. Maternal education was not associated with IQs or Raven's CPM score in our study. It is doubtful that the SES variable and maternal education variable were highly correlated because the variance inflation factor (VIF) did not suggest multicollinearity in any models. In addition, the maternal education variable was still not significant in all models when SES variable was removed. We included mothers who completed grade 6 and those who completed grade 1 to 5 in the same category. Thus, we suspected that the influence of maternal education on intellectual functioning would have been clearly shown if we were able to separate those who completed grade from those who did not complete grade 6. However, available data did not allow us to perform such categorization.

The major strength in our study is that the anthropometric data were available at different time points during childhood. We especially had data in two different time points during the first year of age. Consequently, we were able to separate the influence of early and late infancy growth and were able to detect the periods of growth that are the most important. We used the SLS approach for data analysis. This approach allowed us to

consider growth at later periods without the influence of growth at earlier periods (21). We also were able to follow-up 92% of the participants who completed the measurements during infancy, thus minimizing a selection bias.

There are some limitations in our study. The quality of the HC measurement at birth was uncertain, thus we did not include HC at birth in the analysis. Failure to include these data prohibited us to assess the effect of change in HC at early infancy without the influence of HC at birth. Another limitation is that we did not evaluate the effect of gestational age on intellectual functioning. Gestational age data were retrieved from the record and they were usually estimated from the recall of the last menstrual period. We had high missing values on gestational age variable (17%). Thus, we did not include this variable in our analyses. Among children with gestational age (n = 465), only 11% were preterm births and birth weight of these children ranged from 1900 g to 3500 g. The prevalence of low birth weight (birth weight < 2500 g) was 19% in these preterm children.

We found that only 41% of these children were exclusively breastfed but nearly 50% received breast milk together with water during infancy (data not shown). In addition, about 70-80% of children received complementary foods such as mashed banana, mashed rice, and egg yolk before 4 months of age which was the recommended timing of the introduction of complementary foods at the period of the intervention trial. Since our findings contribute to evidence of a strong and consistent association of growth at early infancy and cognitive abilities, appropriate breastfeeding during early infancy should be the main priority of nutrition interventions in order to promote optimal growth. We analyzed early infancy growth and late infancy separately and found that late infancy

growth was the second most important period of growth related to intellectual functioning at 9 years of age. Thus, our results also imply that safe, adequate, and timely complementary feeding should be promoted along with breastfeeding intervention in order to maintain growth during late infancy (22). Optimal growth during early childhood may enhance early cognitive development which then may result in better cognitive ability later in life.

In conclusion, our study shows that early postnatal growth, particularly during early to late infancy, may be the most important period to successfully impact intellectual functioning at 9 years of age among healthy children. Thus, strategies to provide support for exclusive breast feeding and guidance on appropriate complementary feeding should be emphasized to ensure optimal growth and cognitive development among Thai children.

This study was supported by International Nutrition Foundation/ Ellison Medical Foundation and Mahidol University. We are grateful to all participants, local health personnel, and school teachers for their cooperation. We thank to Emorn Wasantwisut and her colleagues for sharing the data of the intervention trial.

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Table 6.1 Selected characteristics of children (n=560)

Characteristic	Value
Age at cognition test (y)	9.3 (0.3)[1]
Boy, %	50.7
Anthropometry	
At birth	
Birth weight (kg)	3.1 (0.4)
Weight-for-age *Z*-score	-0.4 (0.9)
Birth length (cm), n=462	50.3 (2.7)
Length-for-age *Z*-score	0.3 (1.4)
Head circumference (cm), n=254	32.0 (31.0, 33.0)[2]
Head circumference-for-age *Z*-score	-1.7 (-2.5, -1.2)
At 4 months	
Age (mo)	4.5 (0.5)
Weight (kg)	6.5 (0.8)
Weight-for-age *Z*-score	-0.6 (0.9)
Length (cm)	62.1 (2.2)
Length-for-age *Z*-score	-0.8 (0.9)
Head circumference (cm)	40.6 (1.2)
Head circumference-for-age *Z*-score	-0.8 (0.9)
At 1 year	
Age (mo)	10.4 (0.6)
Weight (kg)	8.1 (0.9)
Weight-for-age *Z*-score	-0.9 (0.9)
Length (cm)	70.6 (2.4)
Length-for-age *Z*-score	-1.0 (0.9)
Head circumference (cm), n=559	43.8 (1.3)
Head circumference-for-age *Z*-score	-0.9 (0.9)
At 9 years	
Age (y)	9.4 (0.3)
Weight (kg)	26.3 (5.4)
Weight-for-age *Z*-score, n=558	-0.9 (1.2)
Height (cm)	129.2 (5.9)
Height-for-age *Z*-score	-0.9 (0.9)
Head circumference (cm)	50.7 (1.3)

[1] Mean (SD) (all such values).

[2] Medians (IQR) (all such values).

Table 6.2 Means of WISC-III IQ and Raven's CPM score at 9 years of age, categorized by socio-demographic variables[1]

Variable	WISC-III IQ			Raven's
	Full scale	Verbal	Performance	CPM score[3]
All Children	93.1 (9.8)[2]	94.2 (9.1)	93.4 (11.6)	21.7 (5.4)
Sex				
Boy, n=284	93.4 (10.0)	93.6 (9.1)	94.5 (12.5)[a]	20.9 (5.4)[a]
Girl, n=276	92.8 (9.6)	94.7 (9.7)	92.3 (10.5)[b]	21.5 (5.2)[b]
Supplementation at infancy				
Placebo, n=139	93.3 (9.9)	93.9 (9.5)	93.9 (12.2)	21.6 (5.3)
Iron, n=147	93.0 (8.7)	93.7 (8.2)	93.7 (10.8)	21.7 (5.5)
Zinc, n=139	92.9 (9.9)	94.0 (9.7)	93.0 (11.3)	21.3 (5.4)
Iron + Zinc, n=135	93.4 (10.6)	95.1 (10.2)	92.9 (12.1)	22.3 (5.5)
Education of mother				
≤ grade 6, n=351	92.4 (9.9)[a]	93.4 (9.3)[a]	92.7 (12.0)	21.4 (5.4)
> grade 6, n=206	94.4 (9.5)[b]	95.4 (9.5)[b]	94.5 (10.9)	22.3 (5.3)
Availability of mother at home				
Present, n=486	93.4 (9.8)	94.5 (9.5)[a]	93.6 (11.5)	21.7 (5.3)
Absent/Occasionally present, n = 74	91.2 (9.7)	91.9 (8.7)[b]	92.2 (12.0)	21.9 (5.3)
SES tertiles				
Low, n=186	90.5 (9.9)[a]	92.0 (9.5)[a]	90.5 (11.8)[a]	20.4 (5.3)[a]
Medium, n=188	92.8 (9.6)[a]	94.2 (9.2)[a,b]	92.8 (11.2)[a]	21.7 (5.2)[b]
High, n=186	96.0 (9.1)[b]	96.2 (9.1)[b]	96.9 (11.0)[b]	23.0 (5.3)[c]
School location				
Rural, n=476	92.2 (9.5)[a]	93.2 (9.1)[a]	92.6 (11.3)[a]	21.3 (5.2)[a]
Urban, n=84	98.3 (9.8)[b]	99.3 (9.3)[b]	97.8 (12.2)[b]	23.9 (5.3)[b]

[1] Different letters denoted significant difference between categories, $P < 0.05$.

[2] Mean (SD) (all such values).

[3] Adjusted for age.

Table 6.3 Association of body size and WISC-III IQ and Raven's CPM score at 9 years of age[1]

	WISC-III IQ						Raven's CPM score	
	Full scale		Verbal		Performance			
	β	SE	β	SE	β	SE	β	SE
Weight, SDS								
At birth	0.5	0.4	0.1	0.4	0.9	0.5	0.2	0.2
At 4 mo	1.4*	0.4	0.8	0.4	1.8*	0.5	0.4	0.2
At 1 y	1.5*	0.4	0.9*	0.4	1.8*	0.5	0.4	0.2
At 9 y	0.1	0.1	0.8*	0.4	0.0	0.5	-0.2	0.2
Length/Height, SDS								
At birth	0.7	0.4	0.1	0.4	1.3*	0.5	0.0	0.3
At 4 mo	1.7*	0.5	1.2*	0.4	2.0*	0.5	0.6*	0.3
At 1 y	1.9*	0.5	1.3*	0.4	2.3*	0.5	0.7*	0.3
At 9 y	1.4*	0.4	1.4*	0.4	1.2*	0.5	0.3	0.2
Head circumference, SDS								
At 4 mo	1.7*	0.4	1.4*	0.4	1.9*	0.5	0.4	0.3
At 1 y	1.7*	0.5	1.6*	0.5	1.7*	0.6	0.5	0.3
At 9 y	1.1*	0.4	1.0*	0.4	1.0*	0.5	0.4	0.2

[1] Adjusted for sex, maternal height, maternal education, availability of mother at home, SES, and school location; SDS, standard deviation score.
* $P < 0.05$.

Table 6.4 Correlation matrices of body size measures at birth, 4 months, 1 year, and 9 years

	Weight			Height			Head circumference	
	4 mo	**1 y**	**9 y**	**4 mo**	**1 y**	**9 y**	**1 y**	**9 y**
Weight								
Birth	0.42*	0.40*	0.24*					
4 mo		0.85*	0.36*					
1 y			0.44*					
Height								
Birth				0.29*	0.28*	0.17*		
4 mo					0.85*	0.49*		
1 y						0.60*		
Head circumference								
4 mo							0.85*	0.68*
1 y								0.78*

* $P < 0.05$

Table 6.5 Association of weight gain and intelligence quotient (IQ) (n=551)

Variable	Full scale IQ						Verbal IQ						Performance IQ					
	2-SLS		3-SLS		4-SLS		2-SLS		3-SLS		4-SLS		2-SLS		3-SLS		4-SLS	
	β	SE	β	SE	β	SE	β	SE	β	SE	β	SE	β	SE	β	SE	β	SE
At birth (SDS)	0.6	0.4	0.6	0.4	0.6	0.4	0.2	0.4	0.2	0.4	0.2	0.4	1.0*	0.5	1.0*	0.5	1.0*	0.5
Early infancy growth (Birth to 4 mo, SDS)	1.2*	0.4	1.3*	0.4	1.3*	0.4	0.8*	0.4	0.8*	0.4	0.8*	0.4	1.5*	0.5	1.6*	0.5	1.6*	0.5
Late infancy growth (4 mo to 1 y, SDS)			0.6	0.4	0.6	0.4			0.4	0.4	0.4	0.4			0.5	0.5	0.5	0.5
Late postnatal growth (1 y to 9 y, SDS)					-0.1	0.4					0.5	0.4					-0.8	0.5
Sex, male	-0.7	0.9	-0.9	0.9	-0.9	0.9	-1.9*	0.8	-2.0*	0.8	-1.8*	0.8	0.6	1.0	0.5	1.0	0.2	1.0
Maternal height (SDS)	0.8*	0.4	0.8	0.4	0.8	0.4	0.8	0.4	0.8	0.4	0.7	0.4	0.8	0.5	0.8	0.5	0.8	0.5
Maternal education, ≤ grade 6	-0.1	0.9	0.2	0.9	0.2	0.9	-0.3	0.9	-0.2	0.9	-0.3	0.9	0.4	1.0	0.5	1.0	0.6	1.0
Availability of mother at home, present	2.0	1.2	2.3*	1.2	2.4*	1.2	2.6*	1.1	2.7*	1.1	2.7*	1.1	1.4	1.4	1.6	1.4	1.6	1.4
SES scores (SDS)	2.0*	0.4	1.9*	0.4	1.9*	0.4	1.2*	0.4	1.2*	0.4	1.1*	0.4	2.5*	0.5	2.5*	0.5	2.6*	0.5
School location, urban	4.0*	1.2	3.9*	1.2	4.0*	1.2	4.5*	1.2	4.5*	1.2	4.4*	1.2	2.6	1.4	2.5	1.4	2.7	1.4

SDS, standard deviation score; weight at birth, 1 SD = 0.4 kg; early infancy growth in weight, 1 SD = 0.7 kg; late infancy growth in weight, 1 SD = 0.5 kg; late postnatal growth in weight, 1 SD = 4.9 kg.
* P <0.05.

Table 6.6 Association of length/height gain and intelligence quotient (IQ) (n=453)

Variable	Full scale IQ						Verbal IQ						Performance IQ					
	2-SLS		3-SLS		4-SLS		2-SLS		3-SLS		4-SLS		2-SLS		3-SLS		4-SLS	
	β	SE	β	SE	β	SE	β	SE	β	SE	β	SE	β	SE	β	SE	β	SE
At birth (SDS)	0.9*	0.4	1.0*	0.4	1.0*	0.4	0.2	0.4	0.3	0.4	0.3	0.4	1.5*	0.5	1.6*	0.5	1.6*	0.5
Early infancy growth (Birth to 4 mo, SDS)	1.7*	0.5	1.9*	0.5	1.8*	0.5	1.3*	0.5	1.4*	0.5	1.3*	0.5	2.0*	0.6	2.2*	0.6	2.2*	0.6
Late infancy growth (4 mo to 1 y, SDS)			1.4*	0.4	1.4*	0.4			0.8	0.4	0.9*	0.4			1.7*	0.5	1.7*	0.5
Late postnatal growth (1 y to 9 y, SDS)					0.4	0.4					0.7	0.4					-0.1	0.5
Sex, male	-1.6	1.0	-1.9*	1.0	-1.7	1.0	-2.4*	1.0	-2.6*	1.0	-2.2*	1.0	-0.6	1.2	-1.1	1.2	-1.2	1.2
Maternal height (SDS)	0.6	0.5	0.3	0.5	0.2	0.5	0.5	0.4	0.3	0.5	0.2	0.5	0.7	0.6	0.3	0.6	0.3	0.6
Maternal education, ≤ grade 6	0.3	0.9	0.4	0.9	0.5	0.9	-0.3	0.9	-0.2	0.9	-0.2	0.9	0.7	1.1	0.9	1.1	0.9	1.1
Availability of mother at home, present	1.8	1.3	1.7	1.2	1.7	1.2	2.0	1.2	1.9	1.2	1.8	1.2	1.1	1.5	0.9	1.5	1.1	1.5
SES scores (SDS)	1.7*	0.5	1.7*	0.5	1.7*	0.5	1.1*	0.5	1.1*	0.5	1.1*	0.5	2.2*	0.6	2.3*	0.6	2.2*	0.6
School location, urban	3.4*	1.3	3.3*	1.3	3.3*	1.3	3.4*	1.3	3.3*	1.3	3.2*	1.3	2.6	1.6	2.4	1.6	2.7	1.6

SDS, standard deviation score; length at birth, 1 SD = 2.7 cm; early infancy growth in length, 1 SD = 2.2 cm; late infancy growth in length, 1 SD = 1.3 cm; late postnatal growth in length/height, 1 SD = 4.7 cm.
* $P < 0.05$.

Table 6.7 Association of head circumference and intelligence quotient (IQ) (n=550)

Variable	Full scale IQ				Verbal IQ				Performance IQ			
	2-SLS		3-SLS		2-SLS		3-SLS		2-SLS		3-SLS	
	β	SE	β	SE	β	SE	β	SE	β	SE	β	SE
Early infancy (4 mo, SDS)	1.8*	0.5	1.8*	0.5	1.5*	0.4	1.5*	0.4	1.9*	0.5	2.0*	0.5
Late infancy growth (4 mo to 1 y, SDS)	0.5	0.4	0.5	0.4	0.6	0.4	0.6	0.4	0.3	0.5	0.3	0.5
Late postnatal growth (1 y to 9 y, SDS)			-0.2	0.4			-0.1	0.4			-0.3	0.5
Sex, male	-1.5	0.9	-1.6	0.9	-2.9*	0.9	-2.9*	0.9	0.0	1.1	-0.1	1.1
Maternal height (SDS)	0.9*	0.4	0.9*	0.4	0.8	0.4	0.8	0.4	1.0*	0.5	1.0*	0.5
Maternal education, ≤ grade 6	0.2	0.9	0.2	0.9	-0.2	0.9	-0.2	0.9	0.5	1.0	0.5	1.1
Availability of mother at home, present	2.4*	1.2	2.4*	1.2	2.8*	1.1	2.8*	1.1	1.5	1.4	1.5	1.4
SES scores (SDS)	2.0*	0.4	2.0*	0.4	1.2*	0.4	1.2*	0.4	2.6*	0.5	2.6*	0.5
School location, urban	3.9*	1.2	4.0*	1.2	4.4*	1.2	4.5*	1.2	2.6	1.4	2.7	1.4

HC, head circumference; SDS, standard deviation score; HC at 4 mo, 1 SD = 1.2 cm; late infancy growth in HC, 1 SD = 0.7 cm; late postnatal growth in HC, 1 SD = 0.8 cm.
* $P < 0.05$.

Table 6.8 Association of weight gain and Raven's CPM score (n=551)

Variable	Raven's CPM score					
	2-SLS		3-SLS		4-SLS	
	β	SE	β	SE	β	SE
At birth (SDS)	0.19	0.23	0.20	0.23	0.19	0.23
Early infancy growth (Birth to 4 mo, SDS)	0.33	0.24	0.34	0.24	0.36	0.24
Late infancy growth (4 mo to 1 y, SDS)			0.16	0.23	0.17	0.23
Late postnatal growth (1 y to 9 y, SDS)					-0.38	0.22
Age at cognition test (y)	2.25*	0.66	2.30*	0.67	2.46*	0.67
Sex, male	1.29*	0.48	1.26*	0.48	1.12*	0.49
Maternal height (SDS)	0.34	0.23	0.33	0.23	0.34	0.23
Maternal education, ≤ grade 6	0.09	0.49	0.12	0.49	0.16	0.49
Availability of mother at home, present	-0.20	0.65	-0.16	0.66	-0.12	0.66
SES scores (SDS)	1.02*	0.25	1.02*	0.25	1.04*	0.25
School location, urban	1.58*	0.67	1.57*	0.67	1.64*	0.67

SDS, standard deviation score; weight at birth, 1 SD = 0.4 kg; early infancy growth in weight, 1 SD = 0.7 kg; late infancy growth in weight, 1 SD = 0.5 kg; late postnatal growth in weight, 1 SD = 4.9 kg.
* P <0.05.

Table 6.9 Association of length/height gain and Raven's CPM score (n=453)

Variable	Raven's CPM score					
	2-SLS		3-SLS		4-SLS	
	β	SE	β	SE	β	SE
At birth (SDS)	0.05	0.26	0.08	0.25	0.07	0.26
Early infancy growth (Birth to 4 mo, SDS)	0.58*	0.29	0.62*	0.29	0.63*	0.29
Late infancy growth (4 mo to 1 y, SDS)			0.49	0.25	0.49	0.25
Late postnatal growth (1 y to 9 y, SDS)					-0.23	0.27
Age at cognition test (y)	1.88*	0.75	2.10*	0.75	2.29*	0.79
Sex, male	1.01	0.55	0.88	0.56	0.76	0.57
Maternal height (SDS)	0.22	0.26	0.12	0.27	0.15	0.27
Maternal education, ≤ grade 6	0.02	0.54	0.07	0.54	0.07	0.54
Availability of mother at home, present	-0.63	0.71	-0.65	0.71	-0.62	0.71
SES scores (SDS)	1.06*	0.27	1.05*	0.27	1.06*	0.27
School location, urban	1.69*	0.75	1.66*	0.74	1.68*	0.74

SDS, standard deviation score; length at birth, 1 SD = 2.7 cm; early infancy growth in length, 1 SD = 2.2 cm; late infancy growth in length, 1 SD = 1.3 cm; late postnatal growth in length/height, 1 SD = 4.7 cm.
* P <0.05.

Table 6.10 Association of head circumference and Raven's CPM score (n=550)

Variable	Raven's CPM score			
	2-SLS		3-SLS	
	β	SE	β	SE
Early infancy (4 mo, SDS)	0.38	0.26	0.38	0.26
Late infancy growth (4 mo to 1 y, SDS)	0.24	0.23	0.24	0.23
Late postnatal growth (1 y to 9 y, SDS)			0.03	0.23
Age at cognition test (y)	2.33*	0.67	2.33*	0.67
Sex, male	1.16*	0.51	1.18*	0.52
Maternal height (SDS)	0.38	0.23	0.38	0.23
Maternal education, ≤ grade 6	0.14	0.49	0.14	0.49
Availability of mother at home, present	-0.18	0.65	-0.18	0.65
SES scores (SDS)	1.01*	0.25	1.01*	0.25
School location, urban	1.60*	0.67	1.59*	0.67

HC, head circumference; SDS, standard deviation score; HC at 4 mo, 1 SD = 1.2 cm; late infancy growth in HC, 1 SD = 0.7 cm; late postnatal growth in HC, 1 SD = 0.8 cm.
* $P < 0.05$.

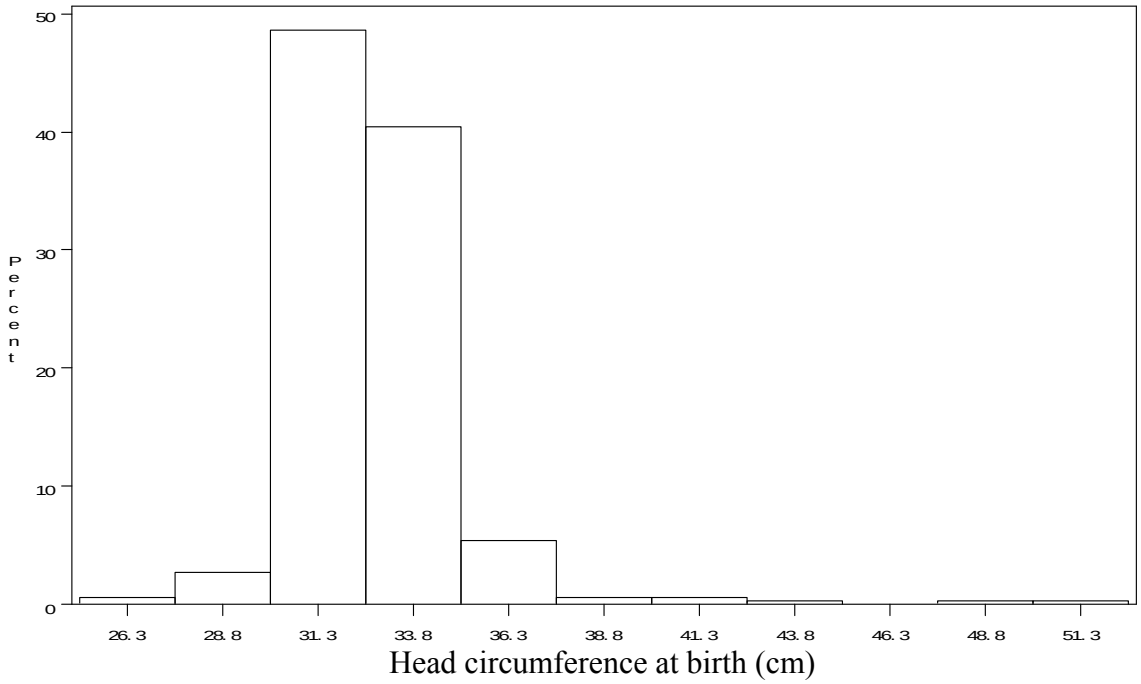

Figure 6.1 Distribution of head circumference at birth

CHAPTER 7
SUMMARY AND CONCLUSIONS

This study was a follow-up study conducted among children in Khon Kaen, northeast, Thailand who previously participated in a randomized controlled trial of iron and zinc supplementation at 4-6 months of age for 6 months (n=560). We explored the effects of supplementation during infancy on cognitive performance and growth at 9 years of age. To our knowledge, this is the first study that has assessed the effects of iron and zinc supplementation during infancy on cognitive and growth outcomes at 9 years of age. We also explored the influence of growth at birth, early infancy, late infancy, and late postnatal on intellectual functioning at 9 years of age.

7.1 Key findings

Long-term effects of iron and zinc supplementation on cognitive performance

We showed that there were no long-term effects of daily iron and/or zinc supplementation during infancy on school outcomes, including the percentages of children who attended nursery school and kindergarten and age at first grade entry. Percentage of children who were attending grade 4 was highest in the iron group but this was due to that children in the iron group were older than those in other groups.

Full scale IQ, verbal IQ, and performance IQ were not significantly different among 4 groups. The IQs of participating children ranged from 92.9 to 93.7 points for full scale IQ, 93.9 to 95.4 points for verbal IQ, and 93.1 to 94.0 points for performance IQ. There were no differences among 4 groups in the WISC-III index scores. Similarly, the Raven's CPM scores were not different among 4 groups and ranged from 21.4 to

22.4. The school performance scores in Thai, English, Mathematics, and Science were not different among groups.

Although iron and zinc are important for optimal brain development and early childhood is a critical of brain growth, this long-term follow-up study did not show any benefits of iron and/or zinc supplementation during infancy on cognitive performance at 9 years of age. Even though their iron and zinc status were improved after iron and zinc supplementation for 6 months, these children could have become deficient again in the second year of life or beyond. In addition, although there is a growth spurt of the brain during the first two years of life, the brain continues to develop at different rates throughout early adulthood (i.e., age 16-19 years) (152). Therefore, providing iron and zinc supplementation only during the early childhood may not be sufficient to optimally impact long-term cognitive development.

It might be too early to detect long-term effect on cognitive performance at 9 years of age. A follow-up study of high protein-energy supplementation during pregnancy and the first 2 years of life (106) showed inconsistent but moderately positive effects of supplementation on cognitive abilities when children were assessed during infancy and preschool periods; however, benefits of supplementation on intellectual functions were larger when the subjects were tested in adolescence and adulthood (140-142).

It is also possible that our children were deficient in other nutrients, such as protein and energy, iodine, folate, vitamin B-12, or omega-3 unsaturated fatty acids, which have been known to be associated with cognitive performance (17, 143). If this is the case, the effect of iron and zinc supplementation may be attenuated.

Long-term effects of iron and zinc supplementation on physical growth

Comparison among intervention groups (iron alone, zinc alone, iron plus zinc, and a placebo) on weight, height, BMI, and MUAC at 9 years of age, adjusted for size at 1 year of age (endline) did not show any significant differences. There were also no differences among intervention groups for WAZ, HAZ, and BMIZ. However, WAZ at baseline modified the effect of supplementation on WAZ at 9 years of age (P=0.038 for interaction). Children with lower baseline WAZ tended to benefit from iron and zinc supplementation. However, results suggest that supplementation may be harmful to those with higher baseline WAZ.

Many studies have investigated the effect of iron and/or zinc supplementation during infancy, but none of these studies have investigated the effects on growth beyond infancy. Nonetheless, these studies reported non beneficial concurrent effects which seem to be in the same direction as our study that showed non positive long-term effects of iron and/or zinc supplementation during infancy on growth at school-age period.

Low prevalence of underweight and stunting at baseline may explain a non beneficial long-term effect of iron and zinc supplementation on growth. Our results were also in part in accordance with an earlier meta-analysis that showed a greater concurrent effect of zinc supplementation on weight among children who were underweight at baseline (20). A positive long-term effect of iron and/or zinc supplementation on WAZ was shown among children with low baseline WAZ, while an adverse long-term effect was shown among children with high baseline WAZ. More supportive evidence is needed to provide a definite conclusion of the long-term effects of iron and/or zinc supplementation at infancy on growth measured at late childhood.

Influence of prenatal and postnatal growth on intellectual functioning at 9 years of age

We analyzed the anthropometric data that were available at different time points from birth to late postnatal. Our findings suggested that early postnatal growth, particularly during early and late infancy, is the most important period of child growth associated with IQ at 9 years as compared to other periods. Gain in weight from birth to 4 months was strongly associated with all IQs at 9 years in which 1 SD gain in weight increased 1.3, 0.8, and 1.6 points in full scale, verbal, and performance IQ, respectively. Gain in length from birth to 4 months was positively associated with all IQs at 9 years. Specifically, 1 SD gain in length from birth to 4 months increased 1.8, 1.3, and 2.2 points in full scale, verbal, and performance IQ, respectively. Gain in length from 4 months to 1 year was also associated with all measures of IQ. HC at 4 months was also significantly associated with IQ at 9 years, 1 SD increased in HC was associated with 1.8 points, 1.5 points, and 2.0 points increment in full scale, verbal, and performance IQ. Effects of size at birth and growth from 4 months to 1 year were smaller and inconsistent. There was no association between growth from 1 year to 9 years and IQ at 9 years.

Our findings confirm the previous longitudinal studies (122, 124, 125, 146) in both developed and developing countries that early postnatal growth was the most important as compared to growth in other periods for cognitive abilities later in life. These studies showed that birth weight was not associated with cognitive abilities at 11 years (125) or at adulthood (124). Gain in length at early postnatal period was more influential on educational achievement (EA) in adult women than birth length or growth in height at late postnatal (124). Growth in HC at early postnatal was more important than

HC at birth and growth in HC at late postnatal on IQ at 8 years of age (122), 9 years of age (146), or EA at adulthood (124).

7.2 Strengths and limitations of the current study

Our study has several strengths. We were able to follow-up 92% of the participants who completed the intervention trial during infancy, thus minimizing a selection bias. We also ensured that those lost to follow-up were similar to those who participated in this study. Students *T* test was performed and the findings did not show significant differences regarding the distribution of supplementation type, sex, birth weight, or baseline biochemical and anthropometric data. Additionally, we found that supplement compliance among our children was high (93-95%). Thus, we can eliminate the factor that might be related to the null findings. Our study was conducted in rural communities and the children who participated in our study were apparently healthy children. When more evidence is available, findings from this study could be generalized to populations in rural communities of developing countries which usually have similar problems of iron and zinc deficiency.

We assessed a variety of cognition skills including both verbal and nonverbal cognitive abilities using the WISC-III and the Raven's CPM. Among WISC-III subtests, arithmetic, digit span, coding, and symbol search were used to measure the performance associated with short-term memory, attention, and reasoning which are known to be affected by iron and zinc deficiencies (46, 53, 132). We also assessed the school performance and other variables related to schooling.

Finally, anthropometric data were available at different time points during childhood, especially two time points during the first year of age. Consequently, we were able to separate the influence of early and late infancy growth and were able to detect the periods of growth that are the most important. We used the SLS approach for data analysis. This approach allowed us to consider growth at later periods without the influence of growth at earlier periods (150).

Our study has several limitations. First, other than cognitive abilities, we did not have the opportunity to measure behavioral and physical activity of the children which have been known to be indirect consequences of nutritional deficiency (12, 48).

We have several issues related to the limitation of the data. Since we did not plan to assess body composition in the intervention trial study, data on anthropometric measures that are needed for body composition calculation were not available. Therefore, we were not able to demonstrate whether there was any long-term benefit of iron and/or zinc supplementation on body composition. Additionally, the quality of the HC measurement at birth was uncertain, thus we did not include HC at birth when analyzing the influence of prenatal growth on IQ. Failure to include these data did not allow us to assess the effect of growth in HC at early infancy without the influence of HC at birth. We also did not have complete information on gestational age and thus cannot evaluate the effect of gestational age on IQ.

7.3 Future studies

The current study did not show long-term effects of iron and zinc supplementation during infancy on cognitive abilities at 9 years of age. However, we do not know whether

these children these children could have become deficient again in the second year of life or beyond when further brain growth occurs, though in a lesser magnitude. Hence, further studies are needed to investigate whether the continuation of iron and zinc supplementation from early childhood through late childhood and adolescence will provide any long-term benefits.

We did not have the opportunity to measure behavioral and physical activity of the children which have been known to be indirect consequences of nutritional deficiency (12, 48). Further studies that included the measurement of behavioral and physical activity would provide additional evidence about the benefit of iron and zinc supplementation in infancy.

Nine years of age may have been too early to detect long-term effect of the supplementation on cognitive performance. Thus, it is necessary to carry out studies at later ages to explore whether benefits will occur when the assessment is performed at an older age.

Since this is a first study to explore long-tem effects of iron and zinc supplementation during infancy on cognitive function and growth later in life, more studies in different settings are needed to confirm our findings.

7.4 Implication of the study finding

Although our study did not show a benefit of iron and zinc supplementation during infancy on intellectual functioning and growth measured at 9 years of age, we found a potential long-term benefit of iron and/or zinc supplementation on WAZ at 9 years of age among those with low WAZ at baseline and a possible negative effect

among those with high WAZ at baseline. Because this is a first study to report long-term effects of iron and zinc supplementation, the findings should be viewed as preliminary evidence. We cannot definitely conclude that iron and zinc supplementation during infancy is not useful for cognitive development, unless we have more evidence to confirm. The preliminary implication that can be obtained from this study is that iron and/or zinc intervention during infancy may not provide the same long-term benefit on growth in all children. Thus, investment in these intervention programs should consider baseline nutritional status of targeted infants and the intervention should be restricted to infants who are at risk of undernutrition in order to ensure the benefit and minimize the adverse effect on growth during late childhood.

Our findings also contribute to evidence of a strong and consistent association of growth at early childhood to intellectual functioning at 9 years of age. This implies that optimal growth during early childhood may enhance early cognitive development which then may consequently result in better cognitive ability later in life. Thus, appropriate breastfeeding during early infancy should be the main priority of nutrition interventions in order to promote an optimal growth. Appropriate complementary feeding practice should also be promoted along with breastfeeding interventions in order to maintain growth during late infancy.

7.5 Summary

In summary, this study is the first study to provide information about the effects of iron and zinc supplementation during infancy on cognitive performance and growth at 9 years of age. Findings from this study did not show long-term benefits of iron and/or

zinc supplementation during infancy on cognitive performance at 9 years of age. However, a positive long-term effect of iron and/or zinc supplementation on WAZ was found in children with lower WAZ at baseline and a negative effect was found in children with higher WAZ at baseline. These findings suggest that iron and/or zinc supplementation should be considered as a possible intervention for improving growth among malnourished children. Our findings also contribute to the mounting evidence that early childhood growth may influence on cognitive abilities later in life. Thus, nutrition intervention programs should emphasize the importance of early childhood nutrition.

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APPENDICES

Appendix 1: Socio-economic, demographic, morbidity, and schooling information

Emory University
Institute of Nutrition, Mahidol University

Long-term effects of iron and zinc supplementation
Socio-economic, demographic, morbidity, and schooling information

Section A: Socio-demographic		
1	ID	_ _ _ _ _ _ _
2	Date of interview (dd/mm/yyyy)	_ _/_ _/_ _ _ _
3	Name and code of the interviewer _____	__
4	Respondent's name _____ Tel _____	
5	Relation of the respondent to the child 1 = Mother 2 = Father 3 = Grandparent 4 = Other relative specify _____ 5 = Other specify _____	___
6	Child's name _____	
7	Child's birth date (dd/mm/yyyy)	_ _/_ _/ _ _ _ _
8	Current address House no. ____Vill no. ___ Village name _____ Subdistrict _____	
9	District 1 = Ubonrat 2 = Banphang 3 = Nampong 4 = Kaosuankwang 5 = Muang	__
10	How many adults (18 years or older) are there in the household?	__ __
11	How many children (younger than 18 years) are there in the household?	__ __
12	Who is the primary caregiver of the child? 1 = Mother 2 = Father 3 = Grandparent 4 = Other relative specify _____	__

	5 = Other specify _____	
13	How old is the primary caregiver? (age in years)	__ __
14	What is the highest grade of primary caregiver? 0 = None 1 = Grade 1 to 6 2 = Grade 7 to 9 3 = Grade 10 to12 4 = Vocational school 5 = University 6 = Other specify _____	__
15	Can the primary caregiver read or write? 1 = Read only 2 = Read and write 0 = No	__
16	What is main occupation of primary caregiver? 0 = Does not work 1 = Housewife 2 = Agriculture/livestock/hunt/fishing 3 = Non-agricultural worker 4 = Clerical duties 5 = Social services 6 = Service provider 7 = Merchant 8 = Professional 9 = Don't know	__
17	How old is child's mother? (age in years; 99 = don't know)	__ __
18	What is the highest grade of mother? 0 = None 1 = Grade 1 to 6 2 = Grade 7 to 9 3 = Grade 10 to12 4 = Vocational school 5 = University 6 = Other specify _____ 9 = Don't know	__
19	Can mother read or write? 1 = Read only 2 = Read and write 0 = No	__
20	What is main occupation of mother? 0 = Does not work 1 = Housewife 2 = Agriculture/livestock/hunt/fishing 3 = Non-agricultural worker 4 = Clerical duties 5 = Social services 6 = Service provider 7 = Merchant	__

	8 = Professional 9 = Don't know	
21	Does mother live with the child? 1 = Yes 0 =No	__
22	How many children does mother have (include target child)? (9 = don't know)	__
23	What is the child's birth order? (9 = don't know)	__
24	How old is child's father? (age in years; 99 = don't know)	__ __
25	What is the highest grade of father? 0 = None 1 = Grade 1 to 6 2 = Grade 7 to 9 3 = Grade 10 to12 4 = Vocational school 5 = University 6 = Other specify _____ 9 = don't know	__
26	Can father read or write? 1 = Read only 2 = Read and write 0 = No	__
27	What is main occupation of father? 1 = Agriculture 2 = Hired laborer 3 = Government official 4 = Trade 5 = Stay at home dad 6 = Other occupation specify _____ 9 = don't know	__
28	Does father live with the child? 1 = Yes 0 =No	__
29	Who was/were the primary caregiver/s of the child at : 0 - 6 mo > 6 - 12 mo > 12 - 24 mo > 24 - 60 mo > 60 mo - present (1 = Mother alone, 2 = Father alone, 3 = Mother and father, 4 = Grandparent/s, 5 = Other relative specify _____, 6 = Other specify _____, 9 = don't know)	___ ___ ___ ___ ___

Section B: Housing characteristics and assets		
1	From what material is the majority of the walls in your house constructed? 1 = Bamboo/grass/leave 2 = Tin 3 = Plywood/ laminated plywood 4 = Wood 5 = Brick 6 = Wood and cement 7 = Cement 8 = Other specify _____	__
2	From what material is the majority of the roof of your house constructed? 1 = Grass/leave 2 = Tin 3 = Tile 4 = Tin and tile 5 = Other specify_____	__
3	From what material is the majority of your floor made? 1 = Soil 2 = Wood 3 = Wood and cement/ceramic 4 = Cement 5 = Ceramic 6 = Terrazzo 7 = Other specify_____	__
4	What is the source of cooking/drinking water? 1 = Rain 2 = Public use river/pond/well 3 = Underground water 4 = Private well 5 = Tap water 6 = Bottled water	__
5	What type of toilet do you use in your house? 1 = Bush 2 = Pit 3 = Toilet 4 = Other specify _____	__
6	The house where you live is connected to an electrical system (receives electricity)? 1 =Yes 0 = No	__
7	The house where you live is connected to a telephone land line? 1 =Yes	__

	0 = No	
8	How many rooms, including a living room, dining room, kitchen, and bedroom, are in the house where you live?	__ __
9	How many rooms do you use for ONLY sleeping? # rooms:	__ __
10	In your house, where is your kitchen located? 0 = No kitchen 1 = In the bedroom 2 = Outside the bedroom, but not separated from other rooms 3 = In separated room, inside or outside of the house	__
11	The house where you live is: 1 = Owned 2 = Borrowed 3 = Rented 4 = Just a guest 5 = Other specify _____	__
Household items		
12	Gas stove 1 = Yes 0 = No	__
13	Electric rice cooker 1 = Yes 0 = No	__
14	Refrigerator 1 = Yes 0 = No	__
15	Electric fan 1 = Yes 0 = No	__
16	Washing machine 1 = Yes 0 = No	__
17	Radio with tape player 1 = Yes 0 = No	__
18	Stereo with CD player 1 = Yes 0 = No	__
19	Television 1 = Yes 0 = No	__
20	Video camera/ VCR/ DVD player 1 = Yes 0 = No	__
21	Computer 1 = Yes 0 = No	__
22	Cell phone 1 = Yes 0 = No	__

Vehicles		
23	Truck or bus 1 = Yes 0 = No	__
24	Car / Van 1 = Yes 0 = No	__
25	Pick-up truck 1 = Yes 0 = No	__
26	Plow machine 1 = Yes 0 = No	__
27	Motorcycle 1 = Yes 0 = No	__
28	Bicycle 1 = Yes 0 = No	__
Business		
29	Store 1 =Yes 0 = No	__
30	Rice mill 1 = Yes 0 = No	__
31	Food shop 1 = Yes 0 = No	__
32	Artisan shop (wood shop, shoe repair store, tailor/ mender, electrical repair shop) 1 = Yes 0 = No	__
33	Mechanic shop (garage) 1 = Yes 0 = No	__
34	Other business 1 = Yes specify_____ 0 = No	__

Section C: Morbidity and eating habit		
1	Have the doctor told you that your child has chronic disease? 1 = Yes specify_____ 0 = No 9 = Don't know	__
2	Has your child been ill at any time in the last 2 weeks with Diarrhea Fever/cold Other illness specify _____ 1 = Yes 0 = No 9 = Don't know	 __ __
3	Does your child have a worm infection? 1 = Yes 0 =No 9 = Don't know	__
4	Has your child ever received iron tablets within the last 6 months - Iron tablet - multi-vitamin and mineral tablet or syrup - Vitamin A capsule - Vitamin C - Anti helminth 1 = Daily 2 = Weekly 3 = Occasionally 0 =No 9 = Don't know	 __ __ __ __ __
	Eating habit	
5	Does your child usually eat breakfast? 1= Always 2 = Sometimes 0 = Never	__
6	Does your child usually eat lunch? 1= Always 2 = Sometimes 0 = Never	__
7	Does your child usually eat dinner? 1= Always 2 = Sometimes 0 = Never	__
8	Does your child usually eat snacks? 1= Always 2 = Sometimes 0 = Never	__
9	Does your child bring his/her own lunch to school? 1= Always 2 = Sometimes 0 = Never	__
10	Does your child bring money to buy lunch or other foods at school? 1= Always 2 = Sometimes 0 = Never	__

11	Does your child normally receive free lunch under school lunch program? 1= Always 2 = Sometimes 0 = Never	__
12	Does your child participate in other nutrition interventions? 1 = Yes specify _____ 0 = No	__
13	How would you rate the child's appetite when compared to other children? 1 = Less than others 2 = Similar to others 3 = More than others	__

Section D: Schooling information			
1	Did your child attend day care center? 1 = Yes 0 = No	__	
2	How old was your child when s/he entered day care center? (0 = did not attend day care center)	__ year __ mo	
3	How long had your child been in day care center? (0 = did not attend day care center)	__ year __ mo	
4	Did your child attend kindergarten school? 1 = Yes 0 = No	__	
5	How old was your child when s/he entered kindergarten school? (0 = did not attend kindergarten)	__ year __ mo	
6	How long had your child been in kindergarten school? (0 = did not attend kindergarten)	__ year __ mo	
7	How old was your child when s/he entered first grade?	__ year __ mo	
8	What grade is your child in now?	__	
9	Which grades did your child repeated in elementary school? (Code # of times; 0 = did not repeat any grade; 8 = has not been in that grade)	Grade	Times
		1	
		2	
		3	
		4	
		5	

Appendix 2: Biochemistry and anthropometry form

Emory University
Institute of Nutrition, Mahidol University

Long-term effects of iron and zinc supplementation
Biochemistry and anthropometry data

Section A. General Information		
1	ID	_ _ _ _ _ _ _
2	Date of blood collection (dd/mm/yyyy)	_ _/_ _/_ _ _
3	Child's name	
Section B. Blood Test		
Have you eaten some food such as rice, noodle, fruit, vegetables, soda, desserts, snacks, or some other food during the last 6 hours?		Yes ____ No ____
When was the last time you eat or drink some food such as rice, noodle, fruit, vegetables, soda, desserts, cookies or some other food?		__ __.__ __
Do you have any illnesses? 1 = Yes specify _____ 0 = No		____
Hemoglobin (g/L)		__ __ __
Serum ferritin (µg/dl)		__ __. __
Serum transferrin receptor (mg/L)		__ __.__
Serum zinc (µg/dl)		__ __.__
C-reactive protein (mg/L)		__ __.__
Section C. Urine test		
Urine Iodine (µg/L)		__ __ __.__
Section D. Anthropometric measurement		
Weight (kg)	__ __ . __	__ __ . __	__ __ . __
Height (cm)	__ __ __ . __	__ __ __ . __	__ __ __ . __
Head circumference (cm)	__ __ . __	__ __ . __	__ __ . __
Arm circumference (cm)	__ __ . __	__ __ . __	__ __ . __
Triceps skinfold (mm)	__ __ . __	__ __ . __	__ __ . __
Subscapular skinfold (mm)	__ __ . __	__ __ . __	__ __ . __

Time of blood taken __ __ . __ __ hrs.