Control of iron deficiency anemia in the first 1000 days of life:

Prevention of impaired child development

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Abstract

Iron deficiency anemia is the most common micronutrient deficiency worldwide. Pregnant women and children constitute risk groups due to their high iron requirements. To provide additional evidence on the long-term impact of iron deficiency in pregnancy and in children under 2 years old on child development, we conducted two longitudinal observational studies. To explore effective and optimal approaches for iron supplementation for pregnant women and in-home fortification for infants and young children, we also performed a randomized controlled trial and an iron absorption study.

We analyzed two years follow-up data of 850 children born to women who participated in a randomised controlled trial of prenatal micronutrient supplementation in western rural China. The prenatal iron deficiency anemia (IDA) group showed significantly lower Mental Development Index (MDI) at 12, 18 and 24 months of age compared to the prenatal non-IDA group of children. The adjusted mean difference in MDI for the three age intervals was 5.8 (95% Confidence Interval [CI] 1.1 to 10.5), 5.1 (95% CI 1.2 to 9.0) and 5.3 (95% CI 0.9 to 9.7). When supplemented with iron/folic acid (60 mg iron), there was no significant difference on MDI between prenatal IDA and non-IDA groups; when supplemented with folic acid alone or multiple micronutrients (30 mg iron), MDI was significantly lower in prenatal IDA group.

The second longitudinal observational study compared social emotional affect and behavior of three groups of non-anemic 4-year-old children: children with IDA in infancy whose anemia was not corrected before 24 months (chronic IDA, n=27); children with IDA in infancy whose anemia was corrected before 24 months (corrected IDA, n=70), and children who were non-IDA in infancy and at 24 months (non-IDA, n=64). Chronic IDA displayed less positive affect, less frustration tolerance, more passive behavior, and more physical self-soothing in the stranger approach and delay of gratification. In contrast, corrected IDA was comparable to non-IDA in terms of behavior and affect. The randomized controlled trial of prenatal supplementation we carried out, involving 5828 pregnant women, suggests that multiple micronutrients are as effective as iron folic acid in increasing maternal hemoglobin concentration, birth weight and mean duration of gestation.

To determine whether iron absorption is enhanced with a mixture of $FeSO_4$ and NaFeEDTA, we conducted an iron absorption study with a crossover design in two groups aged 24 to 31 months old. A complementary food consisting of millet porridge with cabbage, tofu, and pork-filled wheat flour dumplings was fortified with iron as either $FeSO_4$ or NaFeEDTA or a mixture of $FeSO_4$ and NaFeEDTA. We found that an equal mixture of $FeSO_4$ and NaFeEDTA significantly enhanced iron absorption and thus could be a strategy to ensure adequate iron absorption from phytate-containing complementary foods.

Our results combined with other studies show the benefit of giving at least 30 mg iron daily to pregnant women. This can provide guidance to policy on the development of a prenatal supplementation standard in China. Enhanced iron absorption from a mixture of $FeSO_4$ and NaFeEDTA supports the current practice of the use of NaFeEDTA for in-home fortification of complementary food.

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Chapter 1

General Introduction

Evidence indicates that the first 1000 days of life (the period from the woman's pregnancy continuing into the child's second year) is the most critical period as this is where nutritional deficiencies have a significant and often irreversible adverse impact on child survival and growth affecting their ability to learn in school and productivity in later life[1, 2]. Sufficient iron supply and intake during pregnancy and in children under 2 years of age are an important component for nutrition interventions during these critical 1000 days. A causal relationship between iron deficiency and poorer brain function has not been demonstrated yet. Our study thus explored long-term effects of iron deficiency anemia (IDA) during pregnancy and in children under 2 years of age on cognitive function, psycho-motor function and social-emotional behavior of children. Our study results contribute to the evidence of an adverse effect of iron deficiency in the first 1000 days on child development and of the benefits of iron supplementation.

IRON DEFICIENCY

Iron deficiency, defined as a stage in which there is insufficient iron to maintain the normal physiological function of tissues such as the blood, brain and muscles is the most widespread nutrition disorder in the world, affecting more people globally than any other condition[3-5]. In China, iron deficiency is common in women and children. A national survey in 2001 showed the prevalence of iron deficiency (ID) and iron deficiency anemia (IDA) to be 65.5% and 20.8%, respectively, among infants under 12 months, and 43.7%, and 7.8% among children aged 12-36 months[6]. The prevalence of ID and IDA were 42.6% and 19.1% in pregnant women, while 34.4% and 15.1% in non-pregnant women, respectively.

Iron depletion, the first stage of ID, is characterized by a progressive reduction in the amount of storage iron in the liver. Iron-deficiency erythropoiesis, the second stage of ID, is characterized by the exhaustion of iron stores and is also referred to as "iron deficiency without anemia." IDA, the third and final stage of ID, is characterized by exhaustion of iron stores, declining levels of circulating iron, and presence of frank microcytic, hypochromic anemia. The main feature of this stage is a reduction in the concentration of haemoglobin in the red blood cells, arising from the restriction of iron supply to the bone marrow. Decrease in the haematocrit and red cell indices also occur[7].

CRITICAL PERIOD FOR IRON DEFICIENCY

Accumulation of iron by the human foetus begins early in pregnancy, increases dramatically in the third trimester and continues after birth up to 30-50 y of age[8]. Human beings are unable to excrete iron actively[9]. Iron deficiency arises when physiological requirements cannot be met by iron absorption from the diet. Iron is present in many foods, and its intake is directly related to energy intake[10]. Therefore the risk of iron deficiency is highest when iron requirements are greater than energy needs, being the case with infants and young children, adolescents, and menstruating and pregnant women[9]. However, where diets are based mostly on staple foods with little meat intake, iron deficiency may occur throughout the life span[11].

During infancy, rapid growth exhausts iron stores accumulated during gestation and often results in deficiency, if iron fortified weaning foods or iron-fortified formula are not supplied. In many developing countries, plant-based weaning foods are rarely fortified with iron, and the frequency of anemia exceeds 50% in children younger than 4 years[12]. In school-aged children, iron status typically improves as growth slows and diets become more varied. The frequency of iron deficiency begins to rise again, mainly in female individuals, during adolescence, when menstrual iron losses are superimposed with needs for rapid growth[9]. During pregnancy, iron requirements increase three-fold because of expansion of maternal red-cell mass and growth of the fetal-placental unit[13].

In this thesis, we assessed the long-term effects of iron deficiency anemia on child development during the first 1000 days if life, and explored effective and optimal approaches for iron supplementation and iron fortification among pregnant women, infants and young children (Figure 1).

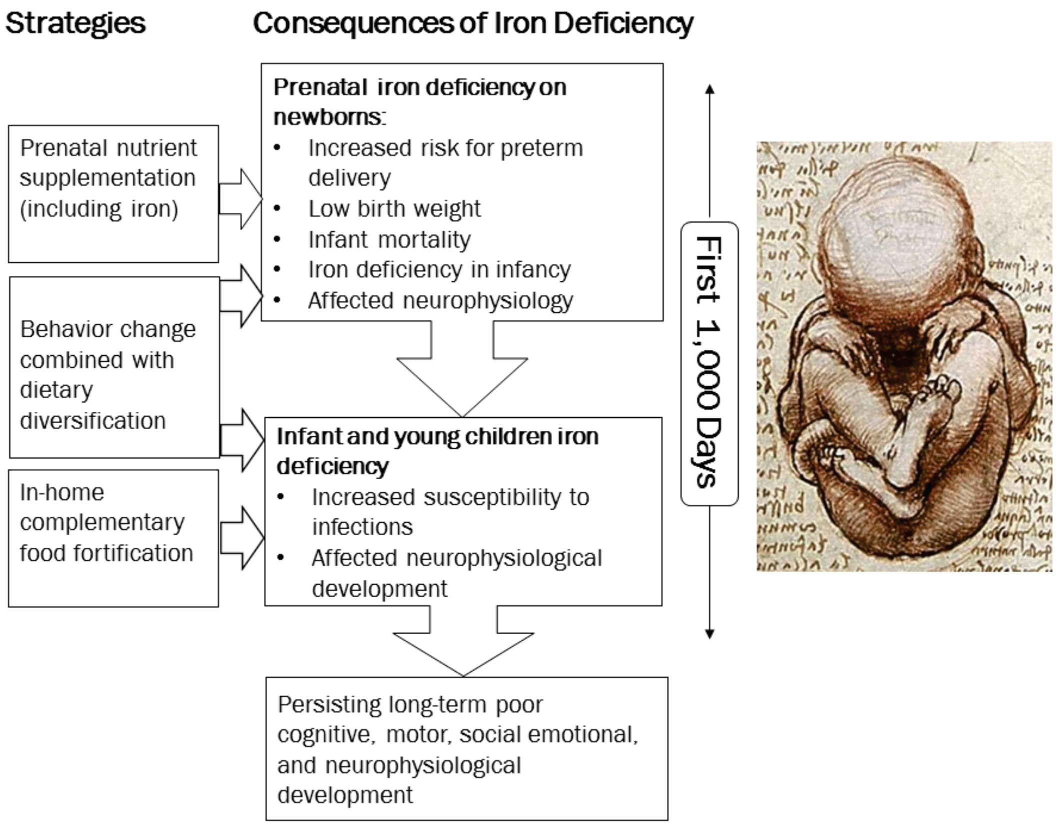

Figure 1: Consequence of iron deficiency and strategies to prevent iron deficiency

HEALTH EFFECTS OF IRON DEFICIENCY

Iron is needed for many essential bodily functions and required by enzymes involved in specific brain functions[8].The high frequency of iron deficiency anemia in the developing world has substantial health and economic costs. In an analysis involving data of ten developing countries, the median value of physical productivity losses per year due to iron deficiency was estimated to be about US$0·32 per head, or 0·57% of the gross domestic product[14]. During the first two trimesters of pregnancy, iron deficiency anemia increases the risk for preterm delivery, low birth weight, infant mortality, and predicts iron deficiency in infants after 4 months of age[15, 16]. It is estimated that anemia accounts for 3.7% and 12.8% of maternal deaths during pregnancy and childbirth in Africa and Asia, respectively[17]. In addition iron deficiency anemia increases susceptibility to infections, mainly of the upper respiratory tract, which happens more often and have a longer duration in anemic than in healthy children[18]. Iron deficiency, even in the absence of anemia, might cause fatigue and reduced work performance in adults[19, 20].

Human and animal studies provided some evidence on the adverse effects of iron deficiency on cognitive and motor development of infants and young children, while it would be premature to conclude that a causal connection exists between iron deficiency per se during development and subsequent cognitive or behavior performance[8]. Further research on the effects of iron deficiency on child development could have a major societal impact in regions where iron deficiency is widespread and poses an adverse impact on individual infants and young children everywhere.

The brain and iron deficiency

Animal model studies have demonstrated that iron is required for normal myelination of nerves, for neuronal metabolic activity and the formation of neurotransmitters, such as dopamine, serotonin and gamma aminobutyric acid (GABA)[21] . Irreversible changes in brain iron distribution can occur in iron-deficient rats even after prolonged iron repletion if the ID occurs during a critical phase of early brain development[22]. A fundamental tenet for considering a biological role of iron in neurodevelopmental delays and poor cognition and behavior is that there is a failure for delivering iron to the brain during particular sensitive periods[23]. There is direct evidence of biochemical abnormalities in brains of iron-deficient infants [22, 24], which demonstrated a slowed nerve conduction velocity using auditory-evoked potential studies in iron deficient infants at six months of age. Despite 12 months of iron therapy that corrected hematological indices of iron status, the slowed nerve conduction velocity remained abnormal and showed no indication of normalization. Significant delays in the waveform of auditory brainstem responses and visual-evoked potentials have been demonstrated in 4-year-old children who had IDA in infancy compared with non-IDA controls. One interpretation is that iron deficiency occurred in these infants during a “critical period” of development resulting in irreversible changes associated with hypo-myelination and changes in monoamine metabolism[25]. A second interpretation is that these are not iron status sensitive measurements and other environmental factors in the first 6 months of the infant’s life impacted neurodevelopment[26, 27]. However it is difficult to directly bridge the animal literature with similar measurements in human infants and children. The similarity of iron metabolism across a number of mammalian species, however, provides some encouragement that the animal literature furnishes a conceptual basis for understanding possible mechanisms whereby dietary iron deficiency alters neural functioning[25].

A number of possible mechanisms have been suggested for a postulated effect of iron deficiency on children’s development[24, 28-30]. Lack of iron might interfere with the metabolism of neurotransmitters, which might either affect psychomotor function and cognitive function directly or might affect scores on tests of psychomotor function and cognitive function indirectly through effects on behavior. In this thesis, we observed long-term effects of IDA in pregnancy and/or IDA in children under 2 years of age on cognitive function, psycho motor function and social emotional behavior of children.

Prenatal iron deficiency anemia and child mental and motor development

Unlike iron deficiency in infancy, perinatal (i. e., late foetal and neonatal) iron deficiency has received little attention, due in large part of previous thinking that infants are protected from maternal iron deficiency unless the mother is markedly anemic; this thinking however is no longer accepted[31-33].

Animal studies demonstrated that proper iron nutrition during perinatal development is critical not only for obtaining adequate levels of brain iron, but also for normal behavioral and motor development in the offspring[34, 35]. During pregnancy, the iron requirements of the pregnant women are increased three-fold to cover needs of expansion of maternal red-cell mass and growth of the fetal-placenta, and maternal anemia develops unless these needs are met[13, 36]. Epidemiologic studies suggest that maternal iron deficiency contributes to reduced fetal iron stores[37-43], and infants born to anemic mothers have low iron stores and are more likely to develop anemia [38, 39, 44-46]. However, knowledge on the impact of prenatal iron deficiency and IDA on mental and motor development of children is limited. To our knowledge, only limited cross-sectional observational studies related perinatal iron deficiency to newborns temperament-like behaviors. One study reported higher levels of irritability in infants whose mothers were iron deficient[47]. In another study, lower levels of neonatal hemoglobin concentration and serum iron were correlated with higher levels of negative emotionality and lower levels of alertness and soothability [48]. Tamura et al (2002) observed associations between impaired language ability, fine motor skills and tractability at five years in children and maternal IDA, indicated by low cord serum ferritin concentration[49].

Intervention study results on prenatal micronutrient supplementation (with iron) and child development are inconclusive. A Nepal study found that maternal prenatal supplementation with iron and folic acid was positively associated with general intellectual ability, some aspects of executive function, and motor function in the offspring at 7 to 9 years of age[50]. A study in Indonesia did not find an impact of prenatal supplementation of iron on functional development of infants[51]. Li Q et al. (2009) reported the follow-up of a randomized trial on prenatal supplementation in rural China[52] and demonstrated the benefit of micronutrient supplementation on mental development of children at 12 months of age[53], however, a positive impact of micronutrient supplementation was not shown in 18 and 24 months follow-up assessments (unpublished data). Apparently, the anemia prevalence of the women in the above studies was still high at the end of pregnancy even after iron supplementation [51, 52, 54], which may have compromised the impact of prenatal iron supplementation on the child development.

Conducting a longitudinal study targeting to women's iron status in pregnancy and their offspring's mental and motor development at age 3 to 24 months may provide convincing evidence of an effect of prenatal iron deficiency on developmental outcomes in young children.

Iron deficiency anemia in children <2 years and long-term effect on social emotional behavior

Numerous cross-sectional studies show lower cognitive and motor test scores in infants with iron deficiency anemia (IDA) compared to peers with good iron status [31, 55-59]. Follow-up studies report persisting lower scores among children who had IDA in infancy, even though they received iron treatment as young children[26, 60-62].

Although mental and motor outcomes have generally received most attention, alterations in the social/emotional domain are also consistently observed in infants with IDA. Lozoff et al. (2006) found that compared to a non-IDA controls, infants with IDA are more likely to be fearful, hesitant/wary, unhappy, inactive, easily fatigued, have less endurance, be in close contact with mothers, or vocalize less[31, 60]. Pre-school follow-up studies have found that children with chronic, severe ID in infancy had poorer cognitive and motor development[63-66], as well as lower levels of alertness, physical activity, positive affect, and verbalization than children with good iron status in infancy[67, 68].

Such behavioral and affective alterations have been interpreted as evidence of "functional isolation"[69]. According to the functional isolation hypothesis, nutritional deficiencies contribute to changes in infants' affect, activity or attention that lead them to seek less stimulation from the physical and social environments[70]. In response to infants' behavior, caregivers may offer less stimulation. Over time the alterations in child and caregiver behavior interfere with the child's normal acquisition of environmental information and adversely affect the child's development[26]. However, there is limited research on long-term effects of early affective and behavioral changes due to IDA. Pre-school follow-up studies have found that children with chronic, severe ID in infancy had poorer cognitive and motor development[63-66], as well as lower levels of alertness, physical activity, positive affect, and verbalization than children with good iron status in infancy[67, 68]. Corapci et al (2006) observed mother-child interaction of 5 year old children and found that children with chronic, severe iron deficiency in infancy had lower levels of alertness, physical activity, positive affect and verbalization at the age of five [71]. But the iron status of the preschool children in above studies is unknown, which is an obvious confounder of these studies.

Further research on the effect of IDA in infancy on social emotion among non-IDA preschool-aged children would provide additional evidence on alterations in the social emotional domain as a consequence of IDA in an early age.

STRATEGIES TO PREVENT AND CONTROL IRON DEFICIENCY ANAEMIA

The importance of preventing and treating iron deficiency to improve cognitive and emotional development at a population level cannot be proven solely by showing a relationship between iron deficiency and development in individual children. It must be shown that iron deficiency is preventable and correctable by feasible public health methods[72].

Three main strategies for prevention and control iron deficiency in populations exist, alone or in combination: education combined with dietary modification and/or diversification to improve iron intake and bioavailability; iron supplementation (provision of extra iron, usually in higher doses, in addition to normal food intake); and iron fortification of foods. These strategies can be implemented for pregnant women in order to improve the situation of the child, or directly implemented for child, or by combining these two supply channels.

Strategies for pregnant women —iron supplementation

Although dietary modification and diversification is the most sustainable approach, change of dietary practices and preferences is difficult and need a long period of time[9]. In addition, foods that provide highly bioavailable iron (such as meat) are often unavailable or unaffordable in developing countries[73]. A strategy that yields results in the shorter run is iron supplementation. Iron supplementation can be targeted to high-risk groups (e.g. pregnant women), and can be cost effective[14]. For oral supplementation, ferrous iron salts (ferrous sulphate and ferrous gluconate) are preferred because of their low cost and high bioavailability[9]. A pooled analysis of data from eight studies of iron-folate supplementation during pregnancy suggested an increase of 12 g/L in hemoglobin and a 73% reduction in the risk of anemia at term[74, 75].

In view of higher requirements during gestation, the World Health Organization (WHO) recommends universal distribution of iron-folic supplements to pregnant women in developing countries to prevent iron deficiency anemia[76]. However, pregnant women are often deficient in several other nutrients in the meantime, all of which can negatively affect their health as well as their infants' health, growth and development across the life course[77]. Multiple micronutrients supplements containing iron and other micronutrients should be more efficient to reduce anemia, because other nutrients that are often lacking in the diets of pregnant women in poor populations, including vitamin A, riboflavin and vitamin B_6 and B_{12}, are also needed for hemoglobin synthesis. Improving maternal status of multi micronutrients could also benefit pregnancy outcome, infant micronutrient stores at birth and breast milk content of micronutrients[78].

To assess the impact of the use of multiple micronutrient supplements compared with iron-folic acid supplementation during pregnancy on health outcomes of new born is important to help policy makers determine optimal approaches to improving maternal and infant health.

Strategies for infants and young children—In-home fortification of complementary feeding

Iron fortification is probably the most practical, sustainable, and cost-effective long-term solution to control iron deficiency at the national level. Fortification with low iron doses is more similar to the physiological environment than is supplementation and might be the safest intervention. Fortification of foods with iron is more difficult than it is with other nutrients[9]. The most bioavailable iron compounds are soluble in water or diluted acid, but often react with other food components to cause off -flavours and colour changes, fat oxidation, or both[79]. In recent years, NaFeEDTA is recommended as an iron fortificant for cereal-based foods since the EDTA moiety protects iron from phytate and additionally prevents Fe-catalysed fat oxidation reactions during the storage of cereal flours [80]. NaFeEDTA is reported to be 2-4 fold better absorbed than $FeSO_4$ from a variety of cereal-based foods [81]. But it is approved as a food additive only at 0.2 mg iron a day as NaFeEDTA per kg bodyweight, which limits its usefulness as a fortificant for infants and children[9]. This translates to a maximum of 2.7 mg iron per day as NaFeEDTA for a child aged 6 months with an average body weight of 7.5 kg [82]. This amount of iron is considered not high enough to be able to use NaFeEDTA alone to provide an adequate amount of iron in a complementary food to meet the iron requirements of a 6 month old child (7.7 mg/day for 6-12 months old children with diets of 12% bioavailability)[83]. One option, which utilizes the enhancing effect of EDTA, but does not exceed the Acceptable Daily Intake of EDTA, is to fortify the complementary food with a mixture of ferrous sulfate ($FeSO_4$) and NaFeEDTA.

Studies on iron absorption of a mixture of ferrous sulfate ($FeSO_4$) and NaFeEDTA among young children may provide additional evidence on in-home fortification of phytate containing complementary food.

RATIONALE AND OUTLINE

Iron deficiency is a common problem among pregnancy women and infants and young children. Among the numerous biological effects of iron, there is considerable evidence that iron is also important for neurological function and development[84]. However a causal relation between iron deficiency and poorer brain function has not been proven. The demonstrated associations between anemia and poor development in correlational or case control studies cannot establish cause-and effect relationships. They provide no information as to the timing of any relationship and it is possible that poor development precedes iron deficiency. In addition, there is considerable evidence that IDA is associated with a large number of socioeconomic and biomedical disadvantages that can themselves affect children's development [26]. Longitudinal observational studies give additional useful information about the long-term prognosis of children with IDA and the types of deficits at different stages of development. Longitudinal observational studies is a first step towards making causal inferences[26].

Meanwhile, there is evidence showing that a high prevalence of iron deficiency anemia in the developing world has substantial health and economic costs[9]. Thus, to explore effective and optimal approaches for iron supplementation and iron fortification among high risk populations (i.e., pregnant women, infants and young children) is of significance for public health.

The main aim of the research presented in this thesis is threefold: i) To explore long-term effects of IDA in pregnancy and IDA in children under two years of age on children's cognitive psychomotor and social emotional development, ii) To understand the impact of pre-natal multiple micronutrients supplementation on health outcomes of newborns, and iii) To investigate iron absorption from a complementary food fortified with a mixture of $FeSO_4$ and NaFeEDTA.

The specific research questions were:

i. Does IDA in pregnancy and/or in children under 2 years of age have a long-term impact on children's cognitive psychomotor and social emotional development?

ii. Are multiple micronutrient supplements as effective as iron-folic acid on newborns health and developmental outcomes?

iii. Will a mixture of $FeSO_4$ and NaFeEDTA as a fortificant of complementary food have a higher absorption compared to $FeSO_4$ alone?

Analysis of data sets from a randomized controlled trial and its follow up on prenatal supplementation (**chapter 2**) and in-home fortification of complementary feeding and its follow up study (**chapter 3**) were used to provide evidence of the above question on long-term impact on children's cognitive and psychomotor function and social emotional behavior of IDA in pregnancy and IDA in children under 2 years of age. A randomized controlled trial comparing the impact of multiple micronutrients supplements and iron-folic acid supplements on health outcome of newborn is described in **chapter 4**, and a laboratory experiment comparing iron absorption of a mixture of $FeSO_4$ and NaFeEDTA to $FeSO_4$ as a fortificant of complementary food based on erythrocyte incorporation of stable iron isotopes is presented in **chapter 5**.

The location of our randomized controlled study and longitudinal studies were in socioeconomically disadvantaged areas of western China, Shaanxi province and Gansu province. The location of iron absorption study was in Yuanshi county, Hebei province.

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Chapter 2

Effect of iron deficiency anemia in pregnancy on child mental development in rural western China

—Follow up of randomized trial of prenatal supplementation

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Submitted

Abstract

Objective Impact of Iron Deficiency Anaemia (IDA) in pregnancy on young child development

Method A two years follow-up of 850 children born to women who participated in a double blind cluster randomised controlled trial of prenatal micronutrient supplementation in western rural China. These women were randomly assigned to receive either daily folic acid, iron/folic acid (60 mg iron), or multiple micronutrients (with 30 mg iron) during pregnancy. Children were categorized into Prenatal IDA group and Prenatal Non-IDA group based on the mother's Haemoglobin in the third trimester. Each group contained 3 subgroups based on mother's treatment: folic acid, iron/folic acid and multiple micronutrients. Bayley Scales of Infant Development were administrated to the children to assess their development at 3, 6, 12, 18 and 24 months of age.

Results Compared to the Prenatal Non-IDA group, the Prenatal IDA group showed significantly lower Mental Development Index (MDI) at 12, 18 and 24 months of age. The adjusted mean difference was 5.8 (95% Confidence interval [CI] 1.1 to 10.5), 5.1 (95% CI 1.2 to 9.0) and 5.3 (95% CI 0.9 to 9.7) respectively. Further analysis showed MDI in the Prenatal IDA and Non-Prenatal IDA groups were similar with supplementation of Iron/folic acid, but significantly lower in the Prenatal IDA group with supplementation of folic acid or multiple micronutrients.

Conclusion Prenatal IDA in the third trimester is associated to mental development of child. However, prenatal supplementation with sufficient iron protects child development even when woman's IDA was not properly corrected in pregnancy.

INTRODUCTION

Young children and women of reproductive age, especially during pregnancy, have increased iron requirements, placing them at increased risk of deficiency and related adverse consequences[1]. Iron is essential for neurotransmission, energy metabolism and myelination in the developing brain[2]. In humans, the brain growth spurt begins in the last trimester of pregnancy and extends through the first two years of life[3]. Numerous studies show lower cognitive and motor test scores in infants with iron deficiency anaemia (IDA) that persist being low even though they received iron treatment as infants [2]. This impact on child development has been demonstrated to be due to reduced brain iron[4].

Brain iron deficiency in the fetus or neonate could be more detrimental than postnatal iron deficiency because of the rapidity of brain growth during in pregnancy[5]. Animal studies demonstrated that proper iron nutrition during perinatal development is critical not only for obtaining adequate levels of brain iron, but also for normal behavioral and motor development in the offspring[6, 7]. During pregnancy, the iron requirements of the pregnant women are increased three-fold to cover needs of expansion of maternal red-cell mass and growth of the fetal-placenta, and maternal anemia develops unless these needs are met[8, 9]. Epidemiologic studies suggest that maternal iron deficiency contributes to reduced fetal iron stores[10-16], and infants born to anemic mothers have low iron stores and are more likely to develop anemia [11, 12, 17-19]. However, knowledge on the impact of Prenatal iron deficiency and IDA on mental and motor development of children is limited.

Study results on prenatal micronutrient supplementation (with iron) and child development are inconclusive. Nepal study found maternal prenatal supplementation with iron and folic acid was positively associated with general intellectual ability, some aspects of executive function, and motor function in offspring at 7 to 9 years of age[20], Indonesia study did not find an impact of supplementation of iron on functional development of infants[21]. Li Q reported the follow-up of a randomized trial on prenatal supplementation in rural China[22] and demonstrated the benefit of MMN on mental development of children at 12 months of age[23], but positive impact of MMN was not shown in 18 and 24 months follow-up assessments (unpublished data). Apparently, the anemia prevalence of the women in above studies was still high at the end of pregnancy even after iron supplementation[21, 22, 24], which may have compromised the impact of iron supplementation on the child development.

Present study followed the same randomized trial with Li Q's study. We followed children till 24 months and analyzed with a different approach to explore relation between iron status in pregnancy and child development. We predict that IDA in pregnancy is associated to the development of child; and iron supplementation in pregnancy is beneficial to child development.

SUBJECTS AND METHODS

This study focused on the follow-up assessments of mental and psychomotor development of children at 3, 6, 12, 18, 24 months of age. These children were born to women involved in a double blind cluster randomised controlled trial of prenatal micronutrient supplementation. The aim of the original project was to evaluate the impact of prenatal micronutrient supplementation on birth weight, duration of pregnancy and perinatal mortality in rural western China during year 2002 to 2006. The details of the trial have been described elsewhere[22]. Briefly, the trial was conducted in two poor rural counties. Villages were randomly assigned to three treatments: MMN, Iron/ folic acid or Folic acid supplementation before recruitment of subjects. The MMN were formulated to contain approximately the WHO/UNICEF recommended dietary allowances for each of 15 minerals or vitamins as follows: 30 mg iron, 400 µg folate, 15 mg Zinc, 2.0 mg copper, 65.0µg selenium, 150.0 µg iodine, 800 µg vitamin A, 1.4 mg vitamin B_1 (thiamine), 1.4 mg vitamin B_2 (riboflavin), 1.9 mg vitamin B_6, 2.6 µg vitamin B_{12}, 5 µg vitamin D, 70 µg vitamin C, 10 mg vitamin E, and 18 mg niacin. The iron/folic acid supplementation contained 60 mg iron and 400 µg of folic acid. The folic acid supplementation contained 400 µg of folic acid.

Figure 1 summarizes the recruitment, randomization and participation of subjects with the trial and this study. All women resident in the project sites who became pregnant during the study period, fulfilled trial selection and filled the consent of this trial were recruited by village doctors. Newly identified pregnant women received an initial prenatal care check-up for baseline information. Altogether 5828 eligible women were recruited in the trial. Among them, 16.7% of the women did not attend the haemoglobin(Hb) check due to losing to follow-up, withdrawing from the trial or fetal losses before third trimester, and the other 28.3% of the women missed the Prenatal care check in the third trimester. Around 55% (3233) of the women attended the haemoglobin check in the middle of the third trimester.

During 2004-2006, a follow-up study was conducted to assess the development of these children. 1286 women with a singleton full term live birth attended the follow-up study. The present study focused on women who tested Hb and attended the follow- up study: 850 women and their children met this inclusion criterion. Among them, 95 (11%) young children missed their first development assessment, 61 (7%) missed the second assessment, 84 (10%), 96 (11%) and 96 (11%) missed their third to fifth assessment respectively. 600(71%) child were assessed on all 5 occasions (Fig.1). The women and their children were categorized into Prenatal-IDA group and Non-IDA group based on the Hb in the third trimester. Each group contained three subgroups based on mother's treatment: MMN, Iron/folic acid and folic acid subgroup.

Data collection

Previous published papers of this trial described data collection during pregnancy and delivery[22], and data collection of the follow-up study[23]. Bayley Scales of Infant Development (BSID) were used as a measure of each child's development. The three parts of BSID are considered complementary: the Mental Scale is designed to assess sensory-perceptual acuities and discrimination, and the ability to respond to these; the Psychomotor Scale is designed to provide a measure of the degree of the control of the body, coordination of the large muscles, and finer motor skills of hands and fingers; and the Infant Behaviour Record (IBR) helps in assessing the nature of the child's social and objective orientation toward his or her environment[25]. BSID was translated into Chinese and locally standardized to become culturally appropriate[26].

The BSID were administered at the village clinic or the child's own home in a standardized manner. Both examiners and participating women were blinded with respect to supplementation group. The child's development and its feeding practices were proposed to be assessed at 3, 6, 12, 18, 24 months of age; however, because of logistical problems, this schedule could not always be followed. If the child was sick, was unavailable, or could not cooperate, then assessment was arranged for a later date within one month. Mental Development crude score (MD) and Psychomotor Development crude score (PD) signify the items that a child passes on the Mental Scale and Psychomotor Scale of the BSID, respectively. Mental Development Index (MDI) and Psychomotor Development Index (PDI) are nonlinear transformations of the MD and PD, by using standard procedures that are based on data for Chinese children[27, 28]. We did not report IBR in this paper.

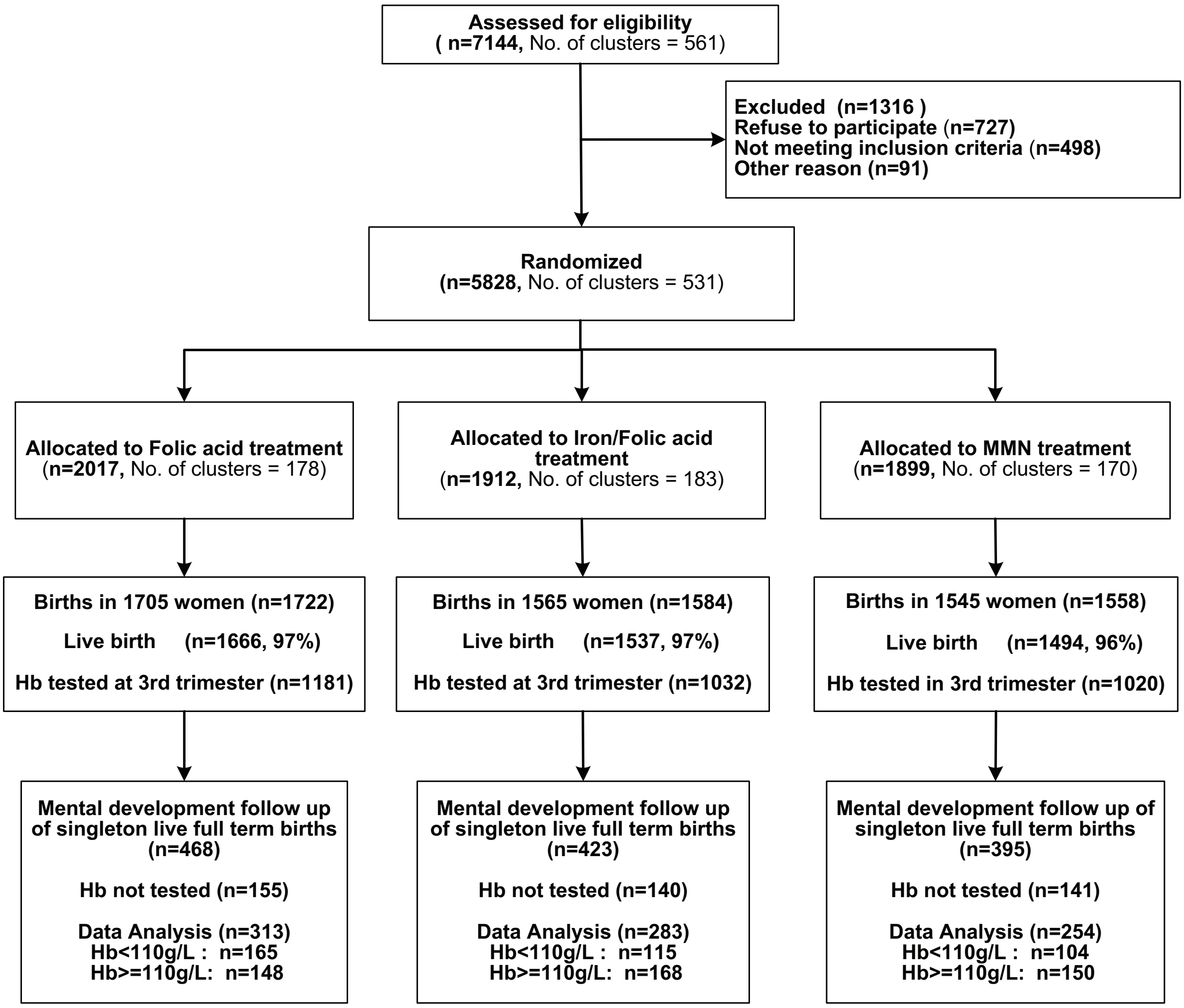

Figure 1 Participants flow chart

Iron status was determined on the basis of Hb at the third trimester by HemoCue portable spectrophotometers (Ängelholm, Sweden). They were calibrated daily and all measurements were made within the temperature operating range of this device (15-30°C) by research team members. The cut-off for anaemia in pregnancy women was Hb <110 g/L[29].

Data analysis

All data were checked manually for completeness and were double-entered into a data management system. We conducted range, extremum and logical checks for accuracy. We compared baseline characteristics between women who were and who were not involved in the present study, and between Prenatal-IDA group and Non-IDA group by using analysis of variance or x^2 tests.

IMPUTE method was used to fill the missing values of MD and PD in each assessment by linear regression between age of children with MD and PD. General linear model (GLM) repeated measurement was used to compare the overall MDI, PDI of the five assessments and MDI, PDI per assessment. The mean differences for the MDI, PDI between the two groups were adjusted for treatment, interaction term between treatment and Hb concentration in the third trimester[23]. We considered baseline characteristics that differed between the two groups as potential confounders in the GLM repeated measurement adjusted model: low birth weight, mother's age at enrolment, gestation weeks when Hb checked, parity of pregnant women, gestation weeks at birth, child's age at assessment.

All analysis was conducted with Stata version 9.2 (Stata/SE 9.2 StataCorp, College Station, TX). All reported p values were 2-tailed and values of <0.05 were considered to be statistically significant.

RESULTS

Baseline characteristics of the women who were and who were not involved in this study are comparable (data not shown). Table 1 shows the baseline characteristics of the pregnant women and their children in the present study. The socio-economic characteristics, women's education, women's BMI at enrolment, mean gestation weeks at enrolment and BMI at enrolment of women were comparable between the two groups. Pregnant women in the Prenatal-IDA group reported significantly older age(p=0.024), greater parity(p=0.025) at enrolment and smaller gestation week at Hb checked(P=0.006). The child birth weight, gestation weeks at birth, gender distribution, breastfeeding rate, formula introduction, prevalence of stunting and underweight of children and age at each assessment were comparable between the two groups except child age at 18 month assessment(p=0.001) breastfeeding rate at 3 months(p=0.013), formula introduction at 24 months(p=0.025).

Table 1. Comparison of baseline characteristics of the women and children in prenatal IDA group and Non-IDA group by iron status in third trimester, Shaanxi Province, China 2004-2006

		Iron status in third trimester		
		Prenatal IDA N=384	Non-IDA N=466	P values
Pregnant women				
Age at enrollment* (yrs)	Mean (SD)	24.9 (4.5)	24.1 (4.5)	0.024
BMI at enrolment (kg/m2)	Mean (SD)	20.8 (2.2)	20.8 (2.3)	0.946
Gestation at enrollment (weeks)	Mean (SD)	13.7 (5.7)	13.5 (5.5)	0.604
Gestation Hb checked* (weeks)	Mean (SD)	32.1 (2.3)	32.6 (2.5)	0.006
Women's education				0.213
	< 3 years	20 (5.2)	19 (4.1)	
	Primary	103 (26.8)	102 (21.9)	
	Secondary	204 (53.1)	280 (60.2)	
	High school+	57 (14.8)	64 (13.8)	
Household wealth				0.751
	Poor	91 (23.7)	112 (24.0)	
	Middle	155 (40.4)	178 (38.2)	
	Wealth	138 (35.9)	176 (37.8)	
Parity at enrolment*				0.025
	0	237 (61.7)	321 (68.9)	
	1	122 (31.8)	123 (26.4)	
	> 2	25 (6.5)	22 (4.7)	

* <0.05 Figures are number (percentage) for remaining variables.

Table 1. Comparison of baseline characteristics of the women and children in prenatal IDA group and Non-IDA group by iron status in third trimester, Shaanxi Province, China 2004-2006 (con't)

		Iron status in third trimester		
		Prenatal IDA N=384	Non-IDA N=466	P values
Gender				0.141
	Boy	244 (64)	273 (59)	
	Girl	140 (36)	193 (41)	
Age of the 5 assessment (months)				
3	Mean (SD)	3.2 (0.2)	3.2 (0.2)	0.495
6	Mean (SD)	6.2 (0.3)	6.2 (0.3)	0.632
12	Mean (SD)	12.3 (0.5)	12.3 (0.4)	0.941
18	Mean (SD)	18.4 (0.5)	18.3 (0.4)	0.001
24	Mean (SD)	24.5 (0.5)	24.4 (0.5)	0.18
Breast feeding (months)				
3		324 (87)	421 (92)	0.013
6		292 (80)	362 (81)	0.567
Formula introduction(months)				
3		46 (12)	47 (10)	0.344
6		58 (15)	65 (14)	0.748
12		77 (20)	93 (20)	0.887
18		108 (28)	117 (25)	0.423
24		100 (26)	89 (19)	0.025
Underweight(months)				
3		15 (4)	14 (3)	0.599
6		8 (2)	14 (3)	0.157
12		15 (4)	14 (3)	0.308
18		8 (2)	9 (2)	0.951
24		8 (2)	9 (2)	0.85
Stunting(months)				
3		46 (12)	47 (10)	0.289
6		69 (18)	75 (16)	0.346
12		42 (11)	47 (10)	0.442
18		54 (14)	65 (14)	0.872
24		54 (14)	61 (13)	0.897

* <0.05 Figures are number (percentage) for remaining variables.

Impact of prenatal iron status on mental development of young children

Table 2 summarizes study findings related to MDI of children during the first 24 months of life. Overall analysis showed significantly lower MDI(P=0.036) in the Prenatal-IDA group. Assessed at individual time point, the Prenatal-IDA group showed significantly lower MDI at 12, 18 and 24 months of age. The adjusted mean MDI difference was 5.8 (95% CI 1.1 to 10.5), 5.1 (95% CI 1.2 to 9.0) and 5.3 (95% CI 0.9 to9.7) respectively.

Table 2. Comparison of mean, standard deviation and 95% confidence intervals of adjusted difference of MDI of children born to mothers enrolled in the prenatal supplementation trial by Prenatal iron status in third trimester, Shaanxi Province, China 2004-2006

		Number	Mean (SD)	Adjusted Difference (95%CI)*	P values*
3 months age	Prenatal IDA	384	118.6 (21.4)		
	Non-IDA	466	118.3 (20.0)	-3.2 (-8.3, 1.9)	0.224
6 months age	Prenatal IDA	384	98.7 (17.0)		
	Non-IDA	466	99.6 (17.4)	1.8 (-2.6, 6.1)	0.42
12 months age	Prenatal IDA	384	103.1 (19.0)		
	Non-IDA	466	105.0 (18.8)	5.8 (1.1, 10.5)	0.016
18 months age	Prenatal IDA	384	98.0 (15.3)		
	Non-IDA	466	100.2 (15.7)	5.1 (1.2, 9.0)	0.01
24 months age	Prenatal IDA	384	87.9 (18.2)		
	Non-IDA	466	90.9 (16.8)	5.3 (0.9, 9.7)	0.017

*Adjusted by treatment, interaction between treatment and gestation Hb, low birth weight, mother age at enrollment, gestation week when Hb checked, parity, gestation week at delivery, and age of children at assessment.

Impact of prenatal iron status on psychomotor development of young children

Table 3 describes PDI of children at the 5 assessment points. No significant differences in PDI (P=0.097) were found between in the two groups.

Modifying effect of micronutrient supplementation in pregnancy on the response of prenatal iron status on child development

We had initially hypothesized that iron supplementation in pregnancy would be beneficial to child development. Accordingly, a subgroup analysis was conducted to explore the modifying effect of micronutrient supplementations in pregnancy on the response of prenatal iron status on child development.

Table 4 and 5 summarized MDI and PDI by prenatal iron status and treatment groups. Prenatal-IDA group children whose mother received prenatal Folic acid or MMN had lower MDI to their peers in Non-IDA group (p=0.046 in Folic acid & p=0.034 in MMN), with significant lower MDI in 3, 18 and 24 months assessment of folic acid subgroups, and in 12, 18 and 24 months assessments of MMN subgroups. While prenatal-IDA group children whose mother received prenatal Iron/folic acid (with 60 mg iron) supplementation had comparable MDI in all the five assessments (P=0.641).

We did not find significant difference PDI between the two groups regardless of treatments.

Table 3. Comparison of mean, standard deviation and 95% confidence intervals of adjusted difference of PDI scores of children born to mothers enrolled in the prenatal supplementation trial by gestation iron status in third trimester, Shaanxi Province, China 2004-2006

		Number	Mean (SD)	Adjusted Difference (95%CI)*	P values*
3 months age					
	Prenatal IDA	384	133.0 (17.0)		
	Non-IDA	466	133.2 (16.7)	-1.7 (-5.9, 2.5)	0.429
6 months age					
	Prenatal IDA	384	110.0 (23.7)		
	Non-IDA	466	111.5 (24.3)	3.4 (-2.6, 9.5)	0.262
12 months age					
	Prenatal IDA	384	98.3 (16.2)		
	Non-IDA	466	100.5 (15.9)	2.5 (-1.5, 6.5)	0.226
18 months age					
	Prenatal IDA	384	106.0 (21.9)		
	Non-IDA	466	107.8 (21.0)	3.3 (-2.1, 8.6)	0.229
24 months age					
	Prenatal IDA	384	103.9 (12.8)		
	Non-IDA	466	103.9 (12.4)	3.8 (0.6, 6.9)	0.019

*Adjusted by treatment, interaction between treatment and gestation Hb, low birth weight, mother age at enrollment, gestation week when Hb checked, parity, gestation week at delivery, and age of children at assessment.

DISCUSSION

Our findings show that IDA in pregnancy is associated to mental development of young children. Further analysis on subgroups shows that the differences on MDI between the two groups only present in Folic acid and MMN subgroups but non-significant suggesting in Iron/folic acid subgroup with 60 mg iron supplemented daily.

This study followed a randomized trial design on prenatal supplementation and explored impact of prenatal IDA on development of children. There is no sample selection bias between this study and the randomized trial. To our knowledge, this is the first longitudinal observation on prenatal iron status and child development. We got consistent results for all the five assessments in children 3-24 months of age. Our study was in a socioeconomically disadvantaged area, with no child iron supplementation after birth. These allowed us to observe the impact of prenatal iron status and iron supplementation on child development.

The study was limited with respect to measurement of iron status. IDA was estimated by Hb in this study. Hb generally overestimates the prevalence of IDA, as it also accounts for anaemia due to other nutritional deficiencies, infections, hemoglobinopathies, or ethnic differences in normal haemoglobin distribution[30]. From results of the baseline survey of this trial showing anaemia prevalence among children less than 2 years, reproductive aged women and adult men of 33.2%, 37.6% and 8.8% respectively (unpublished data) ,

Table 4. MDI by Prenatal iron status in third trimester and treatment groups

		Folic acid		Iron/folic acid		MMN		P values (stratified by treatment)		
		N	Mean (SD)	N	Mean (SD)	N	Mean (SD)	Folic acid	Iron/folic acid	MMN
MDI										
3 months age								0.057	0.763	0.483
	Prenatal IDA	165	116.2 (20.2)	115	120.4 (21.2)	104	120.3 (23.2)			
	Non-IDA	148	121.4 (21.9)	168	117.3 (18.8)	150	116.4 (18.9)			
6 months age								0.487	0.807	0.772
	Prenatal IDA	165	98.1 (16.4)	115	99.0 (17.1)	104	99.3 (18.1)			
	Non-IDA	148	99.3 (16.0)	168	99.0 (17.4)	150	100.4 (18.9)			
12 months age								0.796	0.917	0.030
	Prenatal IDA	165	103.2 (18.2)	115	103.6 (18.4)	104	102.6 (21.0)			
	Non-IDA	148	103.9 (18.8)	168	103.4 (20.0)	150	108.0 (17.2)			
18 months age								0.049	0.341	0.036
	Prenatal IDA	165	97.1 (16.5)	115	100.3 (12.6)	104	96.7 (15.7)			
	Non-IDA	148	100.2 (15.7)	168	98.9 (14.9)	150	101.7 (16.6)			
24 months age								0.042	0.676	0.026
	Prenatal IDA	165	86.7 (17.4)	115	90.9 (15.7)	104	86.6 (21.6)			
	Non-IDA	148	91.1 (17.1)	168	89.9 (16.4)	150	91.8 (17.1)			

*Stratified by treatment and adjusted low birth weight, mother age at enrollment, gestation week when Hb checked, parity, gestation week at delivery, and age of children at assessment.

Table 5. PDI by Prenatal iron status in third trimester and treatment groups

		Folic acid		Iron/folic acid		MMN		P values (stratified by treatment)		
		N	Mean (SD)	N	Mean (SD)	N	Mean (SD)	Folic acid	Iron/folic acid	MMN
PDI										
3 months age								0.140	0.930	0.832
	Prenatal IDA	165	131.2 (16.7)	115	134.2 (18.0)	104	134.7 (16.5)			
	Non-IDA	148	134.3 (18.6)	168	132.6 (17.0)	150	132.7 (14.1)			
6 months age								0.449	0.757	0.361
	Prenatal IDA	165	109.8 (22.4)	115	110.1 (24.2)	104	110.1 (25.2)			
	Non-IDA	148	112.0 (23.2)	168	110.1 (24.6)	150	112.7 (25.1)			
12 months age								0.322	0.167	0.470
	Prenatal IDA	165	98.3 (17.8)	115	98.6 (15.4)	104	98.1 (14.4)			
	Non-IDA	148	100.8 (18.1)	168	100.2 (15.4)	150	100.4 (14.0)			
18 months age								0.063	0.069	0.435
	Prenatal IDA	165	103.8 (22.6)	115	110.7 (19.6)	104	104.5 (22.7)			
	Non-IDA	148	109.8 (21.1)	168	106.3 (22.0)	150	107.6 (19.6)			
24 months age								0.711	0.240	0.063
	Prenatal IDA	165	104.5 (13.4)	115	105.6 (11.2)	104	101.0 (13.2)			
	Non-IDA	148	103.2 (12.2)	168	103.7 (12.2)	150	104.6 (12.8)			

*Stratified by treatment and adjusted low birth weight, mother age at enrollment, gestation week when Hb checked, parity, gestation week at delivery, and age of children at assessment.

we can conclude that iron deficiency was the main cause of anaemia in our study area[31]. Furthermore, for women with adequate iron, Hb start to decline during the early part of the first trimester, reach their nadir near the end of second trimester because of the physiology response, including plasma volume expansion, then gradually rise during the third trimester[32]. Studies among Chinese pregnant women showed Hb did not improve from the second to third trimester, providing evidence that pregnant women's iron stores were not sufficient to continue the expansion of red cell mass in the third trimester[33-35]. Hence, the high prevalence of anaemia in third trimester was mainly due to iron deficiency in China.

We cannot eliminate the possibility that the altered behavior of the prenatal-IDA group in this study was caused by some relevant but unmeasured factor(s), such as maternal depression and other environmental disadvantages, which could account lack of stimulation (leading to altered behavior and development).

Another limitation of this work was the low power to detect changes in the MDI and PDI because the findings presented are from *post hoc*. We have 64% power to detect 5 scores change in MDI and 73% power to detect 4 scores change in PDI with a=0.05. Our results require replication with larger sample size.

The impact on child development in prenatal-IDA group could have occurred during the period of foetal brain development or/and during young children development. Animal models have demonstrated neurometabolic, structural, electrophysiological and behavioural alterations in developing rats subjected to prenatal iron deficiency[36-38]. The postnatal consumption of iron-adequate diets among marginal iron offspring will not fully reverse all of the observed biochemical disturbances[39]. In human, when maternal iron status is poor, the number of placental transferrin receptors increases so that more iron is taken up by the placenta. Evidence is accumulating that the capacity of this system may be inadequate to maintain iron transfer to the fetus when mother is iron deficient[40]. Maternal Hb were significantly correlated with cord blood erythrocyte count, Hb, and haematocrit values, and these were significantly lower when maternal Hb fell low below 110 g/L at 32-35 weeks of gestation[41], new born infants with low cord blood Hb and iron have altered temperament during the first week of life[42]. In our study, the prenatal IDA appears to have impacted fetus brain iron and have adverse effects on neurodevelopment.

Publications have suggested that prenatal IDA is related to reduced fetal iron stores. Infants whose mothers with mild or moderate IDA during pregnancy are at risk of iron deficiency [17, 18], and infants with IDA show poorer cognitive, motor, social-emotional, and neurophysiologic functioning than those without[3, 4, 25, 43, 44]. In our population, children were not provided iron supplementation after birth. Children in the two groups have no differences in terms of feeding practices, growth and other related background characteristics. Probably, more children in prenatal-IDA group suffered IDA due to low fetal iron stores, which can explain delayed mental development of children in Prenatal-IDA group. We did not test children's Hb in our follow-up assessments for ethical considerations.

Our finding that the impacted children development was not presented in the Iron/folic acid group (with 60 mg iron) even when the mothers' IDA was not corrected demonstrated the benefit of sufficiently iron supplementation during pregnancy. Most fetal iron uptake occurs after week 30, fetal and placental iron needs are presumably met by increased efficiency of maternal iron absorption[41]. Iron supplementation in pregnancy improves maternal iron status [41] and can reduce the extent of iron depletion in the third trimester[45] . However, for women who enter pregnancy already with low iron stores, iron supplements fails to prevent iron deficiency[40]. In our randomised trial, the prenatal anaemia prevalence in the third trimester was still high, being 43.1%, 45.1%, and 61% among the MMN, Iron/folic acid and Folic acid groups[22], which showed a high percentage of pregnancy women were with exhaustion of iron stores. Prenatal iron supplementation studies in other poverty areas also showed high prevalence of prenatal anaemia at the end of pregnancy[12, 46]. The compliance and timing of supplementation may explain some of the problem, but in our study the rates of adherence were 93%, 92% and 93% among Folic, Iron/ folic acid and MMN group, and the mean number of supplementation consumed was 165, 166 and 165 respectively[47]. In this context, it seems many women were iron deficiency when entered pregnancy and supplementing iron was not sufficient to cover the needs during pregnancy. Study results of other researches that IDA in pregnancy compromised fetal iron reserves[41, 48], and iron needs of the fetus take priority over maternal requirements[49] support our result on the benefit of prenatal iron supplementation. Previous study[23] followed the same randomized trial[22] and showed benefit of prenatal MMN on mental development on 12 months of age. The inconsistent conclusion with present study is probably because the Prenatal iron status was not considered in previous' analysis.

CONCLUSION AND IMPLICATIONS

In summary, prenatal IDA impacts children mental development. Sufficient iron supplementation during pregnancy is beneficial to mental development of children in areas with poor iron intakes. Our results support the practice of routine iron supplementation during pregnancy in poor rural areas of China.

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Chapter 3

Iron Deficiency Anemia in Infancy and Social Emotional Development in Preschool-age Chinese Children

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Abstract

Objective We aimed to compare affect and behavior of three groups of non-anemic 4-year-old children: children with iron deficiency anemia (IDA) in infancy whose anemia was not corrected before 24 months (chronic IDA) (n=27); children with IDA in infancy whose anemia was corrected before 24 months (corrected IDA) (n=70), and children who were non-anemic in infancy and at 24 months (n=64).

Method Mother and child dyads were invited to a local clinic room. Children's social referencing, wariness, frustration tolerance behavior and affect were observed from a set of laboratory contexts, including free play, stranger approach, novel toy, and delay of gratification. The whole procedure was videotaped. Child affective and behavioral display was coded using a time-sampling (5-second segments) code scheme. Iron status of children was based on fingerstick hemoglobin concentration in infancy, 24 months, and 4 years. Hemoglobin concentration was determined by the cyanomethemoglobin method.

Result Children who had chronic IDA in infancy displayed less positive affect, less frustration tolerance, more passive behavior, and more physical self-soothing in the stranger approach and delay of gratification. In contrast, children whose anemia was corrected before 24 months were comparable to children who were non-anemic throughout infancy in terms of behavior and affect.

Conclusion The results point to the potential benefits of preventing iron deficiency in infancy and treating it before it becomes chronic or severe.

INTRODUCTION

Iron deficiency is considered to be the most common nutritional disorder in the world, and infants are particularly at risk[1]. A survey of Chinese children in 2001 showed the prevalence of iron deficiency (ID) and iron deficiency anemia (IDA) to be 65.5% and 20.8%, respectively, among infants under 12 months and 43.7%, and 7.8%, respectively, among children aged 12-36 months [2].

Numerous studies show lower cognitive and motor test scores in infants with IDA compared to infants with good iron status[3-8]. Follow-up studies report persisting lower scores among children who had IDA in infancy, even though they received iron treatment as young children[9-11]. Although mental and motor findings have generally received the most attention, alterations in the social/emotional domain are also consistently observed in infants with IDA. Compared to non-anemic infants, infants with IDA are more likely to be fearful, hesitant/wary, unhappy, inactive, easily fatigued, have less endurance, be in close contact with mothers, or vocalize less[3, 9].

Such behavioral and affective alterations have been interpreted as evidence of “functional isolation”[12]. According to the functional isolation hypothesis, nutritional deficiencies contribute to changes in infants’ affect, activity or attention that lead them to seek less stimulation from the physical and social environments[13]. In response to infants’ behavior, caregivers may offer less stimulation. Over time the alterations in child and caregiver behavior interfere with the child’s normal acquisition of environmental information and adversely affect the child’s development[14]. Pre-school follow-up studies have found that children with chronic, severe ID in infancy had poorer cognitive and motor development[15-18], as well as lower levels of alertness, physical activity, positive affect, and verbalization than children with good iron status in infancy[19, 20].

This paper included more stressful laboratory contexts to elicit long-term social emotional impact of IDA in infancy. Based on the functional isolation hypothesis and past researches, we hypothesized that affective and behavior differences associated with infancy IDA would be long-lasting. Specifically, we predicted that non-anemic preschool-aged children who had chronic IDA in infancy would show altered affect and behavior compared to those who were non-anemic throughout infancy.

SUBJECT AND METHODS

This study focused on affect and behavior at the 4-year follow-up assessment of a longitudinal complementary food supplement project. The aim of the original project was to evaluate the effects of a complementary food supplement among infants and young children in 5 poor counties of Gansu province in the northwestern part of China during 2001-2003. In each county, 3 villages were randomly chosen. With the help of village doctors, we enrolled all children aged 4-12 months in the villages until the number reached 200 per county. These children were recruited as the experimental group. We selected another 100 children per county as the control group in the nearby villages using the same sampling method. One thousand children in the experimental group received a sachet containing 10 g of nutrient-dense complementary food supplement to be added to their normal diet. The composition of the supplement was as follows: 6 mg iron, 4.1 mg zinc, 385 mg calcium, 0.2 mg vitamin B_2, 7.0 μg vitamin D, 3.8 g protein. The total energy of a sachet was 167kJ. Five hundred children in control group received a sachet of rice flour with added vegetable oil to match the total energy of the experimental supplement. All children were supplemented up to 24 months of age. All were given vitamin A capsules every 6 months[21]. Weight and height measurements were taken every 3 months, and hemoglobin concentration was tested every 6 months. The number of children who attended the 24-month-old assessment in experimental and control groups was 642 and 352, respectively.

In 2004, children recruited in the original study were evaluated at 4 years of age. Weight, height and hemoglobin concentration were measured, behavior and development were assessed. A total of 404 children attended the 4-year follow-up assessment. All methods were reviewed and approved by the Ethics Review Committee of the China CDC, and informed oral consent was obtained from all parents. Details of the original survey and follow-up study have been published elsewhere[21].

The present study focused on children who attended the baseline survey, 24-month assessment, and 4-year follow-up: 232 children met this inclusion criterion. We excluded 71 children who were anemic at the 4-year follow-up assessment, since preschool-aged children with IDA may show altered affect and behavior[22]. Data for 161 children are reported here. They did not differ in background characteristics from children with incomplete data. None of children involved attended preschool or day care and more than 97% of the mothers were farmers.

Twenty-seven children who were anemic before 12 months, still anemic at the 24-month assessment, and non-anemic at the 4-year follow-up were defined as Chronic-IDA-Group. Seventy children who were anemic before 12 months and non-anemic at the 24-month assessment and 4-year follow-up assessment were defined as Corrected-IDA-Group. Sixty-four children who were non-anemic at all time-points were defined as Non-Anemic-Group.

Data Collection

Procedure

Based on cross-cultural research by Chen and colleagues[23] and Goldsmith et al's Assessment Battery[24], we designed a set of laboratory contexts to measure children's social referencing and wariness/inhibition, frustration tolerance and affect. Mother and child were invited to a clinic room and spent 10 minutes in free play. During this period, we provided some toys and puzzle tasks. After free play, a male stranger came in, sat in front of the child, and engaged the child in a scripted conversation for 5 minutes (stranger approach). The stranger then left after telling the child that he would show something special to her/him. The stranger came back with a box, put the box on the table where the child was seated, and then removed the cover of the box. A special toy popped out, and the stranger showed an expression of astonishment (novel toy). In the last episode, the experimenter came in and gave the child a snack, asking the child not to touch or eat it until the experimenter came back again (delay of gratification). The experimenter returned in 1 minute. The whole procedure was videotaped. The mother was present throughout the process and was informed not to give any instructions to the child.

Behavior coding

We coded child affective display using a time-sampling (5-second interval) coding scheme. Positive affect was coded for clear verbal or nonverbal expressions of joy and pleasure in mother-child interactions, such as laughing, smiling, singing happily, jumping with joy, or saying "I am so happy!". Negative affect was coded for the presence of anger, sadness, or crying. Unengaged affect was coded for quiet and uninvolved. Social referencing was coded as looking toward mother or talking to mother during the conversation with stranger, or before touching the novel toy or the snack during the frustration situation. Latency to touch the novel toy, whether being in proximity (within arm length) to mother in stranger approach, whether response to the stranger, and the time spent with passive behaviors in the delay of gratification were coded to index wariness or inhibition behavior. Passive behavior was defined as doing nothing, only looking at the snack. The latency to touch the snack and physical self-soothing in the snack delay situation were also coded. Physical self-soothing included thumb sucking, cloth or hair twisting, and using soft or familiar objects, presumably is for comfort or security.

The videotapes were coded by four Chinese graduate students in psychology who were trained by the second author. Inter-rater agreement averaged 85% for child's affect, social referencing, and inhibited behavior, based on 20% of the sample. Neither the experimenters nor the coders were aware of the children's iron status.

Iron status

Iron status was based on fingerstick hemoglobin concentration at 4-12 months, 24 months, and 4 years. Hemoglobin concentration was determined by cyanomethemoglobin method. The cutoff for anemia was hemoglobin concentration <110 g/L at sea level. Since the study areas were 1300-3000 meters above sea level, the hemoglobin cut off was corrected by the altitude of each county[25].

Statistical analysis

Independent sample *t*-tests and X^2 tests were used to assess differences between the groups in background characteristics. Affective and behavioral outcomes were compared between the Chronic-IDA-Group and Non-Anemic-Group, the Corrected-IDA-Group and Non-Anemic-Group. For affective and behavioral outcomes that were coded as continuous variables (such as latency to touch the novel toy), general linear model (GLM) was used controlling for confounding variables. For the outcomes treated as categorical data (such as percentage of children who ate the snack), we performed logistic regressions to assess the effect of iron group status on affective and behavioral outcomes. Child gender was included as a covariate in all analyses, given that a greater proportion in the Corrected-IDA-Group was male. Child age and mother's education were also considered as potential confounding variables. Weight-for-age Z scores (WAZ) were calculated using EpiInfo (CDC) to determine children's nutritional status. WAZ did not correlate with the outcome variables and therefore was not included as a covariate in the analysis. Statistical significance was set at $p<0.05$. All data were analyzed with SAS (version 9.1).

RESULTS

Descriptive characteristics for the children and their mothers are presented according to iron status group (Table 1). The proportion of males in the Corrected-IDA-Group was higher than that in the Non-Anemic-Group. The percentage of WAZ<-2 in the Corrected-IDA-Group was lower than that in the Non-Anemic-Group at baseline survey and 24-month assessment. There were no other statistically significant differences between the Chronic-IDA-Group and Non-Anemic-Group, and between the Corrected-IDA-Group and Non-Anemic-Group with regard to background characteristics, such as child age, child's growth, mothers' age and education at the 4-year follow-up assessment.

Table 1 Child and family characteristics by iron groups

	Non-Anemic-group (n=64)	Chronic-IDA-Group (n=27)	Corrected-IDA-Group (n=70)
Child characteristics			
Sex,% male	51.6	66.7	77.1[a]
Age, Mo	46.2±3.1	46.6±3.0	45.4±3.3
Waz<-2 (%) (Baseline survey)	9.4	3.7	1.4[a]
Waz<-2% (24-month assessment)	9.8	14.8	7.1[a]
Waz<-2% (4-year follow-up)	4.7	7.4	1.4
Hemoglobin, g/L			
6-12 months	129.2±7.0	110.2±10.3[a]	110.0±7.4[a]
24-month assessment	134.0±6.8	114.9±4.3[a]	131.7±7.4
4-year follow-up	135.4±10.3	133.7±10.6	138.0±11.8
Family characteristics			
Mother's age, Y	29.5±3.0	30.7±3.0	30.0±3.7
Mother education (% attending less than 5 years)	44.4	52.1	58.5

Non-Anemic-Group: Non- anemic prior to 12 months, non-anemic at 24 months, non- anemic at 4 years
Chronic-IDA-Group: Anemic prior to 12 months, persisting anemic at 24 months, non-anemic at 4 years
Corrected-IDA-Group: Anemic prior to 12 months, non-anemic at 24 months, non- anemic at 4 years

Values are expressed as mean ± standard deviation or as a percentage (n) for categorical variables. There were no statistically significant group differences in background characteristics by using the independent t-test for continuous variables and the X^2 test for categorical variables, except for the proportion of males and percentage of WAZ<-2 in the Corrected-IDA-Group vs. Non-Anemic-Group. The proportion of males in Corrected-IDA-Group was higher than that in Non-Anemic-Group. The percentage of WAZ<-2 in Corrected-IDA-Group was lower than that in Non-Anemic-Group.
[a] Significantly different from Non-Anemic-Group, P<0.05

Wariness or inhibited behavior

Children in the Chronic-IDA-Group children spent more time with passive behaviors (p<0.05) than those in the Non-Anemic-Group. There were no statistically significant group differences in the latency to touch the novel toy before or after covariate control. The proportion of children in proximity to mother during stranger approach, and the proportion of children response the stranger's questions did not differ between the two groups. With regard to social referencing, a greater proportion of children in the Chronic-IDA-Group showed social referencing to their mother before talking to the stranger, before and after picking up the novel toy, but the differences were not significant (p>0.05) (Table 2).

We did not find significant differences between the children in the Corrected-IDA-Group and Non-Anemic-Group in terms of wariness/inhibited behaviors.

Frustration tolerance

Compared to Non-Anemic-Group, the Chronic-IDA-Group children's latency to touch/pick up a snack was significantly less (p<0.05), and they spent longer time physically self-soothing (p<0.01). There were no statistically significant differences between children in the Corrected-IDA-Group and Non-Anemic-Group in the above mentioned behaviors. Both the Corrected-IDA-Group and Chronic-IDA-Group showed a higher proportion of children who ate the snack during the observation compared to the Non-Anemic-Group (odds ratio[OR] for children in the Chronic-IDA-Group was 5.50, p<0.05 , OR for children in the Corrected-IDA-Group was 2.36, p<0.05).

Table 2. Child affect and behavior by iron status groups

Outcome variables	Non-Anemic Group (n=64)	Chronic IDA Group (n=27)	Corrected IDA Group (n=70)	Chronic IDA &Non-Anemic Group (95% CI)	Corrected IDA & Non-Anemic Group (95% CI)
Social reference					
Reference mom before talk to stranger, %	50.00%	62.90%	42.90%	1.662 (0.563, 4.676)	0.585 (0.356, 2.066)
Reference mom before picking up the toy, %	46.90%	61.50%	44.90%	2.97 (0.96,9.17)	1.33 (0.54, 3.27)
Reference mom after picking up the toy, %	68.40%	81.30%	54.50%	2.78 (0.51,15.1)	1.05 (0.32,3.43)
Wariness or inhibited behavior					
Child being in proximity (within arm length) to mother during stranger approach, %	48．4％	37.0％	54．3％	0.737 (0.262, 2.074)	1.432 (0.596,3.39)
Latency to touch the novel toy (sec)	27.2±11.4	22.9±16.4	38.0±12.4	NA	NA
Response to the stranger (%)	82.80%	85.20%	78.80%	0.84 (0.195, 3.637)	1.420 (0.182,1.797)
Passive behaviors (sec)	**14.8±4.3**	**23.0±6.6[a]**	**18.1±4.2**	NA	NA
Frustration tolerance					
Latency to touch/pick up snack(sec)	**24.8±7.9**	**7.8±11.4 [a]**	**21.1±3.5**	NA	NA
% of children ate the snack	**15.40%**	**25.90%**	**30.00%**	5.497 (1.004, 30.097)	2.357 (1.012, 5.492)
Physical self-soothing(sec)	**1.5±2.3**	**7.2±1.6[a]**	**4.3±2.7**	NA	NA
Affect					
Positive affect during free play (sec)	54.4±14.1	31.8±21.6	54.7±13.5	NA	NA
Unengaged affect during free play (sec)	205.5±21.6	219.5±32.9	183.6±24.8	NA	NA
Positive affect during stranger approach, %	**28.10%**	**7.40%**	**27.10%**	**0.138 (0.023,0.818)**	**0.459 (0.174,1.215)**
Unengaged affect during stranger approach, %	**50%**	**66.70%**	**44.30%**	**0.284 (0.085, 0.953)**	**0.671 (0.248, 1.818)**
Positive affect during delay of gratification task (sec)	**11.0±3.4**	**4.7 ± 5.2[a]**	**9.7± 3.2**	NA	NA
Unengaged affect during delay of gratification (sec)	43.1±5.2	51.2±7.9	43.7±5.0	NA	NA
Positive affect during novel toy session (sec)	36.9±8.3	27.9±17.5	31.7±14.5	NA	NA
Unengaged affect during novel toy session (sec)	65.4±8.2	73.1±12.4	71.4±9.3	NA	NA

[a] Significant different from Non-Anemic Group, $P<0.05$. NA, not applicable
Values are means±SE for continuous variables, with adjustment for background factors. Adjust means are derived from multiple regression of covariance, controlling for age, gender, maternal education.
Percentages are shown for categorical variables, odds ratio(OR) and 95% confidential intervals (95% CI) are from logistic regression.

Affect

We found significant differences in positive affect during the delay of gratification task between the Chronic-IDA-Group and Non-Anemic-Group, children in the Chronic-IDA-Group spent less time smiling($p<0.05$). During stranger approach, Logistic analysis indicated that the odds of having more positive affect were 76.2% lower for children in the Chronic-IDA-Group compared to children in Non-Anemic-Group ($P<0.05$), and the proportion of children who showed unengaged affect was significantly higher in the Chronic-IDA-Group ($p<0.05$). In the observation of free play and novel toy session, the Chronic-IDA-Group averaged less time smiling than the Non-Anemic-Group, but the difference did not reach statistical significance.

The affective behaviors of children in the Corrected-IDA-Group were comparable to those of children in Non-Anemic-Group.

Negative affect was not analyzed because less than 2% of children cried or fussed during the observation.

DISCUSSION

Our findings support the prediction of long-term social-emotional effects of chronic IDA during infancy. We found that, despite correction of IDA by 4 years of age, children who had chronic IDA in infancy showed less positive affect and frustration tolerance, more passive behavior, unengaged affect, and physical self-soothing than children who were non-anemic throughout infancy. Furthermore, we found that children whose anemia was corrected before 24 months were comparable in behavior to children who were non-anemic throughout infancy.

The present study used more extensive observations than previous studies to examine the affect and behavior of former IDA, non-anemic preschool-age children. We performed a delay of gratification task to assess the effects of IDA on preschoolers' capacity for self-control in the face of temptation[26]. Delay of gratification tasks have not previously been used in iron deficiency studies. Research in other areas has shown that 4-year-old children who are more successful at waiting in delay of gratification situation are more attentive, better able to concentrate, and exhibit greater self-control and frustration tolerance than their peers when they are adolescents [26].

We included novel toy and stranger approach situations specifically to pursue earlier observations of wary, hesitant behavior in IDA infants. Novel toy and stranger approach paradigms have been used to examine individual differences in preschoolers' behavior in reaction to novel situations [27]. Wary or behaviorally inhibited children typically display low approach behavior and stay in close proximity to their mothers when confronted with a range of novel stimuli, including people, objects and situations [22]. There is increasing evidence that this wary/inhibited behavioral pattern is a risk factor for future problems, such as anxiety, depression, and negative self-perceptions of competence [22, 28]. In our study, affect and behavioral alterations seemed especially apparent with increased novelty, unfamiliarity, or stress [29, 30]. We found little difference in affect and behavior during free play or new toy presentation, but affect and behavioral differences became apparent in the stranger approach session and delay of gratification task. We found that the Chronic-IDA-Group children were less successful at waiting, and showed more physical self-soothing, passive behaviors, and less positive affect.

Lozoff et al found preschoolers with IDA showed less social looking with their mothers during a play observation that involved familiar and unfamiliar toys[22]. Our finding on increased social looking in the Chronic-IDA-Group is not directly comparable to Lozoff's results as all children in our study were non-anemic.

Our results require replication in other samples and settings, especially because of our small sample size. Nonetheless, our observations of behavioral alterations in preschool children with chronic IDA in infancy support the findings of other research. A study in France followed children's iron status and Development Quotient (DQ) including postural, coordination, language and sociability Quotient. The study found that IDA at 10 months did not correlate with DQ, whereas hemoglobin concentration at 2 years did; it was positively associated with overall developmental, postural, coordination, and social quotients at 2 years[16]. A longitudinal study in Costa Rica included a structured mother-child interaction task at 5 years. Children with chronic ID in infancy displayed lower levels of physical activity, positive affect, verbalization, and reciprocal interaction, compared to children with good iron status, despite the correction of their IDA in infancy[20]. In early adolescence, mothers and teachers rated them as having more internalizing behavior problems (anxiety/depression, social problems, stc.)[10]. These findings point to the potential "functional isolation" significance of early behavior alterations in children with chronic IDA in infancy. Social-emotional alterations in early iron deficiency children have been interpreted in light of iron's role in dopamine system function[31, 32]. Iron deficiency rats have alterations in dopamine metabolism and exhibit behaviors, they show more anxious-like behaviors, have reduced exploration in new environments, have decreased stereotype, and demonstrate a slower rate of habituation[33, 34]. Studies on the affective changes in iron deficient anemic infants (wariness, hesitance, absence of positive affect) seem to make sense in this context[9]. Neonatal dopamine terminal damage leads to a lifelong hyperreactivity to novel objects and experiences in an unfamiliar environment[32] and seems to have long term effects on context-dependent attention and affective responses[9]. This can partially explain the altered affect and behavior of the Chronic-IDA-Group in the stranger approach session and delay of gratification task.

This study cannot eliminate the possibility that the altered behavior of the Chronic-IDA-Group was due to some relevant but unmeasured factor(s), although the groups were generally comparable in background characteristics, such as maternal depression or other environmental disadvantages, which could account for both poor infant feeding practices (leading to IDA) and lack of stimulation (leading to altered behavior and development).

The study was limited with respect to measurement of iron status. IDA was estimated by hemoglobin concentration. Hemoglobin concentration generally overestimates the prevalence of IDA, as it does not account for anemia due to other nutritional deficiencies (e.g., vitamin A deficiency), infections, hemoglobinopathies, or ethnic differences in normal hemoglobin distribution[35]. However, it appears that iron deficiency was a major cause of anemia in this population; hemoglobin concentration showed an overall increase of 10.5 g/L after the supplementation intervention in infancy [36, 37]. Of the 27 Chronic-IDA-Group children in the follow-up sample who did not correct anemia by the 24-month assessment, 16 did not receive supplementation (control group) and 11 received supplementation. Regarding possible reasons for persistent anemia in these 11 children, it should be noted that the food supplement provided a low dose of iron (6 mg/day). This amount of iron was much lower than WHO recommended dose for IDA treatment (2 mg/kg/day) [25]. It is possible that a higher amount of iron would have corrected IDA in study children by 24 months.

CONCLUSION AND IMPLICATIONS

In summary, this study showed that preschool-age children who had chronic IDA in infancy showed less positive affect and less frustration tolerance, more passive behavior, and more physical self-soothing during a delay of gratification situation at preschool age. Children whose anemia was corrected before 24 months were comparable in social-emotional behavior to children who were non-anemic throughout infancy and early childhood. These results point to the potential benefits of preventing iron deficiency in infancy and treating it before it becomes chronic or severe.

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Chapter 4

Impact of micronutrient supplementation during pregnancy on birth weight, duration of gestation, and perinatal mortality in rural western China: double blind cluster randomised controlled trial

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Abstract

Objective To exam the impact of antenatal supplementation with multiple micronutrients or iron and folic acid compared with folic acid alone on birth weight, duration of gestation, and maternal haemoglobin concentration in the third trimester.

Design Cluster randomised double blind controlled trial.
Setting Two rural counties in north west China.
Participants 5828 pregnant women and 4697 live births.
Interventions Villages were randomised for all pregnant women to take either daily folic acid (control), iron with folic acid, or multiple micronutrients with a recommended allowance of 15 vitamins and minerals.

Main outcome measures Birth weight, length, and head circumference measured within 72 hours after delivery. Neonatal survival assessed at the six week follow-up visit.

Results Birth weight was 42 g (95% confidence interval 7 to 78 g) higher in the multiple micronutrients group compared with the folic acid group. Duration of gestation was 0.23 weeks (0.10 to 0.36 weeks) longer in the iron-folic acid group and 0.19 weeks (0.06 to 0.32 weeks) longer in the multiple micronutrients group. Iron-folic acid was associated with a significantly reduced risk of early preterm delivery (<34 weeks) (relative risk 0.50, 0.27 to 0.94, P=0.031). There was a significant increase in haemoglobin concentration in both iron-folic acid (5.0 g/l, 2.0 to 8.0 g/l, P=0.001) and multiple micronutrients (6.9 g/l, 4.1 to 9.6 g/l, P<0.001) groups compared with folic acid alone. In post hoc analyses there were no significant differences for perinatal mortality, but iron-folic acid was associated with a significantly reduced early neonatal mortality by 54% (relative risk 0.46, 0.21 to 0.98).

Conclusion In rural populations in China antenatal supplementation with iron-folic acid was associated with longer gestation and a reduction in early neonatal mortality compared with folic acid. Multiple micronutrients were associated with modestly increased birth weight compared with folic acid, but, despite this weight gain, there was no significantly reduction in early neonatal mortality. Pregnant women in developing countries need sufficient doses of iron in nutrient supplements to maximise reductions in neonatal mortality.

Trial registration ISRCTN08850194

INTRODUCTION

Neonatal deaths contribute greatly to child mortality in developing countries, and these deaths have steadily increased as a percentage of all deaths of children under the age of 5[1,2]. In China, by 2004 neonatal mortality accounted for more than half of all deaths in children under 5[3]. Low birthweight babies are at higher risk of morbidity and mortality than those of normal birth weight[4] and are also at risk of postnatal growth retardation, with possible adverse long term effects on their physical and cognitive development[5,6]. One of the major causes of low birth weight in developing countries is the poor nutritional status of the mother before and during pregnancy, resulting in restricted fetal growth especially during the third trimester[7].

Poor quality diet and inadequate intake, combined with increased nutrient requirements for placental and fetal growth, can lead to multiple micronutrient deficiencies in pregnancy and contribute to higher rates of low birth weight[8]. To address these multiple deficiency states UNICEF has proposed the use of multiple micronutrient supplements in pregnancy that provide the recommended individual intakes for pregnant women[9].

China is the most populated developing country in the world, and the prevalence of low birth weight varies across different socioeconomic groups and areas. A 1999 survey of low birth weight in China showed a prevalence of low birth weight of 7.6% and 11.8% in “poor” and “very poor” counties, respectively[10]. With an average prevalence of low birth weight of 5.9%, an estimated 1.2 million low birthweight babies are born in China every year.

The only antenatal supplement promoted by the Ministry of Health in China is folic acid to prevent congenital neural tube defects[11]. There are no specific policies or programmes for the distribution of multiple micronutrient or iron-folic acid supplements during pregnancy, even to disadvantaged women. To provide evidence in China to formulate public health policy on nutrient supplementation in pregnancy we conducted a community based cluster randomised controlled trial in a disadvantaged rural population. We assessed the impact of iron-folic acid and multiple micronutrient supplements during pregnancy, compared with folic acid alone, on birth anthropometry, duration of gestation, and maternal haemoglobin concentration in the third trimester. We also conducted a post hoc assessment of the impact of the supplements on perinatal mortality, comprising stillbirths and early neonatal deaths.

METHODS

Experimental design

The trial took place in two poor rural counties in Shaanxi Province of north west China. A pilot study found a low birth rate of 13% and 57% of women with anaemia (haemoglobin <110 g/l) in the third trimester. Because of the scale of this trial we allocated the same treatment to all pregnant women in a given village. In 2001 in the first county there were 14 townships and 234 villages, and in the second county there were 20 townships and 327 villages. Randomisation of villages was stratified by county with a fixed ratio of treatments (1:1:1) and blocking of 15 to ensure geographical balance with an approximately equal distribution of treatments per township.

The randomisation schedule was generated off site with a pseudo-random number generator in SAS version 6 (SAS Institute, Cary, NC). A treatment colour code was assigned to each village based on the treatment allocation schedule. The treatment codes were opened only once all data had been collected and blinded analysis of the primary hypothesis was completed.

Study population and sample size

The study sample consisted of all women resident in the counties who became pregnant between August 2002 and January 2006 and who fulfilled trial selection criteria (see below). Before the start of the trial, we estimated that we needed a sample of 2255 eligible pregnant women per treatment to detect a 25% reduction in low birth weight between either iron-folic acid or multiple micronutrient and folic acid (control) groups, assuming a two tailed test, with $\alpha=0.05$ and $\beta=0.20$, and a prevalence of low birth weight of 9.3% (based on a three month sample in 2000 of births from a county hospital in the study area). We increased the sample size to 2480 pregnant women per group to account for 10% fetal loss and loss to follow-up.

At the start of the trial we also expected that recruitment would take 2.5 years, based on registered live births in the counties in the previous year. Recruitment was slower than expected, however, because many births were in women who lived in large cities in eastern China but who returned to their village to give birth and register their child. After 3.5 years the trial sponsor requested that recruitment be stopped because of limitations with funding. At that stage, January 2006, we had recruited and randomised 5828 eligible women. We estimated that this sample of about 1900 women per treatment would provide 80% power to detect a 50 g difference in birth weight between either iron-folic acid or multiple micronutrients and folic acid (control) groups, assuming a two tailed test, with $\alpha=0.05$ and birth weight standard deviation (SD) of 436 g (based on values observed across all treatment groups). This sample size, however, only had 80% power to detect a 40% reduction in low birth weight between treatment groups, assuming a two tailed test, with $\alpha=0.05$ and a prevalence of low birth weight of 4.6% (prevalence observed across all treatment groups). It also had a similarly low power of 80% to detect a 50% reduction in early neonatal mortality between treatment groups, assuming a two tailed test, with $\alpha=0.05$ and an early neonatal mortality rate of 15 per 1000 live births. We did not adjust for the cluster randomised design in any of these estimates of sample size.

Enrolment and pregnancy surveillance procedures

Village doctors, with support from the township maternal and child healthcare workers, recruited women by active surveillance for pregnancy in women of reproductive age. At the start the village doctor conducted a mini-survey of all women of reproductive age living in their village to identify those who were likely to become pregnant, including newly married women, those not using contraception, or those who said they wanted a child. Informed verbal consent for pregnancy monitoring was obtained by trained village doctors, and consenting women were visited every month and asked about the date of their last menstrual period. Women with periods delayed by more than five days had a urine pregnancy test, and confirmed pregnancies were reported to the township maternal and child health worker. Pregnancies in women resident in the studied townships or counties were passively detected at antenatal clinics in local health facilities. If the diagnosed pregnancy was less than 28 weeks' gestation, informed verbal consent to participate in the trial was sought from the woman and her partner by trained MCH staff from township hospitals.

Newly identified pregnant women were interviewed to record their sociodemographic status and their menstrual, reproductive, medical, and family history. Recruited pregnant women received three free antenatal care checks, at which they were asked about pregnancy complications and underwent a physical examination including blood pressure and weight measurement. Haemoglobin was measured at the third antenatal check. All the information collected during the pregnancy until the six week follow-up visit was recorded in a pregnancy care record book, which served both as a clinical record and data capture instrument, and was collected by trained MCH staff with monitoring by project staff.

Interventions

Villages were randomly assigned for women to receive one of three daily antenatal supplements: multiple micronutrients, iron-folic acid, or folic acid alone (control). The multiple micronutrient supplements were formulated to contain approximately the WHO/UNICEF recommended dietary allowances for each of 15 minerals or vitamins as follows: 30 mg iron, 400 μg folate, 15.0 mg zinc, 2.0 mg copper, 65.0 μg selenium, 150.0 μg iodine, 800.0 μg vitamin A, 1.4 mg vitamin B_1 (thiamine), 1.4 mg vitamin B_2 (riboflavin), 1.9 mg vitamin B-6, 2.6 μg vitamin B-12, 5.0 μg vitamin D, 70.0 mg vitamin C, 10.0 mg vitamin E, and 18.0 mg niacin[12]. Iron-folic acid supplements contained 60 mg iron (twice the amount of elemental iron in the multiple micronutrients) and 400 μg folic acid. The folic acid supplement contained 400 μg of folic acid. The three supplement types were produced by the Beijing Vita Nutritious Products, Beijing, China, and were of identical appearance and packaged in blister packs.

At enrolment, each woman received 15 capsules with instructions to take one capsule daily. The village doctor visited the women every two weeks to provide more supplements and to retrieve the used blister strips and record the number of remaining capsules. The number of supplements consumed throughout the trial was summed to estimate compliance.

Measurement of outcomes

Hospital nursing staff measured birth weight within one hour of delivery. In the six county hospitals and the three largest township hospitals (78% of birth weights) birth weight was measured with an electronic scale (type BD 585, Tanita, Dongguan, Guangdong Province, China) with precision to the nearest 10 g. For births in the 31 smaller township hospitals (10% of birth weights) and for home births (12% of birth weights) birth weight was measured with a baby scale (type RTZ-10A-RT, Wuxi Weigher Factory, Wuxi, China) with precision to the nearest 50 g. For home deliveries, township maternal and child health staff visited the women at home within 72 hours of delivery to measure the baby and gather information on delivery. We excluded from analyses newborn anthropometry collected later than 72 hours after birth. Low birth weight was defined as <2500 g. Birth length was measured to the nearest one millimeter (mm) by using a portable measuring board with fixed head piece. Occipitofrontal head circumference was measured with a tape to the nearest 1 mm.

Gestational age at birth was measured as completed days based on the first day of the last menstrual period, obtained at the baseline interview. Preterm delivery was defined as delivery before 37 completed weeks' gestation and early preterm delivery as before 34 weeks. Small for gestational age babies were defined as those whose weight was below the 10th centile of the gestational age-sex specific US reference for fetal growth[13].

Maternal haemoglobin concentration was measured in capillary blood collected early in the third trimester (gestation 28-32 weeks) from a subsample of 599 pregnant women with a birth outcome, who were consecutively enrolled from 6 July 2004 to 28 October 2005. HemoCue portable spectrophotometers (Ängelholm, Sweden) were used to assay haemoglobin concentration and were calibrated daily and all measurements were made within the temperature operating range of this device (15-30°C). We excluded haemoglobin measurements collected before 6 July 2004 because in winter these measurements were taken below the operating range, potentially resulting in incorrect values. Anaemia in the third trimester was defined as haemoglobin <110 g/l[14].

Village doctors or hospital staff reported fetal losses during pregnancy, birth outcome, delivery information, and neonatal and maternal deaths; maternal and child health staff recorded data with precoded structured forms. Neonatal survival was assessed at the six week follow-up visit.

We defined perinatal deaths as stillbirths (fetuses delivered at 28 weeks' gestation or later with no signs of life and recorded as occurring before the onset of or during labour) plus early neonatal deaths (deaths among liveborn infants occurring within seven days of delivery). Neonatal deaths were defined as deaths among liveborn infants occurring within 28 days of delivery. Project staff re-interviewed all women who had a stillbirth or neonatal death to check the reported information and, for hospital or clinic deliveries, to cross check with medical records.

Statistical analysis

To assess the effectiveness of randomisation we examined the baseline characteristics of the clusters and the individual pregnant women across treatment groups. A wealth index was constructed from an inventory of 16 household assets or facilities with a principal component analysis method[15], and this index was categorised into thirds as an indicator for the poorest, middle income, and richest households. The mean number of supplements consumed and treatment compliance rates (percentage of days that supplements were consumed) were also examined.

All analyses were conducted using the intention to treat principle. We included only liveborn infants in the analyses of birth anthropometry because most stillborn infants were not measured. We estimated mean differences and 95% confidence intervals for birth weight and gestation and adjusted for the effect of randomisation by villages using generalised estimating equation linear models with an independent correlation structure, which is a suitable modelling strategy where there are more than 40 clusters per treatment group.[16,17] The adjusted mean differences in birth weight, birth length, head circumference, and gestation, and their 95% confidence intervals, were computed relative to the folic acid group. Similarly, we calculated adjusted mean differences for newborn anthropometry and gestation at birth and their 95% confidence intervals to compare the multiple micronutrient and the iron-folic acid groups. To adjust for cluster randomisation, we applied generalised estimating equation binomial regression models with log link and exchangeable correlation structures to estimate the relative risks and 95% confidence intervals for low birth weight, small for gestational age, preterm delivery, and anaemia with the folic acid group as the reference and with multiple birth indicator as a cofactor, and similarly to compare the multiple micronutrient and iron-folic acid groups.

We analysed perinatal mortality only in singleton births to prevent bias from the effect of multiple births, which have higher mortality risk. We calculated rates of stillbirth and perinatal death using the number of pregnancies at 28 weeks' gestation as the denominator. Neonatal death rates were calculated with the number of live births as the denominator. Kaplan-Meier survival analysis was used to estimate the survival probabilities of liveborn singleton infants from birth to 28 days and to compare survival across treatment groups. To adjust for the cluster randomisation, we applied generalised estimating equation binomial regression models with log link and exchangeable correlation structures to estimate the relative risks and 95% confidence intervals for stillbirth and perinatal and neonatal death rates with the folic acid group as the reference. Wald χ^2 tests assessed the overall effects of the three treatments for each outcome. We used Stata version 9.2 (Stata/SE 9.2 StataCorp, College Station, TX) for all analyses.

RESULT

Figure 1 shows the flow of participants through the trial. Enrolment began on 1 August 2002 and continued until 9 July 2005; the last baby was born to a trial participant was on 24 January 2006, and all women completed the trial by 28 February 2006. Over the 3.5 year period, there were 7144 confirmed pregnancies from the monitored population: 727 women refused to participate in the trial, 498 did not meet the inclusion criteria, and 91 did not participate for other reasons. We enrolled and randomised 5828 women, but of these 133 women were lost to follow up, 279 stopped taking supplements and refused to continue to participate, and 601 had a spontaneous or induced abortion or other medical condition resulting in fetal loss. There were a total of 4650 pregnancies that resulted in at least one live birth. There were 167 stillbirths, where birth weight was not usually recorded. There were a total of 4697 live births; birth weight was missing or was measured beyond 72 hours after birth in 276. There were 4421 (94%) live births with birth weight available for analysis and 222 perinatal deaths. The final row of boxes in figure 1 show the numbers included in the intention to treat analyses for each of the trial outcomes examined. Across all treatment groups, 7% of the women were enrolled in a village other than their usual place of residence, and this was usually their mother's village.

The sociodemographic characteristics and the anthropometric measurements at enrolment and the cluster and individual level baseline characteristics were balanced by treatment groups (table 1). The reproductive history of the pregnant women was similar across groups and reflected China's "one child policy," with 3585 (61.5%) women having their first pregnancy (table 2). The percentage of women delivering at home was balanced across treatment groups with 9.4% (149) for iron-folic acid group, 12.4% (193) for the multiple micronutrients group, and 11.4% (195) for the folic acid only group. There were 47 pairs of twins and one set of triplets. These multiple births were not balanced across the treatment groups, with 19 pairs of twins (of 1584 births) in the iron-folic acid group, 12 pairs of twins and set of one triplets (of 1558 births) in the multiple micronutrient group, and 17 pairs of twins (of 1722 births) in the folic acid group (P=0.031).

There was a high level of compliance with the supplementation in all treatment groups (table 3). The mean number of doses of supplements consumed per women during pregnancy was 165, and this was similar in each group. About 6% of women consumed fewer than 90 supplements during the pregnancy, and over 80% consumed more than 120 supplements.

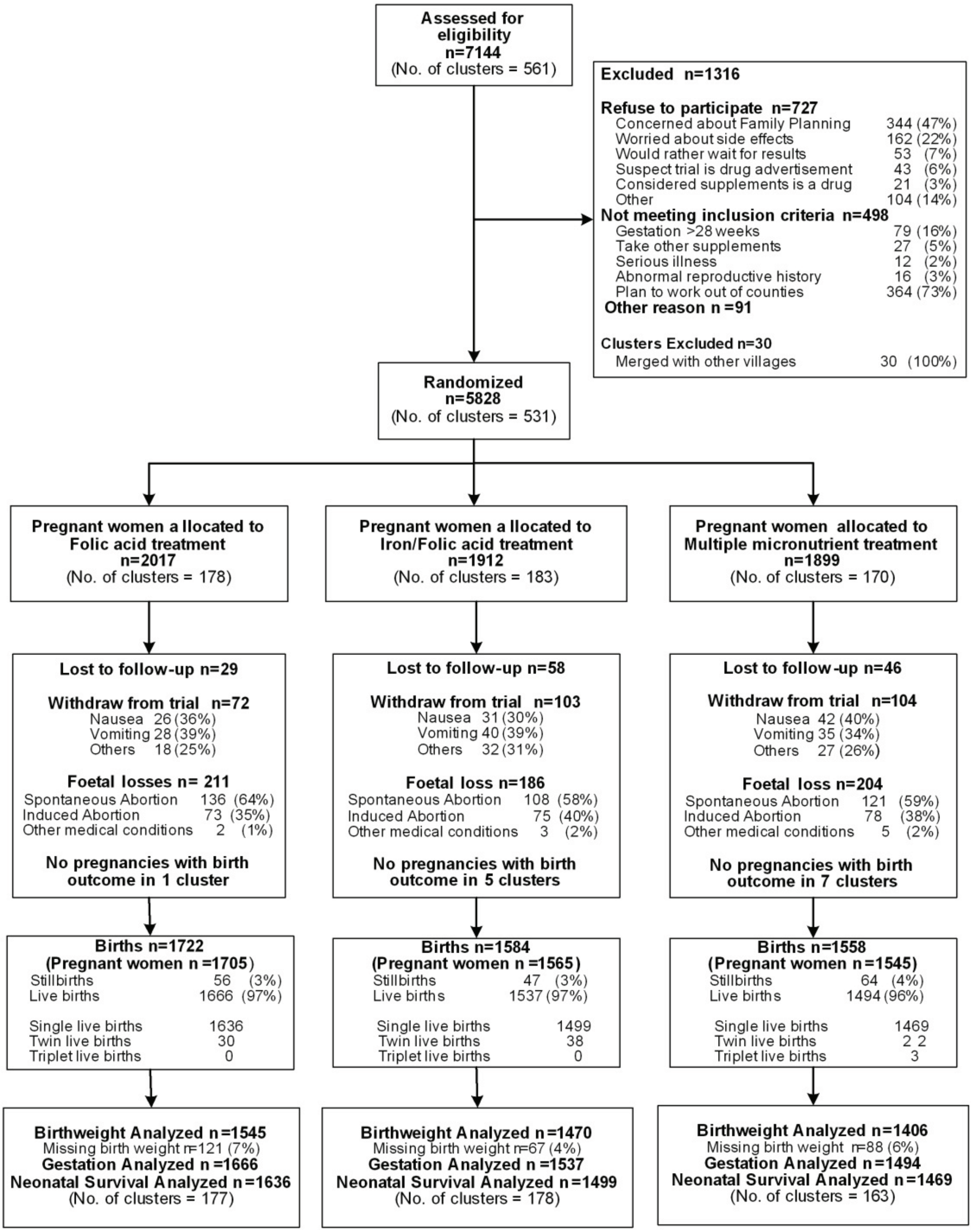

Figure 1 Participant flow chart

Table 1 **Baseline characteristics of clusters, households, and participants by treatment group. Figures are numbers (percentages) unless stated otherwise**

	Folic acid	Iron-folic acid	Multiple micronutrients
No of clusters	178	183	170
Mean (SD) population in clusters in 2001	826 (502)	781 (434)	890 (515)
Mean (SD) births in clusters in 2001	6.8 (5.4)	6.5 (4.4)	7.6 (8.6)
Mean (SD) pregnancies/cluster/year	6.3 (1.1)	6.3 (1.1)	6.0 (1.3)
Mean (SD) No of family members/cluster	4.2 (0.77)	4.2 (0.81)	4.2 (0.89)
Maternal age (years):			
Mean (SD)	24.8 (4.4)	24.8 (4.3)	24.8 (4.4)
15-19	152 (7.5)	153 (8.0)	160 (8.4)
20-24	950 (47.1)	875 (45.8)	867 (45.7)
25-29	564 (28.0)	567 (29.7)	555 (29.2)
30-34	312 (15.5)	277 (14.5)	276 (14.5)
^{3}35	39 (1.9)	40 (2.1)	41 (2.2)
Women's education:			
<3 years	133 (6.6)	100 (5.3)	118 (6.2)
Primary	527 (26.3)	495 (26.0)	542 (28.6)
Secondary	1087 (54.2)	1022 (53.7)	962 (50.8)
High school and above	259 (12.9)	288 (15.1)	271 (14.3)
Women's occupation:			
Farmer	1677 (83.9)	1563 (82.3)	1599 (84.8)
Other	322 (16.1)	337 (17.7)	287 (15.2)
Household wealth index:			
Mean (SD)	–0.03 (1.49)	0.07 (1.53)	–0.03 (1.55)
Poorest third	665 (33.0)	600 (31.4)	671 (35.3)
Middle third	725 (35.9)	639 (33.4)	627 (33.0)
Richest third	627 (31.1)	673 (35.2)	601 (31.7)

Impact of nutrient supplementation on anthropometry and gestation at birth

The intracluster correlation coefficient for birth weight was 0.03 (95% confidence interval 0.015 to 0.052). There was no evidence of an effect of iron-folic acid on mean birth weight (P=0.17) but birth weight was significantly higher (42 g, 7 to 78 g, P=0.019) in the multiple micronutrients group compared with the folic acid alone (table 4). The increase in birth weight observed in the iron-folic acid and the multiple micronutrient groups corresponded with reductions in the risk of low birth weight (<2500 g) compared with folic acid alone of 19% (P=0.20) and 22% (P=0.14), respectively. In a post-hoc analysis of babies who were small for gestational age, there were no significant differences in the proportion across the three treatment groups, although the proportion in the multiple micronutrient group was slightly lower (table 4).

Table 2 Baseline characteristics at enrolment related to pregnancy by treatment group. Figures are numbers (percentages) unless stated otherwise

	Folic acid	Iron-folic acid	Multiple micronutrients
No of pregnant women	2017	1912	1899
Mean (SD) No of pregnancies	1.6 (0.7)	1.6 (0.7)	1.6 (0.7)
Parity:			
0	1223 (60.6)	1184 (61.9)	1178 (62.0)
1	714 (35.4)	669 (35.0)	657 (34.6)
2	80 (4.0)	59 (3.1)	64 (3.4)
Gestation (weeks):			
Mean (SD)	13.8 (5.8)	13.6 (5.6)	13.9 (5.6)
<12	884 (43.8)	835 (43.7)	820 (43.2)
12-15	432 (21.4)	424 (22.2)	424 (22.3)
16-28	701 (34.8)	653 (34.2)	655 (34.5)
Height (cm):			
No of women	1998	1897	1878
Mean (SD)	158.8 (5.1)	158.9 (5.2)	158.7 (5.3)
Weight (kg):			
No of women	1970	1864	1846
Mean (SD)	52.5 (6.0)	52.7 (6.4)	52.7 (6.1)
BMI (kg/m2):			
No of women	1968	1863	1840
Mean (SD)	20.8 (2.2)	20.9 (2.3)	20.9 (2.2)
Mid-upper arm circumference (cm):			
No of women	1983	1884	1872
Mean (SD)	23.1 (2.1)	23.2 (2.2)	23.2 (2.1)

The intracluster correlation coefficient for gestation at birth was 0.02 (0.004 to 0.036). Table 4 shows there was a significant increase in the duration of gestation of 0.23 weeks (0.10 to 0.36 weeks, P=0.001) in the iron-folic acid group and 0.19 weeks (0.06 to 0.32 weeks, P=0.004) in the multiple micronutrient group compared with folic acid. The increase in the mean duration of gestation in the iron-folic acid group corresponded with a 21% reduction in the risk of preterm delivery (<37 weeks) (P=0.13) and a significant 50% reduction in the risk of early preterm delivery (<34 weeks) (relative risk 0.50, 0.27 to 0.94, P=0.031). Compared with folic acid alone, the increase in the mean duration of gestation observed in the multiple micronutrient group corresponded with non-significant reductions in the risk of preterm delivery (<37 weeks) and early preterm delivery (<34 weeks) of 14% (P=0.29) and 30% (P=0.26), respectively (table 4).

The intracluster correlation coefficient for birth length was 0.03 (0.007 to 0.047) and for head circumference was 0.08 (0.050 to 0.101). Table 4 shows that in the iron-folic acid group there was a significant increase in birth length (0.24 cm; 0.02 to 0.46 cm, P=0.03) compared with folic acid alone. There was no significant effect of iron-folic acid on mean head circumference (P=0.21).There were no significant effects of the multiple micronutrients on either mean birth length (P=0.12) or mean head circumference (P=0.80).

Compared with folic acid alone, the difference in mean birth weight, adjusted for multiple births, gestation at delivery, and cluster randomisation, was 12.6 g for the iron-folic acid and 31.0 g for the multiple micronutrients, indicating that the extension of the duration of gestation contributed to a similar increment in mean birth weight for iron-folic acid (11.7 g) and multiple micronutrients (11.3 g) compared with the folic acid.

Impact of nutrient supplementation on maternal haemoglobin concentration

Haemoglobin samples were available for 599 women in the third trimester. The baseline characteristics for these women were balanced across the treatment groups, and there were no significant differences between the women with and without haemoglobin measurements. Also there was no significant difference in the mean gestation at haemoglobin testing (P=0.54), which was 32.0 weeks (SD 2.6), 31.9 weeks (SD 2.8), and 32.2 weeks (SD 3.1) for the folic acid, iron-folic acid, and multiple micronutrient groups, respectively. There was a significant increase in haemoglobin concentration with both iron-folic acid and multiple micronutrients compared with folic acid (P=0.001 and P<0.001, respectively). In both the iron-folic acid and the multiple micronutrient groups, however, more than 40% of the women were still anaemic in the third trimester (table 4)

Table 3. Compliance* and number of doses of supplements consumed by treatment group

	Folic acid	Iron-folic acid	Multiple micronutrients
No who gave birth	1705	1565	1545
No (%) who complied:			
0-25	9 (0.6)	16 (1.0)	18 (1.2)
26-50	24 (1.4)	29 (1.9)	32 (2.1)
51-75	95 (5.6)	110 (7.0)	88 (5.7)
76-100	1577 (92.5)	1410 (90.1)	1407 (91.1)
Mean (SD)	93.4 (12.7)	91.9 (14.8)	92.6 (14.9)
No (%) of supplements consumed:			
<90	105 (6.2)	85 (5.4)	106 (6.9)
91-119	181 (10.6)	160 (10.2)	154 (10.0)
120-179	684 (40.1)	638 (40.8)	630 (40.8)
^{3}180	735 (43.1)	682 (43.6)	655 (42.4)
Mean (SD)	165 (44)	166 (44)	165 (45)

*Compliance calculated by number of actual supplements consumed divided by number of supplements expected to be consumed.

Table 4 Birth anthropometry, gestation and haemoglobin, with adjusted difference or relative risk for comparison of multiple micronutrients or iron-folic acid with folic acid alone

Birth outcomes	No (%*) of infants	Mean (SD)	Adjusted† difference or relative risk (95% CI)	P value
Birth weight (g)‡				
Folic acid	1545	3153.7 (444.9)		
Iron-folic acid	1470	3173.9 (424.4)	24.3 (-10.3 to 59.0)	0.169
Multiple micronutrients	1406	3197.9 (438.0)	42.3 (7.1 to 77.5)	0.019
Birth weight <2500 g				
Folic acid	82 (5.3)	—		
Iron-folic acid	66 (4.5)	—	0.81 (0.59 to 1.12)	0.199
Multiple micronutrients	57 (4.1)	—	0.78 (0.56 to 1.08)	0.139
Small for gestational age				
Folic acid	280 (18.1)	—		
Iron-folic acid	278 (18.9)	—	1.04 (0.89 to 1.22)	0.618
Multiple micronutrients	238 (16.9)	—	0.95 (0.82 to 1.12)	0.549
Gestation at birth (weeks)				
Folic acid	1666	39.63 (1.93)		
Iron-folic acid	1537	39.84 (1.69)	0.23 (0.10 to 0.36)	0.001
Multiple micronutrients	1494	39.82 (1.80)	0.19 (0.06 to 0.32)	0.004
Preterm <37 weeks				
Folic acid	102 (6.1)	—		
Iron/folic acid	76 (4.9)	—	0.79 (0.58 to 1.07)	0.131
Multiple micronutrients	78 (5.2)	—	0.86 (0.64 to 1.14)	0.285
Early preterm <34 weeks				
Folic acid	30 (1.80)	—		
Iron-folic acid	15 (0.98)	—	0.50 (0.27 to 0.94)	0.031
Multiple micronutrients	19 (1.27)	—	0.70 (0.38 to 1.30)	0.259
Birth length (cm)§				
Folic acid	1367	48.8 (2.9)		
Iron-folic acid	1299	49.1 (2.7)	0.24 (0.02 to 0.46)	0.032
Multiple micronutrients	1251	49.1 (2.8)	0.22 (-0.05 to 0.49)	0.117
Head circumference (cm)¶				
Folic acid	1350	33.1 (1.6)		
Iron-folic acid	1278	33.2 (1.6)	0.11 (-0.06 to 0.28)	0.207
Multiple micronutrients	1234	33.1 (1.6)	0.02 (-0.16 to 0.21)	0.8
Haemoglobin (g/l)**				
Folic acid	218	105.3 (14.2)		
Iron-folic acid	193	110.1 (14.8)	5.0 (2.0 to 8.0)	0.001
Multiple micronutrients	188	111.8 (14.1)	6.9 (4.1 to 9.6)	<0.001
Anaemia¶ (<110 g/l)				
Folic acid	133 (61.0)	—		
Iron-folic acid	87 (45.1)	—	0.74 (0.61 to 0.91)	0.003
Multiple micronutrients	81 (43.1)	—	0.72 (0.59 to 0.88)	0.001

*If applicable.
†Adjusted for multiple births and cluster randomisation in general estimating equation linear models.
‡Data missing for 121 in folic acid, 67 in iron-folic acid, 88 in multiple micronutrients.
§Data missing for 299 in folic acid, 239 in iron-folic acid, 243 in multiple micronutrients.
¶Data missing for 316 in folic acid, 260 in iron-folic acid, 260 in multiple micronutrients.
**In subsample of 599 pregnant women with birth outcome who were consecutively enrolled from 6 July 2004 to 28 October 2005.

Impact of supplementation on perinatal mortality

Post hoc analyses showed no significant impact on the risk of perinatal mortality (stillbirths and early neonatal mortality) either in the iron-folic acid (P=0.307) or in multiple micronutrients group (P=0.336) compared with folic acid (table 5).

Post hoc analyses also showed no effect of iron-folic acid compared with folic acid on the stillbirth rate, though there was a non-significant increase of 39% (relative risk 1.39, 0.95 to 2.04) in the multiple micronutrients group. When we combined all fetal losses (spontaneous abortions and stillbirths), the rates were 103.1/1000 pregnancies for folic acid, 93.7/1000 for iron-folic acid, and 111.3/1000 for multiple micronutrients, indicating no evidence of an altered risk for total fetal losses with iron-folic acid (relative risk 0.91, 0.74 to 1.12) or with multiple micronutrients (1.15, 0.94 to 1.40) compared with folic acid.

The post hoc analyses showed a reduction in the risk of early neonatal mortality among infants born to women randomised to receive either iron-folic acid or multiple micronutrients compared with folic acid. The magnitude of the reduction (54%) was significant for the iron-folic acid group (relative risk 0.46, 0.21 to 0.98) compared with folic acid but not for the multiple micronutrients group (29%; relative risk 0.71, 0.36 to 1.39).

Figure 2 illustrates cumulative mortality curves for infants from birth to 28 days, with lower mortality rates for neonates whose mother's received iron-folic acid and multiple micronutrients. Most of the divergence of the mortality curves occurred in the first seven days after birth, after which the curves were about parallel. The overall differences were of borderline significance (P=0.055), but the difference between iron-folic acid and folic acid alone was significant (P=0.032). The other individual treatment comparisons (multiple micronutrients *v* folic acid and multiple micronutrients *v* iron-folic acid) were not significant (P=0.077 and P=0.708, respectively).

There were 73 neonatal deaths, including three pairs of twins. The highest number of neonatal deaths because of preterm delivery was in the folic acid group (11/73, 15.1%) with similar numbers for iron-folic acid (5/73, 6.8%) and multiple micronutrients (6/73, 8.2%) groups. There was also a higher number of deaths from birth asphyxia in the folic acid group (8/73, 11.0%) with the same number in the iron-folic acid (3/73, 4.1%) and multiple micronutrient (3/73, 4.1%) groups.

There were no significant differences between multiple micronutrients compared with iron-folic acid for birth anthropometry, duration of gestation, haemoglobin concentration, or perinatal mortality.

On the basis of our results, the number of women who would need to be treated with iron-folic acid from early in pregnancy would be 6.2 (6.1 to 6.3) to prevent one case of maternal anaemia in the third trimester, 83.3 (83.28 to 83.32) to prevent one preterm delivery <37 weeks, 122 (121.99 to 122.01) to prevent one early preterm delivery <34 weeks, and 125 (124.99 to 125.01) to prevent one early neonatal death. With multiple micronutrients the numbers needed to treat would be 5.6 (5.5 to 5.7), 111.1 (111.08 to 111.12), 188.7 (188.69 to 188.71), and 222 (222.19 to 222.21) respectively.

Table 5 Mortality outcomes (in singleton births)

	Folic acid		Iron-folic acid		Multiple micronutrients		Relative risk (95% confidence interval)*	
	No in group	**Rate/ 1000**	**No in group**	**Rate/ 1000**	**No in group**	**Rate/ 1000**	**Iron-folic acid *v* folic acid**	**Multiple micronutrients *v* folic acid**
Pregnancies with single live or stillbirth:								
Total	1688	—	1546	—	1532	—	—	—
Stillbirths (≥☐28 weeks)	52	30.8	47	30.4	63	41.1	1.01 (0.67 to 1.51)	1.39 (0.95 to 2.04)
Live births	1636	—	1499	—	1469	—	—	—
All neonatal deaths	33	20.2	16	10.7	18	12.3	0.53 (0.29 to 0.97)	0.61 (0.34 to 1.10)
Early neonatal deaths	24	14.7	10	6.7	15	10.2	0.46 (0.21 to 0.98)	0.71 (0.36 to 1.39)
Perinatal deaths	76	45.0	57	36.9	78	50.9	0.84 (0.59 to 1.19)	1.18 (0.85 to 1.63)

*Adjusted for cluster randomisation with general estimating equation binomial model.

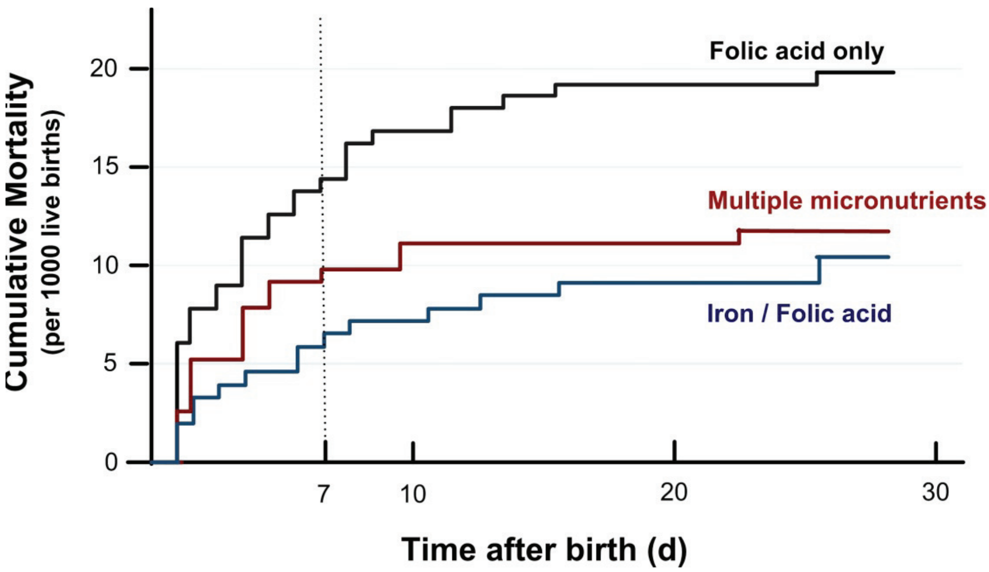

Figure 2 Cumulative mortality curves for infants from birth to 28 days by treatment. Log rank test of difference: P=0.055 for three groups; 0.032 for iron-folic acid *v* folic acid; 0.077 for multiple micronutrients *v* folic acid; 0.708 for multiple micronutrients *v* iron-folic acid

DISCUSSION

In this trial the response to nutrient supplementation in pregnancy varied by outcome. Compared with folic acid alone, multiple micronutrient supplements were significantly associated with increased birth weight, while iron-folic acid had no significant effect. Iron-folic acid supplements were significantly associated with increased birth length. There was a significant increase in the mean duration of gestation in both the iron-folic acid and multiple micronutrients groups compared with folic acid. In post hoc analyses, the iron-folic acid and the multiple micronutrient supplements had no significant effect on perinatal mortality, but there was a significant 54% reduction in early neonatal mortality in women who received supplementation with iron-folic acid compared with folic acid alone, but no significant effects on stillbirths. Both multiple micronutrients and iron-folic acid were associated with significantly increased maternal haemoglobin concentration in the third trimester compared with folic acid, although nearly half of the women in these groups remained anaemic. Overall, we have shown that antenatal nutrient supplementation is associated with increased maternal haemoglobin concentration in late pregnancy, mean birth weight, and duration of pregnancy and reduced preterm delivery and early neonatal mortality.

The effects of iron-folic acid and multiple micronutrients on the duration of gestation and neonatal mortality seem to be related to the iron in these supplements. We tested the impact of two different doses of iron in pregnancy—namely, 60 mg in the iron-folic acid supplements and 30 mg in the multiple micronutrient supplements. The largest impact on neonatal mortality was with the supplement with the highest dose of iron, and we saw a similar pattern for duration of gestation and effects on preterm delivery. In contrast, a recently reported large scale community based trial from Indonesia found no significant differences for neonatal mortality between daily antenatal supplements with iron-folic acid and multiple micronutrients

(WHO/UNICEF formulation as tested in China), which both had the same dose of iron (30 mg)[18]. These findings from Indonesia suggest that the higher neonatal mortality rate we observed in China with multiple micronutrients compared with iron-folic acid cannot be explained by the additional micronutrients but by the different dose of iron in the supplements.

In our trial, the multiple micronutrient supplements were associated with a significant increase in birth weight compared with folic acid, but this did not translate into significant reductions in neonatal mortality. Rather the reduction in neonatal mortality seemed to be related to the increased duration of gestation and the reduced number of early preterm deliveries because of iron in the antenatal supplements. Evidence of an important role of antenatal iron supplements in reducing preterm delivery has been reported from the United States.[19] In the US trial, antenatal iron (30 mg), was associated with a reduced rate of preterm delivery (8% *v* 14%, $P=0.05$) and increased mean birth weight (108 g, $P=0.03$). Longitudinal studies in Brazil[20] and Bangladesh[4] indicate that preterm infants have much higher neonatal mortality than babies with retarded intrauterine growth or those born at term. In Bangladesh, a study of birth outcomes among low birthweight infants reported that 75% of the neonatal deaths were related to preterm deliver[4] . These observational findings suggest a plausible reason as to why the increased mean duration of gestation and reduced preterm delivery (especially early preterm delivery) we observed could be related to a large reduction in neonatal mortality.

Study strengths and limitations

This study was a double blind cluster randomised controlled trial design with a balanced distribution of confounders across treatment groups. Even though randomisation was by village clusters, there were a large number of clusters (531) with about 180 per treatment group and a relatively small number of births in each cluster. Population recruitment of pregnancies allowed complete tracing of fetal losses, including spontaneous and induced abortions and stillbirths, and pregnancy outcomes in both hospital and home deliveries. We used appropriate statistical methods in the data analysis that adjusted for the cluster randomisation.

The location of the study in a socioeconomically disadvantaged area of western China allowed us to examine effects of nutrient supplements in a population with the highest rates of low birth weight and neonatal mortality in the country. Only 2.3% of the enrolled women were lost to follow-up, and 94% of the liveborn infants were weighed within 72 hour of delivery. There was no evidence of under-enumeration of neonatal deaths, with neonatal mortality rates in the folic acid group (20.2/1000 live births) similar to those reported for equivalent rural counties from the national maternal and child health surveillance system (22.5/1000 live births)[3].

The trial was adequately powered to detect small changes in infant anthropometry at birth and the duration of gestation, but one limitation was the low power to detect changes in the prevalence of low birth weight, preterm delivery, and perinatal mortality. The mortality analyses were post hoc rather than a primary hypothesis and we did not have enough data to fully examine the effects of the different supplements on mortality. For example, the non-significant reduction in neonatal mortality associated with multiple micronutrients might have been significant with a larger sample. The outcome data were collected by the local health service staff, although the research team did provide special training on how to measure and record these outcome indicators for the trial.

The balance of treatments by area and therefore by service providers reduced the likelihood of any systematic differences in misclassification of stillbirths and early neonatal deaths by treatment group. The duration of gestation was measured by asking the mother to recall the date of her last menstrual period. The heightened awareness and knowledge about reproduction because of the one child policy in China, however, might have improved the accuracy of the respondent's recall of her last menstrual period and other perinatal events.

Comparison with other studies

Our results help to explain the apparent increase in neonatal and perinatal mortality reported from other trials using the same or similar formulation of multiple micronutrient supplements compared with standard iron-folic acid supplements[21-23]. In two trials from Nepal multiple micronutrient supplementation was associated with a non-significant increase in neonatal and perinatal mortality compared with iron-folic acid[21,22]. In a pooled analysis of these trials neonatal mortality (relative risk 1.52, 1.03 to 2.25) and perinatal mortality (1.36, 1.02 to 1.81) were significantly higher in the multiple micronutrients group compared with iron-folic acid.[23] These findings were interpreted by the authors as an increased risk of perinatal mortality with multiple micronutrient supplements. Our findings showed a significant reduction in neonatal mortality for iron-folic acid (47%) and a non-significant reduction for multiple micronutrients (39%) compared with folic acid. The greater effect on mortality of iron-folic acid compared with multiple micronutrients could account for why comparisons of multiple micronutrients with iron-folic acid give the appearance of an increased risk of neonatal mortality.

There are similarities between our results and the results of other earlier controlled trials of iron supplementation in pregnancy[24,25]. In Niger, a double blind randomised controlled trial assessed the impact of 100 mg iron or placebo in the third trimester on iron status of the mother and newborn and on the anthropometric status of the newborn[24]. The iron supplements were associated with significantly decreased maternal iron deficiency at delivery and at three months after delivery and increased the iron status of the infants at 3 months. Birth length was significantly higher and there was a non-significant increase in birth weight in the iron supplemented group. There were seven fetal and neonatal deaths in the placebo group and one in the iron supplemented group. In the US, a trial of iron supplementation (30 mg/day) versus placebo from 11 weeks' gestation in pregnant women who were not anaemic reported a significantly longer duration of gestation (0.6 weeks) and fourfold reduction in the risk of preterm low birth weight in the iron supplemented women[25]. Our findings were also consistent with those from observational studies that reported an increased risk of preterm delivery in women with iron deficiency anaemia in the first trimester of pregnancy.[26-30] For example, an analysis of perinatal data from Shanghai showed a greater than twofold increase in the risk of low birth weight and preterm delivery in women who had moderate anaemia (90-99 g/l) and a greater than threefold increase for women with severe anaemia (<90 g/l) in the first trimester[29].

The absorption of iron during pregnancy is a complex physiological process, with increasing relative absorption as gestation progresses but with a decrease in the percentage of the dose absorbed as the total dose increases[31]. In our trial, even though the amount of iron in the iron-folic acid supplement was twice the amount in the multiple micronutrient supplement this would not necessarily have led to twice the absorption of iron and therefore twice the dose of iron. The vitamin C in the multiple micronutrient supplements might have enhanced the absorption of iron and also contributed to reducing the difference in the absolute amount of iron absorbed. The response of haemoglobin concentration to the iron-folic acid and multiple micronutrients was similar, but the associated neonatal mortality seemed to be proportionate to the likely relative amount of iron contained in each supplement.

We observed a larger birth weight response in multiple micronutrients group than in the iron-folic acid group. This finding was similar to that observed in other trials comparing iron-folic acid and multiple micronutrients, but the total increase in birth weight in our multiple micronutrients group compared with iron-folic acid group was less than that reported in other studies[22,32,33]. This did not translate into lower neonatal mortality, although it implies improved fetal growth and development, which might confer other health benefits to the newborn, such as improved infant growth, boosted immune responses, and enhanced cognitive development.

We have shown that micronutrient supplements have important health effects for the newborn infant, with a potential to increase birth weight and birth length through increased duration of gestation and direct effects on fetal growth. Reduced neonatal mortality appears to be a response to the iron in the supplements, possibly mediated by increased duration of gestation and lower rates of preterm delivery. We cannot recommend that multiple micronutrients at the currently proposed dose[12] should replace standard iron-folic acid supplements in pregnancy because they seem to provide less protection against preterm delivery and neonatal deaths in populations where iron deficiency in pregnancy is common. An adequate dose of iron is required in micronutrient supplements for pregnant women in developing countries to maximise reductions in neonatal mortality.

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Chapter 5

Mixture of ferric sodium ethylenediaminetetraacetate (NaFeEDTA) and ferrous sulfate: An effective iron fortificant for complementary foods for young Chinese children

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Abstract

Background Ferric sodium ethylenediaminetetraacetate (NaFeEDTA) enhances iron absorption in the presence of phytate. However, the amount of NaFeEDTA that would have to be added to a complementary food to provide the necessary intake of iron for an infant or young child if NaFeEDTA were the sole iron fortificant exceeds the Acceptable Daily Intake (ADI) of EDTA for this age group. EDTA increases iron absorption at a molar ratio EDTA:iron of less than 1:1.

Objective To determine whether iron absorption is enhanced with a mixture of ferrous sulfate ($FeSO_4$) and NaFeEDTA.

Methods Two studies with a crossover design were conducted in separate groups of 14 and 15 children aged 24 to 31 months. A complementary food consisting of millet porridge with cabbage, tofu, and pork-filled wheat flour dumplings was fortified with 2 mg iron as either $FeSO_4$ or NaFeEDTA (study 1) or 4 mg iron as $FeSO_4$ or a mixture of 2 mg each of $FeSO_4$ and NaFeEDTA (study 2).Iron absorption was determined based on erythrocyte incorporation of stable iron isotopes.

Results In study 1, the geometric mean (± SD) iron absorption was 8.0% (3.1, 20.8) and 9.2% (3.1, 27.0) from food fortified with $FeSO_4$ and NaFeEDTA, respectively. In study 2, iron absorption was significantly higher from food fortified with 4 mg iron as 1:1 mixture of $FeSO_4$/NaFeEDTA than from food fortified with$FeSO_4$; the geometric mean iron absorption was 6.4% (3.0, 13.5) and 4.1% (1.9, 8.9), respectively.

Conclusions The enhancing effect of EDTA on iron absorption is less strong in composite meals containing enhancers; nevertheless, the equal mixture of $FeSO_4$ and NaFeEDTA significantly enhanced iron absorption and can be a strategy to ensure adequate iron absorption from phytate-containing complementary foods.

INTRODUCTION

Iron deficiency is the most common nutritional disorder worldwide. Infants and young children are at higher risk due to their high iron requirements during rapid growth and limited intake of bioavailable iron from their diet [1, 2]. A survey in Chinese children conducted in 2001 reported that the prevalence rates of iron deficiency and iron-deficiency anemia in infants under 12 months of age were 66% and 21%, respectively, and 44% and 8% in young children aged 12 to 36 months [3]. Traditionally, infants and young children in China consume homemade complementary foods based on cereals containing mainly nonheme iron of limited bioavailability, which makes it difficult to provide the required amounts of bioavailable iron for this age group. Fortifying the complementary food in the home with an iron-containing micronutrient (and macronutrient) mixture just prior to consumption could be an effective approach for increasing iron intake [4, 5].

Cereal-based complementary foods, however, are particularly difficult to fortify with iron, since they contain significant quantities of phytate, a potent inhibitor of iron absorption [6,7]. Ferric sodium ethylenediaminetetraacetate (NaFeEDTA) is recommended as an iron fortificant for cereal-based foods because the EDTA moiety protects iron from phytate and additionally prevents iron-catalyzed fat oxidation reactions during the storage of cereal flours [8]. NaFeEDTA is reported to be two- to fourfold better absorbed than $FeSO_4$ from a variety of cereal-based foods [9]. Its use for food fortification has been approved by the Joint Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Committee on Food Additives [10]. It is specifically recommended by WHO for fortification of corn flour, soy sauce, and fish sauce [11] and is the only iron compound that is recommended for the fortification of high-extraction wheat flour [12]. A pilot providing multimicronutrients with NaFeEDTA for home addition to foods for infants and young children in rural Gansu Province of China has proven effective in the reduction of iron-deficiency anemia [13].

There is a problem, however, in using NaFeEDTA as the sole iron fortificant in foods for infants and young children. The amount of NaFeEDTA that would have to be added to a complementary food to provide the necessary intake of iron for an infant or young child if NaFeEDTA were the sole iron fortificant exceeds the Acceptable Daily Intake (ADI) of EDTA for this age group. The Joint FAO/WHO Expert Committee on Food Additives has established an ADI of 2.5 mg per kilogram of body weight per day for calcium disodium EDTA, and, from this an ADI of 1.9 mg per kilogram of body weight per day can be calculated for EDTA [14, 15]. This translates to a maximum of 2.7 mg of iron per day as NaFeEDTA for a child aged 6 months with an average body weight of 7.5 kg [16]. This amount of iron is not enough to meet the iron requirements of a 6-month-old child (7.7 mg/day for 6- to 12 month-old children with diets of 12% bioavailability) [17]. One option, which utilizes the enhancing effect of EDTA but does not exceed the ADI of EDTA, is to fortify the complementary food with a mixture of ferrous sulfate ($FeSO_4$) and NaFeEDTA.

The present study investigated iron absorption in Chinese children from a traditional home-made complementary food fortified with two different levels of iron (2 and 4 mg per meal) as $FeSO_4$, NaFeEDTA, and a 1:1 mixture of $FeSO_4$ and NaFeEDTA. We hypothesized that the absorption of iron from NaFeEDTA would be higher than that from $FeSO_4$, and that the higher fortification level (4 mg) would result in the greatest amount of absorbed iron. To our knowledge, this is the first study in young children investigating iron absorption from a home-made complementary food fortified with a mixture of $FeSO_4$ and NaFeEDTA.

SUBJECTS AND METHODS

Subjects

Thirty-five apparently healthy children, 24 to 31 months of age, of both sexes were recruited through the local health center in rural Yuanshi County, Hebei Province, China. Exclusion criteria included regular intake of medications and known gastrointestinal or metabolic disorders. Intakes of vitamin or mineral supplements and fortified foods were discontinued 2 weeks before and during the entire study. The children were treated for intestinal parasites with levamisole hydrochloride (1.5 mg/kg) on three consecutive days about 1 month before the start of the study.

The study protocol was approved by the ethical committee of the National Institute for Nutrition and Food Safety, Beijing, and by the ethical committee at ETH Zurich, Switzerland. The parents of the subjects were informed about the aims and procedures of the study, and oral and written consent was obtained.

Study design

Thirty-five children were randomly assigned to study group 1 (n = 17) or 2 (n = 18). Administration of labeled test meals was done under close supervision on two consecutive days in the morning after an overnight fast. No food or drinks were allowed during the 3 hours following intake of the labeled test meals. One day before administration of the test meals, body weight and height were measured and a baseline venous blood sample was drawn for analysis of hemoglobin concentration. A second blood sample was drawn 14 days after the intake of the second labeled test meal for isotopic analysis. Calculation of iron absorption was based on the shift in the isotopic ratios after a 14-day incorporation period, as described by Walczyk et al. [18].

Stable isotope labels

Labeled $FeSO_4$ ($^{58}FeSO_4$ and $^{57}FeSO_4$) was prepared from isotopically enriched elemental iron (Chemgas, Boulogne, France) by dissolution in diluted sulfuric acid. $Na^{57}FeEDTA$ was prepared from ^{57}Fe-enriched elemental iron by dissolution in hydrochloric acid and dilution in water. The resulting $FeCl_3$ solution was mixed immediately before use with an aqueous Na_2EDTA solution at a molar ratio of 1:1 (Fe:EDTA). Isotopic enrichment was 93.0% for ^{58}Fe and 96.0% for ^{57}Fe.

The test meals were labeled as follows: In study 1, meal A, 2 mg ^{58}Fe (as $FeSO_4$); meal B, 2 mg ^{57}Fe (as NaFeEDTA). In study 2, meal A, 2 mg ^{58}Fe was added as $^{58}FeSO_4$ plus 2 mg Fe as $FeSO_4$ of normal isotopic composition (total 4 mg Fe); meal B, 1 mg ^{57}Fe was added as $Na^{57}FeEDTA$ plus 1 mg ^{57}Fe was added as $^{57}FeSO_4$ plus 1 mg Fe as $FeSO_4$ of normal isotopic composition plus 1 mg Fe as NaFeEDTA of normal isotopic composition.

Test meals

The test meal was a traditional complementary food chosen on the basis of a dietary survey conducted in the rural communities of the study area. The meal consisted of 80 g of millet porridge and 80 g of dumplings with a cabbage, tofu, and pork filling. Millet (whole grain) porridge was prepared as a batch every morning before feeding by weighing the millet and water at a ratio of 1:14, mixing, and boiling for 30 minutes. The wheat flour (low extraction), water, and the filling ingredients needed for 1,000 dumplings were weighed individually. The wheat flour was made into dough by mixing the water and flour. The filling was prepared by mixing the finely chopped cabbage, tofu, and pork meat with oil and spices. Dumplings with the filling inside were prepared as a batch and stored frozen until the day of feeding. The uncooked dumplings contained 22% wheat flour, 17% water, 29% cabbage, 25% tofu, 4% pork meat, 2% oil, and 1% spices. Each morning the dumplings were boiled in water before feeding. For standardization, mineral water was used for food preparation.

Weighed amounts of labeled and unlabeled $FeSO_4$ and/or NaFeEDTA in dilute acid were added to 60 g of millet porridge before feeding. The children consumed the porridge and dumplings with the assistance of a study supervisor. After consumption, each child was then fed an additional 20 g of porridge for cleaning the bowl. Each bowl was rinsed with 20 g of mineral water, and the washings were consumed by the child in order to assure complete consumption of the isotopic labels.

Blood analysis

Hemoglobin was measured in whole blood within 3 hours of blood drawing by the cyanmethemoglobin method (hemoglobin meter 1002 MC). Anemia was defined as a hemoglobin concentration less than 110 g/L [19].

Each isotopically enriched blood sample was analyzed in duplicate for its isotopic composition under chemical blank monitoring. Whole blood samples were mineralized by using a mixture of HNO_3 and H_2O_2 and microwave digestion, which was followed by separation of the sample iron from the matrix by anion-exchange chromatography and a solvent–solvent extraction step into diethyl ether [18]. All isotopic analyses were performed by negative thermal ionization–mass spectrometry (MAT 262; Finnigan MAT, Bremen, Germany) at the Human Nutrition Laboratory at ETH Zurich, Switzerland.

Calculation of iron absorption

Circulating iron was calculated based on the blood volume and hemoglobin concentration. Blood volume was estimated from height and weight according to the method of Linderkamp et al. [20]. For calculations of fractional absorption, 90% incorporation of the absorbed iron into red blood cells was assumed [21].

Food analysis

Food samples were homogenized and freeze-dried before analysis. Duplicate analysis was used to measure native iron and phytate contents in the test meal. The iron content of the test meal was measured with electrothermal flame atomic absorption spectroscopy (AA240Z; Varian, Mulgrave, Australia) after mineralization by microwave digestion (MLS ETHOS; Microwellen Labor System, Leutkirch, Germany) in a mixture of HNO_3 and H_2O_2. The phytate content was measured by a modified method of Makower [22], and inorganic phosphate was determined by the method of Van Veldhoven and Mannaerts [23].

Statistical analysis

The paired *t*-test was used to evaluate data in the iron absorption studies. A p value $< .05$ was considered to indicate a statistically significant difference. Percentage absorption values were converted to logarithms before statistical analysis, and the results were reconverted to antilogarithms to recover the original units

[24]. The results are presented as geometric means ± SD. Height-for-age z-scores (HAZ) and weight-for-age z-scores (WAZ) were calculated with WHO Anthro (version 3, April 2009).

RESULTS

Twenty-nine children (14 boys and 15 girls) completed the study, 14 in group 1 and 15 in group 2. Six children were excluded because they did not completely consume the test meals. Age, anthropometric features, and hemoglobin concentrations of the subjects are summarized in table 1. Stunting (HAZ < – 2) was observed in one child. None of the children were underweight (WAZ < – 2). One child was anemic. One child consumed a zinc supplement, and four children occasionally consumed iron-fortified formula. The consumption of supplement and fortified formula was discontinued prior to the study. Table 2 shows the iron and phytate contents and the molar ratios of phytate to iron in the test meals. Considering both the native iron content of the test meal and the level of fortification iron (2 and 4 mg), the molar ratios of phytate to iron were reduced from 13 (before fortification) to 3.7 and 2.1, respectively. As shown in table 3, in both studies the mean fractional iron absorption from the NaFeEDTA-fortified meals was higher than that from the $FeSO_4$-fortified test meals, but a significant difference was found only in study 2, at a fortification level of 4 mg ($p < .05$). In study 2, the mean amount of absorbed iron was increased from 0.20 mg from the $FeSO_4$-fortified test meal to 0.31 mg from the $FeSO_4$/NaFeEDTA-fortified test meal.

Table 1. Age, anthropometric features, and hemoglobin concentration of the study groups (mean ± SD)

Variable	Study 1 (n = 14)	Study 2 (n = 15)
Age (mo)	28.1 ± 1.4	27.8 ± 2.5
Weight (kg)	12.2 ± 0.9	12.9 ± 1.4
Height (cm)	87.6 ± 4.2	89.7 ± 4.3
Hemoglobin (g/L)	130.5 ± 11.9	127.0 ± 8.3

Table 2. Phytate content of test meals, and iron content and molar ratio of phytate to iron of test meals before and after iron fortification

Variable	Study 1	Study 2
Phytate (mg)	121	121
Iron (mg)		
Prefortification	0.8	0.8
Postfortification	2.8	4.8
Phytate:iron molar ratio		
Prefortification	13	13
Postfortification	3.7	2.7

Table 3. Fractional iron absorption from the test meal fortified with different iron compounds at different levels of iron fortification

Study	Meal A	Fractional iron absorption (%)[a]	Meal B	Fractional iron absorption (%)[a]	B/A ratio	p^b
1 (n = 14)	2 mg Fe as $FeSO_4$	8.0 (3.1, 20.8)	2 mg Fe as NaFeEDTA	9.2 (3.1, 27.0)	1.15	0.19
2 (n = 15)	4 mg Fe as $FeSO_4$	4.1 (1.9, 8.9)	2 mg Fe as $FeSO_4$ + 2 mg Fe as NaFeEDTA	6.4 (3.0, 13.5)	1.56	0.03

a. Values are geometric means ± SD in parentheses.
b. Paired *t*-tests were used to compare differences in absorption on logarithmically transformed data.

DISCUSSION

EDTA is an iron chelator that enhances iron absorption from inhibitory meals by reducing iron binding to phytate at low pH [25]. It is thought that EDTA binds the iron in a soluble complex in the gastrointestinal tract, preventing it from forming insoluble, nonabsorbable complexes with phytate, other dietary inhibitors, or hydroxyl ions [26]. The EDTA moiety per se (added as Na_2EDTA) has been reported to increase iron absorption in adults from $FeSO_4$-fortified rice meals [27] and from $FeSO_4$-fortified wheat and wheat–soy infant cereals [9], and in children from a $FeSO_4$-fortified milk cereal [28, 29]. With a rice meal, a maximum threefold increase in absorption was observed with an EDTA-to-iron molar ratio between 0.25:1 and 0.5:1, compared with only a twofold increase at a 1:1 molar ratio [27]. Hurrell et al. showed that a molar ratio of EDTA to iron of 1:1 was most effective with high-phytate wheat–soy cereal [9]. With our test meals, the total amount of iron (native and fortification) was 2.8 mg in study 1 and 4.8 in study 2, at a molar ratio of EDTA to iron of 0.7:1 and 0.4:1, respectively. This molar ratio is in the range that has been shown to increase iron absorption from phytate-containing meals [27, 30]. It was somewhat surprising, therefore, that only the meal with a molar ratio of EDTA to iron of 0.4:1 significantly increased iron absorption.

The results of the present study can perhaps be explained by meal composition. Most of the previous studies investigated the absorption of NaFeEDTA from meals containing cereal products only. The traditional complementary food given as the test meal in the present study was a composite meal of millet porridge with dumplings containing cabbage, tofu, wheat flour, and a small amount of pork meat. This meal had a modest amount of phytate but also contained enhancing substances, such as ascorbic acid in the cabbage and meat proteins, and other substances that could have counteracted or diluted the effect of EDTA on iron absorption. Ascorbic acid and meat have been reported to counteract the inhibiting effects of phytates on nonheme iron absorption [31-33]. Hallberg et al. demonstrated that iron absorption was increased by 85% by the addition of 20 g of powdered meat and by 39% by the addition of 20 mg of ascorbic acid [31]. The small amount of meat (3 g) and relatively large amount of cabbage (23 g, with an estimated ascorbic acid content of 12 mg [34]) could help dilute the enhancing effect of EDTA, since the enhancing effects of these components are not additive [30]. Tuntawiroon et al. have already reported that other meal components decrease the inhibitory effect of phytate in a rice meal [35]. The apparent discrepancy between studies 1 and 2 could be explained by the other meal components having a stronger effect on absorption from 2 mg of fortification iron than from 4 mg of fortification iron.

This is the first study to compare iron absorption in young children from a homemade complementary food fortified with $FeSO_4$ with iron absorption from the same food fortified with a mixture of $FeSO_4$ and NaFeEDTA. Our findings suggest a benefit in using a combination of $FeSO_4$ and NaFeEDTA (with a final molar ratio of EDTA to iron of 0.4:1) to fortify a composite complementary food. The enhancing effect of this mixture in cereal gruels containing no other meal components is likely to be even greater. An enhanced iron absorption would help meet the high iron requirements of young children with low body weight while not leading to an exposure to EDTA above 1.9 mg daily per kilogram of body weight [15]. With the 1:1 mixture of NaFeEDTA and $FeSO_4$, a maximum of 5.4 mg of iron could be added to the complementary food so as to conform with the ADI (for children with minimum body weight of 7.5 kg). Based on the fractional absorption from our traditional complementary food (6% in study 2), this would provide some 30% of the child's requirement in a single meal. Our study was performed in a county in northern China that is not considered a National Poverty County, and the growth and hemoglobin concentrations of the children were better than the average level for rural children in China. The iron status of the children could not be assessed, but it is estimated to be sufficient based on the hemoglobin concentration. In addition, the test meal used in our studies was less inhibitory than other complementary foods. Ma et al. reported that the molar ratio of phytate to iron was around 5.5 in diets of the Chinese rural population [36], and the positive impact of EDTA on iron absorption would be expected to be higher in these more inhibitory meals. An additional advantage of the mixture is its lower cost. The price of NaFeEDTA is much higher than that of $FeSO_4$, and costs are of major concern in national fortification programs [37].

The present results provide practical suggestions on the use of NaFeEDTA for in-home fortification of complementary foods. The China Ministry of Health issued the General Standard of Fortified Complementary Food Supplement in 2009, which is consistent with the recommendations of the Joint FAO/WHO Expert Committee on Food Additives [15] and allows the use of NaFeEDTA in fortified complementary food. The mixture of NaFeEDTA with other iron compounds was recommended [38]. This study provides support for that recommendation and opens the door for intervention studies with in-home fortified complementary foods to assess effectiveness, acceptability, compliance, cost, and other factors that are needed to evaluate the long-term impact of this strategy.

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Chapter 6

Complementary feeding and growth of infant and young child in China

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Abstract

Objective to provide information on complementary feeding status in China, emphasis the important of complementary feeding promotion, and explore approaches to increase nutritional status of children by improving infant and young children feeding.

Methods 2000 and 2005 China Food and Nutrition Surveillance System (CFNSS) database were used to assess the frequency and quality of complementary food intake of rural children in China. Logistic Regression was applied to analyze the relationship of complementary feeding practice and child growth.

Results The complementary feeding practices improved in rural areas during year 2000 to 2005. While the feeding practices still poor in both rural and poor rural areas. The percentage of children consuming meat/eggs more than 4 times a week among 6-9 months children was only around 30%, for other age groups only around 50% or less. Our study also showed positive impact of daily consumption of meat/eggs, milk, and vegetables/fruit on child growth. OR of no meat/eggs inclusion in complementary feeding for stunting at age 9-24 month was1.392, no non-milk inclusion for stunting was 1.378.

Conclusions Our data on complementary feeding practices providing compelling evidence on the gaps of complementary feeding practices and the importance of improving complementary feeding in rural China. Integrated actions including behavior change on child feeding practice and in-home complementary food fortification should be implemented in more rural areas of China to improve the growth of rural children.

INTRODUCTION

Nutrition of infants and young children affects their health and their physical cognitive and emotional development[1]. Poor maternal nutrition, inadequate child feeding practices and high rates of infection cause stunting, which is difficult to reverse after the age of 2 years[2]. Breastfeeding is a critical factor for improving nutritional status, reducing illness and improving cognitive and social development in young children. Exclusive breastfeeding for the first 6 months of life and continued breastfeeding for 2 years and beyond are closely associated with improved health[3]. Improvements in complementary feeding from 6 months of age enhance linear growth and micronutrient status[4, 5]. Suboptimal feeding practices and poor quality complementary food result in micronutrient deficiencies and stunting among children 6-24 months of age[3] and beyond[2].

In China, despite the significant decrease of malnutrition among children during 1990 to 2005, with the national stunting prevalence of children under 5 years of age decreased from 33.4% to 10.5%, underweight from 19.1% to 6.9%, while the prevalence of stunting and underweight in poor rural areas was 17.5% and 12.3% respectively[6], continues to be unacceptably high. Practical and acceptable methods to improve infants and young children feeding in China especially in poverty areas are urgently needed.

Our study focus on the evaluation of complementary feeding practices thought intake of food items and its frequency in year 2000 and 2005 in rural China, analyzed association of complementary food items intake and its consuming frequency with children' nutritional status (stunting, underweight). The purpose of this study is to provide information on complementary feeding status in China, emphasis the important of complementary feeding promotion, and explore approaches to increase nutritional status of children by improving infant and young children feeding.

MATERIAL AND METHOD

Data sources

2000 and 2005 China Food and Nutrition Surveillance System (CFNSS) database were used in this study. The CFNSS was established in 1990 in order to monitor the food intake of households and nutritional status of Chinese children. The surveillance sites of CFNSS were sampled based on the existing Disease Surveillance Site with 145 sites and the Urban/Rural Social Economic Status Survey of State Statistical Bureau with 600 sites. Comprehensive information could be collected by doing this. In order to be nationally representative and to have sufficient representation of urban, general rural and poor rural areas, respectively, a three stage stratified random sampling method was used. 14 urban sites, 17 general rural sites and 9 poor rural sites, altogether 40 sample sites (county site in rural, district site in urban) were in the CENSS. In each site, two townships (streets in urban) randomly selected, and 2 villages (neighbourhood committees in urban) randomly selected from each township. With the help of village doctors (community doctors in urban), we enrolled all children under-5 years old children in the village (neighbourhood committee) until the number reached 400 per county. The caregivers were interviewed based on a designed questionnaire, which includes household information, child feeding practice. Anthropometric and hemoglobin concentration of the selected children were assessed. Details information on China Food and Nutrition Surveillance System has been described elsewhere[7].

Data analysis

All data were checked manually for completeness and were double-entered into a data management system. We conducted range, extremum and logical checks for accuracy. Child growth was assessed by using z-score of NCHS references recommended by WHO. The complementary feeding practice in children was measured by two variables: the beginning age of complementary food items included to child and the frequency of the complementary food items intake.

X^2 tests were used to assess different proportion of children who consume a food item $\geq$4 times/week times between year 2000 and 2005 by each age groups.
We performed multivariate logistic regression to assess food item and its frequency on stunting and underweight. Child gender, mother's education, family income, migrant mother are considered as potential confounding variables.
Statistical significance was set at $P<0.05$. The data analysis of this study was conducted by SAS 9.1.

RESULT

Complementary feeding practice of rural children in 2000 and 2005

Using database of 2000 CFNSS, complementary feeding practices of 4238 rural children aged 6-24 months were analyzed. Using database of 2005 CFNSS, complementary feeding practices of 4527 rural children were analyzed here. Detailed information was reported in the series reports on 15-year establishment of Chinese Food and Nutrition Surveillance System[7]. The proportion of meat/egg, milk, vegetable/fruit inclusion in complementary feeding by different age groups was listed in table 1,2, 3. Milk is defined as other than breastmilk. From year 2000 to year 2005, the proportions of feeding meat/eggs with more than 4 times per week were increased significantly ($P<0.05$) in all age groups, from 15% to 33% in general rural, and from 15% to 30% in poor rural among children 6 to 9 months of age. from 14% to 46% in general rural, from 20% to 40% in poor rural among 9 to 12 months of age children, For children aged 12 to18 months and 18 to 24 months, the proportions of children inclusion of meat/eggs were also increased significantly. (Table 1).

Table 1. The proportion and frequency of meat/eggs consumption among infants and children with different age groups (%)

Age (month)	6～9 m		9～12 m		12～18 m		18～24 m	
year	2000	2005	2000	2005	2000	2005	2000	2005
General rural								
<1 time/week	38	40	41	24	50	11	54	9
1-3 times/week	47	26	45	30	36	33	38	34
>4 times/week	15*	33*	14*	46*	13*	55*	8*	57*
Poor rural								
<1 time/week	34	47	34	25	42	18	48	19
1-3 times/week	51	24	46	35	48	32	45	32
>4 times/week	15*	30*	22*	40*	10*	50*	7*	50*

P<0.05

The inclusion of milk in complementary feeding showed the same trend. The improvement was even more obvious in poor rural areas. (Table 2).

As for the inclusion of vegetables/fruit complementary feeding, in general there was no improvement except the inclusion more than 4 times a week of 6-9 months poor rural children and 18-24 months general rural children (Table 3).

Table 2. The proportion and frequency of milk consumption among infants and children with different age groups (%)

Age (month)	6～9 m		9～12 m		12～18 m		18～24 m	
year	2000	2005	2000	2005	2000	2005	2000	2005
General rural								
<1 time/week	66	50	65	35	57	28	63	28
1-3 times/week	5	10	4	14	5	15	6	14
>4 times/week	29	38	31*	51*	37*	57*	31*	58*
Poor rural								
<1 time/week	65	34	67	24	63	25	65	31
1-3 times/week	9	13	6	16	7	18	9	17
>4 times/week	26*	53*	26*	60*	30*	58*	26*	53*

P<0.05

Table 3 The proportion and frequency of vegetable/fruit consumption among infants and children with different age groups (%)

Age (month)	6～9m		9～12m		12～18m		18～24m	
Year	2000	2005	2000	2005	2000	2005	2000	2005
General rural								
<1 time/week	40	38	22	21	12	11	15	5
1-3 times/week	19	20	21	23	14	19	26	17
>4 times/week	41	42	58	56	73	70	40*	77*
Poor rural								
<1 time/week	64	33	27	16	17	10	11	4
1-3 times/week	18	20	21	24	20	21	17	18
>4 times/week	18*	47*	41	60	63	69	72	78

P<0.05

Complementary feeding and child growth

Table 4 shows the multivariate logistic regression analysis results on the inclusion of different item of complementary food and children malnutrition (stunting and underweight). To demonstrate the effect of complementary food, we used children aged 9-24 months for analysis. As the 9 months children should have begun complementary feeding for 3 months. No meat/eggs inclusion, no milk inclusion and no vegetables/fruit inclusion increase the risk of 9-24 months child stunting and underweight (P<0.01), except no meat/eggs introduction on underweight (p=0.19).

Table 4. Logistic regression result on infant and young child growth and complementary food items (9~24 m)

	Stunting		Underweight	
	OR	P	OR	P
No Starch inclusion	0.636	<0.01	0.628	<0.01
No milk inclusion	1.378	<0.01	1.189	<0.01
No vegetables/fruit inclusion	1.433	<0.01	1.548	<0.01
No meat/eggs food inclusion	1.392	<0.01	1.118	0.19

Based on the same data, multiple regression analysis was used to evaluate the relationship between the frequency of inclusion complimentary food item and malnutrition of the young children aged 9-24 months. Every independent was laid three levels: based on the frequency of complementary food item included every day, 1 was defined as included every week, 2 was defined as included every month, and 3 was defined as included less than 1 time every month or never have included. The results of the logistic regression analysis showed that three levels of vegetable/fruit, meat/egg food and milk inclusion are predictors of stunting and underweight (P<0.01), except monthly introduction of vegetable/fruit on underweight (Table 5).

Table 5. Logistic regression analysis on infant and young child growth and frequency of complementary foods items (9~24 m)

Food items	Frequency	Stunting		Underweight	
		OR	P	OR	P
Milk	weekly	2.1	<0.01	1.818	<0.01
	monthly	2.842	<0.01	3.094	<0.01
	Seldom/never added	3.088	<0.01	2.54	<0.01
Vegetable/fruit	weekly	1.739	<0.01	1.908	<0.01
	monthly	1.698	0.03	1.566	0.1
	Seldom/never added	1.768	<0.01	1.478	<0.01
Meat/egg	weekly	2.862	<0.01	2.487	<0.01
	monthly	4.131	<0.01	3.632	<0.01
	Seldom/never added	2.546	0	1.963	<0.01

Based on added everyday, 1 was defined as added every week, 2 was defined as added every month, 3 was defined as added less than one time every month or never added.

DISCUSSION

The growth of Chinese children increased significantly during year 1990 to year 2005, while the prevalence of stunting of children in poor rural counties continues to be unacceptably high. The high prevalence of stunting compared with underweight underlines the need to better target interventions to address its direct determinants, including improving overall dietary quality and diversity, rather than focus solely on adequate energy and weight gain[8]. Our data on complementary feeding practices providing compelling evidence on the gaps of complementary feeding practice in rural China and showing the intervention on complementary feeding must be strengthened and expanded.

Compared to year 2000, the frequencies of consuming meat/eggs and milk in rural infants and young children were higher in year 2005. But even in year 2005, the feeding practice still poor in both rural and poor rural areas. The percentage of children consuming meat/eggs more than 4 times a week among 6-9 months children was only around 30%, for other age groups only around 50% or less. Our study also showed positive impact of daily consumption of meat/eggs, milk, and vegetables/fruit on child growth. These results provided evidence to the development of practical communication messages for parents behavior change in rural China. A needs assessment survey in two rural townships in China concluded that it was poor complementary feeding practices rather than inadequate household food resources responsible for the growth faltering in this areas[9], and the follow up education intervention was successful in improving the mothers' infant feeding knowledge and some reported infant feeding practices, maintaining higher breastfeeding rates, decreasing anemia rates and improving infant growth at 12 months of age in the intervention group[10]. Caufield et al (1999) showed that parents' behavior change in improved complementary feeding can result in increased growth among infants[11]. Brown et al demonstrated a positive impact on weight gain in exclusive breastfed and complementary-fed children following the promotion of a variety of improved feeding practices in rural Bangladesh[12]. In Malawi, participating mothers changed their behavior and were able to use existing food resources to improve complementary feeding practices through practical communications [13].

The nutrition requirements (with variations depending on age) from complementary food, for infants that consume average intakes of breastmilk after the age of 6 months , are as follow: energy, 50-70%; protein, 20-45%; vitamin A, 5-30%; thiamine, 50-80%; riboflavin, 50-65%; calcium, 60%, vitamin B_6, 75-88%; Zinc, 85%; and almost 100% for iron[14]. Most infants are physically able to consume family foods by about 12 months; however, their high nutritional requirements relative to body size and small amounts of foods consumed indicate that nutrients-dense foods will still be required into the second and third years of life[15, 16]. Plant-based complementary foods by themselves are insufficient to meet the needs for certain nutrients (particularly iron, zinc and calcium) during the period of complementary feeding. Inclusion of animal products can meet the gap in some cases, but this increases the cost and may not be feasible for the lowest-income groups[16]. Furthermore, the amount of animal products that can feasible be included are generally not sufficiency to meet the gaps in iron calcium and sometimes zinc, Gibson et al evaluated 23 different complementary food mixtures used in developing countries, some of which included animal products. Although most met the protein and energy needs, none met the desired iron density and few met the desired calcium and zinc density[17]. Thus strategies to optimize nutrient intake from locally available foods may need to be coupled with other approaches in order to fully address the problems of micronutrient malnutrition[16]. Fortification of staple foods will not address the micronutrient deficiencies of infants and young children because of the small amounts they consume relative to their high requirements. Target fortification or the products of complementary foods fortified with micronutrients and of an adequate micro- and micronutrients is one approach to help meet infants and young children nutritional requirements during this vulnerable period[15]. An effectiveness trial conducted in 5 poor counties of Gansu province in the northwestern part of China during 2001-2003 to evaluate the effects of a complementary food supplement among infants and young children. Village doctors were responsible to delivery supplements and provide knowledge on infant and young children feeding. After 18 months project implementation, the Length for age Z score (LAZ) and hemoglobin concentration increased more among children in nutrient-dense complementary food supplement group[18, 19].

Integrated actions including behavior change on child feeding practice and in-home complementary food fortification should be implemented in more rural areas of China to improve the growth of rural children.

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Chapter 7

General Discussion

Iron deficiency anemia (IDA) is the most common micronutrient deficiency worldwide. Pregnant women and children constitute risk groups for developing IDA due to the higher iron requirements for placenta development, fetal growth and child growth and brain development. Many reports have shown an association between IDA and impaired cognitive and psychomotor development in infants and young children[1]. However, a causal relation between iron deficiency and poorer brain function has not been demonstrated. In addition, there is ample evidence that the high frequency of iron deficiency anemia in the developing world has substantial health and economic costs[2]. It is therefore of public health significance to explore effective and optimal approaches for iron supplementation and iron fortification among high risk populations (i.e., pregnant women, infants and young children).This thesis addresses the above outstanding areas, aiming at i) exploring the long-term effect of IDA in pregnancy and IDA in children under 2 years on cognitive and psychomotor function and social emotional behaviour, ii) understanding the impact of prenatal multiple micronutrient supplementation on health outcomes of newborns, and iii) investigating iron absorption from a complementary food fortified with a mixture of $FeSO_4$ and NaFeEDTA.

This chapter will review the main findings, discuss limitations of the methods used, propose areas for further research and finally formulate implications relevant to public health.

MAIN FINDINGS

The main findings of this thesis are summarized in Table 1. The human brain is vulnerable during critical periods of development, including the last trimester of fetal life and the first 2 years of childhood-a period of rapid brain grown termed the “brain growth spurt”[3]. We found that iron deficiency anemia in the third trimester of pregnancy is associated with impaired mental development of the child up to 24 months of age. Prenatal iron supplementation with sufficient iron protects child development even when the IDA of the mother was not properly corrected during pregnancy (chapter 2). Preschool aged non-iron deficient and anaemic children who had chronic IDA in the first 2 years of life showed affected social emotional behavior. In contrast, behavior and affect of children whose anemia was corrected before 24 months were comparable to that of children who were non-iron deficient and anemic throughout the first 2 years of life (chapter 3). Our results indicate that adverse effects can be reduced and/or prevented with iron supplementation during critical periods of brain development. WHO recommends universal distribution of iron-folic acid supplements to pregnant women in developing countries to prevent iron deficiency anemia. Our study suggests that multiple micronutrients are just as effective as iron-folic acid in reducing anemia and good adherence can be achieved with multiple micronutrients during pregnancy (chapter 4). In-home fortification of complementary food is an effective approach to provide additional iron to infants and young children in developing countries. An equal mixture of $FeSO_4$ and NaFeEDTA significantly enhanced iron absorption and can be a strategy to ensure adequate iron absorption from phytate-containing complementary foods (chapter 5).

METHODOLOGICAL ISSUES

This section addresses methodological issues which may potentially influence the conclusions presented in this thesis, namely selection bias, information bias and confounding.

Selection Bias

Selection bias encompasses any systematic differences between comparison groups and may lead to spurious associations. Selection bias could occur as a result of non-random selection for example due to refusals (non-response) to participate in the study associated with particular characteristics related to the outcome of the study[4]. In this thesis, selection bias could have occurred in the longitudinal observational studies in chapter 2 and 3, the intervention study in chapter 4 as well as in the laboratory study reported in chapter 5.

Table 1 Main findings of the study

Chapter 2 Prenatal iron deficiency anaemia in the third trimester of pregnancy is associated to mental development of child.	**Chapter 4** Prenatal multiple micronutrient supplementation (with iron) is associated with increased Hb in late pregnancy, birth weight and reduced preterm delivery.
Chapter 3 Preschool-aged non-iron deficiency anemia children who had chronic iron deficiency anemia in infancy showed affected social emotional behavior.	**Chapter 5** Mixture of sodium Iron EDTA and ferrous sulfate have a higher iron absorption compared to ferrous sulfate.

Chapter 2 is a secondary analysis of 2 years follow-up data of a study population that participated in a randomized controlled trial on prenatal supplementation to assess the cognitive and psychomotor development of children. In total, 5828 eligible Chinese women from a rural area were recruited in the original randomized controlled trial. From them, 1286 women with a singleton full term live birth attended the follow-up study. Among them, hemoglobin samples in the third trimester were available in 850 women. There are possibilities that our sample does not reflect the original study population introducing selection bias. However, there were no significant differences in baseline characteristics between participating and not-participating women. As for the baseline characteristics of the prenatal-IDA group and non-IDA group in our study, the socio-economic characteristics, mean number of gestational week and BMI at enrolment of women were comparable between the two groups. Pregnant women in the prenatal-IDA group reported significantly older age (p=0.024), greater parity (p=0.025) at enrolment and shorter gestation at the time haemoglobin concentration was checked (P=0.006). The child birth weight, the number of gestational week at birth, gender distribution, breastfeeding rate, formula introduction, prevalence of stunting and underweight of children and age at each assessment were comparable between the two groups except for child age at 18 month assessment (p=0.001), breastfeeding rate at 3 months (p=0.013), formula introduction at 24 months (p=0.025). The observed differences in baseline characteristics for some of the variables may have impacted our outcome. However, we took a conservative approach by adjusting the outcome estimates for these variables in the data analysis. Although we took into account the most relevant baseline variables, it cannot be ruled out that some unmeasured participant characteristics related to the outcome of the study might still have led to minor bias in the estimates.

In addition, among the 850 children, 95 (11%) missed their first development assessment, 61 (7%) missed the second assessment, 84 (10%), 96 (11%) and 96 (11%) missed their third to fifth assessment respectively. In total, 600 (71%) children were assessed on all 5 occasions. We used multiple imputation to fill the missing values in each assessment. Imputation produced outputs for each complete dataset and a pooled output increased the power of our study[5] and reduced the potential risk of non-response[4]. We found similar trends of the pooled results compared to the complete case analysis of our data and the results were similar to what has been found in other studies[5]. The pooled results are considered to provide valid statistical inferences compare to those provided by simple imputation or complete case analysis[6, 7].

Chapter 3 is also a secondary analysis of a dataset from a multiple stage cluster sampling trial on fortified complementary food supplementation and its follow-up study [8]. The sample size of the original trial was 1478 children aged 4-12 months. Of these, 1094 children attended the 24-month-old assessments. For the 4-year follow up, we subsampled children by random selection of villages because of limitation of funding. As a consequence, from the original samples, only 404 children were involved in the social emotional observations at 4 year follow-up. Similarly, only those children who attended the baseline survey, 24-month assessment, and 4-year follow-up, and being non-anemic at 4-year follow up assessment were reported in chapter 3 (N=161). When selection criteria are used to exclude subjects in analysis, there is a possibility, as mentioned before, that the sample does not reflect the study population introducing selection bias. However, data analysis showed there was no significant difference in baseline characteristics between the children excluded and involved. As for the child and family characteristics of the study groups, the proportion of males in corrected-IDA group was higher than that in non-IDA group. The percentage of WAZ less than -2 in the corrected-IDA group was lower than that in non-IDA group at baseline and 24-month assessment. There were no other statistically significant differences between the chronic-IDA group and the non-IDA group, or between the corrected-IDA group and the non-IDA group with regard to background characteristics, such as child age, child's growth, mothers' age and education at the 4-year follow-up assessment. The above differences between the groups are not likely to have introduced a selection bias because these variables are unlikely to be related to our outcomes; similarly, we took a conservative approach by adjusting for these variables in the data analysis. Therefore we have confidence in our findings.

Chapter 4 is a double blind cluster randomized controlled trial using cluster (village) randomization. The trial took place in two poor rural counties in Shaanxi province of north west China. There were 34 townships and 561 villages in the two counties. The randomization of villages was stratified by county with fixed ratio treatments (1:1:1) and blocks of 15 villages to ensure geographical balance with an approximately equal distribution of treatment per township. The socio-demographic and the anthropometric characteristics at enrolment and the cluster and individual level baseline characteristics were balanced by treatment groups. Only 2.3% of the enrolled women were lost to follow up with no differences among the treatment groups. The rate of adherence was more than 75% among more than 90% of women and similar for the three treatment groups. There was no evidence of under-enumeration of neonatal deaths, with neonatal mortality rates in the folic acid group (20.2/1000 live birth) similar to those reported for equivalent rural counties from the national maternal and child health surveillance system (22.5/1000 live birth)[9]. So selection bias in this trial is assumed to be minimal.

In the laboratory study reported in **Chapter 5,** iron absorption of a fortified complementary food was determined based on erythrocyte incorporation of stable iron isotopes. Thirty-five apparently healthy children (24-31 months) of both sexes from 3 villages were recruited through the local health center in Yuanshi County of Hebei province. Exclusion criteria included regular intake of medications and known gastrointestinal or metabolic disorders. Twenty nine young children (14 boys, 15 girls) completed the studies. Six children were excluded as they did not completely consume the test meals. The children in this study are reflecting the general population of apparently healthy children in rural areas. Selection bias in this laboratory study is not very likely.

Information bias

Information bias involves misclassification of participants with respect to disease or exposure status. Information bias in our studies could have occurred regarding the assessment of child development (chapter 2 and chapter 3), definition of IDA (chapter 2 and chapter 3), and due to measurement error (chapter 4 and chapter 5).

Assessment of child development

Bayley Scale of Infant Development (BSID) was applied to assess children's development in **Chapter 2.** BSID provides indicators of mental development, motor development and indirectly behavior. It is a global measure of development and the most commonly used outcome measure in infants and young children who are aged 2 month to 2.5 years [10, 11].Wide recognition in developmental testing, the high-coefficient of reliability, and test-retest reproducibility have favored its use[12]. BSID was translated into Chinese and locally standardized to ensure culturally appropriateness [13]. The Bayley test is measuring very different functions in children of different ages, for example, the language items become increasingly important in the second year of age. If lower Bayley scores were noted in ID infants or if scores increased with iron treatment, the scores themselves would provide little guide to what specific functions were altered[14]. The Bayley scales are advised for assessing current developmental status and not for predicting future abilities[1]. These disadvantages of Bayley are not relevant to our observational study, because we compared the scores of children of the same age.

The BSID was administered at the village clinic or the child's own home in a standardized manner. In our study, the child's development was proposed to be assessed at 3, 6, 12, 18, 24 months of age; however, because of logistical problems, this schedule could not always be followed. If the child was sick, was unavailable, or could not cooperate, then assessment was arranged for a later date within one month. The later application of the BSID could have introduced bias in the assessment of child development, although we adjusted for age during each assessment in the data analysis.

The study reported in **chapter 3** used extensive behavioral videotaped observations to determine whether preschool children that had chronic IDA when they were below 2 years of age showed alterations in affect and activity. Based on cross-cultural research by Chen and colleagues[15] and Goldsmith et al.[16], we designed a set of observations in a laboratory setting to measure the child's social referencing and wariness/inhibition, frustration tolerance and affect. Mother and child were invited to a clinic room for the laboratory observations which were videotaped. The videos were coded by four Chinese graduate students in psychology, who were unaware of the iron status of the children. Inter-rater agreement averaged 85% for child's affect, social referencing, and inhibited behavior, based on 20% of the sample. The behavioral observation was multifaceted, entailing behavioral ratings and direct quantitative observations during play and developmental testing and has been verified on testing behavioral differences between preschool children affected by IDA in infancy[17]. The possibility of information bias on children's social emotional development is therefore limited.

Definition of iron deficiency anemia

Iron deficiency anemia was estimated according to hemoglobin concentration in **chapter 2, 3** and **4.** This assessment is a potential limitation of our studies. Hemoglobin concentration generally overestimates the prevalence of IDA, because it does not account for anemia attributable to other nutritional deficiencies (e.g., vitamin A deficiency), infections, hemoglobinopathies, or ethnic differences in normal hemoglobin distribution[2]. In population groups in which iron deficiency has been identified as the primary cause of anemia, hemoglobin is a very useful indicator of tissue iron deficiency. However, when many causes of anemia coexist, additional iron specific indicators are required to diagnose or survey iron deficiency[18]. The baseline survey of **chapter 2** and **chapter 4** showed the anaemia prevalence among children less than 2 years, reproductive aged women and adult men of 33.2%, 37.6% and 8.8% respectively. From the much lower anaemia prevalence of adult men, we can conclude that iron deficiency was probably the main cause of anaemia in our study area[19]. Furthermore, for women with adequate iron, Hemoglobin concentration starts to decline during the early part of the first trimester, reaches its nadir near the end of second trimester because of the physiologic response, including plasma volume expansion, then gradually rises during the third trimester[20]. Studies among Chinese pregnant women showed that Hemoglobin concentration did not improve from the second to third trimester, providing evidence that pregnant women's iron stores were not sufficient to support the expansion of red cell mass in the third trimester[21-23]. Hence, the high prevalence of anemia in the third trimester was mainly due to iron deficiency in China. As in **chapter 3,** hemoglobin concentration showed an overall increase of 10.5 g/L after iron supplementation [8, 24]. It appears that iron deficiency was a major cause of anemia in this population.

Confounding

Confounding is said to occur when the influence of factors other than iron status may explain the apparent association between iron and behavior, thereby affecting the internal validity of the study[11]. There is considerable evidence that IDA is associated with a large number of socioeconomic and biomedical disadvantages that can themselves affect children's development such as low socioeconomic status, lack of stimulation in the home, including lack of maternal warmth; poor maternal education, and intelligence quotient (IQ); maternal depression; frequent absence of father; low birth weight and early weaning; parasitic infection; elevated blood lead levels; and undernutrition [25].

Chapter 2 and **chapter 3** explored the impact of IDA in first pregnancy and/or chronic IDA in less than 2-year-olds on child development. In **chapter 2**, potential confounders, such as household wealth, women's education, women's Body Mass Index (BMI) at enrolment, child birth weight, gestation weeks at birth, gender distribution, prevalence of stunting and underweight of children at assessment were comparable between the prenatal IDA and non-IDA group. We adjusted by multivariate analysis the outcome estimates for women's age at enrolment, gestation weeks when Haemoglobin concentration was checked, parity at enrolment, age at the 5 assessments, feeding practice. We cannot eliminate the possibility that the altered behavior of the prenatal-IDA group in this study was caused by some relevant but unmeasured factor(s), such as maternal depression and other environmental disadvantages, which could account for lack of stimulation (leading to altered behavior and development). So, this would possibly leave room for some residual confounding leading to under- or overestimation of the findings.

Similarly, in **chapter 3**, children's age, mother's age and mother's education, Hemoglobin concentration at 4-year follow up are comparable between chronic IDA and non-IDA group, and between corrected IDA and non-IDA group. In the analysis, we adjusted for the factors that were different between the groups, such as proportion of male children in corrected IDA group which was higher than that in the non-IDA group, the percentage of underweight which was lower in corrected IDA group than that in the non-IDA group at baseline survey and 24- month assessment. Although statistical control of these factors improves the comparability of the study groups, it is inherently inadequate because it cannot account for some unmeasured factors, such as social economic status, maternal depression, father's absent and as yet unrecognized confounders[11]. So, again we cannot be completely confident that the estimates are unbiased because some residual confounding might still have affected the study results.

Chapter 4 is a double blind cluster randomized controlled trial, thus most of the confounding factors would have been balanced and thereby controlled by successful randomization. Even though randomization was by (village) clusters, there were a large number of clusters (531) and a relatively small number of births in each cluster. Therefore, we may consider this as a trial with individual randomization. The randomization by village was stratified by county with fixed ratio treatments (1:1:1) and blocks of 15 villages to ensure geographical balance with an approximately equal distribution of treatment per township.

EXTERNAL VALIDITY

External validity indicates the extent to which the results of an internally valid study can then be generalized to other populations and circumstances[11]. It is important to discuss our findings in the context of results of other studies and to reach a synthesis leading to conclusions about the current state of knowledge.

Prenatal iron deficiency anemia and long-term impact on cognitive and psychomotor function of children

Chapter 2 of this thesis explored the impact of prenatal IDA on cognitive and psychomotor function of children at 3, 6, 12, 18 and 24 months of age. The prenatal IDA group showed significantly lower Mental Development Index (MDI) scores at 12, 18 and 24 months of age compared to the prenatal non-IDA group. To our knowledge, this is the first longitudinal study which followed children for 2 years after birth.

Poorer cognitive, motor and/or social emotional functioning was observed while infants and young children were iron deficient. But maternal iron status during pregnancy was unknown in the studies on child mental development. It is thus unclear whether the observed effects are due to both pre- and postnatal iron deficiency or postnatal deficiency only. The brain is at its most vulnerable during critical periods of development, including the last trimester of fetal life and the first 2 years of childhood-a period of rapid brain grown termed the “brain growth spurt”[3]. During fetal development, iron is prioritized to red cells at the expenses of other tissues, including the brain[26]. When maternal iron deficiency occurs, the number of placental transferrin receptors increases so that more iron is taken up by the placenta. Evidence is accumulating that the capacity of this system may be inadequate to maintain iron transfer to the fetus when the mother is iron deficient[27], which caused decreased brain iron[28] [29]or/and IDA in the offspring[30]. Wachs et al (2005) found that newborn infants with low cord blood Hemoglobin concentration and iron have altered temperament during the first week of life[31]. Similarly, the prenatal IDA probably has impacted fetus brain iron and caused adverse effects on neurodevelopment in our study.

Research suggests that prenatal IDA is related to reduced fetal iron stores. Infants whose mothers with mild or moderate IDA during pregnancy are at risk of iron deficiency [32, 33]. Infants with IDA show poorer cognitive, motor, social-emotional, and neurophysiologic functioning than those without[25, 34-37]. In our population, children were not provided iron supplementation after birth. Children in the two groups have no differences in terms of feeding practices, growth and other related background characteristics. Probably, more children in the prenatal-IDA group suffered IDA due to low fetal iron stores, which can explain delayed mental development of children in the prenatal-IDA group.

We conducted a secondary analysis of follow-up data of an existing dataset. The low power to detect changes in the MDI and PDI is a limitation in our study. We had 64% power to detect a change of 5 scores in MDI and 73% power to detect a change of 4 scores in PDI with an a=0.05. Our results require therefore replication with larger sample sizes.

Iron deficiency anemia in children under 2 years and long-term effect on social emotional behavior

The study in **chapter 3** used more extensive videotape observations than previous studies to examine the long-term effect of chronic IDA in children under 2 years on social emotional behaviour. We found that, despite correction of IDA when children had reached the age of 4 years, children who had chronic IDA before 2 years old showed less positive affect and frustration tolerance, more passive behavior, unengaged affect, and physical self-soothing than children who were non-IDA throughout infancy and young child period. Furthermore, we found that children whose anemia was corrected before 24 months were comparable in behavior at 48 months to children who were non-anemic throughout infancy and young child period.

Our observations of behavioral alterations in preschool children with chronic IDA in infancy and young child period support the findings of other research. A study in France followed children's iron status and Development Quotient (DQ) including postural, coordination, language and sociability quotients. Dommergues et al (1989) found that hemoglobin concentration did not correlate with DQ in 10-month-old children, whereas hemoglobin concentration at 2 years did; hemoglobin concentration was positively associated with overall developmental, postural, coordination, and social quotients at 2 years[38]. A longitudinal study in Costa Rica included a structured mother-child interaction task at 5 years. Children with chronic ID in infancy displayed lower levels of physical activity, positive affect, verbalization, and reciprocal interaction, compared to children with good iron status, despite the correction of their IDA in infancy[39]. These children were followed up to early adolescence, and mothers and teachers rated those with IDA in infancy as having more internalizing behavior problems (anxiety/depression, social problems, etc.)[40].

Social-emotional alterations in young iron deficient children have been interpreted in the light of iron's role in the neurotransmitter function of dopamine in the brain [41, 42]. Iron deficient rats have alterations in dopamine metabolism and show more anxious-like behaviors, with reduced exploration in new environments, with decreased stereotyping, and demonstrate a slower rate of habituation[43, 44]. Studies showing affective changes in iron deficient anemic infants (wariness, hesitance, absence of positive affect) seem to make sense in this context[45]. Neonatal dopamine terminal damage leads to a lifelong hyper-reactivity to novel objects and experiences in an unfamiliar environment[42] and seems to have long term effects on context-dependent attention and affective responses[45]. This can partially explain the altered affect and behavior of the chronic IDA group in the stranger approach session and delay of gratification task of our study.

Our results indicate that adverse effects on social emotional behavior by iron deficiency could be prevented and/or reversed with iron before iron deficiency becomes severe or chronic. Our results emphasize the importance of protecting the developing brain from iron deficiency.

Comparison of the impact of prenatal multiple micronutrient and iron-folic acid supplementation on new born health outcomes

Chapter 4 showed the result of a double blind cluster randomized controlled trial. Compared with folic acid alone, multiple micronutrient supplementations were significantly associated with increased birth weight; there was a significant increase in the mean duration of gestation in both the iron-folic acid and multiple micronutrients groups; both multiple micronutrients and iron-folic acid were associated with significant increased maternal Hemoglobin concentration in the third trimester.

A meta-analysis of 12 randomized controlled trials comparing multiple micronutrients with daily iron-folic acid supplementation during pregnancy (including our trial in China) supports our findings [46], concluding that multiple micronutrient supplements were as effective as the iron-folic acid supplements in terms of the hemoglobin response, even though they contained only half the dose of iron (30 mg). Good adherence can be achieved with prenatal micronutrient supplements during pregnancy when the supply is guaranteed and mothers are counseled positively. Multiple micronutrients increase mean birth weight and reduce the incidence of low birth weight. These findings confirm the conclusions of many other studies over the last two decades that women will consume iron, as well as multiple micronutrients, as long as they have access to them and are adequately counseled on their use[46-48].

In our study, the pregnant women's anemia prevalence in the third trimester was still high, being 43.1%, 45.1% and 61% among the multiple micronutrients supplementation group and iron-folic acid group folic acid group respectively. This has also been seen in many effectiveness trials over the years. It may be that initiating supplementation in pregnancy is too late for many women, especially those with pre-existing anemia[46, 49]. A study conducted in Vietnam showed that the use of weekly iron-folic acid prior to and during pregnancy was associated with better iron status in the first and second trimesters of pregnancy and with reduced prevalence of low birth weight compared with pregnant women who only received daily iron-folic acid supplementation during pregnancy[49]. However, studies by Ekstrom et al. in Bangladesh comparing weekly and daily iron supplements found that a maximum hemoglobin effect was achieved by just 40 tablets each containing 60 mg of iron, whether taken as a daily or a weekly regimen. However, anemia still persisted in these Bangladeshi women, even when the maximum hemoglobin response had been reached, with 20% still affected in the weekly and 14% in the daily regimes[50]. This suggests that other factors are involved in the causality of the anemia besides just iron availability. One possible reason could be the high burden of infections. The WHO/UNICEF program guidance for the control of anemia recommends the treatment of infections[51]. Furthermore, studies in Kenya[52], South Africa[53] and Vietnam[54] all show that deworming, together with iron supplementation, resolves the problem of anemia better than either treatment alone. More studies are still needed in this area.

Iron absorption with a mixture of $FeSO_4$ and NaFeEDTA as a fortificant of complementary food

Chapter 5 is the result of two studies using a crossover design conducted in separate groups of 14 and 15 children aged 24 to 31 months. A complementary food consisting of millet porridge with cabbage, tofu, and pork-filled wheat flour dumplings was fortified with 2 mg iron as either $FeSO_4$ or NaFeEDTA (study 1) or 4 mg iron as $FeSO_4$ or a mixture of 2 mg each of $FeSO_4$ and NaFeEDTA (study 2). Iron absorption was determined based on erythrocyte incorporation of stable iron isotopes. It demonstrated that the equal mixture of $FeSO_4$ and NaFeEDTA significantly enhanced iron absorption of homemade complementary food.

The EDTA has been reported to increase iron absorption in both adults and children from $FeSO_4$ fortified meals[55-58]. With a rice meal, a 3-fold increase in absorption was observed with an EDTA to iron molar ratio between 0.25 and 0.5:1, and a 2-fold increase was observed at a 1:1 molar ratio[55]. With our test meals, molar ratio of EDTA to iron is of 0.7:1 in study 1, and 0.4:1 in study 2. These molar ratios are in the range of other results showing increase of iron absorption from phytate containing meals [55, 59]. It was somewhat surprising therefore that only the meal with the molar ratio of EDTA to iron of 0.4:1 has significantly increased iron absorption.

The results of the present study can perhaps be explained by meal composition. Most of the previous studies investigated the absorption of NaFeEDTA from meals containing cereal products only. Our test meals had a modest amount of phytate but also contained enhancing substances, such as ascorbic acid in cabbage and meat proteins, and other substances which could have both counteracted or diluted the influence of EDTA on iron absorption. Ascorbic acid and meat have been reported to counteract the inhibiting effects of phytates on non-heme iron absorption[60-62]. Hallberg et al. also demonstrated that the effect of 20 g powdered meat showed an increase in iron absorption of 85%, 20 mg ascorbic acid lead to an increase of 39% [60]. The small amount of meat (3 g) and relatively large amount of cabbage (23 g, with an estimated ascorbic acid content of 12 mg [63]) consumed could help dilute the enhancing effect of EDTA[59]. Tuntawiroon et al have already reported other meal components to decrease the inhibitory effect of phytate in a rice meal [64]. So the apparent discrepancy between studies 1 and 2 could be explained by the different meal components having a stronger effect on absorption from 2 mg fortification iron than from 4 mg fortification iron.

Chapter 5 was performed in a county of northern China that is not considered a national poverty county, and the hemoglobin concentrations of the children were better than the average level for rural children in China. In addition, the test meal used in our studies was less inhibitory than other complementary foods. The positive impact of EDTA on iron absorption would be expected to be higher in these more inhibitory meals.

Complementary feeding practices in rural China: optimize nutrient intake from locally available foods combined with complementary food fortification

Chapter 6 used 2000 and 2005 Food and Nutrition Surveillance data to analyze the status of complementary feeding practices and the association of child growth with consumption of food items such as meat/eggs, milk and vegetables/fruit in rural China. Compared to year 2000, the frequencies of consuming meat/eggs and milk in rural infants and young children were higher in year 2005. But even in year 2005, the feeding practice still poor in both rural and poor rural areas. For example, the percentage of children consuming meat/eggs more than 4 times a week among 6-9 months children was only around 30%, for other age groups only around 50% or less. Our study also showed positive impact of daily consumption of meat/eggs, milk, and vegetables/ fruit on child growth. These results provided evidence to the development of practical communication messages for parents behavior change in rural China. A needs assessment survey in two rural townships in China concluded that it was poor complementary feeding practices rather than inadequate household food resources responsible for the growth faltering in this areas[65], and the follow up education intervention was successful in improving the mothers' infant feeding knowledge and some reported infant feeding practices, maintaining higher breastfeeding rates, decreasing anemia rates and improving infant growth at 12 months of age in the intervention group[66]. Caufield et al (1999) showed that parents' behavior change in improved complementary feeding can result in increased growth among infants[67]. Brown et al demonstrated a positive impact on weight gain in exclusive breastfed and complementary-fed children following the promotion of a variety of improved feeding practices in rural Bangladesh[68]. In Malawi, participating mothers changed their behavior and were able to use existing food resources to improve complementary feeding practices through practical communications [69].

The high energy and nutrient requirements relative to body size and the capacity to only consume small amounts of foods indicate that nutrient-dense foods must be provided from 6 months until the second and third years of life[70, 71]. The plant-based complementary foods by themselves are insufficient to meet the needs for certain nutrients (particularly iron, zinc and calcium) during the period of complementary feeding.

The energy and nutrient requirements (with variations depending on age) from complementary food for infants consuming average intakes of breastmilk after the age of 6 months , are as follows: energy, 50-70%; protein, 20-45%; vitamin A, 5-30%; thiamine, 50-80%; riboflavin, 50-65%; calcium, 60%, vitamin B_6, 75-88%; Zinc, 85%; and almost 100% for iron[72]. Inclusion of animal products can meet the gap in some cases, but this increases costs and may not be feasible for the lowest-income groups[71]. Furthermore, the amount of animal products that can feasibly be included are generally not sufficient for iron, calcium and sometimes zinc. Gibson et al. evaluated 23 different complementary food mixtures used in developing countries, some of which included animal products. Although most met the protein and energy needs, none met the desired iron density and few met the desired calcium and zinc density[73]. Thus, especially for pregnant women and young children who are at high risk of IDA, strategies to optimize nutrient intake from locally available foods may need to be combined with other approaches such as complementary food fortification in order to fully address the problems of micronutrient malnutrition[71].

CONCLUSIONS

Our longitudinal observational studies provided additional evidence to the long-term prognosis of IDA in pregnancy and/or IDA in children under 2 years on child development, in addition to well established evidence on preterm labour, low birth weight and infant mortality. Our study results suggest that prenatal IDA impacts infants' and young children's mental development. Furthermore, preschool-aged children who had chronic IDA in infancy, which was not corrected till 2 years old showed affected social emotional development. While children whose IDA was corrected before 24 months were comparable in behavior to children who were non-IDA throughout infancy and young child period.

From our study it can be concluded that:

1. The association of IDA during the first critical 1000 days of live has long-term impact on child development
2. Multiple micronutrients with at least 30 mg iron are as effective as iron-folic acid in reducing IDA during pregnancy
3. Equal mixture of $FeSO_4$ and NaFeEDTA as a fortificant of complementary food significantly enhanced iron absorption of homemade complementary food.

PUBLIC HEALTH IMPLICATIONS

In humans, there is compelling evidence that children less than 24 months old with iron deficiency anemia are at risk for poorer cognitive, motor, social emotional and neurophysiological development in both the short and long run[36]. Existing trials on iron supplementation among infants and young children in developing countries uniformly show benefits of iron, especially on motor development and social emotional behavior[36]. These results indicate that adverse effects can be prevented and/or reversed with iron earlier in development or before iron deficiency becomes severe or chronic[36]. The issue of optimal timing of intervention also pertains to prenatal iron deficiency. Animal studies with iron repletion, even earlier, corresponding to the third trimester in pregnant women, suggest complete correction of regional and total brain iron[74, 75] and neurotransmitter function[74], but not dendritic organization in the hippocampus[75]. These human and animal studies indicate that the ability of iron repletion to correct brain iron deficits and some brain-behavior effects depends on timing[36]. Our study results further emphasized the importance of protecting the developing brain from iron deficiency by increase iron supply and intake during pregnancy and in the child less than 24 months old. Causal inferences between iron deficiency and adverse effects on children's development cannot be made based on current study results.

There is a possibility that IDA is acting as a marker for other underlying factors which affected children's development[14]. Programmatic recommendations, as is well recognized, cannot always wait until there is perfect evidence for action, which may never come[46]. Furthermore, sufficient data has shown that the high frequency of iron deficiency anemia in the developing world has substantial health economic costs. Iron deficiency anemia increases the risk of preterm labour, low birth weight, infant mortality, and predicts iron deficiency in infants after 4 months of age[76]. Iron deficiency anemia increases susceptibility to infections, mainly of the upper respiratory tract of children. Iron deficiency might increase the risk for chronic lead poisoning in children exposed to environmental lead[77]. So, iron deficiency is really a public health problem among pregnant women and children in developing countries where monotonous, plant-based diets provide low amount of bioavailability iron. Strategies on prevention and control of iron deficiency, in particular among high-risk populations with higher iron requirements, are needed (Figure. 1)

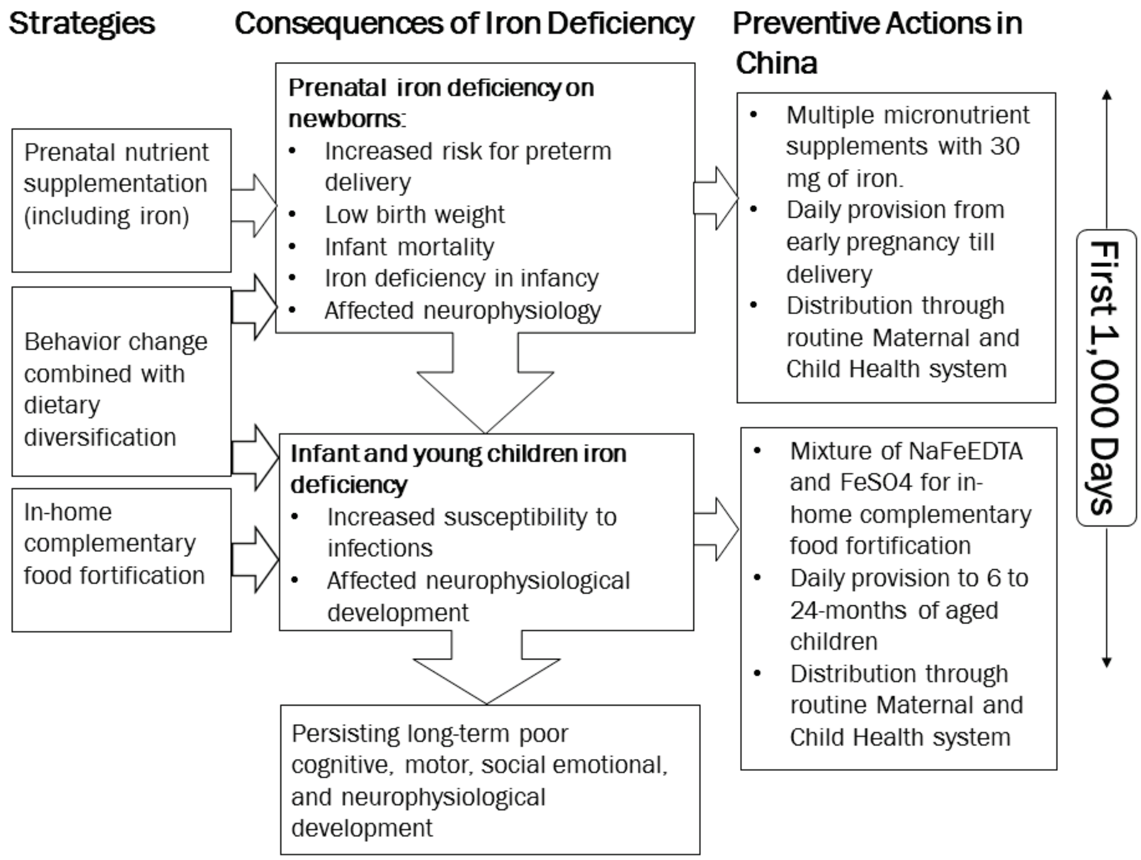

Figure1: Consequences of iron deficiency and preventive actions in China

In China there are no specific policies or programs for the distribution of multiple micronutrient or iron-folic acid supplementation during pregnancy, even to disadvantaged women. WHO recommends universal distribution of iron-folic acid supplements to pregnant women in developing countries to prevent and treat iron-deficiency anemia[78]. Our research results and the meta-analysis of 12 randomized controlled trials (including our trial in China)[46] suggests that the daily provision of multiple micronutrients is an optimal approach for pregnant women in developing countries including China in terms of health and development outcomes of newborns and young children. China's current legislation allows only 1/3 to 2/3 of the recommended recommend nutrient intake (RNI) for iron to be provided by daily supplementation[79]. The maximum of extra iron that can be provided to pregnant women daily is 10, 17 and 23mg at first, second and third trimester respectively. Our study results combined with the results of other studies[46] show the benefit of giving at least 30 mg iron daily to pregnant women on prevention of IDA, which can provide guidance to policy maker to develop a new standard for prenatal nutrient supplementations. In our study, the routine Maternal and Child Health system ensured successful implementation. The challenge in a large public health program is to achieve high-quality, consistent counselling with good coverage, effect monitoring system through this routine Maternal and Child Health system.

Our study on iron absorption with a mixture of $FeSO_4$ and NaFeEDTA as a fortificant of complementary food provide practical suggestions on the use of NaFeEDTA for in-home fortification of complementary food. The Ministry of Health in China issued the Standard of Fortified Complementary Food Supplement (CFS) in 2009, in which a mixture of NaFeEDTA with other iron compounds was recommended [80, 81]. The standard of CFS is consistent with the recommendations of the Joint FAO/WHO Expert Committee on Food Additives [82] on the use of NaFeEDTA in fortified complementary food, with an ADI of 2.5 mg per kilogram of body weight per day for calcium disodium EDTA, and, based on this an ADI of 1.9 mg per kilogram of body weight per day can be calculated for EDTA [82, 83]. This translates to a maximum of 2.7 mg of iron per day as NaFeEDTA for a child aged 6 months. Several effectiveness trials on in-home fortified complementary food with 2.5 mg of NaFeEDTA and 5 mg of other iron compounds are conducted in China since then. The CFS products are distributed through routine Maternal and Child Health system freely. The effectiveness trials received high coverage of compliance and positive results (personal communication). CFS has also attracted considerable interest from the Ministry of Health and other official bodies in China in free supply of CFS for poor rural children in China's poverty counties. However the quality control of CFS, timely supply, high quality counselling, effective monitoring system should be addressed before it can be expanded nationwide.

FUTURE RESEARCH

Considering our study findings and research by others, the following research is proposed for the near future.

1. Continued research into the causal relationship of iron deficiency and child behaviour

The demonstrated associations between anemia and poor child development in correlational or case control studies cannot establish cause-and effect relationships. Longitudinal observational studies give additional useful information about the long-term prognosis of children with IDA and the types of deficits at different stages of development. However, they also cannot provide evidence of a causal relationship. The most accurate way of pinpointing iron deficiency as a cause of poor development is to conduct a double-blind, randomized, controlled trial and to demonstrate that correcting or preventing IDA (with confounding factors such as intestinal worm and infections addressed in both groups) will result in improved children's development. The randomized controlled trials are extremely difficult and expensive to run. They need large samples to have adequate statistical power, even in populations in which the prevalence of IDA is high [25]. The subjects must be followed for a long period with information on iron status and child development. Currently there are too few randomized trials of adequate size and appropriate design to make firm conclusions on a causal relationship. In addition, researchers are reluctant to use a placebo group in the field of iron deficiency on ethical grounds. This is the main reason we do not have final answers to the important question of whether iron treatment can benefit the development of IDA children[25].

2.Development of better measurement tools to assess child development

The Bayley test was sensitive to initial differences between IDA and non-IDA groups in previous studies[25], however, it tests very different functions in children of different ages. Therefore, if lower Bayley scores were noted in ID infants or if scores increased with iron treatment, the scores themselves would provide little guidance to what extent specific functions were altered[14]. Previous research has emphasized the importance of measuring speed of information processing, accuracy of discrimination and conceptual learning instead of global developmental scores to get a better understanding of the effects of ID on cognitive function[84]. There are many well established and widely used tests in rodents that target various aspects of learning, memory, attention and emotion, while so few of these tests have been used to examine effects in iron-restricted animals. It is worthwhile for psychologists and nutritionists working together on the development of better measurement tools to be used in the assessment of iron deficient children. The use of much more refined behavior and cognitive measures will provide the necessary evidence for defining the “sensitive period” in infant development and the potential that iron remediation will be successful[85].

3.Explore the causality of anemia

The results of our prenatal nutrient supplementation trial (chapter 5) showed participants remained anemic even though they were taking seemingly adequate amounts of supplemental iron. This has also been seen in many other effectiveness trials on prenatal supplementation over the years[46]. The effectiveness trials on fortified complementary food among infants and young children in China also found anemia still persist in around 20% of children after 18 months intervention (unpublished data). These suggest that other factors are involved in the causality of the anemia beside just iron availability, such as worm infections. The research result on the causality of anemia in a region can provide evidence for integrated actions in a region and better resolve the anemia problem.

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Summary

Summary

Evidence indicates that the first 1000 days of life (the period from the woman's pregnancy continuing into the child's second year) is the most critical period. This is where nutritional deficiencies have a significant and often irreversible adverse impact on child survival and growth affecting their ability to learn in school and productivity in later life. Sufficient iron supply and intake during pregnancy and in children under two years of age are an important component for nutrition interventions during these critical 1000 days. A causal relationship between iron deficiency and poorer brain function has not been demonstrated yet. In addition, there is ample evidence that the high frequency of iron deficiency anemia (IDA) in the developing world has substantial health and economic costs. It is therefore of public health significance to explore effective and optimal approaches for iron supplementation and iron fortification among high risk populations (i.e., pregnant women, infants and young children).

This thesis addresses some of the above outstanding areas, aiming at i) exploring the long-term effect of IDA in pregnancy and IDA in children under 2 years on cognitive and psychomotor function and social emotional behaviour, ii) understanding the impact of prenatal multiple micronutrient supplementation on health outcomes of newborns, and iii) investigating iron absorption from a complementary food fortified with a mixture of $FeSO_4$ and NaFeEDTA.

The human brain is vulnerable during critical periods of development, including the last trimester of fetal life and the first 2 years of childhood - a period of rapid brain growth termed the "brain growth spurt". We found that iron deficiency anemia in the third trimester of pregnancy is associated with impaired mental development of the child before 24 months of age. The children whose mothers had iron deficiency anemia showed a significantly lower Mental Development Index (MDI) at 12, 18 and 24 months of age. The adjusted mean difference was 5.8 (95% Confidence interval [CI] 1.1 to 10.5), 5.1 (95% CI 1.2 to 9.0) and 5.3 (95% CI 0.9 to 9.7) respectively. Further analysis showed MDI in the prenatal IDA and non-prenatal IDA groups were similar with supplementation of iron-folic acid (60 mg iron), but significantly lower in the prenatal IDA group with supplementation of folic acid or multiple micronutrients. Prenatal iron supplementation with sufficient iron protects child development even when women's IDA was not properly corrected during pregnancy (**chapter 2**).

In a second study, we compared social emotional affect and behavior of three groups of non-anemic 4-year-old children: children with IDA in infancy whose anemia was not corrected before 24 months (chronic IDA, n=27); children with IDA in infancy whose anemia was corrected before 24 months (corrected IDA, n=70), and children who were non-IDA in infancy and at 24 months (n=64). Children's social referencing, wariness, frustration tolerance behavior and affect were observed in a laboratory setting. The whole procedure was videotaped. Child affective and behavioral display was coded using a time-sampling (5-second segments) coding scheme. Preschool aged children who had chronic IDA in the first 2 years of life showed affected social emotional behavior. In contrast, children whose anemia was corrected before 24 months were comparable to children who were non-iron deficient and anemic throughout the first 2 years of life in terms of behavior and affect (**chapter 3**).

Overall, our results indicate that adverse effects can be reduced and/or prevented with iron supplementation during critical periods of brain development. WHO recommends universal distribution of iron-folic acid supplements to pregnant women in developing countries to prevent and treat iron deficiency anemia. However, pregnant women are often deficient in several other nutrients concurrently, all of which can negatively affect their own health as well as their infants' health, growth and development across the life course. Multiple micronutrient supplements containing iron and other micronutrients should be more efficient to help to reduce anemia, because other nutrients often lacking in the diets of pregnant women in

poor populations, including vitamin A, riboflavin and vitamin B_6 and B_{12}, are also needed for hemoglobin synthesis. Improving maternal status of multiple micronutrients could also benefit pregnancy outcome, infant micronutrient stores at birth and breast milk content of micronutrients.

In a randomized double blind controlled trial described in **chapter 4,** we examined the impact of prenatal supplementation with multiple micronutrients or iron-folic acid compared to folic acid alone on birth weight, duration of gestation and maternal hemoglobin concentration in the third trimester. In total 5828 pregnant women were involved. The results suggest multiple micronutrients are as effective as iron folic acid in increasing maternal hemoglobin concentration, birth weight and mean duration of gestation. Our study also shows good adherence can be achieved with multiple micronutrients during pregnancy.

Using 2000 and 2005 Food and Nutrition Surveillance data, we found that complementary feeding practices are suboptimal in both rural and poor rural areas in China, although significant improvements in practices have been made from year 2000 to year 2005. The percentage of consuming meat/eggs more than 4 times a week among 6-9 months children was only 30%, for other age groups only around 50% or less. The high energy and nutrient requirements relative to body size, the capacity to only consume small amounts of foods indicate that nutrient-dense foods must be provided from 6 months until the second and third year of life. Inclusion of animal products can meet the energy and nutrients gap in some cases, but this increases costs and may not be feasible for the lowest-income groups. Furthermore, the amount of animal products that can feasibly be included are generally not sufficient for iron, calcium and sometimes zinc. Thus strategies to optimize nutrient intake from locally available foods may need to be combined with other approaches such as complementary food fortification in order to fully address the problems of micronutrient malnutrition (**chapter 6**).

In-home fortification of complementary food is an effective approach to provide additional iron and other nutrients to infants and young children in developing countries. To determine whether iron absorption is enhanced with a mixture of $FeSO_4$ and NaFeEDTA, we conducted an iron absorption study with a crossover design in two groups with children aged 24 to 31 months. A complementary food consisting of millet porridge with cabbage, tofu, and pork-filled wheat flour dumplings was fortified with 2 mg iron as either FeSO4 or NaFeEDTA (study 1) or 4 mg iron as $FeSO_4$ or a mixture of 2 mg each of $FeSO_4$ and NaFeEDTA (study 2). Iron absorption was determined based on erythrocyte incorporation of stable iron isotopes. In study 1, the geometric mean iron absorption(±SD) was 8.0% (3.1, 20.8) and 9.2% (3.1, 27.0) from food fortified with $FeSO_4$ and NaFeEDTA, respectively. In study 2, iron absorption was significantly higher from food fortified with 4 mg iron as 1:1 mixture of $FeSO_4$/NaFeEDTA than from food fortified with $FeSO_4$ only; the geometric mean iron absorption was 6.4% (3.0, 13.5) and 4.1% (1.9, 8.9), respectively. We concluded that the equal mixture of $FeSO_4$ and NaFeEDTA significantly enhanced iron absorption and can be a strategy to ensure adequate iron absorption from phytate-containing complementary foods (**Chapter 5**).

The studies in this thesis provide further evidence on the association between IDA during the first critical 1000 days of live and the long-term impact on child development. Our research results and the meta-analysis of 12 randomized controlled trials (including our trial in China) suggest that the daily provision of multiple micronutrients is an optimal approach for pregnant women in developing countries including China in terms of the health outcome of newborns. The benefits of giving at least 30 mg iron daily to pregnant women shown in our study as well as by others can provide guidance to policy makers on the development of a prenatal nutrient supplementation standard in China. Moreover, enhanced iron absorption from a mixture of $FeSO_4$ and NaFeEDTA as a fortificant supports China's current practice on the use of NaFeEDTA for in-home fortification of complementary food.

Considering our study findings and research by others, the following research is proposed for the near future. 1) Continuing research into the causal relationship of iron deficiency and child behaviour; 2) Developing of better measurement tools to assess child development; 3) Exploring the causality of anemia in the region.

Samenvatting

Samenvatting

De eerste 1000 levensdagen van een kind (vanaf de zwangerschap tot in het 2e levensjaar) vormen de meest kritische periode waarin voedingstekorten een significante en vaak onomkeerbaar negatieve impact hebben op de groei en overlevingskansen van een kind. Dit heeft gevolgen op het leer- en werk vermogen tijdens het latere leven. Het bereiken van een voldoende ijzer inname tijdens de zwangerschap en bij kinderen onder de twee jaar vormen belangrijke componenten van voedingsprogramma's gericht op deze eerste 1000 levensdagen. Een causaal verband tussen ijzertekorten en hersenontwikkeling is nog niet eerder aangetoond. Aangezien het bewezen is dat ijzertekorten in ontwikkelingslanden een direct negatief gevolg heeft voor de volksgezondheid en de economie, is het belangrijk om naar effectieve en optimale aanpakken voor ijzer supplementatie en fortificatie te zoeken voor de risicogroepen (zoals zwangeren en kinderen tot 2 jaar)

Dit proefschrift beschrijft de bovenstaande problematiek, met als doel i) het verkennen van het lange termijn effect van ijzertekorten tijdens de zwangerschap en de eerste 2 levensjaren op cognitie, psychomotorische ontwikkeling en sociale-emotionele gedrag, ii) het begrijpen van de impact van een voedingssupplement met meerdere micronutriënten op gezondheid van pasgeborenen en iii) het onderzoeken van de mate van ijzerabsorptie uit bijvoeding gefortificeerd met een mengsel van $FeSO_4$ en NaFeEDTA.

Het menselijke brein is kwetsbaar tijdens periodes van ontwikkeling, met name tijdens het laatste tremester van de zwangerschap en de eerste 2 levensjaren: een periode die gekenmerkt wordt door snelle hersengroei. Wij zagen dat anemie door ijzergebrek tijdens het 3e tremester van de zwangerschap geassocieerd is met verminderde mentale ontwikkeling van het kind tot 2 jaar. Kinderen van moeders met bloedarmoede door ijzertekort (IDA) hadden een significant lagere mentale ontwikkelingsscore (MDR) bij 12, 18 en 24 maanden. Het gemiddelde gecorrigeerde verschil in score was respectievelijk 5.8 (95% (CI 1.1-10.5), 5.1 (95% CI 1.2 - 9.0)en 5.3 (95% CI 0.9 - 9.7). Verdere analyse gaf aan dat bij suppletie met ijzer en foliumzuur (60 mg ijzer) de verlaging in MDR score gelijk was in prenatale IDA en niet-IDA groepen IDA. Echter bij suppletie met alleen foliumzuur of met meerdere micronutriënten was de MDR score significant lager in de prenatale IDA groep. Prenatale ijzer suppletie met voldoende ijzer beschermt de ontwikkeling van het kind zelfs als de moeder nog bloedarmoede had tijdens de zwangerschap (**hoofdstuk 2**)

In een tweede studie hebben we de sociale emotionele ontwikkeling van 3 groepen 4 jaar oude kinderen zonder bloedarmoede vergeleken: (1) kinderen met bloedarmoede door ijzergebrek tijdens de eerste 2 levensjaren waarbij de bloedarmoede niet was gecorrigeerd voor de leeftijd van 2 jaar (chronische IDA, n=27); (2) kinderen met bloedarmoede door ijzergebrek tijdens de eerste 2 levensjaren waarbij de bloedarmoede wel was gecorrigeerd voor de leeftijd van 2 jaar (gecorrigeerde IDA, n=70); en (3) kinderen zonder bloedarmoede door ijzergebrek in de eerste 2 levensjaren (niet-IDA, n=64). Het sociaal-emontioneel gedrag van de kinderen (gericht op 'social referencing', '*wariness','frustration tolerance behavior and affect'*) werd geobserveerd in een laboratorium setting en opgenomen op videotape. Tijdens de analyse werden elke 5 seconden in de geobserveerde periode gecodeerd. Kinderen in de peuterschool leeftijd met chronische bloedarmoede tijdens de eerste twee levensjaren vertoonden een veranderd sociaal-emotioneel gedrag . Het sociaal-emotioneel gedrag van kinderen waarbij de bloedarmoede was gecorrigeerd voordat ze 2 jaar oud waren, daarentegen, kwam overeen met dat van kinderen die geen bloedarmoede door ijzergebrek hadden (**hoofdstuk 3**).

Over het geheel tonen onze resultaten aan dat nadelige effecten gereduceerd en/of voorkomen kunnen worden met ijzer suppletie tijdens kritieke perioden van hersenontwikkeling. De Wereld Gezondheidsorganisatie (WHO) beveelt universele distributie van ijzer-foliumzuur supplementen aan bij zwangere vrouwen in ontwikkelingslanden om bloedarmoede door ijzertekorten te voorkomen en te behandelen. Echter, zwangere vrouwen hebben vaak tekorten in meerdere andere nutriënten, die allen een negatieve invloed hebben op hun eigen gezondheid, als ook op de gezondheid, groei en ontwikkeling van hun kinderen gedurende het hele leven. Multi-micronutriënt supplementen die zowel ijzer als andere micronutriënten bevatten, zouden efficiënter moeten zijn in het reduceren van ijzertekorten, omdat andere nutriënten die nodig zijn voor hemoglobine synthese (zoals vitamine A, riboflavine en vitamine B_6 en B_{12}) ook vaak ontbreken in de voeding van zwangere vrouwen in arme populaties. Het verbeteren van de maternale status van meerdere micronutriënten kan ook ten goede komen aan zwangerschapsuitkomst, de micronutriënten opslag bij kinderen direct na de geboorte en de micronutriënten concentratie in moedermelk.

In een gerandomiseerde, dubbel geblindeerde gecontroleerde studie, beschreven in **hoofdstuk 4**, hebben we de impact op geboortegewicht, duur van de zwangerschap en maternale hemoglobine concentratie in het derde trimester onderzocht van prenatale suppletie met meerdere micronutriënten of met ijzer-foliumzuur ten opzichte van alleen foliumzuur. In totaal waren 5828 zwangere vrouwen betrokken. De resultaten wijzen erop dat suppletie met meerdere micronutriënten even effectief is als met ijzer-foliumzuur in het verhogen van de maternale hemoglobine concentratie, geboortegewicht en gemiddelde duur van de zwangerschap. Onze studie liet ook zien dat goede therapietrouw in suppletie met meerdere micronutriënten tijdens zwangerschap bereikt kan worden.

Gebaseerd op de data van de 2000 en 2005 'Food and Nutrition Surveillance' in China, ontdekten we dat bijvoeding in zowel rurale als arme rurale gebieden in China suboptimaal is, alhoewel significante verbeteringen zijn bereikt in de periode 2000-2005. Slechts 30% van de 6-9 maanden oude kinderen consumeerde meer dan 4 keer per week vlees/eieren. Voor andere leeftijdsgroepen was dit percentage slechts 50% of minder. De, gezien de lichaamsgrootte, relatief hoge energie- en nutriëntvereisten en de capaciteit om alleen kleine hoeveelheden voedsel te consumeren, geven aan dat nutriënt-dichte voedingsmiddelen verstrekt moeten worden vanaf 6 maanden tot het tweede en derde levensjaar. Verhoging van consumptie van dierlijke producten zou wellicht de energy- en nutriënten tekorten kunnen verlichten, maar dit verhoogt de kosten en is mogelijk niet haalbaar voor de laagste inkomensgroepen. Verder is de hoeveelheid dierlijke producten die mogelijk toegevoegd kan worden over het algemeen niet voldoende voor ijzer, calcium en soms zink inname. Dus strategieën om de nutriënt opname uit lokaal beschikbare voedingsmiddelen te verbeteren zouden gecombineerd moeten worden met bijvoorbeeld aanvullende fortificering om micronutriënten ondervoeding volledig aan te kunnen pakken (**hoofdstuk 6**).

Het thuis fortificeren van bijvoeding is een effectieve manier om de inname van ijzer en andere nutriënten te verhogen. Om te onderzoeken of ijzer absorptie wordt verhoogd door gebruikmaking van een mengsel van $FeSO_4$ en NaFeEDTA hebben we een ijzer absorptie studie uitgevoerd met een cross over design in 2 groepen met kinderen van 24 tot 31 maanden. Bijvoeding bestaande uit een gierst pap gecombineerd met gefrituurde deegflapjes gevuld met kool, tofu en varkensvlees verrijkt met 2 mg ijzer in de vorm van $FeSO_4$ of in de vorm van NaFeEDTA (studie 1) of met 4 mg ijzer in de vorm van $FeSO_4$, of een mix in de vorm van 2 mg $FeSO_4$ en 2 mg NaFeEDTA (studie 2). IJzerabsorptie was berekend gebaseerd op het inbouwen van stabiele isotopen in erythrocyten. In studie 1, was de gemiddelde ijzer absorptie (± SD) 8.0% (3.1,20.8) en 9.2% (3.1,27.0) van voedsel gefortificeerd met $FeSO_4$ en met NaFeEDTA respectievelijk. In studie 2 was de ijzer absorptie significant hoger van voedsel gefortificeerd met 4 mg ijzer in de vorm van een 1:1 mix van $FeSO_4$ en NaFeEDTA dan van voedsel gefortificeerd met $FeSO_4$ alleen; de gemiddelde ijzerabsorptie was 6.4% (3.0, 13.5) en 4.1% (1.9, 8.9), respectievelijk. Wij hebben geconcludeerd dat een mengsel van $FeSO_4$ en NaFeEDTA, op basis van verhouding 1:1, de ijzerabsorptie significant verbetert en dat dit een goede strategie is om een adequate ijzer absorptie van phytaat bevattend bijvoeding te bewerkstelligen (**hoofdstuk 5**).

De studies beschreven in dit proefschrift dragen bij aan het bewijs van de relatie tussen bloedarmoede door ijzer tekort gedurende de eerste 1000 levensdagen en het lange termijn effect op de ontwikkeling van het kind. Onze resultaten en de meta analyse van 12 gerandomiseerde onderzoeken (waaronder ons onderzoek in China) suggereren dat een dagelijkse suppletie met meerdere micronutriënten een optimale aanpak is voor zwangere vrouwen in ontwikkelingslanden waaronder ook China, ter verbetering van gezondheid en ontwikkeling van pasgeborenen. De voordelen van het geven van tenminste 30 mg ijzer dagelijks aan zwangere vrouwen, niet alleen bewezen in onze studie maar ook door anderen, kunnen beleidsmakers helpen bij het maken van een standaard aanbeveling voor prenatale suppletie in China. Bovendien bevestigt de verhoogde ijzer absorptie van een mengsel van ijzer in de vorm van $FeSO_4$ en NaFeEDTA de huidige praktijk in China ten aanzien van het gebruik van NaFeEDTA voor thuis fortificering van voedsel voor kinderen.

Naar aanleiding van onze resultaten en die van anderen vinden wij het belangrijk dat er meer onderzoek gedaan wordt naar het causale verband tussen ijzer tekort en het gedrag van het kind; dat er betere meet methoden ontwikkeld worden om de ontwikkeling van het kind te meten; en dat de oorzaken van anemia in de regio verder onderzocht worden.

总　结

总结

证据表明一个人从其母亲怀孕到将其养育至两岁的最初 1000 天是生命中最为关键的时期。这段时间内的营养缺乏将对儿童的生存和成长产生显著而且经常是不可逆转的负面影响，不仅影响到儿童在学校期间的学习能力，还将对其成年后的劳动生产力产生影响。母亲在孕期、婴幼儿在两岁以内摄入和补充足够的铁是生命最初 1000 天中重要的营养干预手段之一。然而，铁缺乏和脑功能受损之间的因果关系目前还未被科学证明，虽然大量证据表明，发展中国家缺铁性贫血的高发与其健康成本及经济花费成正比。因此探讨铁补充、铁强化对特殊人群（如孕妇和婴幼儿）的干预效果和补充方法具有重大的公共卫生意义。

该论文对上述领域的部分问题进行了阐述，主要包括 i) 探讨孕期和两岁以下婴幼儿缺铁性贫血对儿童智力、心理活动能力及社交情绪行为的长期影响； ii) 探讨孕期多维营养素补充对新生儿出生时健康结果的影响； iii ）研究 NaFeEDTA 和 $FeSO_4$ 混和作为铁强化剂强化婴幼儿辅食的铁吸收率。

人类大脑在孕晚期及儿童两岁之前的发育迅速而敏感，通常被称为“大脑发育突增”。我们研究发现孕晚期缺铁性贫血与儿童 24 月龄之前大脑发育受损相关。母亲在孕期发生缺铁性贫血的儿童组，在 12 月龄，18 月龄和 24 月龄时，其 MDI 显著低于母亲在孕期没有贫血的儿童组。经过调整的均值差别分别是 5.8（95%可信限 [CI] 1.1 到 10.5）5.1 (95% CI 1.2 到 9.0) 和 5.3 (95% CI 0.9 到 9.7)。进一步分析显示无论孕期是否贫血，如果孕期补充铁和叶酸（60 mg 铁），两组儿童的 MDI 没有区别，但是如果孕期补充叶酸或多营养素（30 mg 铁）, 孕期母亲贫血的儿童组 MDI 显著低于孕期母亲不贫血的儿童组。因此孕期充足的铁补充能保护儿童脑发育，即使孕妇缺铁性贫血在孕期没有得到完全纠正（第二章）。

在第二个研究中，我们比较了三组非贫血的 4 岁儿童的社交情绪和行为，三组儿童分别为婴幼儿期贫血到 24 月龄还未被纠正的（慢性缺铁性贫血组 n=27）; 婴幼儿期贫血到 24 月龄时得到了纠正的（贫血纠正组 n=70）；婴幼儿期及 24 月龄时均不贫血的（非贫血组 n=64）。通过录像观察评价儿童的社会参照能力，行为抑制性，自我控制能力。每 5 秒钟对儿童的情绪和行为进行编码。结果发现，2 岁以前有慢性缺铁性贫血的儿童，在 4 岁时其社会情绪和行为表现为受损。与其相反，贫血纠正组儿童，到 4 岁时其社会情绪和行为与非贫血组儿童类似（第三章）。

综上， 我们的研究结果显示在脑发育的关键时期进行补铁可以减少和预防由于缺铁对脑发育的影响。 世界卫生组织建议在发展中国家为孕妇广泛提供铁和叶酸以预防和治疗缺铁性贫血。实际上，孕妇往往同时缺乏多种营养素，这些 营养素的缺乏不仅影响孕妇自身健康，更会影响其孩子一生的健康、生长和发育。包含铁和其它微量营养素在内的多种营养素补充剂在减少贫血方面效果会更好，因为除铁以外，贫困家庭的孕妇膳食中还缺乏其它营养素，包括维生素Ａ，核黄素和维生素 B_6和 B_{12}，而这些营养素对血红蛋白的形成也是不可或缺的。改善妇女多种微量营养素状况有益于孕妇健康，同时有益于婴儿出生时微量营养素的储备以及母乳中微量营养素的含量。

第四章论述了一个双盲控制的随机试验，我们验证了孕期补充多维营养素，补充铁叶酸及只补充叶酸对婴儿出生体重，怀孕持续时间和孕晚期血红蛋白的不同影响。共计 5828 名孕妇被纳入了研究。结果显示，在增加孕妇血红蛋白方面，孕期补充多维营养素和补充铁/叶酸效果一样。 我们的研究同时显示孕妇对多种营养素有很好的依从性。

根据 2000 年和 2005 年食物和营养监测数据，我们发现尽管自 2000 年到 2005 年婴幼儿辅食添加方面有了很大的改善，但中国贫困农村地区的辅食添加仍不理想。6－9 月龄儿童每周添加肉或蛋类 4 次以上的比例仅仅为 30%，其他月龄组也仅仅达到 50%或更少。婴幼儿胃容量小，能吃得食物少，必须提供给 6 至 24 月龄甚至 36 月龄儿童高能量和高营养密度的食物。动物性食物的摄入在某种程度上可以满足能量和营养素的要求，但这会增加花费，对最低收入的家庭并不适宜。另外，所能提供和摄入的动物性食物的数量一般不能提供足够的铁、钙，有时还有锌等。因此，鼓励依据当地食物增加营养素摄入同时，还应与其他的策略联合，例如添加营养素强化辅助食品以更好地解决因微量营养素缺乏导致的营养不良问题（第六章）。

家庭内辅助食品强化是提供更多铁的有效手段，为了确定 $FeSO_4$ 和 NaFeEDTA 混和是否可以增加铁吸收率，我们利用交叉设计对两组 24 - 31 月龄儿童进行了铁吸收研究。研究一中，儿童食用由小米粥和白菜、豆腐、猪肉馅饺子组成并强化了 2 毫克 $FeSO_4$ 或 2 毫克 NaFeEDTA 的辅食 ，研究二中，儿童食物相同但强化了 4 毫克 $FeSO_4$ 或强化了 $FeSO_4$ 和 NaFeEDTA 各 2mg。铁吸收状况以铁稳定性同位素的红血球合成计定 。研究一显示，以 $FeSO_4$ 强化和 NaFeEDTA 强化的辅助食品铁吸收率平均值(± SD)分别 为 8.0% (3.1, 20.8) 和 9.2% (3.1, 27.0)。研究二显示 ，由 $FeSO_4$ 和 NaFeEDTA 1：1 混和的 4mg 铁强化的铁吸收率显著高于只用 $FeSO_4$ 强化的；他们的铁吸收率分别是 6.4% (3.0, 13.5)和 4.1% (1.9, 8.9)。结论是等比例混和的 $FeSO_4$ 和 NaFeEDTA 显著提高了铁吸收率，是从高植酸辅助食品中获得足够铁的有效方法（第五章）。

该论文中的研究为证明生命最初 1000 天出现缺铁性贫血和儿童发育长远影响之间的联系提供了进一步证据。我们的研究结果和对 12 个随机控制研究（包括作者在中国所做的研究）的荟萃分析显示了每日给孕妇提供多维营养素是改善发展中国家包括中国新生儿健康的理想策略。我们的研究以及其他研究结果显示的每日为孕妇提供 30mg 铁可以为国家制定孕期营养素补充政策提供指导。同时，通过 $FeSO_4$ 和 NaFeEDTA 混和作为铁强化剂可以提高铁吸收率这个研究结论论证支持了中国目前正在实施的利用 NaFeEDTA 进行家庭内强化辅助食品的实际做法。

鉴于我们的研究结论和其他人的研究，建议今后进行以下方面的进一步研究： 1） 继续进行铁缺乏和儿童行为之间因果关系的研究； 2） 开发更好的测量工具以评价儿童发育状况； 3）探讨地区内贫血的原因。

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Curriculum Vitae

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CHANG Suying was born on 1st July 1966 in Handan City of Hebei Province, China. After finishing her secondary school education, she started her Bachelor's study at the Department of Preventive Medicine in Hebei Medical University in 1985, and received her Bachelor's Degree in Preventive Medicine in 1990. She worked in Municipal Sanitary and Anti-epidemic Station of Handan City, Hebei Province for two years. In 1992, she became a Master student at the Institute of Nutrition and Food Hygiene, Chinese Academy of Preventive Medicine (CAPM) on nutrition and food safety. She received her Master of Science in Medicine in July 1995.

From July 1995, she began to work at the Institute of Nutrition and Food Hygiene, CAPM in Beijing. From 1995-2001, she worked as an Assistant Research Fellow and was involved in several nationwide research projects such as "National Nutrition Survey", "Food and Nutrition Surveillance of China" for field work and database setup and data analysis.

From 2001 to 2002, she worked concurrently as a Nutrition Consultant for UNICEF on nutrition related programs. She provided support for the double-blind randomly controlled trial entitled "The Efficacy and Feasibility Trial of Micronutrient Supplementation for Pregnant Women" by developing protocols and preparatory work for the trial. After that, she was seconded by the Division of NCDs and Nutrition Management of the Department of Disease Prevention and Control, the Ministry of Health for half year as a Program Officer to coordinate national nutrition programmes on themes like wheat flour fortification, soy sauce fortification, micronutrients supplement of pregnant women, development of national nutrition regulation etc..

From 2003 to 2007, she worked as Associate Research Fellow in Chinese Center for Disease Control and Prevention (China CDC) – formerly known as CAPM. She also worked as the Office Manager of International Life Sciences Institute (ILSI) Focal Point in China. During this period, she was the Coordinator of the program named 'The Application of NaFeEDTA Fortified Soy Sauce in the Control of Iron Deficiency in China' supported by Global Alliance on Improved Nutrition (GAIN). She also worked as the Principal Investigator for several projects, such as "Implementation of Nutrition School Meals in Poor Rural Areas", "Effectiveness of Iron Fortified Soy Sauce on Emotion and Cognitive Performance of 3-6 years Anaemic Children in China" and "Follow-up Study on the Lasting Effect of Home-level Fortified Complementary Food Supplement for Complementary Feeding".

From November 2007 till present, Ms. Chang is employed as a Nutrition Specialist at the UNICEF China office, where she is responsible for the planning, implementation, monitoring and evaluation of the UNICEF China office supported nutrition programme. One of her working focuses is to push forward infant and young child feeding, the implementation of fortified complementary food and multi-nutrients supplementation among children and pregnant women to prevent and control of iron deficiency and malnutrition.

In Oct 2008, Ms. Chang passed the assessment of the Ministry of Health and was awarded the title Senior Research Fellow. Ms. Chang began her sandwich PhD program in the Division of Human Nutrition at Wageningen University in the Netherlands since 2006. She was selected by the International Nutrition Foundation who supported Ms. Chang with fellowships as the Featured Fellow in commending her work during her PhD program.

MAIN PUBLICATIONS

1. **Chang S,** Huang Z, Ma Y, Piao J, Yang X, Zeder C, Hurrell R, Egli I, Mixture of ferric sodium ethylenediaminetetraacetate (NaFeEDTA) and ferrous sulfate: An effective iron fortificant for complementary foods for young Chinese children. Food and Nutrition Bulletin, 2012, Vol 33, 2, 111-116.
2. **Chang S**, Wang L, Wang Y, Brouwer I, Kok F, Lozoff B, Iron-deficiency anemia in infancy and social emotional development in preschool-aged Chinese children, Pediatrics 2011;127:e927–e933.
3. **Chang S,** He W, Chen C, Complementary feeding and growth of infant and young child in China. Biomedical and Enviromental Sciences. 2008, Vol.21, 264-8
4. **Chang S**, He W, Chen C, The research on knowledge and attitude to multi-micronutrients supplements intakes among childbearing aged women in rural China. Journal of Hygiene Research, 2007, Vol.36(6) 725-726.
5. **Chang S**, He W, Chen C The Growth Characteristics of Chinese children under 5 in the past 15 years. Journal of Hygiene Research, 2006, Vol.35(6) 768-771.
6. **Chang S**, Chen C, He W Analysis on the changes of nutritional status in China–The Improvement of complementary feeding amang chinese infants and young children in the past 15 years. Journal of Hygiene Research, 2007, Vol36(1) 207-209.
7. **Chang S**, He W, Jia F Chen C Anemia Status of Chinese children. Journal of Hygiene Research, 2007, Vol36(1) 210-212.
8. **Chang S,** Cao Y, Ma G Marketing and shopping behavior on infants and young children food in rural China. Chinee Journal of Health Education. 2007, Vol.23(4) 244-247.
9. **Chang S,** Ji C The relationship between high blood pressure with overweight and obesity of 7-17 years old Chinese children and adolescents. Chinese Journal of School Health. 2006 Vol.27(8) 652-653.
10. **Chang S.** Complementary Food fortification in China. Journal of Hygiene Research 2003, Vol.32 Suppl. 46-49.
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12. **Chang S.** Ge K. Zhai F. Jia F, Lin H Dietary intake of some micronutrients in different provinces of China. Journal of Hygiene Research 1999. Vol.28 (6) 364-366.
13. **Chang S.** Ge K. Zhai F. Jia F. Xu Q. The analysis of nutritional factors on Anemia in Chinese Adult. Acta Nutrition Sinica, 1998. Vol.20(2) 132-137.
14. **Chang S.** Chang Y .The impact of children development and its analysis method. Foreign Medical Sciences. 1997. Vol.24(1) 40-43.
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16. Ge K. **Chang S.** Du S. Definition and measures of child malnutrition. Biomedical and Environmental Sciences, 2001 Vol.14(3) 283-291.
17. Ge K. **Chang S.** Dietary intake of some micro-nutrients in China. Biomedical and Environmental Sciences, 2001, Vol.14(3) 318-324.
18. Fu Z. **Chang S**. Exclusive breastfeeding and growth of infants under 4 months in China. Journal of Hygiene Research Vol. 29 2000; 275-278.
19. Ge K. **Chang S.** The Food and nutrition of Chinese people. The Chinese food industry from 1949 to 1999. Publishing house of encyclopedia. Beijing, 1999, 311-314.
20. Ge K. **Chang S.** Dietary intake of micronutrients of Chinese inhabitants. Acta Nutrition Sinica, 1999, Vol.21(1) 1-6.
21. Wu Y, Li X, **Chang S**, Liu L, Zou S, Hipgrave D, Variable Iodine Intake Persists in the Context of Universal Salt Iodization in China, The Journal of Nutrition, 2012, doi: 10.3945/jn.112.157982.
22. Chen C, Wang Y, **Chang S**, Effect of In-home Fortification of Complementary Feeding on Intellectual Development of Chinese Children. Biomedical and Environmental Sciences, 2010, Vol 23, 83-91.

23. Chen C, He W, **Chang S.** The Changes of the attributable factors of child growth. Journal of Hygiene Research. 2006, Vol.35(6), 765-767.
24. He W, Chen C, **Chang S.** Analysis on the nutritional status of children of out-for-job mothers. Journal of Hygiene Research. 2007, Vol.36(1), 214-215.
25. Chang Y. Xian Z. He W. **Chang S.** Ma H. Chen C. Child malnutrition in China—Present status and Changing trend. Biomedical and Environmental Sciences.1996 Vol.9. 164-180.

OVERVIEW OF COMPLETED TRAINING ACTIVITIES

Meetings and workshops

- Seminar on Rights Based Approach to Food, Wageningen University, The Netherlands, 2006
- Conference on Obesity and related Chronic Disease of China, Beijing, 2006
- Conference on Nutrition Requirement of Infants and Children and complementary Food in China, Beijing 2007
- East Asia Leaders Group Meeting, Flour Fortification Initiative(FFI), Ho Chi Minh City, Viet Nam, 2009
- Micronutrients Forum, Beijing, China, 2009
- Meeting on Implementation in Asia of the Recommendations on Wheat Flour Fortification. UNICEF, WHO, FFI, Manila, Philippines, 2009
- Training workshop on infant and young child feeding of HIV/AIDS positive mothers, Kunming, China, 2009
- Workshop on nutrition intervention in emergency, Beijing, China, 2009
- National seminar on students meals in rural schools, Beijing, China, 2010
- National Workshop on nutrition improvement in China, Beijing, China, 2010
- International Conference on Early Childhood Development, Beijing, China, 2011.

General courses

- Academic writing, Wageningen University, The Netherlands, 2006
- English Grammar in Use, Wageningen University, The Netherlands, 2006
- Working with endnote, Wageningen University, The Netherlands, 2008
- English training, Beijing, 2010-2011
- Training Workshop on laboratory analysis of Vitamins (I and II), Beijing, China, 2006 and 2007

Optional

- Research proposal development, Wageningen University, The Netherlands, 2006
- Literature study program, Wageningen University, The Netherlands, 2006, 2007, 2008, 2010, 2011
- Literature study program, ETH, Institute of Food, Nutrition and Health, Human Nutrition Laboratory, Zurich, Switzerland, 2009.

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