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Infant Anemia and Micronutrient Status

Studies of Early Determinants in Rural Bangladesh

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Abstract

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Anemia and micronutrient deficiencies in infancy are common in low-income settings. These are partly due to maternal malnutrition and may impair child health and development. We studied the impact of maternal food and micronutrient supplementation, duration of exclusive breastfeeding (EBF), growth and infection on infant anemia and micronutrient status.

In the MINIMat trial in Matlab, Bangladesh, pregnant women were randomized to Early or Usual promotion of enrolment in a food supplementation program and to one of three daily micronutrient supplements. Capsules containing 400µg folic acid and (a) 30 mg iron (Fe30Fol), (b) 60 mg iron (Fe60Fol), (c) 30 mg iron and other micronutrients (MMS) were provided from week 14 of gestation. Capsule intake was assessed with the eDEM device recording supplement container openings. Blood samples (n=2377) from women at week 14 and 30 were analyzed for hemoglobin (Hb). Duration of EBF and infant morbidity was based on monthly maternal recalls. Infants were weighed and measured monthly. Blood samples (n=1066) from 6-months-old infants were analyzed for Hb and plasma ferritin, zinc, retinol, vitamin B12 and folate.

In women, Hb increase per capsule reached a plateau at 60 Fe60Fol capsules, indicating that nine weeks of daily supplementation produced maximum Hb response. Anemia was common (36%) at capsule intakes >60 indicating other causes of anemia than iron deficiency.

In infants, vitamin B12 deficiency prevalence was lower in the MMS (26.1%) than in the Fe30Fol group (36.5%), (p=0.003) and zinc deficiency prevalence was lower in the Usual than in the Early group. There were no other differential effects of food or micronutrient supplementation on infant anemia or micronutrient status. Infants exclusively breast-fed for 4-6 months had a higher mean plasma zinc concentration (9.9±2.3 µmol/L) than infants exclusively breast-fed for <4 months (9.5±2.0 µmol/L), (p< 0.01). No other differences in anemia, iron or zinc status were observed between EBF categories. Infection, low birth weight and iron deficiency were independent risk factors for infant anemia. Regardless of studied interventions, prevalence of anemia (43%), deficiency of zinc (56%), vitamin B12, vitamin A (19%) and iron (22%) in infancy was high and further preventive strategies are needed.

Keywords: Micronutrients, Anemia, Iron Deficiency, Zinc Deficiency, Vitamin A Deficiency, Vitamin B12 Deficiency, Folic Acid Deficiency, Infant, Pregnancy, Dietary Supplementation, Hemoglobin, Dose-Response Relationship, Exclusive Breast Feeding, Bangladesh

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List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I. Eneroth H, Persson LÅ, El Arifeen S, Ekström EC. Dose-effect of iron-folic acid and multiple micronutrient supplemenetation on hemoglobin in pregnancy *Manuscript.*

II. Eneroth H, El Arifeen S, Persson LÅ, Lönnerdal B, Hossain MB, Stephensen CB, Ekström EC. Maternal multiple micronutrient supplementation has limited impact on micronutrient status of Bangladeshi infants compared with standard iron folic acid supplementation. *Journal of Nutrition 2010*; 140(3):618-24

III. Eneroth, H, El Arifeen S, Persson LÅ, Kabir I, Lönnerdal B, Hossain MB, Ekström EC. Duration of exclusive breastfeeding and infant iron and zinc status in rural Bangladesh. *Journal of Nutrition* 2009; 139(8):1562-1567

IV. Eneroth H, Persson LÅ, El Arifeen S, Ekström EC. Infant anemia is associated with infection, low birth weight and iron deficiency in rural Bangladesh *Acta Paediatrica* 2011; 100(2):220-5.

The original articles II-IV have been reprinted with permission from the respective publishers.

Contents

Abbreviations

ARI	Acute respiratory infection
BMI	Body mass index
CI	Confidence interval
CNC	Community nutrition centre
CRP	C-reactive protein
EBF	Exclusive breastfeeding
Fe30Fol	30 mg iron and 400µg folic acid supplement
Fe60Fol	60 mg iron and 400µg folic acid supplement
HAZ	Height-for-age z-score
Hb	Hemoglobin
ICDDR,B	International Centre for Diarrhoeal Disease Research, Bangladesh
ID	Iron deficiency
IQR	Interquartile range
IUGR	Intra uterine growth retardation
MINIMat	Maternal and Infant Nutrition Interventions, Matlab
MMS	Multiple micronutrient supplement
LBW	Low birth weight
NBW	Normal birth weight
OR	Odds ratio
SD	Standard deviation
SES	Socio-economic status
SGA	Small-for-gestational age
VAD	Vitamin A deficiency
WAZ	Weight-for-age z-score
WHZ	Weight-for-height z-score
WHO	World Health Organization

Introduction

Maternal and infant macro- and micronutrient status have consequences for health in infancy and later in life. Through their diverse functional roles in the body, micronutrients are essential for development, growth and immune function. In a disadvantaged setting, pre- and postnatal nutritional factors may cause poor micronutrient status of the infant. In these studies, the determinants of anemia and status of iron, zinc, vitamin A, vitamin B12 and folate in infancy are explored.

Epidemiology of anemia and micronutrient deficiencies in infancy

Anemia is a worldwide problem, where more than 30% of the world's population is affected, with infants and pregnant women at particularly high risk (1). Iron deficiency is believed to be the most common cause of anemia (2), but other determinants could also be important in the etiology. Iron deficiency is prevalent in infants and young children, especially in low-income settings (1). There is increasing evidence from zinc supplementation trials that zinc deficiency in children is a large problem in many countries (3). A third of pre-school children and 15% of pregnant women in the world are estimated to be vitamin A deficient (4). Infants are at risk for vitamin A deficiency and thus vitamin A supplementation is recommended (5). Furthermore, infants are at risk of developing vitamin B12 deficiency where maternal vitamin B12 deficiency is a problem (e.g.for example on the Indian subcontinent) (6). Folate deficiency, on the other hand, is not common in breastfed infants.

Consequences of anemia and micronutrient deficiencies in infancy

Because of the rapid development and growth in infancy, infants are particularly vulnerable to anemia and micronutrient deficiencies. Iron deficiency anemia is associated with delayed motor- and neurodevelopment in children (7, 8). Zinc deficiency in infancy can lead to impaired immune function and impaired growth and development (9, 10). Vitamin A is essential for immune function and its deficiency contributes to child mortality (11). Vitamin B12 deficiency in infancy may have serious effects on neurological development (12, 13). Folate deficiency affects the immune system and children with low folate status have higher risk for acute lower respiratory morbidity (14).

Determinants of micronutrient status in infancy

The theoretical framework of this study illustrates the direct determinants of infant micronutrient status (Figure 1). Micronutrient status of infants aged 6 months depends on pre- and postnatal factors. Maternal micronutrient status, prenatal growth and incorporation of micronutrients into body stores are the prenatal determinants. Postnatal determinants are adequacy of micronutrient intake from breast milk and from complementary foods if such foods have been given, postnatal growth and the number and severity of infections. Socioeconomic status (SES) of the family, food security and food allocation within the household, health care seeking behavior and parental education are other factors that could influence infant micronutrient status. These contextual factors act through the direct determinants and are not discussed in detail in this thesis. The pathways in which micronutrient status is determined differ between iron, zinc, vitamin A, vitamin B12 and folate.

Micronutrient status at birth

Iron stores in a newborn depend on iron status of the pregnant woman. These stores should cover most of the needs for development and growth during the first 6 months of life. Maternal iron status influences transfer of iron to the fetus (15) and maternal iron depletion is associated with reduced fetal iron stores (16, 17). Male newborns have lower cord blood ferritin than female newborns (18, 19) and it is assumed that gender differences are due to hormonal factors as they appear already at birth (20). Infants born with a low birth weight (LBW) have lower iron and zinc status at birth (21), as well as higher requirements of zinc (9) and might have lower micronutrient intake because of lower appetite (22). No sex differences in cord plasma zinc have been observed (23). Infants are born without significant stores of vitamin A (24). Maternal status of vitamin B12 is associated with newborn stores of the vitamin (25, 26). The stores of vitamin B12 in the newborn are enough for the infant's first year if the mother is not deficient in the vitamin (27).

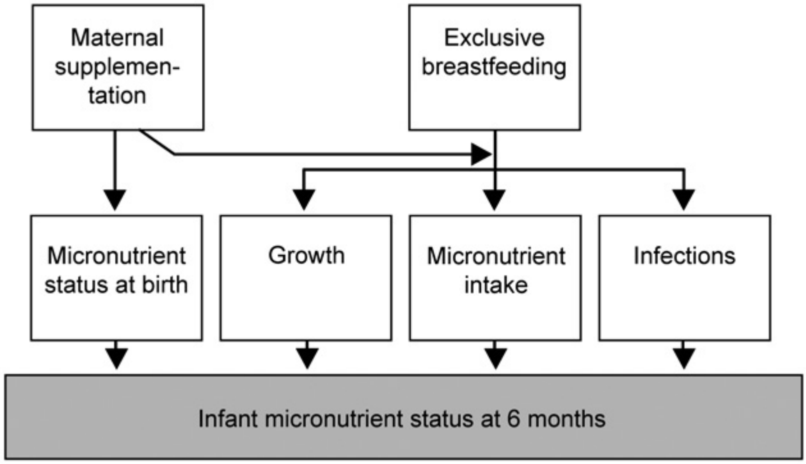

Figure 1. Determinants of infant micronutrient status.

Growth

The high growth velocity in infancy could contribute to the high requirements of micronutrients during this early period of life. When associations between growth and micronutrient status are evaluated, it is difficult to establish causality. High growth velocity seems to increase the risk for low ferritin concentrations in well nourished children (28, 29). The impact of zinc supplementation on growth is well established (30), whereas potential impact of growth on zinc status is not. We have not found accounts of an association between growth velocity and vitamin A status. Vitamin B12 status of infants seems independent of growth velocity (31).

Micronutrient intake

Breast milk alone can provide all the required nutrients for infants to 6 months of age (32). In settings where mothers are malnourished and infants may be deficient in micronutrients at birth, it is especially important that a complementary food provides the infant with additional micronutrients at about 6 months. However, in poor settings, complementary foods are often low in micronutrients (33). Breastfeeding influences iron and zinc status by two means: first, directly by providing a higher intake of iron and zinc compared with complementary foods in many settings and second, indirectly by preventing infections that lead to increased needs and losses of micronutrients (33). Even if the main source of iron in infancy is the prenatally acquired stores, some iron from a bioavailable source is needed even during these first months (34). Bioavailability of iron and zinc in breast milk is good, even in infants receiving complementary foods (35). Bioavailability of iron is higher from breast milk than from other foods (36) and breast-fed infants with iron deficiency upregulate the absorption of iron (37). The concentration of iron and zinc in breast milk does not depend on the maternal micronutrient status (38). In severe anemia, iron concentration in breast milk may be compromised (17).

Vitamin A status at 6 months of life is to a great extent a consequence of dietary intake as infants are born without stores of vitamin A. Colostrum is especially important because of its high content of vitamin A. Vitamin A concentration in breast milk depends on the mother's vitamin A status (39). Breast-fed children with vitamin B12 deficient mothers can develop deficiency because breast milk content of B12 is associated with serum levels of the mother (40). Folate concentrations in breast milk are not associated with maternal folate status (41).

Infection

Infection increases the requirements for micronutrients. This increase can be due to zinc losses in faeces, which is caused by increased stools (42) or increased urinary losses of retinol at times of infection (43). When the child recovers, there are increased needs of micronutrients for catch-up growth. Acute respiratory infections can range from the common cold to pneumonia and are the most common type of illness during infancy. Diarrhea, especially persistent diarrhea also contributes significantly to child mortality in low-income settings. Helminth infections are a common cause of anemia and micronutrient deficiency in older children but not common at this early age (44).

Interventions to improve micronutrient status of infants

Interventions to improve micronutrient status of infants include supplementation in pregnant women, supplementation for infants and improved infant feeding practices. Maternal supplementation and exclusive breastfeeding (EBF) are recommended universally and will be the focus of this thesis. Iron supplementation for infants at risk of becoming iron deficient is targeted to LBW infants and there is no systematic screening for LBW in this study setting.

Supplementation in pregnant and lactating women

Pregnancy increases the requirements of certain nutrients because of the growth of maternal and fetal tissue. Routine universal prenatal supplementation with iron and folic acid is recommended by the World Health Organization (WHO) (45). Pregnant women are at high risk of developing anemia: for instance, it is estimated that 48% of pregnant women in South Asia are anemic (1). Anemia during pregnancy is associated with adverse birth outcomes. However, high hemoglobin (Hb) is associated with negative consequences for mother and child and may act as an indicator of insufficient hemodilution.

Because multiple micronutrient deficiencies are reported from low income settings (46, 47) it has been suggested that a combination of micronutrients could further improve maternal nutrition and infant outcome compared with routine iron-folic acid supplementation. The rationale for this argument is that nutrition deficiencies interact, i.e. deficiency of one micronutrient can affect the utilization of another. There can be positive effects such as increased iron utilization if vitamin A and vitamin C are given together with iron. Previous research has shown that Hb increase was greater in women supplemented with vitamin A and iron together (48). However, a negative effect on bioavailability (e.g. of zinc supplementation on iron absorption) is also possible. There is no uniform recommendation of multiple micronutrient supplementation during pregnancy, except in emergency settings (49). A prenatal supplement containing 13 other micronutrients in combination with iron and folic acid has been developed (50). This supplement, sometimes referred to as the "UNIMAP" supplement, has been evaluated in several countries (51). The effects of this and other multiple micronutrient supplements in comparison with iron-folic acid supplements, have been evaluated with birth weight (52, 53), neonatal mortality (54), and maternal micronutrient status (55) as outcomes. To our knowledge, the effect of this combination of multiple micronutrients on infant anemia and micronutrient status has not been evaluated.

It is suggested that daily antenatal iron supplementation increases Hb concentration in the blood of the pregnant woman (56). No difference in hematological status during pregnancy has been observed when comparing iron-folic acid supplements with multiple micronutrient supplements (55). Traditionally, Hb in pregnancy has been evaluated as a result of allocation to a regimen. Studies that have looked at actual capsule intake have found a plateau in Hb increase per iron supplement capsule (57, 58). A far as we know, this type of analysis, evaluating Hb increase per ingested capsule has not been done with multiple micronutrient supplements.

In pregnancy, energy requirements increase with about 1420 kJ per day in the 2nd trimester and approximately 1880 kJ per day in the 3rd trimester (59). Food supplementation in pregnant women in low income countries has been associated with higher birth weights (60-62) and because LBW is associated with low iron and zinc stores at birth, prenatal food supplementation could potentially improve iron and zinc status of the infant.

Exclusive breastfeeding

The positive effects of EBF on child health and reduction of child mortality are well documented (63). EBF is protective against infections because of the high intake of immune factors from breast milk and because it eliminates the risk of bacterial contamination from other foods. Since 2001, WHO recommends EBF in the first 6 months of life (32). Adequate and safe complementary food is crucial to fulfill micronutrient requirements later in infancy, but not before 6 months of age according to WHO infant feeding recommendations. However, it is debated whether a duration of EBF for 6 months, as compared with earlier introduction of complementary foods, conveys a larger risk for micronutrient deficiencies in settings with high prevalence of maternal macro- and micronutrient deficiencies and LBW infants (32, 64, 65). Of particular concern is anemia and iron status, but zinc may also be a limiting nutrient. Previous results indicate lower Hb concentrations (66-68), higher risk for anemia (66, 69) and lower plasma ferritin concentrations (67, 68) with longer duration of EBF or predominant breastfeeding. However, these studies were carried out in settings where infant formula or iron fortified baby foods were provided or are likely to be part of the diet of infants who are not exclusively breast-fed. It is reported from Zambia that infants who were exclusively breast-fed had higher Hb than infants who received complementary foods (70). Thus, data on the association between EBF and anemia and iron status are inconclusive and therefore conclusions for recommendations in low-income settings cannot be drawn from extant data.

Rationale for the studies of this thesis

Because anemia and micronutrient deficiencies may have detrimental effects on infant health and development, strategies to prevent such deficiencies are needed. These strategies should be based on knowledge about the etiology of anemia and micronutrient deficiencies. To implement efficient public health interventions to prevent this problem, a number of knowledge gaps need to be filled. The MINIMat trial enabled these epidemiologic studies with a large sample and careful adjustments for potential confounders.

Hb response to prenatal iron supplementation has mainly been assessed as an outcome of a full regimen. In paper I we will take the approach previously used for iron supplementation and evaluate the Hb concentration per supplement capsule consumed. We will further compare the Hb response to capsule intake of the currently recommended iron-folic acid supplement with a multiple micronutrient supplement and a supplement with a lower dose of iron. It has not previously been described whether pregnant women reach a plateau in Hb concentration with multiple micronutrient supplements and if that plateau differs from that previously described for the currently recommended iron-folic acid supplement. Knowledge about the maternal Hb response to different types of supplement can guide policy on type and dosage of supplementation for women during pregnancy.

Where maternal malnutrition is common and many infants are born with LBW, maternal multiple micronutrient supplementation may ameliorate the risk of infant anemia and micronutrient deficiencies. However, the effect of this maternal supplementation sometimes referred to as the UNIMAP in comparison with iron-folic acid supplementation on infant micronutrient status has not been previously evaluated. Before implementing maternal supplementation with multiple micronutrients, this aspect has to be considered (Paper II).

EBF is important for the health of the child, but adequacy of 6 months EBF for iron and zinc status is debated. There is a need for assessment of risk for iron and zinc deficiency according to duration of EBF also in low-income settings where iron fortified baby foods are not common (Paper III).

Anemia in infancy is often assumed to be due to iron deficiency, but the etiology is probably highly specific to setting. To find the most efficient measures to prevent anemia in this specific setting, we explored several of the potentially most important contributors to anemia in infancy and assessed their relative importance (Paper IV).

Aim of the thesis

The overall aim of this thesis is to study pre- and postnatal determinants of infant anemia and micronutrient status in the context of a high prevalence of maternal malnutrition and low birth weight.

The specific objectives of the thesis are:

1. To evaluate the response in hemoglobin concentration per ingested capsule of iron-folic acid- and multiple micronutrient supplements in pregnant women (Paper I)
2. To assess whether type of prenatal micronutrient supplement or timing of food supplementation affects infant iron, zinc, vitamin A, vitamin B12 and folate status differentially (Paper II)
3. To determine the association between duration of exclusive breastfeeding and infant anemia and iron and zinc status at 6 months of life (Paper III)
4. To investigate what factors contribute to infant anemia in this population including micronutrient deficiencies, growth and infections (Paper IV)

Methods

This thesis builds on data from the study "Maternal and Infant Nutritional Interventions in Matlab" (MINIMat, ISRCTN16581394) carried out in Bangladesh. MINIMat is a randomized community-based trial combining nutritional interventions directed to the mother and with the aims to improve maternal health, reduce the high frequency of LBW and improve infant growth and development.

Study setting

Bangladesh is a densely populated country in South Asia. The country has one of the highest prevalences of LBW in the world (22%) (71). LBW can be due to premature birth or intrauterine growth retardation (IUGR). The latter is the most common cause in Bangladesh, resulting in infants born small for gestational age (SGA) (72). A major cause of this high prevalence of IUGR is widespread maternal malnutrition.

The government of Bangladesh provides underweight (Body mass index, BMI <18.5 kg/m^2) pregnant women in many districts with a food supplement through the National Nutrition Program. In this ongoing program, enrolled women consume this food supplement 6 days a week at a Community Nutrition Centre (CNC). All pregnant women, regardless of nutritional status are recommended a daily supplement of 60 mg iron and 400 μg folic acid. Oral contraceptives (used by 29% (73)) are sometimes combined with iron supplementation. Vitamin A supplements are distributed to 12-24 mo old children in twice-yearly campaigns (74) and the coverage of postpartum vitamin A supplementation of women is low (75). The mean duration of any breastfeeding is 33 months and 89% of Bangladeshi children are breast-fed for some time (73). Among infants less than 6 months 43% are exclusively breast-fed (73) and there is evidence that breastfeeding counseling can increase the rates of exclusive breastfeeding substantially (76). Under five mortality is 54 per 1000 live births and on the decline (77). The most common causes of under 5 mortality are neonatal causes and infectious diseases such as acute respiratory infections (ARIs) and diarrhea (73).

Matlab is an upadzila (a sub-district) in Comilla south east of the capital Dhaka. The area lies in a fertile, yearly flooded river delta where most people rely on fishing and agriculture for a living. Many families are land-less and work as day laborers in agriculture. Traditionally, women have low status in this society and their mobility can be constrained. Women are regarded as the main caretakers of children and responsible for household chores. Few women participate in income earning activities but many women in poor households work in the fields.

The population of Matlab is about 200 000 and live in about 140 villages. Matlab is part of the Health and demographic surveillance system run by ICDDR, B and consists of one region that receives ICDDR, B health care and another region that receives governmental health care. Residents of the area where ICDDR, B is responsible for health care were eligible for enrolment in the MINIMat study. The health care services are provided by one hospital and four clinics serving approximately 100 000 people.

Subjects and randomization

When community health research workers from ICDDR, B, who visit Matlab households monthly, identified a pregnant woman, they asked her to consider participating in the MINIMat study. Women who were in gestational week <14 and living permanently in the area were eligible. Allocation to the interventions was individual and done in permuted blocks of 12 women. The study included randomization to the nutritional interventions in a 2 by 3 design. The nutritional interventions thus included randomization to six groups: to Early versus Usual inclusion in a food supplementation program and to receive one of three types of micronutrient supplements. Enrolled women visited a sub-centre clinic at approximately 14 weeks of gestation and had a blood sample taken and analyzed for Hb concentration (by HemoCue®). Women with Hb <80 g/L were excluded from the study and referred to Matlab hospital. Women with Hb ≥80g/L received capsule bottles with micronutrient supplements from the community health research workers who refilled the bottles monthly. At about week 30 of pregnancy, women who were still in the study were randomized to receive EBF counseling or the standard package of health education.

Food supplementation

The food supplement was available at a CNC 6 days per week. The supplement consisted of locally produced food items: roasted rice powder, roasted pulse powder, molasses and soybean oil. Women's group participants produced the supplement packages to be mixed with water and consumed at a CNC. The energy content of the supplement was 2500 kJ and it did not contain added micronutrients. Women who had been randomized to Early food supplementation were promoted to start attending the food supplementation program immediately after confirmation of pregnancy. Each CNC was notified about which women were pregnant and who should be enrolled in the program at what time. Women who were randomized to the Usual timing of food supplementation were not promoted to start attending the food supplementation program in the first trimester. Thus, women in the Usual group enrolled in the food supplementation program at the time of their own choice, most commonly at the end of the second trimester.

Micronutrient supplementation

The micronutrient supplements were distributed to study participants during pregnancy and 3 months post-partum. Capsules of the three supplements looked the same and they were distributed in bottles with letter codes, (3 different codes for each type of supplement). Randomization and codes were blinded to women, data collectors and the study teams until statistical analyses of main outcomes were performed.

The three types of micronutrient supplements were:

a) 30 mg iron+ 400 μg folic acid or (Fe30Fol)
b) 60 mg iron+ 400 μg folic acid (60FeFol)
c) 30 mg iron+ 400 μg folic acid + 13 other micronutrients (MMS) (Table 1)

Table 1. Content of one MMS capsule.

Nutrient	**Component**	**Amount**
Vitamin A	Retinyl acetate	800 μg RE
Vitamin D	Cholecalciferol	5 μg
Vitamin E	α-tocopherol acetate	10 mg
Vitamin C	Vitamin C	70 mg
Vitamin B-1	Thiamin mononitrate	1.4 mg
Vitamin B-2	Riboflavin	1.4 mg
Niacin	Niacin	18 mg
Vitamin B-6	Pyridoxine hydrochloride	1.9 mg
Vitamin B12	Cyanocobalamin	2.5 μg
Folic acid	Folic acid	400 μg
Iron	Iron fumarate	30 mg
Zinc	Zinc sulphate	15 mg
Copper	Copper sulphate	2 mg
Selenium	Sodium selenite	65 μg
Iodine	Potassium iodide	150 μg

Exclusive breastfeeding counseling

At week 30 of pregnancy, women in the study were randomized to receive either the standard package of maternal and child health education or extensive counseling on EBF from trained counselors. The counselors were trained in a 40-hour training program based on a translated version of "Breastfeeding counselling: a training course" (78). The counselors then visited the women in week 30 and week 34 of pregnancy, within 3 days of delivery, 15 days after delivery and at 1, 2, 3 and 4 months after delivery. The importance of EBF to 6 months of life was stressed during counseling. The counselors focused on decision-making, motivation, problem-solving skills and how to manage obstacles and influences from the surrounding that could affect breastfeeding practices negatively. The regular health message, delivered by health care staff at postnatal clinic visits with less individualized support, included information on the benefits of breastfeeding.

Data collection during pregnancy

The main points of data collection during pregnancy are outlined in Figure 2. At approximately week 8 of pregnancy trained data collectors interviewed enrolled women about their age, parity and educational level. Weight and height of the women were measured and maternal underweight was defined as a BMI <18.5 kg/m^2. Based on a number of items available in the household, an asset score was created by the study team as an indicator of SES (79). Women were thereafter visited monthly during pregnancy.

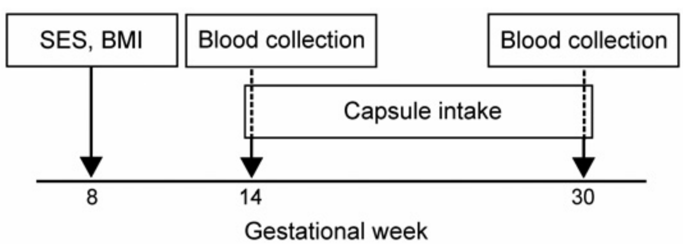

Figure 2. Data collection during pregnancy. SES (Socio-economic status), BMI (Body mass index).

Infant follow-up

The main points of data collection during infancy are outlined in Figure 3. Because of a birth notification system, data on sex, weight and length of the newborns could be collected within 72 hours for most infants. Infants weighing <2500 g at birth were classified as LBW and those with birth weight ≥2500 g as normal birth weight (NBW). SGA was defined as infants with birth weight less than 10 percentiles from the average birth weight for any given gestational age in a reference population (80). All women who gave birth to a child weighing >1000 g, (birth weight measurement taken within 72 hours) were invited to the infant follow-up study. Information on infant feeding, morbidity and anthropometry from infants was collected every month up to 6 months by trained interviewers.

Infant feeding was assessed with a recall of foods given to the infants during the last month. Interviewers probed for breast milk, plain water, fruit juice, milk other than breast milk, other liquids, semi-solids, solids and vitamins and medicines. Women were asked to recall if the foods in question were given during the first or second half of the month. We used the WHO definition of EBF (81). Infants who had been given breast milk only or in combination with vitamins and medicines were categorized as being exclusively breast-fed for each 15-day period up to the introduction of other foods. From the variable that described duration of EBF in days based on the 15-day periods, we then categorized EBF <4 months and EBF 4-6 months. Morbidity recalls were done monthly up to 6 months. Mothers were asked to recall if the infant had had fever, cough, rapid breathing, chest indrawings or unusually loose or frequent stools during the past 7 days. We used mothers' own recall of morbidity to define diarrhea, cough, fever, chest indrawings and difficult breathing. ARI was defined as the combination of fever and cough or difficult breathing. Current infection was defined as a C-reactive protein (CRP) >10 mg/L (82). Data collectors trained in anthropometry visited families monthly to weigh and measure the length of their infants. They used SECA scales (GmbH) and locally manufactured length boards. We defined wasting as weight-for-height z-score (WHZ) <-2, stunting as height-for-age z-score (HAZ) <-2 and low weight-for-age as weight-for-age z-score (WAZ) <-2, using the WHO Anthro software to compare growth of these infants with a reference population.

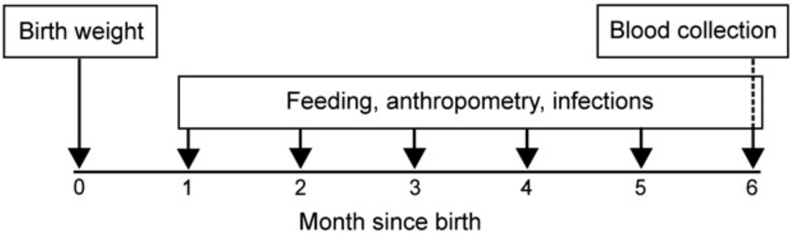

Figure 3. Data collection during infancy.

Collection and analyses of blood samples

At about week 14 and week 30 of pregnancy, blood samples were taken from the women in the study (Figure 2). Because Hb is reduced through the normal process of hemodilution, the cut-off to define anemia in pregnancy was set to Hb <110 g/L (1). At 6 months after birth, 4.5 ml venous blood was collected from the infants (Figure 3). Ill infants were excluded (n=52). Hb was assessed locally (Hemocue®) while analyses of plasma ferritin, plasma zinc, plasma retinol, plasma vitamin B12, plasma folate and plasma CRP were carried out at Department of Nutrition at University of California, Davis. The cut-off values to define anemia and micronutrient deficiencies in infancy are described in Table 2.

Table 2. Indicators used in this study to define anemia and studied micronutrient deficiencies in infancy.

Condition	Indicator	Cut-off(s)
Anemia	Blood hemoglobin	<110 g/L(2) and <105 g/L(83)
Iron deficiency (ID)	Plasma ferritin	<12 µg/L (2) and <9 µg/L (83)
Zinc deficiency	Plasma zinc	<9.9 µmol/L (84)
Vitamin A deficiency (VAD)	Plasma retinol	< 0.70 µmol/L (85)
Vitamin B12 deficiency	Plasma vitamin B12	<150 pmol/mL (86)
Folate deficiency	Plasma folate	<6.8 nmol/mL (87)

Assessment of adherence to supplement intake

The micronutrient bottles in the study were equipped with a microprocessor inside the cap (eDEM®) that recorded the date and time of every opening of the cap. This information was downloaded into a computer when capsule bottles were collected from the women. The number of openings from when the bottle was distributed to the women to their week 30 clinic visit was included in the analysis (Figure 2). To assure that baseline Hb data coincided with the supply of capsules, cases where micronutrients were supplied 7 days before or after the clinic visit at week 14 were excluded. Data of more than three openings for one day were set to three openings to avoid overestimation of capsule intake.

Statistical analyses

In this section, the methods used to assess associations between main independent and dependent variables are outlined. Descriptive statistics, adjustments for confounding and stratified analyses were performed as described in each of the four papers of this thesis.

In Paper I, we used locally weighted scatterplot smoothing (loess) to describe the relation between Hb concentration gestational week 30 and number of micronutrient capsules consumed. Linear regression models were used to assess the Hb dose-response to capsule intake. The plots were used to identify at what capsule intake Hb concentration no longer increased. The capsule intake category (1 for <60 and 0 for ≥60) and the interaction between capsule intake category and capsule intake were then tested in a linear regression model.

In Paper II, differences in infant Hb and micronutrient concentrations between maternal supplementation groups were assessed using Analysis of variance (ANOVA). Linear regression models were then used to adjust for elevated CRP where appropriate. Prevalence of infant anemia and micronutrient deficiencies between supplementation groups were assessed with chi-square tests and logistic regression models.

In Paper III, linear and logistic regression models were used to adjust for confounding in analyses evaluating the impact of duration of EBF on anemia, iron and zinc status.

In Paper IV, logistic regression models were developed to evaluate the factors associated with anemia in infancy.

Statistical analyses were performed in SPSS 17.0 (Paper I), SPSS 14.0 (Paper II-IV), SPSS Inc, Chicago, Illinois, USA and Stata 9.2, StataCorp LP, College station, Texas, USA (Paper II and III).

Ethics

Community health research workers from ICDDR, B informed eligible women about MINIMat at a household visit. Consent was obtained in a two-step procedure: At the household visit, oral consent was obtained in conjunction with a pregnancy test. Women who gave primary consent were invited to a clinic visit where written consent was obtained. Women were informed that there would be no negative consequences if they declined to participate in the study or in certain study activities. Some women used their right to refuse clinic visits and blood samplings, although they continued to take part in the interviews. Appropriate measures were taken to assure confidentiality.

The Ethical Review Committee of ICDDR, B reviewed and approved the study protocol in October 2000. We requested a review from the regional ethics committee in Uppsala but were advised not to send in an application to the regional committee because there were no additions to the research proposed in the original protocol approved by ICDDR, B.

Results

General characteristics

Women in the study claimed to be between 14 and 50 years at enrollment and parity was 0-10. At enrollment 27% of the women were malnourished. The woman with the highest education had 16 years in formal school, but one third of the women had no formal schooling (Table 3).

In our sample, 30% of the infants were born with LBW and 57% were SGA. At 6 months of age, 6% of the infants were wasted and 25% were stunted. As indicated by elevated CRP, 14% of the infants had an infection or inflammation when the blood sample was taken at 6 months after birth.

Anemia and micronutrient status in infancy

The concentrations of blood Hb and plasma micronutrients of the infants at 6 months of age were generally low, except for plasma folate (Table 4). We found high rates of anemia, Hb <105 g/L (47%) and deficiency of zinc (57%) and vitamin B12 (31%) in particular. Moreover, VAD (23%) and ID plasma ferritin <9 µg/L (20%) were prevalent, whereas folate deficiency was not common (1%) (Figure 4).

Blood Hb and plasma micronutrients concentrations were lower in LBW infants than in those born NBW, with the exception of plasma folate (Table 4). LBW infants had higher prevalence of anemia, ID, VAD, zinc deficiency and B12 deficiency than NBW infants (Figure 4).

Infants with elevated CRP had higher prevalence of anemia, zinc deficiency and VAD and lower prevalence of ID. Prevalences when infants with elevated CRP were excluded were: Anemia (43%) (224/525), ID (22%) (202/903), zinc deficiency (56%) (506/903) and VAD (19%) (165/881) (Figure 4). Of the infants with normal CRP, 65% (341/525) had Hb <110 g/L and 30% (274/911) had plasma ferritin <12 µg/L. Vitamin B12 and folate deficiency prevalences were not affected by exclusion of infants with elevated CRP.

Table 3. Characteristics of women and infants in the study (n=1066)[1].

Characteristic	
Maternal age, y	26.3±6.2
Maternal BMI[2], kg/m^2	20.2±2.6
Maternal Hb[3], g/L	116±12
Maternal Education	
No education	338 (31.7)
1-5 y	257 (24.1)
≥6 y	471 (44.4)
Parity[4]	
0	335 (31.5)
1-2	524 (49.2)
≥3	206 (19.3)
Capsules taken to wk 30[5]	84 [55-104]
Food packages consumed to wk 34[6]	82 [49-112]
Male infant	551 (51.7)
Birth weight, g	2697±404
WAZ[7]	1.1±1.0
WHZ[8]	0.31±1.1
HAZ[8]	1.3±1.0

[1]Means±SD, n (%) or median [IQR]
[2]BMI was available for n=1064
[3]Hb wk 14 was available for n=988
[4]Parity was available for n=1065
[5]eDEM capsule count was available for n=939
[6]n=1063
[7]n=1042
[8]n=1037

Table 4. Blood Hb and plasma micronutrient concentrations of infants in the whole population and in infants with low birth weight (LBW)[1].

	All		LBW		*p*
	n		*n*		
Hb, g/L	796	105 [97-112]	207	100 [94-108]	<0.001[2]
Ferritin, µg/L	1058	21.9 [10.9-43.2]	313	16.5 [6.9-32.2]	<0.001[3]
Zinc, µmol/L	1050	9.6 [8.2-10.9]	308	9.1 [7.8-10.5]	<0.001[2]
Retinol, µmol/L	1026	0.87 [0.72-1.06]	299	0.84 [0.67-1.03]	0.020[2]
Vitamin B12, pmol/L	1033	202 [132-316]	303	185 [118-303]	0.030[3]
Folate, nmol/L	1031	30.6 [(22.2-38.8]	303	31.1 [21.8-38.6]	0.74

[1]Values are median [IQR]
[2]Difference from normal birth weight assessed with t-test
[3]Difference from normal birth weight assessed with non-parametric test

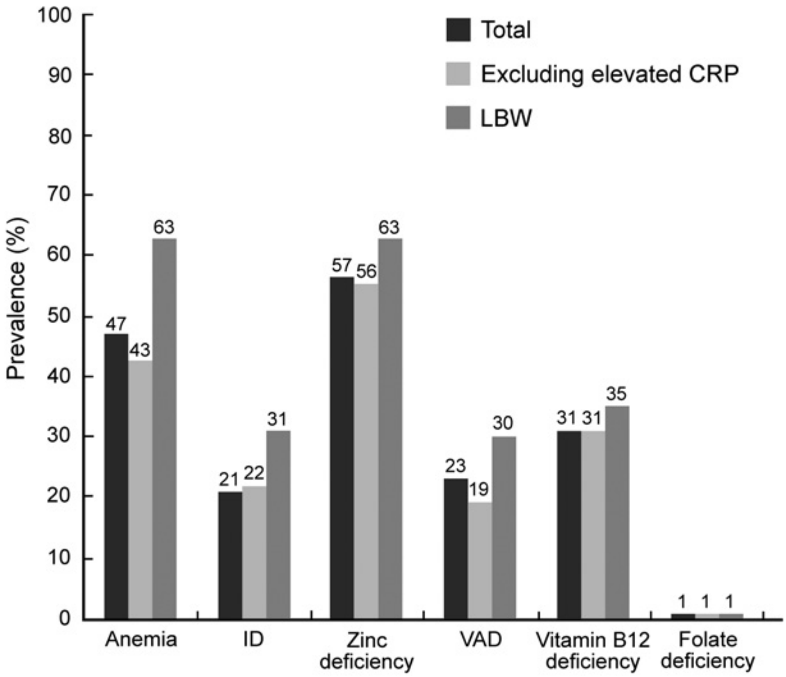

Figure 4. Prevalence of anemia and iron deficiency (ID), Zinc deficiency, Vitamin A deficiency (VAD), Vitamin B12 deficiency and Folate deficiency in the total population, in infants with normal C-reactive protein (CRP) and in infants with low birth weight (LBW).

Maternal hemoglobin response to micronutrient supplementation during pregnancy (Paper I)

In Paper I, 2377 pregnant women were included in our sample. At baseline the prevalence of anemia was 30% (709/2377). Hb concentration increased with increasing consumption of micronutrient capsules for all types of prenatal supplements (β=0.09, p<0.001) after adjustment for SES, baseline Hb, food supplementation and time between capsule supply and follow-up.

The scatterplot (Figure 5) suggested a nonlinear pattern in which the Hb response per ingested capsule was largest initially, leveling out at 60 capsules. By linear regression modeling, disregarding type of supplement, we tested the difference in response between the first 60 capsules taken and the proceeding capsules consumed by introducing an interaction variable "intake category". A significant interaction was found (p=0.01), confirming a change in dose-effect that was mainly driven by Fe60Fol (0.16-0.15, p=0.006). The change in effect was less dramatic for MMS (p=0.19) and absent in Fe30Fol (p=0.81). The effect per capsule in the lower range of capsules intake (<60 capsules) was lower in Fe30Fol and MMS groups than in the Fe60Fol group. There was no difference between Fe30Fol and MMS.

The Hb increase per capsule was larger in women who were anemic at baseline than in non-anemic women. There was no further dose response to capsule intakes ≥60 capsules for anemic or non-anemic women. Mean Hb concentration was 115±12 g/L at follow-up and prevalence of anemia was still high at 36% (850/2377), especially in initially anemic women (56%) (399/709).

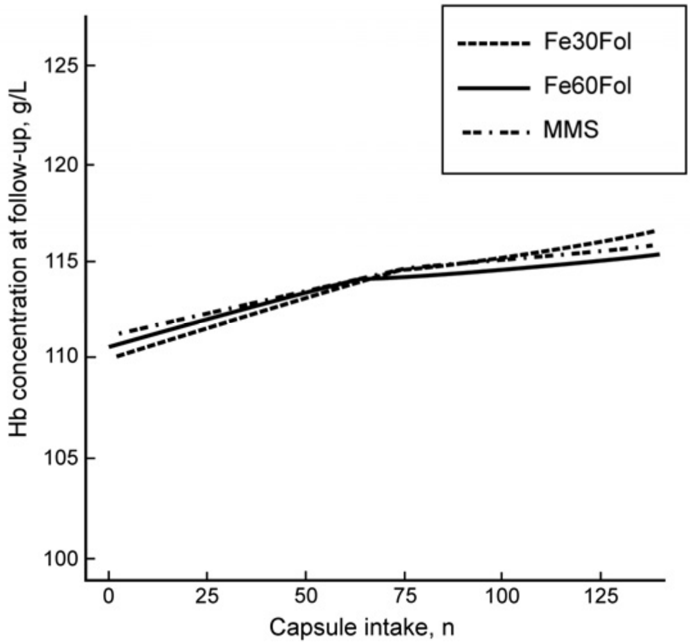

Figure 5. Hemoglobin (Hb) concentration at follow-up at about wk 30 in pregnancy and capsule intake, by type of supplement.

Maternal food and micronutrient supplementation and infant micronutrient status (Paper II)

The number of infants included in this analysis was 1066 and sample size for the different biomarkers in the randomization groups ranged from 203 to 357. No interactions between time of initiation of food supplementation and type of micronutrient supplementation were found on any indicator of infant anemia or micronutrient status. Despite the interventions with food and micronutrients, deficiencies of iron, zinc and vitamin B12 in infancy were prevalent in this population (Figure 4). Plasma vitamin B12 concentration was higher in the MMS group, 221[145-324] pmol/L than in the

Fe30Fol group, 190[123-305] pmol/L and the Fe60Fol group, 197[126-322] pmol/L (*p*=0.049). We found a lower prevalence of vitamin B12 deficiency in the MMS group (26%) than in the Fe30Fol group (37%) (*p*=0.003). Zinc deficiency was more common in the group of infants whose mothers were randomized to Early start of food supplementation (60%) than in the Usual food supplementation group (54%) (p=0.046). Concentrations of Hb, plasma ferritin, plasma zinc and plasma retinol were similar across micronutrient and food supplementation groups. Despite the different iron content of the capsules, there was no difference in anemia or micronutrient status between infants born to women randomized to the Fe60Fol or Fe30Fol group.

Duration of EBF and infant anemia, iron and zinc status (Paper III)

Blood samples analyzed in Paper III were n=1032 for plasma zinc, n=1040 for plasma ferritin and n=791 for Hb. Infants who were exclusively breast-fed for 4-6 months had a higher mean plasma zinc concentration (9.9±2.3µmol/L) than infants who were exclusively breast-fed for <4 months (9.5±2.0µmol/L), (*p*<0.01). This association only appeared in the NBW strata and prevalence of zinc deficiency did not differ between EBF categories. Duration of EBF was not associated with concentration of plasma ferritin or Hb or with prevalence of iron deficiency or anemia in any strata. Additional risk for anemia and deficiency of zinc and iron with a longer duration of EBF was assessed with logistic regression, but not significant for any condition. All analyses were adjusted for allocation of mother to EBF and food and micronutrient interventions. Prevalence of anemia and iron and zinc deficiency was high, regardless of EBF duration.

Determinants of anemia in infancy (Paper IV)

Because we selected one full year of data collection to have a sample equally representative of all seasons, the sample in study IV consisted of 580 infants (n=265 anemic and n=315 non-anemic) Potential determinants associated with infant anemia in the bivariate analyses are presented in Table 5. The factors that were studied but not found to be associated with anemia in the bivariate analyses were deficiency of zinc, vitamin B12 and folate, occurrence of diarrhea or ARI during 4-6 months, wasting at 6 months and weight or length gain 0-6 months. We found that iron deficiency and current infection, as indicated by elevated CRP, and LBW were independently associated with increased risk for anemia at 6 months in the final analysis adjusted for sex of the infant and maternal anemia at baseline (Table 5). We calculated the population attributable proportion of anemia to 15% attributable to ID, 13% attributable to elevated CRP and 21% attributable to LBW. We also noted an effect of season on anemia, not mediated by micronutrient deficiencies, birth weight or infection.

Table 5. Logistic regression models with predictors of infant anemia at 6 months: Iron deficiency (ID), Vitamin A deficiency (VAD), Elevated C-reactive protein (CRP), Low birth weight (LBW), Small-for-gestational-age (SGA), Stunting and Low weight-for-age.

	Bivariate analyses OR (95% CI)[1]	**Model 1, n=580 OR (95% CI)[2]**	**Model 2, n=532 OR (95% CI)[3]**
ID	2.4 (1.6, 3.6)	2.6 (1.7, 4.0)	2.2 (1.4, 3.6)
VAD	2.0 (1.4, 3.0)	1.6 (1.0, 2.4)	1.6 (1.0, 2.5)
Elevated CRP	2.5 (1.5, 4.0)	2.6 (1.5, 4.3)	2.7 (1.6, 4.7)
LBW	2.4 (1.6, 3.5)	2.0 (1.3, 2.9)	2.3 (1.5, 3.5)
SGA	1.7 (1.2, 2.4)		
Stunting	1.8 (1.2, 2.6)		
Low weight-for-age	1.6 (1.1, 2.5)	1.4 (0.91, 2.2)	1.2 (0.73, 1.6)

[1]Unadjusted
[2]Adjusted for variables in the model
[3]Adjusted for variables in the model, sex of infant and maternal anemia week 14

Discussion

The micronutrient supplements increased Hb in pregnant women in a dose-response manner; and Fe60Fol, Fe30Fol and MMS all produced an Hb increase per capsule. Fe60Fol produced the maximum potential Hb at an intake of 60 capsules, which was not the case with Fe30Fol or MMS. Hb concentration increased per ingested capsule for both anemic and non-anemic women, with a higher increase per capsule in anemic women. The prevalence of anemia was still high after supplementation despite a response leveling effect to intakes above 60 capsules. (Paper I). The prevalence of anemia and micronutrient deficiencies was high in 6-month-old infants in this population (Paper II and Paper III). Infant vitamin B12 concentration was different between micronutrient supplementation groups, with the highest concentration found in the MMS group (Paper II). Infants in the MMS group had a lower prevalence of vitamin B12 deficiency than infants in the Fe30Fol group (Paper II). Prevalence of zinc deficiency was higher in infants born to women who were randomized to Early promotion of food supplements than in infants in the Usual food supplementation group (Paper II). Infant plasma zinc concentration was positively associated with an EBF duration of 4-6 months when compared with a shorter duration of EBF (Paper III). Other than that, no differences in hemoglobin or plasma micronutrient concentrations, prevalence of anemia or micronutrient deficiencies were observed between maternal micronutrient or food randomization groups (Paper II) or EBF categories (Paper III). Anemia in infancy was associated with iron deficiency, low birth weight, signs of infections and season of birth (Paper IV).

Methodological Considerations

Validity

The careful planning and implementation of the MINIMat study assure the validity and reliability of the data presented in this thesis. Interviewers and data collectors were trained and the questionnaires were pre-tested. Equipment for length and weight measurements was routinely calibrated and anthropometric measurements were done twice for each participant and visit.

Internal validity is also strengthened by the thorough assessment of infant feeding. To help the caretaker remember foods given to the infant, we used a list of types of infant foods to probe for in the monthly recall. A 24-hour recall of foods given to the infant is the recommended method to assess current prevalence of EBF (81). Such short recalls, and long recalls since birth are equally poor in predicting EBF duration (88). The assessment of EBF in the MINIMat study was found to accurately reflect EBF practices at the group level in an investigation using stable isotope technique to detect water from non-breast milk sources in a subset of infants (89).

The capsule-counting device eDEM is regarded as the best available method to measure adherence (90). As for most other methods, such as capsule count and recall of capsule intake, overestimation may be an issue. By restricting eDEM count of >3 per day to capsule intake of three, we reduced overestimation due to playing with or displaying the capsule bottle to others.

We were able to measure several possible confounders in the relation between the exposures and infant micronutrient status. We did not have data on potential intakes of supplements for infants. However, the confounding from this factor is probably limited as iron supplements for infants were not available at the market at the time of the study and the use of zinc supplementation in diarrhea episodes is still limited (73).

The random allocation of women to the nutritional interventions was successful and there were no baseline differences between groups. Selection bias could have been introduced when some women refused blood sampling of their infants. The comparison between dropouts and those with a blood sample suggests that this effect on external validity was limited, as differences were small. However, there could have been differences in behavior between participants with complete data and those who dropped out from the study.

Outcome assessment

Anemia and micronutrient status were assessed by measuring biomarkers in blood and plasma. The indicators of micronutrient status used in this study are some of the most commonly used in population studies. Still, they each have their limitations. Cut-off values for deficiency of micronutrients in infancy are often extrapolated from cut-off values for older age groups. We have used the cut-offs suggested in the recent literature, sometimes with the addition of well recognized cut-off values for the sake of comparability with other studies. The knowledge of reliable biomarkers and appropriate cut-offs to define anemia and micronutrient deficiencies in infancy are still unsatisfactory.

Hb is an iron-containing oxygen transporting protein. When iron levels are low, Hb concentration in the blood falls. There are several other potential reasons to decreased Hb concentration, but because Hb is easy to measure, it is commonly used as an indicator of iron status. Ferritin is the storage form of iron that reflects the total body content of iron. In infancy, it has been used as an indicator for a long time (91). Plasma zinc reflects zinc intake in healthy adults and is the most commonly used biomarker to asses zinc status in response to zinc supplementation (92). Because of the homeostatic regulation of plasma zinc, it is not a reliable indicator of an individual's zinc status but only of a response to zinc supplementation and the risk of zinc deficiency in populations (93). Plasma zinc depends on time of the day and time since the last meal, although such differences seem less in young children than in adults (94). Plasma retinol is one of the recommended biochemical methods to assess response to vitamin A interventions (95). The guidelines for assessing vitamin A status in populations are now being updated.

Blood Hb, plasma ferritin, plasma zinc and plasma retinol are acute phase indicators (96). In individuals with infection or inflammation, plasma ferritin can appear higher and Hb and plasma zinc and plasma retinol lower than if infection or inflammation was not present. In individuals with infection or inflammation, ID can be masked and anemia, zinc deficiency and VAD overestimated. To deal with this kind of over- and underestimation, infants with elevated CRP can be excluded, or adjustments for elevated CRP can be made in statistical models. The most correct way to present prevalences of anemia and micronutrient deficiencies is after exclusion of infants with elevated CRP. In this study, exclusion of infants with elevated CRP led to a decrease in prevalence of anemia and VAD with 4 percentage points. The estimates of ID and zinc deficiency were affected by only 1 percentage point each. Thus, in our study, plasma retinol and blood Hb seemed most affected and plasma ferritin less affected than expected from the literature. In the multivariate analyses, we controlled for CRP as an indicator of falsely elevated or decreased biomarker concentrations by adding the variable CRP in the statistical models. There is a possibility that a part of the differential effect of the interventions on micronutrient status was taken out if there was an increase in infection or response to infection as a result of the MINIMat interventions. We used a semi-quantitative CRP with the cut-off set at >10 mg/L and thus some infants with sublinical infection may have gone unnoticed. CRP increases quickly at the onset of infection and declines within 1-2 days. Another acute phase protein, α-acid glycoprotein (AGP), rises more slowly and declines 5-6 days after infection onset. Ideally, several acute phase indicators should be used to capture all stages of subclinical infection or inflammation.

Plasma B12 is a widely used and effective biomarker to evaluate vitamin B12 supplementation in adults (97). Plasma folate is a reliable indicator of dietary folate intake, but red cell folate measures may be more reliable in intervention studies (87). The measurement of vitamin B12 and folate in plasma is complicated by the sex and age variations in concentrations of the circulating vitamins (86). Plasma vitamin B12 and folate have previously been used to evaluate vitamin B12 and folate status of infants (98, 99).

Sample size

Because the MINIMat study was designed to evaluate the impact of nutritional interventions on birth weight, the sample size calculations were made on the basis of finding a difference in birth weight. We calculated the differences in infant micronutrient status that we could detect with the smallest sample size in any supplementation group in any of our analyses (n=202, Hb concentration in Paper II). Using this sample size, 80% power and 95% probability we were able to detect a difference of 0.3 SD between supplementation groups. Difference=$\sqrt{}$ $((1.96+0.84)^2 * 2 * SD^2)/n$. We judge that a smaller difference than 0.3 SD is of limited public health importance and do not consider limited sample size as a plausible reason for non-significant results.

The differences in sample size in Paper I-IV depend on the number of participants with available exposure and outcome measurements. In paper I-III, all available participants with measurements were used while in paper IV, a selection based on a full year of study recruitment was performed.

Generalizability of findings

The sub-district of Matlab is relatively typical of the Bangladesh delta land, with poor rice farmers, widespread malnutrition and high occurrence of infectious diseases. However, because of the higher coverage of maternal and child health services in this area, indicators of maternal and child health (e.g. perinatal and infant mortality) are a bit lower than in other areas served by the regular government program (100). Anemia and deficiencies of zinc, vitamin B12 and folate were common in early pregnancy in this study (101). All women received food supplements during pregnancy and all received at least iron-folic acid supplements. Although women participants in our study had a poor nutritional status, the interventions to improve their nutritional status were more intensive than in many other low-income settings.

One should be cautious in generalizing from our findings to populations with different infectious disease patterns. The micronutrient requirements and metabolism are likely to be different, such as in settings where HIV (102) or malaria is common (103).

Prevalence of anemia and micronutrient deficiencies

In this study, anemia prevalence was 36% in women at about gestational week 30. The national estimate of anemia in pregnant women in rural areas is 47% and anemia in pregnancy is considered a severe public health problem in Bangladesh (1).

Infants in this study had high prevalence of anemia, zinc deficiency, vitamin B12 deficiency, VAD and ID. These high prevalences are not unique for this study, but common in low-income countries. Depending on the particular setting, age of infants at blood sampling and biomarker cut-offs applied, large variations have been reported. In this study 43% of infants had Hb <105g/L and 65% had Hb <110g/L. Even higher rates of anemia in 6-month- old infants in another site in rural Bangladesh were reported (55% with Hb <105 g/L) (104). The national estimate of anemia in 6-59-month-old children in rural areas is that 47% have Hb <110g/L (1). It is clear that childhood anemia is a severe public health problem in the country in that prevalence exceeds 40% (1). We found that 22% of the infants had plasma ferritin <9 μg/L and 30% <12 μg/L. With the latter cut-off applied, 9% of the studied infants were deficient in Vietnam (105), 25% in Honduras and 37% in Ghana (106). We report a higher prevalence of zinc deficiency (56%), than in many studies (105, 107, 108). The high prevalence of zinc deficiency among women in the MINIMat study (101) could, together with the high prevalence of infections have contributed to the unusually high rate of infant zinc deficiency in this study. A similar prevalence of VAD (about 20%) was found in Vietnamese infants as in our study (105). VAD is considered a severe public health problem in Bangladesh, where the national estimate of VAD prevalence in pre-school children is 22% (4). A similarly high prevalence of infant B12 deficiency as in our study (31%) was also found in India (36%) (98). In that same study, 6% of the infants had low folate levels (98) which is higher than in our study (1%), but nevertheless probably not of large public health importance.

Maternal micronutrient supplementation

Effect on maternal Hb

For all three types of prenatal supplement, the maternal Hb concentration increased with increasing capsule intake at low intakes. The maximum Hb response to iron supplementation does not seem to prevent anemia. A potential explanation is that anemia in this population is due to causes other than ID. Iron deficiency anemia was uncommon at week 14 (101), but low Hb concentrations could be associated with ID in late pregnancy because of increased iron requirements. It is also possible that a part of the anemia in this population is physiological of pregnancy. The Hb concentration of anemic women increased more per ingested supplement capsule than for non-anemic women, which was expected because of more efficient iron absorption (109).

A lower capsule intake was required for Fe60Fol than Fe30Fol and MMS to reach the maximum Hb response per ingested capsule. This event is probably due to the lower iron content in Fe30Fol and MMS. Furthermore, studies suggest that the total amount of iron needed is less than the recommended dose if taken daily (57, 110). Our results indicate that 9 weeks of daily supplementation would be sufficient for a maximum Hb response. Possibly, the other nutrients in the MMS (such as vitamins A, B and C) are of such low doses that an additional effect on Hb to that from iron-folic acid is not observed (55).

One way to reduce anemia and iron deficiency during pregnancy is to supplement women at risk before pregnancy and the increase in requirements it conveys. This strategy has been successful in Vietnam (111) but pre-pregnancy iron supplementation did not produce lower prevalence of anemia or improve iron status during pregnancy in Bangladesh, despite high prevalence of anemia pre-pregnancy (112, 113).

Effect on infant micronutrient status

To our knowledge, this is the first study to evaluate the UNIMAP multiple micronutrient supplement against iron-folic acid supplementation with infant micronutrient status as an outcome. We expected an impact on infant iron and zinc status through an increase in status at birth, on vitamin B12 status through status at birth or higher vitamin B12 content of breast milk and on vitamin A through higher vitamin A concentrations in breast milk. We found limited effects and therefore have sought an explanation as to why the provision of MMS to women during pregnancy and lactation did not affect the micronutrient status of the infant. In this setting, both women (101) and infants (Paper II-III) were deficient in a number of nutrients (e.g., vitamin B12, vitamin A and zinc). Thus it is reasonable to assume a potential benefit from supplementation of these nutrients. It is possible that the amount of nutrients in the supplement was too low to prevent deficiency, especially because micronutrient needs are greater in pregnancy. Some trials have assessed the effect of combinations with higher doses of multiple micronutrients on maternal micronutrient status, but to our knowledge, effects on infant micronutrient status have not been assessed. There may be a methodological explanation underlying the lack of an additional effect of MMS when compared with iron-folic acid. Absence of biomarker responsiveness is an unlikely explanation of the lack of response to MMS in that the biomarkers used in this study have been responsive to supplementation in other studies.

It is possible that MMS did improve micronutrient status at birth but the effect disappeared by the time of blood sampling of the infants at 6 months of age or that an effect appeared later when the infant had been weaned.

The role of multiple micronutrients

In previous research, multiple micronutrients during pregnancy have failed to improve maternal anemia and iron status above the effects of iron-folic acid only. Furthermore, the effect on other micronutrients is limited (55). Our results indicate that an iron supplement with 60 mg iron and folic acid is superior to a multiple micronutrient supplement with 30 mg iron in increasing Hb per capsule. Further, MMS during pregnancy did not improve micronutrient status of infants compared to an iron supplement with 60 mg iron and folic acid. The small effects in our study do not support a change from the recommended iron-folic acid to MMS of women during pregnancy and lactation as a means to increase infant iron, zinc, vitamin A, vitamin B12 or folate status. So far, it seems as though MMS in pregnant women has little or no effect on maternal and infant micronutrient status (55). Although some studies have discussed whether prenatal multiple micronutrients may increase the risk of neonatal death under certain conditions, a recent meta-analysis did not find any positive effect on infant survival; nor did the meta-analysis demonstrate any increased risk of perinatal or neonatal death (54). However, based on some positive effects on birth outcomes, it is suggested that multiple micronutrients replace iron-folic acid supplements in women during pregnancy (114). There may also be other benefits of multiple micronutrient supplementation for women during pregnancy and for their offspring (115-117). The future role of multiple micronutrient supplementation as a public health intervention is still unclear.

Maternal food supplementation

We found that the prevalence of zinc deficiency was higher in infants whose mothers were randomized to starting food supplementation Early than in infants whose mothers were randomized to Usual start. The underlying mechanism to this unexpected finding is not clear. One hypothesis is that increased maternal food intake resulted in higher zinc requirements in the infants. It is also possible that the food supplement that was low in micronutrients replaced micronutrient rich foods so that maternal zinc intake was reduced in the Early food supplementation group. More information is needed to draw conclusions on mechanisms and potential policy implications of this finding.

Exclusive breastfeeding

In Paper II, the impact of duration of EBF on infant micronutrient status was studied. Concern has been expressed about the adequacy of EBF for iron and zinc status in vulnerable children, such as those with LBW. Our main conclusion was that infants do not have a higher risk for iron deficiency when they are exclusively breast-fed for 4-6 months than when they are exclusively breast-fed for <4 months. While a higher risk for anemia with longer duration of EBF (66) or full breastfeeding (69) is reported, lower rates of anemia in exclusively breastfed infants than in infants receiving complementary foods have also been observed (119). Theoretical calculations suggest that exclusively breast-fed infants who are born small are at risk of iron depletion at 3 months when cord clamping practices are not optimal (120). Most previous studies have compared shorter and longer duration of EBF in countries where the use of fortified formula and commercial baby foods is more common as replacement foods than they are in Bangladesh. The complementary foods most commonly given in Matlab are low in micronutrients (33). We found slightly higher concentrations of zinc in plasma in the infants who were exclusively breast-fed for the longest duration. The underlying mechanisms could be that EBF protects against diarrheal infections that negatively influence zinc status. Another possible explanation is that the phytate content of introduced foods may have limited zinc absorption from breast milk (118). In conclusion, promoting EBF for 6 months does not seem to confer a risk of a higher prevalence of anemia or deficiencies of iron and zinc in this setting.

A limitation of this study was that we did not have information to evaluate other aspects of infant feeding in addition to EBF. It may have been worthwile to look at the quality and diversity of foods introduced to the infants but our feeding assessment did not allow for this type of evaluation.

Determinants of anemia in infancy

Because we knew that anemia in women in the beginning of pregnancy was not associated with ID, it was natural to question the assumption that anemia in infancy is primarily due to ID. Of the factors studied, LBW, ID and elevated CRP were the predictors of anemia. These three predictors are difficult to disentangle from each other: LBW, for instance, increases the risk for infections and for ID. It is not clear what undelies the increases risk for anemia with LBW if it is not explained by ID or infection. Probably, all aspects of infection and micronutrient deficiencies were not captured in our categorical variables. We do not know what underlies the effect of season of birth on infant anemia. Food availability and infectious disease pattern are two factors likely to contribute to seasonal variations. Seasonality was also found in maternal anemia (101). The practical implication of this is that one should take seasonality into account when interpreting biomarkers of micronutrients.

It is also important to look at what we did not find. We expected an association between infant anemia and vitamin B12 status because of the link between B12 deficiency and anemia in women at baseline (101). The association between vitamin A and anemia observed in one of the bivariate analyses disappeared when CRP was added to the model. This finding confirms the effect of vitamin A as an acute phase reactant. Further, we expected an association between sex of the infant and anemia, especially since prevalence of anemia was significantly higher in males than in females. Probably, the other factors (e.g., LBW and ID) explained some of the differences that could be contributed to sex differences.

Strategies to prevent infant anemia and micronutrient deficiencies

In this study population, prevalence of micronutrient deficiencies was high and the evaluated interventions only had a limited impact on infant micronutrient status. Our results infer that prevention of infections and LBW might be equally important as iron supplementation to control anemia in infancy. LBW infants are at highest risk of developing micronutrient deficiencies and interventions that reduce the prevalence of LBW, such as through improved maternal nutrition, should be prioritized. Both health care services and health care seeking for children could be improved and home-based care is a key issue. Zinc supplementation is recommended during diarrhea episodes (121) and this intervention, if uptake would increase, has the potential to prevent many episodes of diarrhea and ameliorate the risk of subsequent zinc deficiency.

Iron supplementation is recommended to all LBW infants, but in many parts of the world, LBW screening is not in place and blood sampling to screen for iron deficiency not practiced. Because there are several issues with providing iron supplement to iron replete children, including higher risk for infections and slower growth (122), it is important not to take lightly the distribution of iron supplements in areas where only some of the children are iron deficient. Because infants may suffer from several micronutrient deficiencies (47), multiple micronutrient supplementation has also been suggested (115).

To avoid that micronutrient deficiencies develop beyond 6 months of age, timely introduction of high quality complementary foods is crucial. Micronutrient content of complementary foods is low in Bangladesh (33) as is food diversity (123). One-quarter of the infants of age 6-9 months are not given complementary foods at all (73) and thus at high risk of developing micronutrient deficiencies. Interventions directed at increasing the awareness of the importance of timely and adequate complementary foods should be prioritized and preferably linked to other infant feeding interventions such as promotion of EBF to 6 months.

Strategies to improve maternal nutrition often include nutritional education, provision off supplements and fortification of staples. Women in Bangladesh are aware of increased nutritional requirements during pregnancy, but are unable to increase their intakes (124). Therefore, it is important to address social and economic barriers to improve women's nutrition. Beyond nutrition interventions, a range of measures can increase wealth, food security and decision-making power of women. Ideally, however, nutrition interventions should also aim for these outcomes so that women in Bangladesh and elsewhere are able to increase their food intake during pregnancy and obtain adequate health care when necessary for themselves and their offspring.

Conclusions

Micronutrient deficiencies and anemia are public health problems in infants in this setting. Anemia at 6 months of life is explained to a similar extent by iron deficiency, low birth weight and current infection as represented by elevated C-reactive protein.

In pregnant women, a plateau in Hb concentration per ingested micronutrient capsule was reached after an intake of 60 capsules of 400µg folic acid and 60 mg iron, indicating that 20 weeks of daily supplementation would be more than the required dose to produce a maximum Hb response. Anemia in late pregnancy was common in women with capsule intake above 60, indicating other causes of anemia than iron deficiency in this population.

We found no adverse effects on infant anemia or micronutrient status with the use of a lower dose of iron (30 mg) in a maternal supplement when we compared this dose to the standard dose 400µg folic acid and 60 mg iron. The use of a maternal multiple micronutrient supplement as compared with a supplement with iron-folic acid may have a beneficial effect on infant vitamin B12 status. We found no other benefits of maternal multiple micronutrient supplements over iron-folic acid supplements on these outcomes.

In our study, infants whose mothers were encouraged to enrol in a food supplementation program early in the pregnancy had a higher prevalence of zinc deficiency than infants of women who started the food supplementation at the time of their choosing. More information is needed to ascertain the underlying mechanisms and potential impact of this finding.

Exclusive breastfeeding for 4-6 months was not associated with prevalence of anemia, iron deficiency or zinc deficiency. Thus, it seems “safe” to promote Exclusive breastfeeding to 6 months without risking an even higher prevalence of anemia and iron and zinc deficiency in this setting where the use of fortified baby foods is likely to be low.

Summary in Swedish/Sammanfattning på Svenska

Anemi och mikronäringsämnesbrister hos spädbarn är vanliga i låginkomstländer och beror delvis på mödrarnas näringsstatus. Barn som har brist på järn, zink, vitamin A eller vitamin B12 riskerar försämrad tillväxt, utveckling och immunförsvar. Anemi hos spädbarn antas ofta bero på järnbrist. Barnens status av mikronäringsämnen påverkas av hur mycket av dessa som tillförts under fosterstadiet, tillförts via bröstmjölken till ammade barn, tillväxthastighet och förekomst av infektioner. Exklusiv amning är när barnet inte får någon annan mat eller dryck än bröstmjölk. Världshälsoorganisationen (WHO) rekommenderar exklusiv amning till 6 månaders ålder. Det har diskuterats huruvida barn som föds underviktiga och till mödrar med näringsbrister riskerar järn- och zinkbrist vid 6 månaders exklusiv amning.

I projektet MINIMat i Matlab på landsbygden i Bangladesh gav man kosttillskott till gravida kvinnor för att förbättra deras egen och deras barns hälsa. Gravida kvinnor randomiserades till ”Early” den grupp som uppmuntrades till tidig start i ett program som erbjöd ett mattillskott under graviditeten, eller till ”Usual” där ingen uppmaning om tidig start gavs utan kvinnorna i denna grupp började ta del av mattillskottet när de själva ansåg att det var lämpligt. Kvinnorna randomiserades också till att få en av tre typer av kapslar med mikronäringsämnen från graviditetsvecka 14 till 3 månader efter barnets födelse:
a) 60 mg järn och 400 μg folsyra (Fe60Fol)
b) 30 mg järn och 400 μg folsyra (Fe30Fol) eller
c) 30 mg järn och 400 μg folsyra samt 13 andra mikronäringsämnen (MMS).
Kapslarna distribuerades i burkar vars lock innehöll en mikroprocessor (eDEM) som räknade antalet gånger som burken öppnades. Detta gav ett mått på hur många kapslar som kvinnorna tagit. Blodprov togs från kvinnorna vid vecka 14 och vecka 30 och analyserades för hemoglobin (Hb). Efter att barnen fötts intervjuades kvinnorna varje månad om vilka livsmedel som deras barn ätit samt om barnen haft tecken på infektioner. Barnen vägdes och mättes varje månad. Vid 6 månaders ålder togs ett blodprov från barnen och detta analyserades för Hb (ett mått på anemi), plasma ferritin (ett mått på järnstatus), zink, retinol (ett mått på vitamin A status), vitamin B12, folat samt akutfasindikatorn CRP (ett mått på infektion).

I artikel I undersökte vi hur koncentrationen av Hb vid graviditetsvecka 30 ändrades per kapsel med mikronäringsämnen för 2377 kvinnor. Vi fann att Hb ökade med ökat intag av kapslar för Fe30Fol, Fe60Fol samt för MMS vid låga intag. Vid intag av >60 kapslar Fe60Fol skedde ingen ytterligare ökning av Hb. Detta tyder på att 20 veckor av dagligt tillskott av järn och folsyra som rekommenderas av WHO kan vara mer än vad som behövs för att uppnå maximalt Hb-svar på järntillskott. Även vid höga intag av kapslar var förekomsten av anemi hög (36 %), något som tyder på att det finns andra orsaker till anemi än järnbrist i den här populationen.

I artikel II jämförde vi barnens (1066 individer) mikronäringsämnesstatus i de olika randomiseringsgrupperna. Prevalensen av anemi (43 %) samt brist på zink (56 %), vitamin B12 (31 %), vitamin A (19 %) och järn (22 %) var hög. Få barn hade folatbrist (1 %). Bland de barn vars mödrar hade fått MMS hade färre brister på vitamin B12 (26 %) än bland dem vars mödrar fått Fe30Fol (36 %) (p=0.003). För de andra vitaminerna och mineralerna fanns inga statistiskt signifikanta skillnader. Vår slutsats är att det inte var av avgörande betydelse för barnens vitamin- och mineralstatus vilka kapslar mödrarna fått. Det var vanligare med zinkbrist hos barn vars mödrar fått mattillskottet tidigt än bland barn vars mödrar var i gruppen som startade senare. De bakomliggande faktorerna och de möjliga följderna av detta resultat är ännu oklara.

I artikel III jämförde vi anemi samt status av järn och zink hos barn som blivit ammade exklusivt i mindre än 4 månader med barn som hade ammats exklusivt i 4-6 månader. Det visade sig att anemi och järnstatus var lika i de båda grupperna, medan de barn som ammats exklusivt i 4-6 månader hade något högre zinkstatus (9.9±2.3 µmol/L) än barn som ammats exklusivt i en kortare period (9.5±2.0 µmol/L), (p<0.01). Det beror troligtvis på att den kost som ges till barnen i Bangladesh innehåller låga mängder av järn och zink och berikad bröstmjölksersättning är ovanligt. Vår slutsats är att det under dessa förhållanden inte medför en större risk för brist av järn och zink att amma exklusivt i 4-6 månader.

I artikel IV beskrev vi hur anemi var associerat med pågående infektion och låg födelsevikt i lika hög grad som med järnbrist. För att minska andelen anemiska spädbarn behövs åtgärder för att förhindra infektioner och för att förebygga låg födelsevikt.

Sammanfattningsvis var anemi och brister av mikronäringsämnen vanligt förekommande bland 6-månader gamla barn på landsbygden i Bangladesh. Åtgärder, utöver de som studerats i avhandlingen, är nödvändiga för att förbättra barnens nutritionsstatus.

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