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Effects of Pre- and Postnatal Nutrition Interventions on Child Growth and Body Composition

The MINIMat Trial in Rural Bangladesh

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Abstract

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Nutritional insults and conditions in fetal life and infancy may influence later growth and body composition as well as the development of chronic diseases in adult life. We studied the effects of maternal food and micronutrient supplementation and exclusive breast-feeding counseling on offspring growth 0-54 months and body composition at 54 months of age. We also validated and developed equations for a leg-to-leg bioimpedance analyzer in order to assess body composition of Bangladeshi children aged 4-10 years.

In the MINIMat trial in Matlab, Bangladesh, pregnant women were randomized to Early (around 9 weeks) or a Usual invitation (around 20 weeks) to food supplementation and to one of three daily micronutrient supplementations with capsules of either 30 mg Fe and 400 µg folic acid, or 60 mg Fe and 400 µg folic acid, or multiple micronutrient supplements (MMS) (15 micronutrients including 30 mg Fe and 400 µg folic acid). They were also randomized to exclusive breastfeeding counseling (EBC) or to usual health messages (UHM). Growth of their children was measured from birth to 54 months, when body composition also was assessed.

There were no differences in background characteristics across the different intervention groups. There was no differential effect by prenatal interventions on birth weight or length. Early invitation to food supplementation reduced stunting from early infancy up to 54 months for boys (average difference 6.5 percent units, 95% CI=1.7 to 11.3, p=0.01), but not for girls (average difference 2.4 percent units, 95% CI=-2.2 to 7.0, p=0.31). MMS resulted in more stunting than standard Fe60F (average difference 4.8 percent units, 95% CI=0.8 to 8.9, p=0.02). Breast-feeding counseling prolonged the duration of exclusive breastfeeding (difference 35.0 days, 95% CI 30.6-39.5, p<0.001). Neither the pregnancy interventions nor the breast-feeding counseling influenced body composition at 54 months.

Early food supplementation in pregnancy reduced the occurrence of stunting in boys 0-54 months, while prenatal MMS increased the proportion of stunting. Early food and multiple micronutrient supplementation or exclusive breastfeeding intervention provided to rural Bangladeshi women during pregnancy did not affect offspring body composition at 54 months of age. The effects on postnatal growth suggest programming effects in early fetal life.

Keywords: body composition, child growth, exclusive breast feeding, food supplementation, multiple micronutrients, pregnancy, programming

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Dedication

To my parents and family

List of Papers

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

I Khan AI, Kabir I, Ekström EC, Asling-Monemi K, Alam DS, Frongillo EA, Yunus M, Arifeen S, Persson LÅ. Effects of prenatal food and micronutrient supplementation on child growth from birth to 54 months of age: a randomized trial in Bangladesh. Nutr J. 2011 Dec 8;10:134.

II Khan AI, Hawkesworth S, Hossain D, Arifeen S, Moore S, Hills AP, Wells JC, Persson LÅ, Kabir I. Body composition of Bangladeshi children: comparison and development of leg-to-leg bioelectrical impedance equation. J Health Popul Nutr. 2012 Sep; 30 (3):281-290.

III Khan AI, Kabir I, Hawkesworth S, Ekström EC, Arifeen S, Frongillo EA, Persson LÅ. Early food and/or multiple micronutrient supplementation in pregnancy does not affect body composition in offspring at 54 months: follow-up of the MINIMat randomized trial, Bangladesh. *Manuscript.*

IV Khan AI, Hawkesworth S, Ekström EC, Arifeen S, Frongillo EA, Yunus M, Persson LÅ, Kabir I. Effects of exclusive breastfeeding intervention on child growth and body composition: The MINIMat trial, Bangladesh. *Manuscript.*

Contents

Abbreviations

AFA	Arm fat area
AMA	Arm muscle area
BMI	Body mass index
CI	Confidence interval
CNC	Community nutrition centre
EBF	Exclusive breastfeeding
FM	Fat mass
FFM	Fat free mass
Fe30F	30 mg Fe fumarate + 400 µg folic acid
Fe60F	60 mg Fe fumarate + 400 µg folic acid
HAZ	Height-for-age z-score
icddr,b	International Centre for Diarrhoeal Diseases Research, Bangladesh
IUGR	Intrauterine growth retardation
MINIMat	Maternal and Infant Nutrition Interventions in Matlab
MMS	Multiple micronutrient supplements
LBW	Low birth weight
OR	Odds ratio
SD	Standard deviation
SES	Socio-economic status
WAZ	Weight-for-age z-score
WHZ	Weight-for-height z-score
WHO	World Health Organization

Introduction

Conditions in early life are important for the development of certain chronic diseases in later life (1). The Developmental Origins of Health and Disease (DOHaD) hypothesis states that alterations in nutrition and endocrine status during fetal life and early infancy result in developmental adaptations that permanently change structure, physiology and metabolism, thereby predisposing individuals to disease in later life (2, 3). However, most of the evidence has been based on observational studies where birth anthropometry (e.g. birth weight) has been used as a proxy indicator for fetal nutrition. In those studies poor fetal nutrition could not be separated from other factors that cause intrauterine growth restriction (4). During pregnancy, the fetus is solely dependent on maternal intake and nutritional stores, mostly fat, for its energy. Poor maternal nutrition during pregnancy implies a risk of poor nutrition availability to the fetus. Thus, intra-uterine life and infancy has been identified as a possible critical period for the development of a variety of health outcomes in later life (1). Fetal and early post-natal under-nutrition induce processes that may increase the risk for coronary heart disease, stroke, type 2 diabetes, and metabolic syndrome (5, 6). Systemic reviews have shown that breastfeeding (particularly when exclusive) may be associated with lower blood cholesterol concentrations in later life (7). Early growth patterns may also induce long-term effects on body composition by impacting on hormonal axes that regulate childhood growth. Thereby body composition may play a key role in the 'programming' of such diseases (8). There is growing evidence that weight, diet, and physical activity patterns are even programmed early in life (9). In the case of undernutrition there is a critical window of opportunity for effective preventive action that starts from around conception period (10). This gives a unique opportunity to target preventive interventions focusing on the peri-conception period, the time of the greatest potential for lifelong effect.

Maternal nutrition and fetal development

Poor maternal nutrition, especially in the periconceptual period has been related to adverse birth outcomes (11, 12). The association between maternal nutrition and birth outcome is influenced by many socioeconomic, biologic, and demographic factors, which may vary from population to population

(13) and is more likely to involve multiple rather than single nutrient deficiencies (14). Every year about 3.6 million infants die during the neonatal period (15). Among them, two thirds of these deaths occur in sub-Saharan Africa and in South-Asia. More than a third of child deaths are thought to be attributable to maternal and child undernutrition and about half of the infants are born with intrauterine growth retardation (IUGR) in low-income countries, with the highest prevalence in south Asia and parts of sub-Saharan Africa (16). The prevalence of malnutrition in Bangladesh is very high (17) and millions of children and women suffer from one or more forms of malnutrition including low birth weight, wasting, stunting, underweight, Vitamin A deficiencies, iodine deficiency disorders and anemia. Malnutrition frequently follows the life cycle from one generation to the next, as malnourished mothers give birth to low birth weight infants, who struggle to develop and thrive. Malnourished girls often grow up to become malnourished mothers and the vicious cycle continues (18). Although Bangladesh has made good progress in child health and nutrition in the past decades, but still 41% of children below five years of age suffer from moderate to severe stunting, an indicator of chronic malnutrition (19). Micronutrient deficiencies, especially iron deficiency that results in nutritional anemia in children and women remains a public health problem (20). Poor intake of foods rich in iron and multiple infections have resulted in high rates of anemia among pregnant women and children less than two years (21, 22). Children of malnourished mothers are born with low birth weight, fail to grow normally, and face a higher risk of disease and premature death (23). Studies analyzing intergenerational effects on fetal growth have shown that maternal nutrition is strongly related to a baby's birth weight (18, 24). Malnourished mothers also face a higher risk of complications and death during pregnancy and childbirth (25, 26).

Maternal nutrition is generally considered as an important factor for fetal growth, development and size at birth, and is influenced by the quality of the mother's diet before and during pregnancy (12, 25). The usual diet of poor women in low-income countries is frequently deficient not only in energy and protein but also in different vitamins and trace elements (27-29). Inadequate intake and poor quality of diet, combined with increased nutrient requirements for placental and fetal growth, can lead to multiple micronutrient deficiencies in pregnancy. Micronutrient deficiencies may be the result from inadequate intake of fruits, vegetables, fish and meat, and also infections can contribute further to this deficiency. Pregnant women in those settings frequently have deficiencies of iron, vitamin A, zinc, vitamin B-12, riboflavin, vitamin D and vitamin E (30). The usual diet in rural Bangladesh is relatively monotonous and low in energy content (31). Rice is the main staple food, which is usually eaten with green-leafy vegetables and sometimes a small amount of fish. Consumption of meat and other animal products is very seldom. Maternal energy intake in an earlier study in

Matlab, rural Bangladesh, at around 5 – 7 months of pregnancy was estimated at 1464 kcal per day (32). This low energy intake was found to be typical for pregnant women in rural Bangladesh, and was in agreement with an assessment a few decades earlier (31). Energy intake assessment is often subject to under-reporting by the subjects (33). However, the energy intake reported in these poor and malnourished women may be adequately reported since their dietary pattern consists of a limited number of food items and an almost fixed meal pattern (two to three meals per day), and usually lack of any snack foods or drinks, as well as no tradition or facilities for women to eat outside home.

Evidence suggests that the timing of poor nutrition in pregnancy is also important. There are critical windows during fetal development where the consequences for the offspring depend on the stage of fetal development and the vulnerability of the fetus at that specific time (34). This has been shown in animal studies where maternal nutrient restriction from the first week of pregnancy throughout gestation has resulted in an increase of adipose tissue in the offspring despite a decrease in birth weight, while nutrient restriction in late pregnancy only was associated with reduced adipose tissue without a change in birth weight (35). In a human study from Guatemala maternal weight gain during mid-pregnancy was associated with infant birth weight, length and head circumference, while weight gain in late pregnancy was associated with birth weight only, suggesting that the timing of nutritional influences in fetal life is reflected in different growth patterns (36). Additionally, analysis of the Dutch famine data demonstrates that the fetal response is dependent on whether the fetus was exposed to famine in early, mid or late gestation (37).

Maternal nutrition both carries short and long-term health consequences (Figure 1) that include increased risks of fetal, neonatal and infant death and impaired postnatal growth, immune function and intellectual development (38). Observational studies have shown that both gestational weight gain and prenatal energy intake are positively associated with fetal growth (25). Maternal nutritional deficiencies have different effects on fetal growth that depends upon the stage of fetal development. Experimental animal studies and observational human studies have demonstrated the importance of nutritional insults that occur in the earliest embryonic stages to subsequent fetal growth and birth outcomes (39, 40).

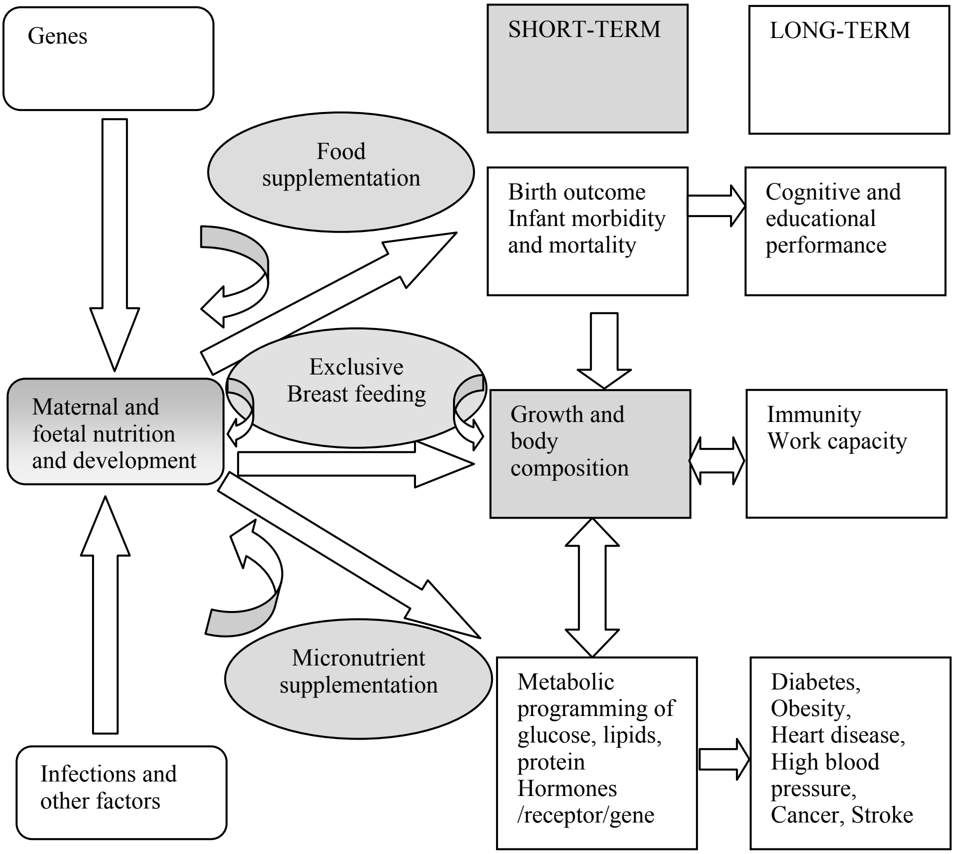


Figure 1. Conceptual framework of the short-term and long-term effects of early nutrition (adapted from: The State of the World Children 2001, UNICEF). The shaded items are included in the current studies

Animal studies have indicated that fetal growth is mostly affected by maternal dietary intake (particularly deficiencies of protein and micronutrients) during the peri-implantation stage and the stage of rapid placental development (41, 42). Thus, there are reasons to advocate improved diet during pregnancy, and may be especially in the periconceptional period, as well as during lactation, as a continuum of improved nutrition that may affect maternal, fetal, and infant health as well as later health and disease (43).

The Developmental Origins of Health and Disease (DOHaD)

The relevance of nutrition during pregnancy and in early infancy and its long-term effect on later health of the offspring is highlighted in the Developmental Origins of Health and Disease (DOHaD) hypothesis (3, 44, 45). This hypothesis proposes that influences in early life may lead to physiological modifications predisposing an individual to an increased risk of developing chronic disease in later life. Early-life conditions influence patterns of growth, body composition, and later risk of non-communicable chronic diseases (46). Thus exposure during early life is reportedly predictive of a variety of health outcomes, including body size, body composition and the risk of later conditions and diseases such as hypertension, stroke, type 2 diabetes, obesity and other cardio-vascular diseases (1, 38). In addition, studies of the Dutch famine have shown associations between the timing of gestational undernutrition and different risk profiles for coronary heart disease and other chronic health problems (47).

There is an ongoing epidemic in low- and middle-income countries of nutrition-related chronic diseases such as diabetes, cardiovascular diseases, and cancers (48). This is a particular concern in the context of low-income countries that are undergoing a rapid nutrition transition, where micronutrient deficiencies remain widespread, stunting is common, fruit and vegetable consumption is low, and obesity and related chronic diseases such as diabetes and heart disease are advancing as leading causes of morbidity and mortality (49). Currently, stunting is the most prevalent form of undernutrition in a worldwide perspective, especially in low-income countries. In 2010 the prevalence of stunting among children before 5 years of age was 38% in Africa (for a total of 60 million children), 28% in Asia (for a total of 100 million children), and 14% in the Latin-American region (for a total of 7 million children) (50, 51). The Asian countries account for almost 60% of the total amount of stunted children worldwide. Evidence from countries undergoing a rapid nutrition transition is also emerging. In Brazil, women in the lowest quartile of height had significantly higher odds of hypertension than women in the highest quartile, controlling for other risk factors (52). A recent systematic review and meta-analysis showed evidence of a rising secular trend of hypertension and prevalence of type 2 diabetes in Bangladesh (53). Another study done in Bangladesh identified short stature and high body mass index as independent risk factors for glucose intolerance in both men and women (54). There is also evidence for an early occurrence of chronic disease risk factors among stunted children. For example, a study among Jamaican children showed that stunting during the first two years of life was associated with an increased risk of high systolic blood pressure by 7-8 years of age, controlling for confounders (55). The effect of stunting is not only restricted to the first few years of life but extends throughout childhood and beyond. In terms of long-term body composition, birth length has been positively associated with attained adult height and fat-free mass. It has been suggested that stunted children would have a predisposition to develop obesity and metabolic complications later in life (56, 57). Some researchers have raised concern that improved nutrition in utero may increase fat mass and therefore in itself be a risk factor for later diseases, such as coronary heart disease (58).

There is some evidence that programming of body composition in early life may play a key role in the developmental origins of chronic diseases (8). Weight gain during infancy and childhood also appears to be associated with later body composition. The association between growth and later body composition indicates that early nutrition may be the underlying mechanism (1, 8). The distribution of body fat is also important in relation to later risk of chronic diseases; abdominal fat distribution is associated with metabolic disturbances (59). Thus body composition may play a role in the programming of such diseases; itself being programmed by early growth and by being a mediator of the programming process (8). A better characterization of the body composition in early age may improve the understanding of fetal origins of adult disease. In India it has been shown that small, light mothers give birth to small babies who preserve body fat to such an extent that it may lead to later insulin resistance (60). This so-called 'thin-fat baby phenomenon' could potentially be exaggerated or be prevented by nutritional interventions. It is therefore of great importance within the DOHaD research to add nutrition interventions to the current dominance of observational epidemiology, and to include assessments of body composition in the assessment of outcomes.

Most of the prenatal nutrition intervention studies have only included birth weight as outcome. There are very few human prenatal intervention studies that follow child growth and body composition longitudinally, evaluating long-term outcomes. In a randomized trial in Madura, East Java, a prenatal higher energy supplementation resulted in significantly heavier children up to the age of 24 months and increased height throughout the first 5 years when comparing with those who received a low-energy supplement (61). Two recent studies have suggested that prenatal multiple micronutrient supplementations may result in a modest but significantly increased growth up to two years (62, 63). As women in disadvantaged settings may sufferer from both macro- and micronutrient deficiencies, it is important to investigate whether combinations of nutritional interventions in pregnancy may be needed for favorable short- and long-term outcomes in the offspring.

Interventions to improve maternal and child nutrition and health

Food supplementation

Nutritional status of the mother is known to be an important determinant of fetal growth, size at birth, and postnatal growth. Previous studies have highlighted the need of improving maternal diet. Additional energy is required for growth and maintenance of the fetus, placenta, and maternal tissues during pregnancy. Energy is the main nutritional determinant of gestational weight gain. Deficiencies of other specific nutrients may also restrict gestational and fetal weight gain (64). Thus, the Food and Agriculture Organization/World Health Organization/United Nations University recommended on the basis of theoretical calculations that during pregnancy women increase their energy intake by 85 kcal/day in the first trimester, 285 kcal/day in the second trimester, and 475 kcal/day in the third trimester (65). The main purpose of maternal and fetal nutrition during pregnancy is to achieve appropriate energy intake (in the form macronutrients) and also ensure the intakes of specific nutrients (like vitamin and minerals) to meet maternal and fetal requirements (66).

Prenatal food supplementation has been used as one of the strategies to improve maternal nutritional status as well as fetal development where food insecurity and maternal malnutrition are prevalent (67-71). These interventions have had mixed results (28, 67-73) due to variations in maternal nutrition status, amount and composition of supplements, seasonal differences of food availability, and also some other factors that may influence maternal nutritional status and fetal growth (74). A food supplementation intervention carried out among women in Guatemala during pregnancy and lactation resulted in significantly increased birth weights (40, 67). The latest Cochrane review concluded that providing balanced energy and protein supplements to undernourished pregnant women increased birth weight (mean difference +38 g, 95% CI 0 to +75 g) and resulted in fewer stillbirths (25). An inadequate amount of food intake of poor dietary quality combined with the increased nutrient requirements for placental and fetal growth can result in multiple micronutrient deficiencies in pregnancy that may adversely affect pregnancy outcomes (16, 25).

Timing of food supplementation

The optimal timing of food supplementation to malnourished pregnant women is unclear. Little is known about the effect of timing of food supplementation during pregnancy on size at birth and subsequent child growth as well as on other functional outcomes. Maternal weight gain during pregnancy gives only a general reflection of fetal growth. Fetal weight increases exponentially in the third trimester, while the overall rate of maternal weight gain is usually constant after the first trimester. In the first two trimesters as much as 90% of the fat is deposited in maternal stores (75). Healthy urban Guatemalan women who delivered term newborns were followed during pregnancy; maternal nutritional status in the beginning of gestation and the rate of fat gain early in pregnancy were the two indicators strongly associated with birth weight (75). Fat deposited in early pregnancy acts as a reserve for the last trimester's caloric demands. Animal experiments indicate that the timing of undernutrition in pregnancy gives different responses as to size at birth, placental size, and development of endocrine responses (34). Studies of the Dutch 'hunger winter' (76) have shown that maternal undernutrition during pregnancy is associated with deficits in mean birth weight of approximately 300–500 g. According to Dutch data timing of undernutrition during pregnancy suggests that the majority of this birth weight deficit can be attributed to famine exposure during the third trimester (76). Although specific human studies are scarce, a healthy diet starting from early pregnancy, especially the periconceptional period, including sufficient intake of macro- as well as micronutrients, is likely of importance for the prevention of several adverse pregnancy outcomes (77, 78).

Multiple micronutrient supplementations

Adequate maternal micronutrient status is critical at the time of conception and during pregnancy. Multiple micronutrient supplementations (MMS) during pregnancy may be a one of the strategies to prevent adverse pregnancy outcomes (12, 43). Multiple micronutrient supplements were developed and recommended for trial purposes by UNICEF, WHO, and United Nations University (UNIMMAP); supplements that contain approximately one Recommended Dietary Allowance of 15 micronutrients including 30 mg iron and 400 µg folic acid (79). Over the past decade, a series of randomized controlled trials that compared the MMS with the usual iron-folic acid supplement has been carried out in Bangladesh (80), China (81), Indonesia (82, 83), Nepal (84), Pakistan (85), Burkina Faso (86), Guinea-Bissau (87), and Niger (88). Meta analysis of these trials reported a small positive effect of MMS on birth weight (pooled estimate: +22.4 g; 95% CI: 8.3, 36.4 g) and a reduction in the prevalence of LBW (pooled OR: 0.89; 95% CI: 0.81, 0.97) (89).

On the other hand, there is a lack of knowledge about the potential long-term effects on child growth and other health outcomes. A follow-up of a MMS trial in Nepal showed that, at a mean age of 2.5 y, children of women who had taken the MMS were on the average 204 g heavier (95% CI: 27, 381 g) and had a slightly lower systolic blood pressure than the control subjects, but occurrence of underweight, stunting, or wasting were not significantly different between the two groups (62). In the MINIMat trial in Bangladesh, MMS in combination with an early invitation to prenatal food supplementation substantially reduced under-5 mortality (80) and the prevalence of vitamin B-12 deficiency at 6 mo was lower in the MMS group (26.1%) than in the IFA group (36.5%) (90), but there was no overall difference in motor development between the groups at the age of 7 mo (91). An effect on mortality by the MMS was also observed in a cluster-randomized trial Indonesia, in which infants of women who consumed the MMS had an 18% reduction in early infant mortality (up to 90 d postpartum) compared with infants whose mothers had received an IFA supplement (RR: 0.82, 95% CI 0.70-0.95) (83). In contrast, no effect on mortality in the first 2 years of life was found in a MMS trial in Guinea-Bissau, but that study was not powered for the assessment of such an outcome (92). None of these studies, apart from the MINIMat trial (the trial of this thesis) assessed infant growth and nutritional status longitudinally. Another randomized controlled trial in Burkina Faso compared the MMS to IFA (86) and found that birth weight (52 g; 95% CI: 4, 100 g), birth length (3.6 mm; 95% CI: 0.8, 6.3 mm), arm circumference (1.2 mm; 95% CI: 0.2, 2.3 mm), and chest circumference (2.8 mm; 95% CI: 0.1, 5.6 mm) were all significantly higher in the MMS group.

There is a lack of knowledge of the possible combined effect of prenatal food and multiple micronutrient supplementations on several offspring outcomes, including postnatal growth. One recent randomized trial in Burkina Faso showed that prenatal multiple micronutrient-fortified food supplements resulted in a higher birth length in comparison with a multiple micronutrient supplement alone (93).

Exclusive breastfeeding

The positive effects of exclusive breast-feeding (EBF) on child health are well documented (94). Studies in high- as well as low-income countries have shown that nutritional status of young infants is well maintained if the infants are exclusively breastfed up to 6 months of age (95). EBF has protective effects against infections because of the high content of immune factors in breast milk and the elimination of bacterial contamination from other foods. WHO recommends EBF in the first 6 months of life since 2001 (96). Timely introduction of adequate and safe complementary food is crucial to respond to macro and micronutrient requirements after 6 months of age, according to WHO infant feeding recommendations. Mothers who feed their children exclusively by breast milk not only provide optimal nourishment, but also space pregnancy and childbirth (97). The rapid growth in infancy and early childhood contributes to the high requirements of nutrients during this early period of life. Breast milk alone can reportedly provide all the required nutrients up to 6 months of age (96). Infant growth data from low-income countries indicate that a decline of growth after 6 months of age suggest lack of appropriate feeding practices as well as a burden of infectious disease episodes (98).

The long-term effects of breastfeeding are more difficult to assess, but there are epidemiological data suggesting that breastfeeding is associated with reduced risk of obesity and related metabolic disorders in later life (99). Systematic reviews of observational studies have found that breastfeeding practices, particularly exclusive breastfeeding, are associated with later lower blood cholesterol (7) and may be protective against the development of obesity (100) and diabetes in later life (101). Some studies, however, have not been able to demonstrate any associations between breastfeeding and anthropometric or body composition indices (102, 103). There are relatively few trials addressing these issues. A large randomized trial of breastfeeding promotion in Belarus, which successfully resulted in a large increase in exclusive breastfeeding in the intervention group, showed no effect on levels of obesity at 6.5 years of age (103). In contrast, two randomized trials of infant formula of different composition demonstrated that a nutrient-enriched diet in infancy increased fat mass later in childhood, supporting a causal link between infant feeding and a later risk of obesity (104). Thus, mainly results from observational studies suggest that there are beneficial effects of breastfeeding that are extended beyond infancy.

Rationale for the studies of this thesis

A good start in life is important and maternal nutritional status during pregnancy has repeatedly been demonstrated to be associated with pregnancy outcomes (105). The major part of the evidence for the DOHaD hypothesis is derived from studies where birth weight is used as a proxy measure for nutritional state in pregnancy and most of the epidemiological studies investigating associations between prenatal influences and adult chronic disease are register-based; retrospective cohorts performed in high-income settings. However, the DOHaD hypothesis is of particular relevance for low-income settings where poor nutrition in early life is common and, at the same time, a rapid nutrition transition is ongoing and the occurrence of chronic diseases in adult life is increasing. A number of prenatal nutritional supplementation trials have been carried out, but few have investigated effects beyond the neonatal period. In spite of animal experimental evidence and observations from famines, there are no previous trials investigating the effect of timing of the prenatal nutrition intervention. Further, no trials so far have investigated the effect of timing of the food supplementation combined with different micronutrient alternatives on offspring outcomes including postnatal growth. The Maternal and Infant Nutrition Intervention in Matlab (MINIMat) trial, where random allocation of time at start of prenatal food supplementation was combined with different micronutrient alternatives is a unique resource for improved knowledge of programming of later health and disease risk. This research area is particularly important for countries such as Bangladesh where the burden of both fetal growth restriction and infectious disease mortality still is heavy and where there is a rapid emergence of overweight, insulin resistance and chronic diseases such as hypertension and type II diabetes. The MINIMat trial is a population-based randomized food and micronutrient intervention in rural Bangladesh (International Standard Randomized Controlled Trial, number ISRCTN16581394). Pregnant mothers were randomly allocated to an early or usual timing of invitation to food supplementation and to three different micronutrient alternatives. MINIMat women were also randomized to exclusive breastfeeding counseling or usual health messages. The offspring of this study has been extensively followed-up and the current thesis is based on detailed anthropometric measurements from birth to 54 months of age, when body composition assessment also was performed.

Aim and objectives

The overall aim of this thesis, based on the MINIMat trial in rural Bangladesh, was to study the effect of timing of prenatal food supplementation and different alternatives of micronutrient supplementation as well as of an exclusive breast-feeding counseling intervention on offspring growth and body composition.

Specific objectives:

1. To analyze the effects prenatal multiple micronutrient supplementations and an early invitation to prenatal food supplementation on physical growth 0-54 months of the offspring (paper I)

2. To develop a prediction equation for Bangladeshi children 4-10 years with the Tanita TBF 300A analyzer for body composition determination by using the deuterium dilution technique as a reference method (Paper II)

3. To evaluate the effects of prenatal early invitation to food and/or multiple micronutrient supplementation on offspring body composition at 54 months of age (Paper III)

4. To study the effect of randomization to exclusive breast feeding counseling in addition to prenatal food and micronutrient supplementation on childhood growth and body composition at 54 months of age (Paper IV)

Methods

Study area

Bangladesh is a densely populated country in South Asia with a high but in recent years declining prevalence of LBW, 22 % (106, 107). The trial that forms the basis for this thesis, Maternal and Infant Nutrition Interventions in Matlab (MINIMat, ISRCTN16581394), was conducted in Matlab, where a health and demographic surveillance system (HDSS) covers a population of about 220,000 people. Matlab is a rural sub-district situated in a river delta prone to frequent flooding, 57 km southeast of the capital Dhaka in Bangladesh. The main economic activities in the area are farming and fishing. The general diet consists largely of rice and vegetables with the addition of lentils and beans. Fish is usually consumed while meat is eaten seldom. In that area the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), runs a HDSS (Figure 2) that records health and demographic information on a monthly basis since 1966, as well as a central hospital and four connected subcenters that provide health care to the resident population in the areas. icddr,b has divided the Matlab HDSS into two areas: the Maternal and Child Health and Family Planning Programme (MCH-FP) area (70 villages) and a government service area (79 villages). In the MCH-FP area, the female community health research worker (CHRW) visits each household every month providing intensive health-care services (e.g. family planning counseling, immunisation, distribution of a menu of contraceptives, vitamin A capsules and safe delivery kits). In the government service area, the government provides family planning and basic health-care services. All pregnant women of the icddr,b service area were eligible for enrolment of this study.

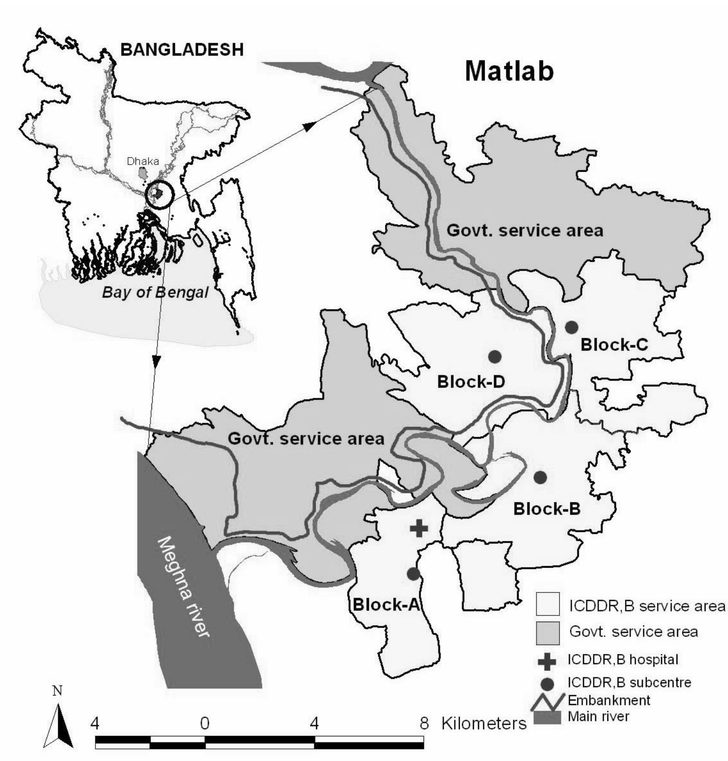


Figure 2. The location of icddr,b health and demographic surveillance area, with the study area (Block A to D) within the sub-district of Matlab

Study design and participants

Pregnant women who resided in the icddr,b service area within the HDSS were eligible. Community Health Research Workers (CHRW) visited the households on a monthly basis to ask women about their menses. Women who reported missing a menstrual period for two weeks or more was offered a pregnancy test, and if positive and fulfilling eligibility criteria they were invited to participate in the MINIMat trial. A woman was enrolled in the MINIMat trial if the following eligibility criteria had been met: fetus was viable, gestational age < 14 weeks by ultrasound examination; the woman had no severe illness and consented for participation. In total, 4,436 pregnant women were enrolled in the MINIMat trial between November 2001 and October 2003 and their children were followed up until March 2009 for the analyses presented in this thesis.

The primary outcomes of the MINIMat trial were to analyze the effects of prenatal food and micronutrient supplementation on birth weight, infant mortality and maternal hemoglobin in the third trimester. Several secondary outcomes were defined, including infant and child growth, morbidity, micronutrient status and cognitive development. The papers included in this thesis are based on exposure data from pregnancy and infancy and anthropometric outcome data from birth to 54 months of age, and body composition data at 54 months as illustrated in Table 1.

Table 1. Description of study methods in the four different papers

	Study design	Sample size	Exposure	Study outcomes
Paper I	Trial	3267	Food and micronutrient supplementation in pregnancy	Postnatal child growth
Paper II	Methodology paper	200	Tanita analyser for fat-free mass determination using the deuterium dilution technique as the reference method	Comparison and development of bioelectrical impedance equation
Paper III	Trial	2290	Food and micronutrient supplementation in pregnancy	Child body composition at 54 months
Paper IV	Trial	2168	Food and micronutrient supplementation in pregnancy. Exclusive breastfeeding counselling	Child growth and body composition at 54 months

Interventions

In the MINIMat trial, eligible women were randomly allocated to the two food and three different micronutrient interventions and also included exclusive breastfeeding counseling intervention focused on the promotion of exclusive breast feeding for six months.

Food supplementation

Enrolled pregnant woman of the MINIMat trial were randomly assigned to two food supplement groups (Early invitation: immediately after identification of pregnancy or Usual invitation: at the time of their choosing, i.e., usual care in this community). Food supplementation came from the ongoing government-supported national programme that provided an energy-protein supplement for all pregnant women. In Bangladesh, a government nutrition program provided food supplements to pregnant women with BMI < 18.5 kg/m^2 at the time of data collection. In the MINIMat trial this food supplement was offered to all pregnant women irrespective of nutritional status as assessed by body mass index. The food supplement was locally produced and made available through community nutrition centres (CNC) 6 d/week. The supplement that was provided in plastic packets to be mixed with water contained 80 g roasted rice powder, 40 g roasted pulse powder, 20 g molasses and 12 mL (6 g) soybean oil, which provided 608 kcal (2.85 mJ). Food supplementation continued up to end of pregnancy.

Early invitation food supplement group: Women were encouraged to begin food supplementation as soon as possible after identification of pregnancy, usually around week 9.

Usual invitation food supplement group: Women started food supplementation at the time of their own choosing, as was the usual practice in this community, commonly in the second trimester of pregnancy, in this trial around week 20.

Table 2. Composition of the three micronutrient supplements provided in the MINIMat trial, Bangladesh.

Nutrient	Form	Fe30F	Fe60F[a]	MMS[b]
Iron, mg	Ferrous fumarate	30	60	30
Folic acid, µg	Folic acid	400	400	400
Vitamin A, µg	Retinol	—	—	800
Vitamin B-1, mg	Thiamin HCl	—	—	1.4
Vitamin B-2, mg	Riboflavin	—	—	1.4
Vitamin B-6, mg	Pyridoxine	—	—	1.9
Vitamin B-12, mg	Cyanocobalamine	—	—	2.6
Niacin, mg	Nicotinamide	—	—	18
Vitamin C, mg	Ascorbic acid	—	—	70
Vitamin D, IU	Cholecalciferol	—	—	200
Vitamin E, mg	a-Tocopherol acetate	—	—	10
Zinc, mg	Zinc sulfate	—	—	15
Copper, mg	Copper sulfate	—	—	2
Selenium, µg	Sodium selenite	—	—	65
Iodine, µg	Potassium iodide	—	—	150

[a]Usual capsule composition provided to all pregnant women in Bangladesh
[b]UNICEF/UNU/WHO recommended preparation

Micronutrient supplementation

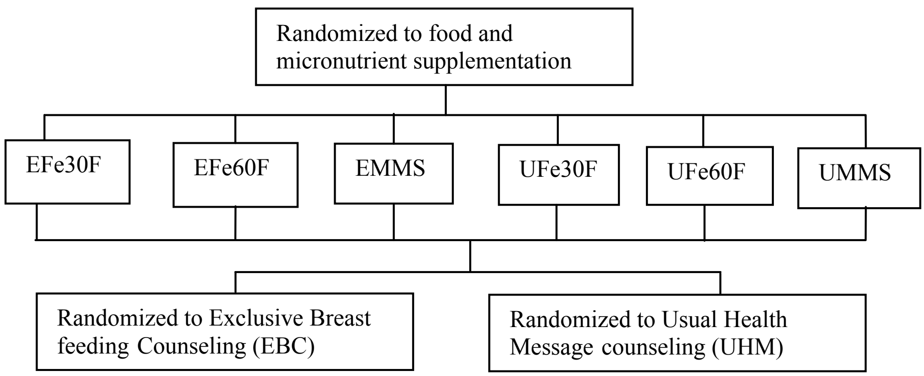
Enrolled pregnant woman of the MINIMat trial were randomly assigned to one of three micronutrient groups: Capsules containing a) 30 mg Fe fumarate + 400 µg folate (Fe30F), or b) 60 mg Fe (fumarate) + 400 µg folate (Fe60F), or c) multiple micronutrient supplements (MMS). The MMS group received 15 different vitamins and minerals (Table 2). The three types of micronutrient supplements taken daily looked identical, and were distributed in special pill bottles. Each bottle contained 35 tablets and was provided at home during monthly home visits by interviewers. The micronutrient supplements were offered to the enrolled women at the clinic visit at 14 weeks of gestation and supplementation continued up until 3 months post-partum.

Exclusive breast-feeding counseling intervention

All enrolled pregnant women with viable fetuses were individually randomized around 30 weeks of gestational age to receive either counseling on exclusive breastfeeding (EBC) by trained counselors or to receive standard/usual health messages (UHM) delivered by the Govt/icddr,b health staff. All breast feeding counselors were trained utilizing a 40-hours (4 hours daily for 10 days) WHO/Unicef Breastfeeding Counseling Training module, which was translated in local (Bangla) language, and tested and adopted to the local culture (108). All breast-feeding counselors were recruited from the local community (Matlab study area), married with at least one child and had their own breastfeeding experience, and a bachelor's degree (14 years of schooling). Within the breast feeding counseling intervention group, women received counseling in 8 sessions: 2 sessions during last trimester of pregnancy, 1 session within 7 days after delivery, and 5 sessions at monthly intervals up to 6 months after delivery. The importance of exclusive breastfeeding to 6 months of life was stressed during counseling. The women randomized to the UHM group received the basic and usual health messages on breastfeeding practices, which are given by the regular government health staff or icddr,b staff at postnatal clinic visits with less individualized support, and these messages included: benefits of colostrum, advice on EBF for 6 months, and advice to start of complementary feeding from 6 months along with continuation of breastfeeding until two years. EBC group got the usual health messages plus the additional counseling inputs given by the counselors. In the EBC group, counseling skills were taught by demonstration and role play and included listening to mothers, learning about their difficulties, assessing the position and attachment of babies during breastfeeding, building mothers' confidence, giving support, and providing relevant information and practical help when required. Counselors were free to make or receive additional contacts, if required. Counseling was given at home and one-to-one basis, also key family members were included. The duration of each counseling visit was typically 20-40 minutes depending at what stage of lactation the mother was in, and individual needs. Usually first 2-3 visits were longer to build the mothers' confidence and later part of counseling visit were shorter just to give support to mothers and reinforce the health messages.

Randomization

The enrolled pregnant women were individually randomized to the two food supplementation groups and to one of the three micronutrient alternatives as well as women in the study were randomized to receive either exclusive breastfeeding counseling (EBC) or usual health message (UHM) (Figure 3). A computer-generated register of study identity numbers with random assignment of food and micronutrient groups was created. The micronutrient supplementation was double-blinded while the food supplementation was allocated randomly but not masked. The papers included in this thesis are based on data from different data collection time points. The study design and outcomes in the four different papers are described in table 1.



E=Early invitation food supplementation, U=Usual invitation food supplementation; Fe30F = 30 mg iron and 400 µg of folic acid; Fe60F = 60 mg iron and 400 µg of folic acid; MMS = multiple micronutrients, 15 micronutrients including 30 mg iron and 400 µg of folic acid;

Figure 3. Randomization to prenatal food and micronutrient supplementation and exclusive breastfeeding counseling interventions in the MINIMat trial, Bangladesh

Data collection and measurements

Data during pregnancy and follow-up was collected at scheduled home visits and sub-center clinic visits. From November 2001 to October 2003 a total of 4,436 pregnant women were enrolled in the MINIMat study and a follow-up until March 2009 is reported in this thesis. On enrollment, maternal weight and height was measured. Trained data collectors interviewed the women about their age, parity, educational level, employment and other socio-economic information at the time of enrollment during a home visit. In February 2007 to March 2009, at 54 months of age, children were tracked by use of their identity code within the Matlab HDSS and their address, which had been updated through the monthly surveillance, if the family had moved within the study area. The initial visit at home included repeated informed consent and a socioeconomic interview, which included information on family wealth (i.e., assets) and household structure. Data collection was performed at 4 local sub-centres run by icddr,b in the Matlab area and a health worker from the study accompanied the participants to the study location early in the morning, following an overnight fast. All measurements were conducted by one of two teams, which consisted of a medical doctor, a nurse, and a laboratory technician assisted by trained field staff. Each observer performed the same measurements, and each measurement was conducted at a similar time of the day. For the purpose of this thesis, only data on children aged around 54 months for whom anthropometry and body composition measurements were available and were analyzed. The total number of children in this follow-up was 2735.

For the methodological study, 200 children (102 male and 98 female) aged 4-10 years were enrolled. Study subjects included a convenience sub-sample of children enrolled in the MINIMat study, who were 4-5 years old, and their older siblings up to 10 years of age.

Anthropometry at birth was part of the primary outcomes of this trial. Weight and length/height were thereafter measured every month up to 1 year, thereafter every 3 months to 24 months and again at 54 months of age. Birth anthropometry was usually performed within 72 hours after birth. All birth weights were measured by SECA electronic or beam scales (UNICEF Uniscale; SECA Gmbh & Co, Hamburg, Germany) with a precision of 0.01 kg. Maternal weight and height were measured on enrollment at around 8 week of gestation. Maternal weight was measured by an electronic scale (Uniscale; SECA) with a precision of 0.10 kg and height was measured to the nearest 0.1 cm by using a stadiometer. A locally manufactured, collapsible length board with a precision of 0.1 cm was used to measure the recumbent length of the newborn.

At 54 months follow-up, body weight was recorded to the nearest 0.1 kg using a digital scale (TANITA HD – 318, Tanita Corporation, Japan) in light clothing and bare feet. The scale was checked on each study day with a standard 20 kg weight. After removing shoes, height was measured to the nearest 0.1 cm by using a daily-calibrated freestanding stadiometer Leicester Height Measure (Seca 214, UK). Weighing equipment was calibrated on a daily basis with standard weights. Refresher training of the interviewers on methods to collect data and perform anthropometric measurements was conducted periodically. Weight and length/height measurements were converted to weight-for-age, length/height-for-age, and weight-for-length/height Z-scores (SD scores) according to the WHO Multicentre Growth Reference Study child growth standards (109). We used Anthro 2007 (WHO Anthro for personal computers, version 2, 2007: Software for assessing growth and development of the world's children. Geneva: WHO, 2007 http://www.who.int/childgrowth/ software/en/) for this conversion.

Mid-upper-arm circumference (MUAC) was measured to the nearest 0.1 cm with a non-elastic metric measuring tape at the midpoint of the upper arm, with the arm hanging straight by the subject's side. Skinfold thickness was measured in triplicate to the nearest 0.2 mm at four sites (biceps, triceps, subscapular, and suprailiac) using Holtain callipers (Holtain, Crymych, UK). All skinfold measurements and MUAC were performed in the same order on the left side of the body. The average of three measurements at each site was used to measure skinfold thickness. Anthropometric measurements (height, weight, MUAC) were conducted by one field research assistant, while skinfold and body composition measurements were performed by the study nurse.

Bioelectrical impedance analysis

Body composition was further assessed by leg-to-leg bioelectrical impedance analysis (BIA) using Tanita TBF-300MA Body Composition Analyzer (Tanita Corporation, Tokyo, Japan). The BIA measurements were made adhering to the manufacturer's guidelines and at a measurement frequency of 50 kHz. Height, sex, and age were entered manually, while weight was recorded automatically using 0.5 kg as an adjustment for clothes weight in all subjects. The Tanita software uses inbuilt prediction equations to estimate fat and fat free mass. These inbuilt prediction equations are based on Caucasian populations of aged 7 and older and their validity may be questioned when applied to other ethnic groups (110) and in younger ages. In a validation study conducted in Matlab, the equations were found to be inaccurate in predicting fat-free mass (FFM) and fat mass (FM) compared to assessment by deuterium oxide dilution technique (paper II). We therefore predicted FFM from our own equation, which was derived in this population in a sub-sample of 200 children aged 4-10 years using deuterium oxide dilution as a reference method (paper II).

Measurement of Total Body Water (TBW) using deuterium dilution

The isotope dilution technique using deuterium oxide (D_2O) was used to estimate total body water (TBW). The deuterium dilution method to obtain the percentage of body fat has been fully described by Colley (111). In brief, this method measures the dilution in the body of a known orally ingested dose of the isotope deuterium (D_2O). Participants orally consumed a dose equating to 0.05 g/kg body weight of D_2O (10%). Pre-dose deuterium abundance was obtained from one fasted saliva sample collected on the study day. Saliva (approximately 1 mL) was collected from the study children chewing on a ball of cotton wool, which was then squeezed into a syringe to extract the saliva. The subjects were instructed to refrain from any food or fluid at least 30 min before the post-dose saliva samples, which were collected at 3 h and 4 h after the administration of deuterium. During the equilibration period, children remained in the specified location with the study team present. All saliva samples were stored at -20^{0}C until shipped to Queensland University of Technology, Australia, for analysis. The enrichment of the D_2O in the pre-dose and 3 h, and 4 h post-dose samples was assessed using isotope ratio mass spectrometry, and TBW was subsequently calculated. The hydration fraction of FFM was assumed to be dependent on the age and sex of the child as described by Lohman (112, 113) and ranged between 76.2% and 78.3%. Weight measured in the early morning in a fasted state was used for all calculations, and fat mass was calculated as the difference between FFM and body weight.

Selection of sample and power calculation

The main MINIMat trial was designed to evaluate the effect of nutritional interventions on birth weight and survival of the offspring and maternal hemoglobin as primary outcomes. The original sample size calculations were made on the basis of finding a difference in birth weight. As this thesis reports secondary outcomes (child growth and body composition), we calculated the differences in child growth that we were able to detect with the given sample size (n = 2735) for paper 1 in any supplementation group in our analyses. For this sample size, 80% power and 95% probability we were able to detect a difference in anthropometric measurements of 0.2 SD score between 6 food and micronutrient supplementation groups or difference of 0.12 SD score for 2 food groups. Paper III-IV reports the follow-up of child body composition at 54 months. For this sample size (n=2290 children), with 80% power and 95% probability, we were able to detect a difference of 0.19 standard deviation (SD) score between any two of the six food and micronutrient supplementation groups and a difference of 0.11 SD score between the two food groups.

The differences in sample size in paper I-IV depend on the number of participants available with exposure and outcome measurements. All available participants measurements were used, except in methodology paper (paper II), where the arbitrary selection of 200 children (102 boys and 98 girls, aged 4-10 years) was a convenience sub-sample of children used for this sub-study.

Statistical analysis

Baseline and follow-up characteristics, including socioeconomic status indicators, maternal literacy and education, occupation, maternal nutritional status, age, and parity were compared across intervention groups. All singletons newborns were included in the intention-to-treat analysis. The outcome variables were: measurements of birth size (i.e., birth weight, length) and child growth up to 54 months and body composition (FM, FFM) at 54 months follow-up. Means and standard deviations (SD) were calculated for continuous variables, while proportions were calculated for categorical variables. Descriptive statistics were stratified by sex and age. Values were expressed as means and standard deviations (SD). Differences between sexes were assessed by independent t-tests. Differences across age categories were tested for males and females separately using one-way analysis of variance. Paired t-tests were used to detect differences in body composition obtained using the inbuilt prediction equations supplied with the Tanita system compared to the deuterium oxide dilution technique for each sex separately as well as combined. The bias and limits of agreement (mean difference±1.96 SD) in relation to deuterium oxide dilution were assessed with the Bland–Altman method (114). Differences between categorical variables were compared using chi-square tests. T-tests and analysis of variance with post hoc Bonferroni corrections was used to compare group differences. Comparison between the different supplementation groups, mean height-for-age Z-scores and occurrence of stunting throughout the 5 y follow-up was made using a general linear modeling of repeated-measurements ANOVA procedure. Stunting in each follow-up visit was the within-subject factor and a food and/or micronutrient supplementation group was the between-subject factor.

To create novel prediction equations for estimating FFM in this population, the FFM values derived from the deuterium oxide dilution were used as the reference method and impedance values were obtained from the Tanita system. The equations were generated by linear regression analysis and impedance index ($height^2$/impedance) was fitted as the primary predictor in the basic model, which was then developed by adding age, sex and weight as further predictors.

The effect of randomization to food, micronutrient and exclusive breast-feeding interventions on body composition at 54 months was evaluated by an intention-to-treat analysis using linear regression. Most statistical analyses were performed using SPSS (version 14, 17.0; SPSS Inc., Chicago, IL, USA), PASW statistics 20.0 (IBM Corporation, Somers, NY, USA), while we used Analyse-it (version 2.22) free software for Bland-Altman plot analyses.

Ethical considerations

In the study area icddr,b runs a central hospital and four connected sub-centers that provide health care to the resident population in the areas. Women and their children who found to be anemic or suffered from any other ailment were managed or referred appropriately. Appropriate measures were taken to assure confidentiality. In this trial, the micronutrient supplementation was double-blinded while the food supplementation was allocated randomly but not blinded. Food supplementation came from the ongoing government-supported national programme. All women received one of the 3 types of micronutrient pills. Placebo groups could not be added due to ethical and practical reasons. Women were informed that there would be no negative consequences if they declined to participate in the study or in certain study activities, and that this would not influence the provision of health services by the organization. Some women refused clinic visits and blood samplings, although they continued to take part in the interviews.

The MINIMat trial as well as the follow-up of the children at 54 months was reviewed and approved by the icddr,b Ethical and Research Review Committees. In addition, an ethics application was submitted to the Regional Ethical Board in Uppsala. As the Review Board is limited to research conducted in Sweden, only the part of the research protocol that was conducted in Sweden was considered and approved. Written informed consent was obtained from all participating mothers and also obtained from the parents/guardian of each participating child.

Results

General characteristics

Women who participated in the MINIMat trial were between 14 and 50 years of age at enrollment and their parity varied from 0 to 10. At enrolment, 27% of the women were malnourished (BMI <18.5 kg/m^2), 30% of the infants were LBW (birth weight <2500 g) and 8% of the children were pre-term (born <37 weeks of gestation). General characteristics of the participating women and their children are shown in Table 3.

Participation

There were 3267 singleton infants with birth anthropometry born by the 4436 women enrolled into the MINIMat trial. Details of losses before measurement of birth anthropometry and losses from follow-up after birth anthropometry up to the 54-months are shown in paper I. Losses to follow-up before birth anthropometry did not differ among the intervention groups (p=0.676). Of the 3267 live singleton births in the MINIMat trial, body composition at 54 months was measured in 2290 children, representing 70% of the original trial live births; 2526 children completed anthropometry measurements at 54 months. Losses to follow-up did not differ across the intervention groups. The distribution of pregnant women and their children in the different supplementation groups and the numbers lost to follow-up are shown in paper I and III.

A total of 3214/4436 (72.45%) mothers were randomized to the breast feeding intervention; of which 2845 women had a live birth (1417 in EBC group, and 1428 were in UHM group). At 54 months, 2168 children completed anthropometry (1092 in EBF counseling group and 1076 in usual health message group). The distribution of pregnant women and their children for the EBC and UHM groups and the numbers lost to follow-up are shown in paper IV.

Table 3. Characteristics of women and children participated in the MINIMat trial

Characteristics	
Women and children (n=3267)	
Maternal age (yrs)	25.82±5.92
Maternal BMI (kg/m^2) at 8 weeks' gestation	20.15±2.66
Gestational age (wks) when birthing	38.75±1.73
Maternal education (% illiterate)	31.1
Maternal occupation (% housewives)	91.6
Parity (%)	
0	31.9
1	28.1
≥2	40.1
Birth weight (kg)	2693.90±410.52
Birth length (cm)	47.70±2.18
Boy child (%)	51
54 months follow-up: Children (n=2526)	
Age (mo)	54.73±1.90
Weight (kg)	13.75±1.66
Height (cm)	99.79±4.28
BMI (kg/m^2)	13.77±0.97
MUAC (cm)	15.21±1.10
Head circumference(cm)	47.95±1.49
Skinfolds (mm)	
Biceps	4.88±1.04
Triceps	7.24±1.48
Sub-scapular	5.16±1.07
Suprailiac	5.02±1.46
Body composition	
FFM (kg)[a]	12.15±1.26
FM(kg)[a]	1.73±0.59
BF(%)[a]	12.25±3.25
AFA(cm^2)[b]	5.12±1.26
AMA(cm^2)[b]	18.09±2.63

Values are given as means ± standard deviation (SD) or percentages where indicated

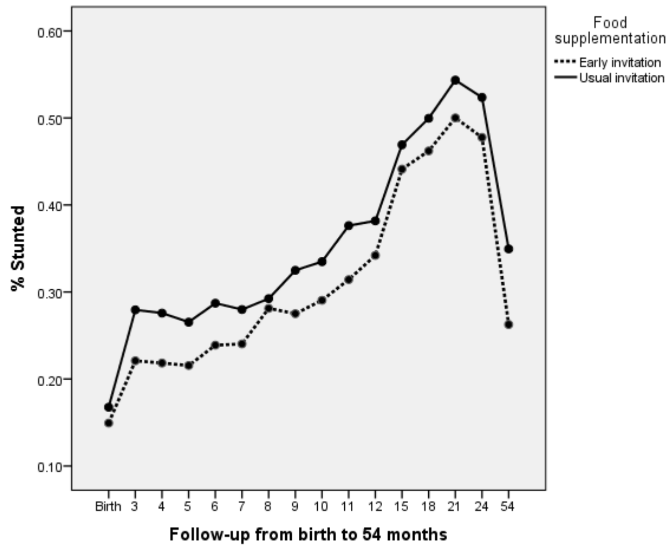
AFA = arm fat area; AMA = arm muscle area; BF = body fat; BMI = body mass index; FFM = fat-free mass; FM = fat mass; MUAC = mean upper-arm circumference; SD = standard deviation

[a]FFM and FM were derived from BIA (Tanita TBF-300MA; Tanita Corporation, Tokyo, Japan) using PoP-specific equations (115)

[b]Both AFA and AMA derived from triceps skinfold thickness and MUAC measurements (116)

Food and micronutrient supplementations and child growth from birth to 54 months

There were no significant differences in the characteristics of mothers and households across the different food and micronutrient supplementation intervention groups. A longitudinal analysis of linear growth was performed using repeated-measures analysis (Paper I). There was no interaction between food and micronutrient supplementation on linear growth. An early invitation to prenatal food supplementation to pregnant mothers resulted in significantly reduced proportion of stunting (mean difference 4.5 percent units, 95% CI=1.2 to 7.8, p= 0.01). In contrast, MMS supplementation resulted in significantly more stunting in comparison with Fe60F (mean difference 4.8 percent units, 95% CI=0.8 to 8.9, p=0.02) (Figure 4). The effect of early vs. usual invitation to food supplementation on frequency of stunting was significantly shown for boys but not for girls (Table 4). The increased proportion of stunting in the MMS group was also more expressed among boys (Table 4). In Table 5 the effects are shown of food and micronutrient supplementation on child growth from 0 to 54 months, stratified by maternal BMI groups, using BMI 18.5 as cut-off level, as the original food supplementation by the government of Bangladesh used this cut-off for selecting malnourished women for the food supplementation program. Among mothers with higher BMI (BMI>=18.5) stunting was less frequent (p=0.05) in early invitation food group (in comparison with usual invitation), while this was not significant among mothers in the lower half of the BMI distribution (p=0.10). This trend was also found when maternal BMI using the cut-off level as median 19.7 shown in Paper I. Further, the negative effect on linear growth by MMS was also shown in the strata with higher maternal BMI, but not significantly different in the group where the mothers had lower BMI (Table 5).



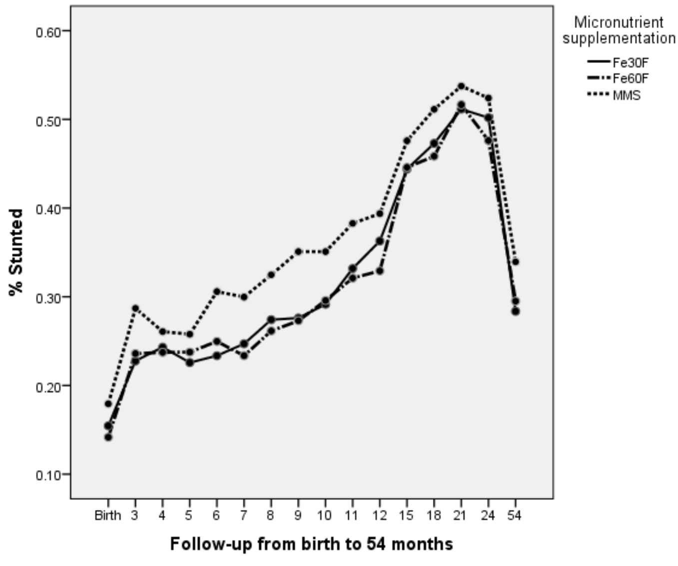


Figure 4. Effects of food and micronutrient supplementations on child linear growth (stunting) from birth to 54 months

Table 4. Effects of prenatal food and micronutrient supplementations on linear growth (16 anthropometric assessments) from birth to 54 months of age, stratified for sex of the child. General lineal models, repeated-measures analyses.

		BOYS					**Girls**				
Randomised intervention		n	HAZ mean (95% CI)	P-value[1]	Stunting, mean% (95% CI)	P-value[1]	n	HAZ mean (95% CI)	P-value[1]	Stunting, mean% (95% CI)	**P-value[1]**
Food supplementation	Early invitation (E)	439	-1.55 (-1.64, -1.46)	0.20	31.9 (28.6-35.2)	0.01	400	-1.50 (-1.58, -1.42)	0.21	29.8 (26.5-33.0)	0.31
	Usual invitation (U)	405	-1.63 (-1.72, -1.54)		38.4 (34.9-41.8)		390	-1.57 (-1.66, -1.49)		32.1 (28.9-35.4)	
Micronutrient supplementation	Fe30F	275	-1.50 (-1.61, -1.39)	0.06	32.5 (28.3-36.7)	0.01	243	-1.52 (-1.63, -1.42)	0.80	31.1 (26.9-35.2)	0.82
	Fe60F	289	-1.58 (-1.69, -1.47)		32.5 (28.4-36.6)		277	-1.52 (-1.62, -1.42)		30.0 (26.1-33.9)	
	MMS	280	-1.69 (-1.80, -1.58)		40.3 (36.2-44.5)		270	-1.56 (-1.66, -1.46)		31.8 (27.8-35.7)	
Interaction Food*Micronutrients	E-Fe30F	142	-1.44 (-1.60, -1.29)	0.70	28.8 (23.0-34.7)	0.84	116	-1.51 (-1.66, -1.35)	0.58	31.2 (25.2-37.2)	0.52
	E-Fe60F	149	-1.57 (-1.72, -1.42)		30.3 (24.6-36.0)		142	-1.44 (-1.56, -1.30)		27.0 (21.5-32.4)	
	E-MMS	148	-1.63 (-1.78, -1.48)		36.5 (30.8-42.2)		142	-1.55 (-1.69, -1.41)		31.1 (25.7-36.6)	
	U-Fe30F	133	-1.56 (-1.72, -1.40)		36.2 (30.2-42.2)		127	-1.54 (-1.69, -1.40)		30.9 (25.1-36.7)	
	U-Fe60F	140	-1.58 (-1.73, -1.43)		34.8 (28.9-40.6)		135	-1.60 (-1.74, -1.46)		33.1 (27.5-38.6)	
	U-MMS	132	-1.76 (-1.91, -1.60)		44.1 (38.1-50.2)		128	-1.58 (-1.72, -1.43)		32.5 (26.7-38.2)	

Abbreviations: HAZ=Height for Age Z-score; Fe30F = 30 mg iron and 400 µg of folic acid; Fe60F = 60 mg iron and 400 µg of folic acid; MMS = multiple micronutrients, 15 micronutrients including 30 mg iron and 400 µg of folic acid; [1] Test of between-subjects effects.

Table 5. Effects of prenatal food and micronutrient supplementations on linear growth (16 anthropometric assessments) from birth to 54 months of age, stratified for level of maternal BMI. General lineal models, repeated-measures analyses.

		Mother's BMI < 18.5 in early pregnancy					**Mother's BMI >= 18.5 in early pregnancy**				
Randomised intervention		n	HAZ mean (95% CI)	P-value[1]	Stunting, mean% (95% CI)	P-value[1]	n	HAZ mean (95% CI)	P-value[1]	Stunting, mean% (95% CI)	P-value[1]
Food supplementation	Early invitation (E)	229	-1.67 (-1.79, -1.55)	0.18	36.1 (31.4-40.7)	0.10	606	-1.47 (-1.54, -1.40)	0.26	28.9 (26.2-31.6)	0.05
	Usual invitation (U)	220	-1.79 (-1.90, -1.67)		41.7 (37.0-46.4)		574	-1.53 (-1.60, -1.46)		32.7 (30.0-35.5)	
Micronutrient supplementation	Fe30F	146	-1.74 (-1.88, -1.59)	0.99	38.9 (33.1-44.7)	0.81	370	-1.43 (-1.52, -1.34)	0.02	29.0 (25.6-32.4)	0.02
	Fe60F	157	-1.72 (-1.86, -1.58)		37.6 (32.0-43.1)		407	-1.48 (-1.56, -1.39)		28.8 (25.5-32.1)	
	MMS	146	-1.72 (-1.87, -1.58)		40.2 (34.4-46.0)		403	-1.59 (-1.68, -1.51)		34.6 (31.3-37.9)	
Interaction Food*Micronutrients	E-Fe30F	68	-1.71 (-1.92, -1.49)	0.69	36.4 (27.9-44.9)	0.93	188	-1.39 (-1.52, -1.27)	0.94	27.7 (22.9-32.5)	0.86
	E-Fe60F	81	-1.69 (-1.89, -1.49)		35.3 (27.6-43.1)		209	-1.44 (-1.56, -1.32)		26.2 (21.7-30.8)	
	E-MMS	80	-1.61 (-1.81, -1.42)		36.5 (28.7-44.3)		209	-1.58 (-1.70, -1.46)		32.7 (28.2-37.3)	
	U-Fe30F	78	-1.77 (-1.97, -1.57)		41.3 (33.4-49.3)		182	-1.46 (-1.59, -1.33)		30.3 (25.4-35.2)	
	U-Fe60F	76	-1.75 (-1.96, -1.55)		39.8 (31.8-47.8)		198	-1.52 (-1.64, -1.39)		31.3 (26.7-36.0)	
	U-MMS	66	-1.83 (-2.01, -1.62)		43.9 (35.3-52.5)		194	-1.61 (-1.73, -1.49)		36.5 (31.8-41.2)	

Abbreviations: HAZ=Height for age Z-score; Fe30F = 30 mg iron and 400 µg of folic acid; Fe60F = 60 mg iron and 400 µg of folic acid; MMS = multiple micronutrients, 15 micronutrients including 30 mg iron and 400 µg of folic acid; [1] Test of between-subjects effects.

Development of leg-to-leg bioelectrical impedance equation

The characteristics of body composition from the deuterium oxide dilution technique were compared with the Tanita scales inbuilt prediction equations in a sub-sample (n=66) of age 7 to 10 years. For boys, TBW (mean difference=0.62 kg, 95% CI=0.28 to 0.97, and p=0.001) was underestimated by the Tanita system. For girls, there was no difference in TBW measured by both deuterium dilution and the Tanita system. However, FM was underestimated (mean difference=1.06 kg, 95% CI=0.16 to 1.97, p=0.023) and FFM was overestimated (mean difference= -1.30 kg, 95% CI= -1.95 to -0.64, p=<0.001) by the Tanita system.

Linear regression analysis was used to develop new prediction equations for FFM. First, the impedance index was added as the only independent variable, and this basic model with variables height and impedance explained 83% of the variance (adjusted R^2=0.83, standard error=1.14, p<0.001) in FFM estimated by the deuterium oxide dilution method. However, in the model further by adding weight, age and sex improve the fit (adjusted R^2=89%). The best fit equation to predict FFM from linear regression modeling was achieved by adding weight, sex and age to the basic model, bringing the adjusted R^2 to 89% (standard error=0.90, p<0.001) (paper II).

Food and micronutrient supplementation and child body composition at 54 months

In paper III, we further assessed the effect of randomization to food and micronutrient supplementation on child nutritional status and body composition at 54 months by an intention-to-treat analysis using unadjusted and adjusted linear regression models. There were no differences in anthropometry or body composition variables between children in the early and the usual start of food supplementation groups. There was no interaction between the food and micronutrient supplementation on child body composition at 54 months (FFM p=0.649; FM p=0.695). Furthermore, the mean of weight, height, BMI, MUAC and head circumference, skinfold thickness and body composition, assessed by BIA, including FFM, FM, percentage BF (BF%) did not differ across intervention groups at 54 months of age (paper III).

Exclusive breast feeding intervention and child growth and body composition at 54 months

In paper IV, we assessed the effect of randomization to exclusive breast-feeding counseling on child growth trajectory from birth to 54 months and body composition at 54 months follow-up by intention-to-treat analysis. Breast feeding counseling intervention was effective in promoting exclusive breast feeding amongst women in the intervention arm; mean duration of EBF in days in exclusive breast feeding counseling group was 111 days compared to 76 days in usual health message group (mean difference: 35.0, 95% CI: 30.6-39.5, $p < 0.001$). There was no effect of counseling for exclusive breast-feeding on child growth trajectories and body composition at 54 months of age. There was an interaction between the breastfeeding intervention and type of prenatal micronutrient supplementation on linear growth of children from birth to 54 months in that prenatal multiple micronutrients (vs. 60 mg iron and folate) combined with EBC (vs. UHM) had a slower linear growth (mean difference -0.17 SD score, $p<0.01$). There was no interaction between the breastfeeding counseling and the food supplementation on these outcomes at 54 months. Mean anthropometry and body composition measurements at 54 months are shown in table 6.

Table 6. Anthropometry and body composition of children with breast-feeding counseling and usual health message groups in the MINIMat trial at 4.5 years of age

	Exclusive breast feeding counseling (EBC) group	**Usual health message (UHM) group**
Characteristics		
Nutritional status	n=1092	n=1076
Weight (kg)	13.70±1.65	13.73±1.61
Height (cm)	99.57±4.22	99.65±4.25
BMI (kg/m^2)	13.78±.99	13.80±.93
MUAC (cm)	15.21±1.11	15.23±1.07
Head circum (cm)	47.92±1.50	47.96±1.45
WHZ	-1.31±.86	-1.29±.80
HAZ	-1.57±.92	-1.56±.92
WAZ	-1.81±.87	-1.79±.85
BMI-for-age z score	-1.20±.83	-1.18±.77
Wasted (n %)	218/1081(20.2)	195/1067(18.3)
Stunted (n %)	347/1081(32.1)	340/1067(31.9)
Underweight (n %)	451/1081(41.7)	428/1068(40.1)
Skinfold		
Biceps	4.91±1.04	4.89±1.04
Triceps	7.25±1.49	7.30±1.46
Subscapular	5.18±1.10	5.20±1.01
Suprailiac	4.99±1.49	5.03±1.41
Body composition	n=997	n=973
FFM (kg)[a]	12.10±1,24	12.12±1,22
FM(kg)[a]	1.73±0.59	1.73±0.58
Body Fat (%)[a]	12.31±3.28	12.32±3.19
AFA(cm^2)[b]	5.13±1.28	5.16±1.23
AMA(cm^2)[b]	18.10±2.65	18.12±2.55

Values are given as means ± standard deviation (SD) or n/N (percentages) where indicated

AFA = arm fat area; AMA = arm muscle area; BF = body fat; BMI = body mass index; FFM = fat-free mass; FM = fat mass; MUAC = mean upper-arm circumference; SD = standard deviation

[a]FFM and FM were derived from BIA (Tanita TBF-300MA; Tanita Corporation, Tokyo, Japan) using PoP-specific equations (115)

[b]Both AFA and AMA derived from triceps skinfold thickness and MUAC measurements (116)

Discussion

In this thesis we have shown that infant and child growth may be influenced by prenatal nutrition interventions – both positively and negatively. Children born to mothers who received an early invitation to food supplementation (around 9 weeks of gestation) were not having a differential birth weight and length as compared to the mothers who started the supplementation with the usual program timing (around 20 weeks), but were less likely to become stunted in infancy and childhood. In contrast, children born to mothers who received multiple micronutrients in comparison with the standard iron-folate program had significantly more stunting during the first five years of life and these effects were primarily seen in boys. Neither the pregnancy nutrition interventions nor the breast-feeding counseling intervention had any effect on body composition at 54 months. The effects on infancy and childhood linear growth suggest fetal programming by the prenatal nutrition interventions.

Methodological considerations

To ensure good quality of data and internal validity, all questionnaires were pretested in the study area. Interviewers and data collectors were carefully trained and supervised in interview techniques and data collection methods, and appropriate refresher training was conducted throughout the study. Weight and length/height equipment was routinely calibrated and anthropometric measurements were done twice for each visit.

All pregnant women within the study area were invited to participate in the study if they met eligibility criteria. Refusal rate was low and almost all pregnant women resided in the study area accepted to participate. After randomization to the intervention groups out-migration from the study area was the main reason for loss to follow-up.

Adherence to supplement of food and micronutrient intake

During monthly home visits the participating women were asked a series of questions in order to assess the adherence to the food supplementation during the past 30 days. 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 women. The capsule-counting eDEM is regarded as the best available method to measure adherence (117).

By design those allocated to the early invitation to food supplementation consumed more packages of supplement (mean difference 30 packages from enrolment to week 30 examination) (80). On average the participants took 77 micronutrient capsules from week 14 to 30. MMS groups took fewer capsules than the other groups and the early invitation food group also took fewer micronutrient capsules on the average (80).

As reported in the main outcome publication, constipation was most common possible adverse reaction to the micronutrient supplementation (30%), followed by nausea (18%), and heartburn (10%) (80). There was no difference in reported frequency of these common side effects between the micronutrient groups. While less common but a more severe side effect vomiting occurred to a larger extent in MMS than in Fe60F and Fe30F.

Outcome assessments

Anthropometric indices (WHZ, HAZ, WAZ) and body composition (FFM, FM, FM%) were used as indicators of child growth. Weight and length/height measurements were converted to SD scores according to the WHO Multicentre Growth Reference Study child growth standards (109), enabling comparisons with child growth results from other settings.

Body composition assessments provide sometime a more accurate description of changing nutritional status than measurements of the traditional indices (118). In some clinical settings, abnormalities in the relative contribution of FM and FFM to weight loss or weight gain can be used as indicators of underlying pathology. For example, relatively large losses of FFM can be indicative of a cachectic process, whereas more normal composition of loss might indicate simple starvation process (119). Body composition was assessed by use of Tanita TBF-300MA bioelectrical impedance analyzer, which has inbuilt software for predicting equations to estimate fat and fat free mass. In a validation study conducted in the same population, the inbuilt standards were found to be inaccurate at predicting fat and fat free mass compared to deuterium oxide dilution (paper II). We therefore predicted FFM and FM from our own equation, which was derived in this population using deuterium oxide dilution as a reference method (paper II) and used as outcome variables (paper III-IV) at 54 months follow-up.

Generalizability of findings

This trial and related studies were conducted in rural Bangladeshi women in a typical low socio-economic setting, where malnutrition was prevalent. The participating women, who were recruited at an average of 9.5 (SD 2.2) weeks of gestation, had a mean weight of 45 kg and height 150 cm (80). Matlab area has a dominance of poor rice farmers. Because of a higher coverage of maternal and child health services in this area, maternal and child health indicators are a bit more favorable as compared to other areas served by the regular government program (120). The findings in this thesis from the four studies might be relevant for other areas in Bangladesh and for neighboring low-income countries, as well as for other settings with similar poor socio-economic conditions and levels of malnutrition. All studies were population based, and since all eligible women were included they may represent pregnant women in such a setting. The study setting has a research infrastructure that increases data quality substantially, but may also influence health seeking behavior, i.e. higher utilization of services. However, this should not result in any differential effect on outcomes, and the proportion delivering in clinics and hospital was the same across intervention groups (80).

Effects of prenatal interventions on child growth

Early invitation to food supplementation had a significant effect on linear growth in infancy and childhood. A study done by Kusin JA *et al.* in Indonesia reported that a high-energy supplementation during pregnancy reduced the prevalence of childhood stunting throughout the first five years as compared to a low-energy supplementation (61). Our study suggests that early invitation to food supplementation during pregnancy prevented stunting. It may be worthwhile to compare the effect sizes of the MINIMat interventions with other postnatal nutrition interventions that have aimed at improving child growth, particularly stunting. An analysis of interventions to prevent stunting in 36 countries showed that nutrition education and counseling of complementary feeding and other supportive strategies could reduced stunting by 19.8% at 12 m, 17.2% at 24 m and 15.0% at 36 months (12). In our study stunting was reduced by 13% from birth to 54 months of age (difference: 35.3-30.8=4.5 percent units, i.e. a reduction by 13%) when invitation to food supplementation was done early in pregnancy, i.e. at around 9 weeks of gestation, as compared to the usual practice, which had an average start at 20 weeks of gestation. In conclusion, this early prenatal food supplementation reduced infancy and childhood stunting with an effect size similar to complementary feeding interventions – in addition to the effects on infant and child mortality that was presented in our main outcome paper (80).

The World Health Organization (WHO) recommends iron and folic acid supplementation to women during pregnancy as a part of routine antenatal care. In our study, we also assessed the effect of multiple-micronutrient supplementation with iron and folic acid supplementation, and failed to observe significant added benefit of multiple-micronutrient supplements over iron and folic acid supplementation for postnatal growth. On the contrary, we found significantly more stunted children in the MMS group as compared to the iron-folic acid supplementation group.

A recent systematic review of prenatal MMS trials reported a mean improvement on birth weight of 52.6 g (121). There is limited information on effects on infant and child growth by prenatal MMS. One recent randomized trial in Burkina Faso found that prenatal MMS reduced the stunting rate during infancy by 27%, but this effect was no longer seen by 30 months of age (122). One nonrandomized, non-blinded trial in Vietnam of prenatal MMS supplementation reported an association with lower prevalence of stunting in the offspring (63). Combined micronutrient supplementations, mainly iron and zinc, have been evaluated in infants and young children. An Indonesian study in infants suggested that single supplementation with zinc or iron improved growth, but that combined supplementation with iron and zinc had no significant effect on child growth or development (123). However, a review paper on iron and zinc supplementation trials suggested, that although interactions between iron and zinc may occur, so far no strong evidence was available that this had an important influence on functional outcomes (124). Animal experiments have explored the possibility that prenatal combined iron and zinc supplementation may influence the later regulation of absorption and micronutrient status in the offspring (125). Also other programming effects on the endocrine system may have long term effects on child growth (126, 127). Due to the blinding of the micronutrient intervention it is unlikely that postnatal care, e.g. feeding practices, play a role in the MMS effect on stunting.

Child body composition: using age- and population-specific prediction equations

A variety of established methods have been used for the measurement of body composition, including air-displacement plethysmography (ADP), dual-energy X-ray absorptiometry (DXA), underwater weighing (densitometry) and magnetic resonance imaging (MRI) (128, 129). However, these methods are expensive, not easily portable, time-consuming, and require highly trained operators that make them unsuitable for low-income country field settings. In epidemiological and field studies, predictions of body fatness are often made from anthropometric measurements, including body mass index (BMI), waist circumference, waist-hip ratio and skinfold thickness. These techniques do not precisely characterize body fat or muscle mass and there is a variation across age, sex and ethnic groups (130, 131). Further disadvantages of these techniques include a high degree of both intra- and inter-observer variation and acceptability of the measures in different populations (132).

In the methodology paper (paper II), the ability of the inbuilt equations from the Tanita TBF-300A system to assess body composition of rural Bangladeshi children was investigated with deuterium oxide dilution as a reference method. The equations for predicting FFM and TBW in this population for the age interval 4-10 years were developed with impedance index, age, sex, and weight included.

Effects of prenatal food- and micronutrient supplementation on child body composition

No other study has examined the effect of prenatal food supplementation and multiple micronutrients on child body composition. We have shown that neither an early invitation to prenatal food supplementation (vs. usual timing) nor multiple micronutrient supplements (vs. iron-folate supplements) nor any combination of the two resulted in any differences in body composition at 54 months – in spite of effects on infant and child linear growth.

One study from Peru found that addition of zinc to prenatal folic acid + iron supplementation neither had effect on birth anthropometric measures nor on body composition of the newborn (133) but maternal zinc supplementation in this population was associated with offspring larger average growth measures beginning in month 4 and continuing through month 12 (134). In a recent randomized antenatal micronutrient trial in Nepal (135) the authors found that maternal supplementation with folic acid + iron + zinc resulted in an increase in mean height and a reduction in mean triceps skinfold thickness, sub-scapular skinfold thickness and AFA, but no significant differences between groups were found in mean weight or BMI-for-age z-scores, waist circumference or AMA at 6–8 years of age. Prenatal dietary supplementation of rural Gambian women did not affect body composition of the offspring at 11-17 years of age (136). Thus, our study confirms the findings in other prenatal food or micronutrient supplementation that in spite of effects on child growth and other health outcomes there is weak evidence for an effect on body composition in childhood by such prenatal interventions.

Timing in pregnancy

The timing of prenatal nutrition interventions may influence the size and scope of the response in the offspring - depending on the timing of critical periods in pregnancy (34). This has been observed both in animal experimental studies (35) and in human observational studies (47). The Dutch famine study has analyzed the effects of prenatal undernutrition on later health. Early gestation appeared to be the most vulnerable period, in that children exposed to poor nutrition in early gestation were observed to have a less favorable lipid profiles in adult life, while poor nutrition exposure in late pregnancy was associated with decreased glucose tolerance (37). In our studies, we observed differential effects of timing of prenatal nutrition interventions. The early initiation of food supplements around 9 weeks of pregnancy resulted in less stunted children from birth to 54 months follow-up compared to usual initiation of food supplements around 20 weeks of pregnancy. Another human study from Guatemala has shown that maternal weight gain during mid-pregnancy was associated with infant birth weight, length and head circumference, while weight gain in late pregnancy was associated with birth weight, suggesting the importance of timing of nutritional influences in fetal life (36). This may be due to beneficial effects on the organ development occurring at this time (137). The placenta seems to play an important role in the effects of timing with observed differences in placental size and efficiency depending on the gestational time of undernutrition (47). This has been suggested as a compensatory mechanism that potentially could improve the transfer of nutrients and oxygen to the fetus. Few efforts have so far been made in early life by nutritional or other lifestyle interventions in order to favorably influence the developmental trajectories at a stage when developmental plasticity is great (138). The MINIMat trial is a contribution to this field.

Exclusive breastfeeding intervention

In paper IV, the effect of exclusive breast-feeding counseling on childhood growth and body composition in the offspring of the MINIMat trial at 54 months of age was studied. Exclusive breastfeeding counseling intervention resulted in a substantial increase in the duration of exclusive breastfeeding, although maternal randomization to exclusive breastfeeding counseling intervention compared to usual health message group was not associated with child linear growth and body composition at 54 months.

The Promotion of Breastfeeding Intervention Trial (PROBIT) - a cluster-randomized trial of a breastfeeding promotion intervention in the republic of Belarus - found no significant effect on height, body mass index, waist or hip circumference, triceps or subscapular skinfold thickness at 6.5 years (139). The long-term effects of breastfeeding are more difficult to assess, systematic reviews of observational studies have found that breastfeeding practices, particularly exclusive breastfeeding, are associated with lower blood cholesterol (7) and may be protective against the development of obesity (100). There are also studies that are not able to demonstrate any associations between breastfeeding and anthropometric or body composition indices (102, 103). In MINIMat trial, exclusive breastfeeding counseling intervention was nested into a prenatal food and multiple micronutrient supplementation, and an analysis of interaction showed that the combination of multiple micronutrient supplementation and exclusive breastfeeding counseling resulted in a small negative effect on linear growth and higher frequency of stunting 0-54 months of age. This may point at potential later negative effects by prenatal multiple micronutrient supplementation (125), as well as the quality and quantity of complementary feeding and its consequences for growth and health outcomes (12).

Most of what we know about the issue of infant feeding practices and later outcomes has been based on observational analysis. The added value of our study is the randomized trial design and the effect on EBF duration.

Sex difference

Sex differences in fetal and infant growth are well recognized (140, 141), but the underlying mechanisms are poorly understood. Cell divisions among male embryos occur more rapidly than those of female embryos (142). Social factors may also influence infant growth; boys are often given preference in food allocation within the families in Bangladesh (143). There is also evidence of sex differences in embryonic metabolism starting from the blastocyst stage (144). In our study we found less stunted children in early food group (compared to usual group) and more stunted children in MMS group (compared to Fe60F). This was evident only in boys and not in girls (paper I). These observations suggest that male fetuses may be both more susceptible to negative influences, and more responding to positive influences. There is also other evidence of sex-specific vulnerability in early fetal growth (145). Animal data have shown an increased risk for cardiac dysfunction among prenatally protein restricted males, compared to females (146), as well as greater sensitivity to maternal body mass index (BMI) and glucose status during human pregnancy (147) in male fetus compared to the female fetus. Data from the Dutch hunger winter families showed an association between undernutrition in fetal life and increased serum cholesterol and triglycerides levels in adult women, but not in men (148). In addition, there is a sex-differential in the effect by prenatal multiple micronutrient supplementation on birth weight, with greater sensitivity in females (84). It is also known that male fetuses demonstrate more rapid growth that makes them more vulnerable in utero (149, 150). Further, male fetuses are more sensitive to women's nutritional status during pregnancy and therefore more susceptible to nutritional constraints, whereas girls appear to be more affected by nutritional status over a lifetime (151). Our results further support the notion that boys are more vulnerable on maternal nutrition in utero, and that boys benefited more from the early start of food supplements than girls.

Previous results from the MINIMat trial

The results presented in this thesis should be related to the previous findings of the MINIMat trial. There was no difference in mean birth size between any of the food and micronutrient supplementation groups (80). However, the early invitation to food supplementation combined with MMS reduced infant mortality rate considerably (16.8/1000 live births as compared to usual invitation with Fe60F 44.1/1000 live births). In the five-year follow-up the early invitation-MMS group had an under-5 year mortality rate of 18/1000 live births, for the U-Fe60F group this was 54/1000 and the corresponding HR was 0.34 (95% CI 0.18-0.65) (80).

Anemia was present in 28% of the women, 55% were zinc deficient, 46% were vitamin B-12 deficient and 18% were folate deficient. Anemia was not associated with iron deficiency but rather with vitamin B-12 deficiency (152). The prevalence of vitamin B-12 deficiency in the offspring was lower in the MMS group (26.1%) than in the Fe30F group (36.5%) ($P = 0.003$). The prevalence of zinc deficiency at 6 months of age was lower in the usual food supplementation group (54.1%) than in the early food group (60.2%) ($P = 0.046$) (90). Infants who exclusively breast-fed 4–6 months had a higher mean plasma zinc concentration (9.9 ±6 2.3 μmol/L) than infants who exclusively breast-fed <4 months (153).

In addition, maternal early food supplementation group resulted in a more favorable lipid status in the children at 54 months compared to usual food group. Prenatal MMS lowered the children's glucose, insulin, HOMA-IR, and growth factors compared to Fe60F (154).

Conclusions

Maternal and child malnutrition is a major public health problem globally, and especially in South Asia (16). An early invitation to food supplementation in comparison to the usual timing of food supplementation in pregnancy reduced the occurrence of stunting during 0-54 months in boys, but not in girls. Prenatal MMS, in contrast, increased the proportion of stunting in boys. The effects of these prenatal nutrition interventions on postnatal growth suggest programming effects in early fetal life. Neither the early invitation to food supplementation, nor the multiple micronutrient supplementations, nor the exclusive breastfeeding counseling had an effect on body composition at 54 months of age.

In the national program, only pregnant women with BMI < 18.5 kg/m^2 were invited to receive the food supplements. But in our study we found that the offspring of women with BMI > 18.5 kg/m^2 benefitted from the early food supplementation.

Previous research from this cohort has observed that early food supplementation in combination with MMS resulted in substantially decreased infant and under-five mortality (80). In addition, the early invitation of food supplementation had favourable effects on the lipid profile compared to usual food supplementation in the offspring in early childhood (154). Putting these results together, it clearly shows the beneficial effects of an early invitation to food supplementation and that the addition of MMS may reduce mortality but increase the tendency of infant and child stunting. The use of MMS resulted in more stunting and levels of both insulin and insulin-like growth factors were indicative of slower growth (154). Thus, the MINIMat interventions may result in important positive results for child health – but also some negative effects. The MINIMat trial cohort is currently followed-up at the age of 10 years. These different trial outcomes point at the necessity to consider not only one, but several health outcomes when judging the public health consequences of an intervention.

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