Maternal and child nutrition in rural Bangladesh:

Special reference to the effect of dietary fat supplementation on vitamin A status

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Maternal and child nutrition in rural Bangladesh: Special reference to the effect of dietary fat supplementation on vitamin A status
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Subject headings: dietary fat, vitamin A, pregnancy, energy intake, diarrhoea, infant, children, growth

Propositions

1. Low dietary fat intake is one of the constraints of the diet of rural Bangladeshi women and an increased fat intake during late pregnancy and lactation has beneficial effects on the maternal vitamin A status in early lactation. Fat intake should be increased to improve vitamin A status in rural Bangladesh *(this thesis)*.

2. Pregnant and lactating women in rural Bangladesh are in critical energy balance and therefore energy intake should be increased throughout this stage of the life cycle to prevent obvious depletion of maternal energy stores after each pregnancy and lactation *(this thesis)*.

3. Local complementary foods in rural Bangladesh should have a minor role in infant nutrition during the first six months of infancy.

4. Among diarrhoeal illnesses, dysentery (bloody diarrhoea) has the most detrimental effect on child nutrition, therefore diarrhoea control programmes should include special preventive measures against dysentery.

5. Widespread malnutrition in the developing world is the outcome of an intergenerational cycle of poverty, undernutrition and disease. Therefore, to intervene in this vicious cycle, nutrition throughout the life cycle should not focus on just one particular age group.

6. Nutrition is the cornerstone that affects and defines the health of all people, rich and poor. *Gro Harlem Brudtland, Director General, WHO.*

7. Knowledge is of two kinds. We know a subject ourselves or we know where we can find information upon it. *Samuel Johnson (1709 - 1784).*

8. The human race is governed by its imagination. *Napoleon Bonaparte (1769 - 1821).*

Propositions belonging to the thesis entitled *"Maternal and child nutrition in rural Bangladesh: Special reference to the effect of dietary fat supplementation on vitamin A status"*.
Dewan Shamsul Alam

Wageningen, 19 June 2001.

To my wife, Daisy and our beloved children Diaz, Bushra and Ron who served as constant source of inspiration;

- *And to all mothers and their infants who made the greatest contributions to this work.*

Content

Maternal and child nutrition in rural Bangladesh:
Special reference to the effect of dietary fat supplementation on vitamin A status

Abstract

Maternal and child nutrition in rural Bangladesh:
Special reference to the effect of dietary fat supplementation on vitamin A status

Ph.D. thesis by Dewan S. Alam, Division of Human Nutrition and Epidemiology, Wageningen University, the Netherlands. June 19, 2001

The prevalence of maternal and child malnutrition in Bangladesh is one of the highest in the world. It is estimated that 50% of women of childbearing age suffer chronic energy deficiency (BMI<18.5), nearly half of infants are born with a low birth weight (<2.5 kg), and about 60% of preschool children are stunted. Protein energy malnutrition and deficiencies of micronutrient often coexist in pregnant and lactating women, in breastfed infants and in young children,

This thesis reports the effect of daily supplementation with 18 g dietary fat from mid/late pregnancy until six months postpartum on the vitamin A status of mothers and their infants. The validity of a specific dietary assessment method to identify pregnant and lactating women at risk of vitamin A deficiency also has been examined and maternal energy intake during pregnancy and lactation was studied. Growth of infants in relation to breastfeeding practices, exclusive or non-exclusive, was assessed. Finally, the effect of diarrhoeal morbidity on growth of young children was examined. All studies were performed in rural Bangladesh.

Results show that daily supplementation with 18 g of dietary fat has a beneficial effect on maternal vitamin A status during early lactation. Those supplemented with fat had relatively higher serum β-carotene and lutein concentrations than those not supplemented. Measurement of vitamin A intake by the 24-hour vitamin A-focused recall was shown to be more reliable than the measurement by a food frequency questionnaire in identifying groups, groups not individual pregnant and lactating women at risk of vitamin A deficiency. Maternal energy intake during pregnancy and lactation was found to be low. The women gained insufficient weight during pregnancy and 48% delivered low birth weight infants. Women also lost 1 kg of body weight during the first six months of lactation. All infants were still breastfed at 6 mo, 70% and 37% were exclusively breastfed at 3 and 6 mo, respectively. There were no differences in morbidity and growth between the exclusive and non-exclusively breastfed infants however the growth of the exclusively breastfed infants more closely resembled that of WHO breastfed reference infants. In young children, dysentery (bloody diarrhoea) showed a significant negative association with ponderal and linear growth both in the 3 mo and 1 yr growth periods.

From these findings it can be concluded that dietary fat supplementation may have a significant role in improving the vitamin A status of populations with a low fat intake and who derive their vitamin A mainly from provitamin A carotenoids. Poor maternal energy balance in otherwise poorly nourished women during pregnancy probably contributed to the extremely high prevalence of low birth weight in the community studied. What is lacking, however, is data on energy expenditure and that is required to precisely define the level of energy inadequacy. The similarity in growth pattern between our infants and that of WHO breastfed reference infants suggests that this growth pattern (i.e. the growth rate) might be physiologically optimal in this population, except that they start at a lower level due to low birth weight. This difference in growth, i.e. poorer weight, therefore is mainly attributable to the intrauterine rather than postnatal factors. It is also concluded that Infant and child nutrition can be substantially improved by preventing dysentery in this community.

1

General Introduction

Malnutrition, in one form or other, affects one third of the total global population. However, the burden is disproportionately distributed between high and low-income countries. In Bangladesh, malnutrition is highly prevalent across all age groups. Nearly 50% of women of childbearing age suffer chronic energy deficit and nearly half of the infants are born with low birth weight (1). Common forms of malnutrition include protein energy malnutrition (PEM), and micronutrient malnutrition, which affect much of the population, of which preschool children, pregnant and lactating women and breastfed infants are the most vulnerable. Among most common micronutrient deficiencies, vitamin A deficiency remains as a significant public health problem in many developing countries, including Bangladesh. While vitamin A deficiency is most prevalent among preschool children, a significant proportion of women of childbearing age are believed to have vitamin A deficiency (2). It is estimated that 1-2 million pregnant women may be at risk of vitamin A deficiency in South Asia alone (3). The consequences of maternal malnutrition are enormous and include higher risk of maternal mortality and morbidity, and increased risks of impaired growth and development of their fetus, premature delivery and low birth weight (LBW) infants. Such LBW infants, breastfed or not, also are at greater risks for poor survival, growth and development (4). Intrauterine growth retardation might also increase risk of diabetes, hypertension and coronary heart disease in later life (5-8)

Other major maternal nutritional problems are inadequate dietary energy intake during pregnancy and lactation which results in inadequate pregnancy weight gain and inadequate maternal nutritional status at entry and during the course of lactation (9;10). Maternal and fetal undernutrition are extremely common in Bangladesh. Moreover children who survive the fetal transition and infancy often suffer from growth retardation due to environmental conditions, predisposing them to diarrhoeal diseases, on average three episodes per child per year (11;12).

This chapter aims to provide background information on the major research issues addressed in this thesis, namely: a) vitamin A deficiency in pregnant and lactating women and a possible role of dietary fat supplementation on vitamin A status of women and their breast fed infants in a population with low dietary fat intake (**Chapter 2**); b) testing of a vitamin A specific dietary assessment method to identify pregnant and lactating women at risk of vitamin A deficiency (**Chapter 3**); c) an evaluation of maternal energy stress during pregnancy and lactation, and the implications on pregnancy outcome and maternal nutritional status (**Chapter 4**); d) infant feeding practices and growth performance during the first six months of life (**Chapter 5**); and the effect of diarrhoea on growth of young children in rural

Bangladesh (**Chapter 6**). At the end of this introductory chapter an outline of the thesis is presented.

VITAMIN A DEFICIENCY

Prevalence and magnitude of the problem

Vitamin A deficiency continues to be a major public health problem in many developing countries with preschool aged children and women of child-bearing age the most vulnerable (13). It is estimated that approximately 3 million children develop clinical vitamin A deficiency (xerophthalmia) every year of which a quarter to half a million suffer blinding eye lesions, and about 60% of those who become blind die within one year (3). South Asian countries disproportionately contribute to the total global burden of vitamin A deficient populations, where 1.6 of 3.3 million clinically deficient children live (14). A more subtle but consequential form, subclinical deficiency (defined as serum retinol <0.70 μmol/L) is also associated with increased risk of mortality and morbidity (15). Subclinical deficiency affects 75 to 250 million preschool aged children globally (3). In Bangladesh, the prevalence of xerophthalmia (night blindness) among preschool aged children is estimated to be 1.5% which is above the cut-off level set by WHO for the problem to be of public health significance (16). It is estimated that 30,000 preschool aged children become blind each year due to vitamin A deficiency in Bangladesh. However, a more recent survey showed substantial improvement in vitamin A status in young children (HKI & INFS 2000). While young children have been the main focus of vitamin A deficiency programs because of the extreme consequences observed in that group, other important vulnerable groups include pregnant and lactating women (2;17;18).

Intervention studies in developing countries have shown that vitamin A supplementation substantially reduces childhood mortality (19-21). It is estimated that vitamin A supplementation alone in deficient populations can reduce the risk of childhood mortality approximately 25% (22). An earlier review estimated that a total of 1.3- 2.5 million deaths of children under the age of four can be prevented globally each year simply by improving their vitamin A status (23). Vitamin A deficiency during pregnancy is associated with nightblindness and elevated risk of maternal mortality. Supplementation with vitamin A or β-carotene in deficient populations may substantially reduce the risk of maternal mortality (24) and morbidity (25). Apart from its impact on maternal health and survival, vitamin A deficiency in pregnant and lactating women has important implications of vitamin A nutriture of their fetus and breastfed infants (4;26;27).

Vitamin A and Carotenoids

The term "Vitamin A" is a generic descriptor for retinoids exhibiting qualitatively the biologic activity of retinol (28). In diet, vitamin A is available as preformed vitamin A mainly and/or as provitamin A carotenoids which are converted to vitamin A. Sources rich in preformed A are foods of animal origin which primarily include liver, fish liver oil, milk, butter, eggs, and foods fortified with retinol. Dark green leafy vegetables, yellow fruits and vegetables are major sources of provitamin A carotenoids. Although more than 500 carotenoids are known to exist, only about 50 of them are labeled as provitamin A carotenoids, which have biologic activity of vitamin A (29). Among the carotenoids, β-carotene has the highest vitamin A activity and normally predominates in the diet (30). Other important provitamin A carotenoids include α- and γ-carotenes, and β-cryptoxanthin, but their contribution to vitamin A activity is much less than that of beta-carotene (31). Due to their chemical structure, some carotenoids such as lycopene and lutein have no vitamin A activity but can serve as an indicator of carotenoid intake and absorption (32).

Vitamin A activity is commonly expressed as retinol equivalent. When provitamin A carotenoids are ingested within a mixed meal, it is estimated that 6 μg of β-carotene or 12 μg other provitamin A carotenoids is converted to1 μg retinol (33). However, these conventionally used conversion factors are being challenged by new data that show these traditional conversion factors overestimate true vitamin A intake from plant sources (34). Data from more recent studies suggest that vitamin A activity depends largely on the matrix of the dietary sources of β-carotene. For example, about 26 μg of β-carotene from vegetables and carrots or about 12 μg from yellow or orange fruit are required for 1 μg retinol (35). A more recent report proposed a conversion factor of 21 μg for β-carotene for 1 μg retinol when obtained from a mixed diet (36)

Both preformed vitamin A and provitamin A carotenoids are fat soluble and their absorption is dependent on the concurrent presence of fat in the diet and on normal lipid digestion (31). However, absorption of provitamin A carotenoids is more dependent on dietary fat than preformed vitamin A due to their non-polar characteristics (32;37). When carotenoid intake is within a moderate range (<15 mg/d), 30% - 50% is normally absorbed in the presence of fat (28). For example, carotenoid absorption can be as low as 5% when dietary fat intake is as low as 7 energy% or less (32). After absorption of β-carotene, a substantial amount is converted to retinol mainly in the intestinal mucosa (31). However, preformed vitamin A is more easily and efficiently absorbed (70-90%) than provitamin A carotenoids (37;38). Therefore, dietary fat intake is likely to have a more important role in determining vitamin A status in populations that depend mainly on provitamin

A carotenoids for their dietary vitamin A intake compared to populations who derive their vitamin A mainly from animal sources.

Vitamin A status during pregnancy and lactation: relationship with fetal and infant vitamin A status.

Adequate maternal vitamin A status and dietary intakes during late pregnancy are necessary for optimal vitamin A transfer to the fetus (4). At birth, infants have low plasma retinol concentrations (39) as well as relatively very low vitamin A stores in the liver (40). Therefore, the maintenance of adequate vitamin A status and accrual of hepatic stores during the neonatal and post neonatal periods depend on the vitamin A supplied through breast milk, especially in exclusively breastfed infants. Maternal breast milk vitamin A concentration is of greatest importance for infants in developing countries where breast milk serves as the exclusive source of vitamin A during infancy and continues to be a major source even in early childhood (41;42). A study in rural Bangladesh showed that infants who were weaned between 6-12 months of age received only 3.2 μg retinol equivalent per day (43). According to current recommendations, infants during their first six months of life should receive at least 180 μg retinol equivalent per day as a basal requirement and double this amount is recommended for safe levels of intake (44). In developing countries, where breast milk retinol concentration is frequently low (27;45), vitamin A deficiency may develop quite early in infancy. The vitamin A status of most newborn in developing countries is marginal or poor, and infants of mothers with inadequate status appear to be at particular risk (46;47). A recent study in Bangladesh demonstrated that 60% and 65% of primarily breastfed infants had relatively low vitamin A status according to serum retinol concentration and Relative Dose Response (RDR) criteria respectively (48).

The relationship between maternal and infant vitamin A status is further established by results from supplementation trials in lactating women. In Indonesia, maternal supplementation during lactation with a single dose of 300,000 I.U. significantly improved maternal and infant vitamin A status (49). A similar intervention resulted in decreased infant mortality in Bangladesh (50), suggesting an indirect benefit of maternal vitamin A supplementation during the postpartum period. In contrast, supplementation of lactating women with β-carotene increased β-carotene concentration in breast milk but not retinol concentration (51). A more recent study reported that supplementation of lactating women with β-carotene, both in the form of pure β-carotene or as fruits and vegetables, enhances vitamin A status of the mother-infant pairs suggesting a strong association between maternal and infant status (52). Several studies have reported that maternal dietary intake during pregnancy and lactation influences breast milk vitamin A concentration (4;45;53), whereas others found no association (54).

Dietary sources and vitamin A status

Dietary sources of vitamin A critically influence vitamin A status of populations. Vitamin A deficiency is more often observed in populations who largely depend on provitamin A carotenoids from plant sources for their vitamin A status compared to those who derive their dietary vitamin A from preformed animal sources. Severe vitamin A deficiency more commonly exists in areas where vitamin A intake from animal sources is less than 40% (33). Data on daily intake of vitamin A suggest that populations in Asian countries have an adequate intake, however, it should be noted that the contribution of animal sources is very low (**Figure 1**). In fact, in some Asian countries provitamin A carotenoids are the exclusive source of dietary vitamin A intake. In Bangladesh, nearly the total intake (99%) of vitamin A is derived from plant sources (43). In some Asian countries, this dietary pattern often coexists with the high prevalence of clinical vitamin A deficiency ((55). Poor bioavailability of

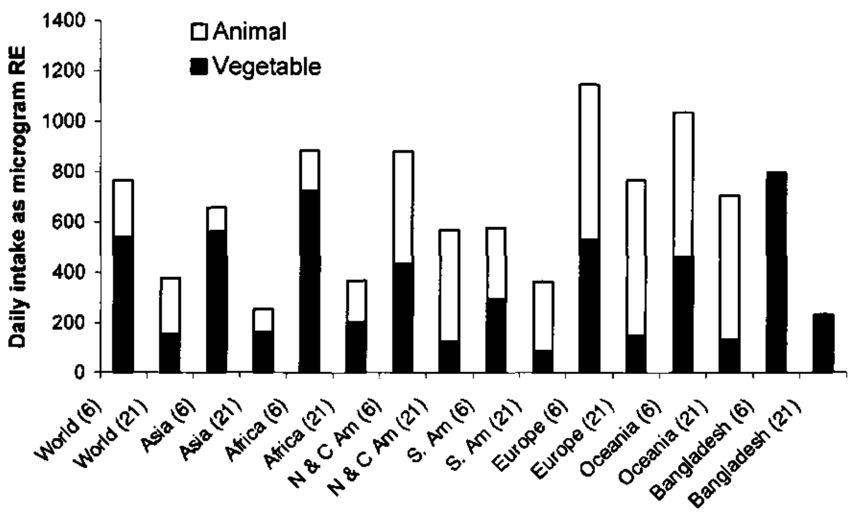

Figure 1. World and regional pattern of food sources of vitamin A. Number in the parenthesis indicate conversion factor used for 1 microgram retinol equivalent.

Sources: FAO/WHO 1988; West 2000; Zeithlin 1992 (Bangladesh data) provitamin A carotenoids along with intestinal parasitosis have been identified as the key limiting factors that determine poor vitamin A status of these populations (34;35;56).

Dietary fat intake and vitamin A status

Dietary fat is essential for the absorption and assimilation of vitamin A and provitamin A carotenoids (28). Intervention studies in young children have shown a positive effect of dietary fat supplementation on vitamin A status. However, the amount required for optimal bioavailability is not clear. A study in school children in Rwanda showed that the addition of 18 g fat (olive oil) with raw or grated carrots resulted in a five-fold increase in absorption of beta-carotene compared to children not given any fat supplement (57). In another controlled dietary intervention trial, children fed with cooked spinach supplemented with 5 g or 10 g of fat demonstrated a significant increase in serum retinol concentration by 5-7 µg/dL, however, improvement in serum retinol concentration did not differ between 5 and 10 g supplementation (58). Based on this study it is often quoted that at least 5 g fat is required for the optimal absorption of provitamin A carotenoids from foods (34). Another study in Indonesia demonstrated a significant improvement in vitamin A status in vitamin A deficient preschool children when supplemented with carotenoid rich foods and 18 g dietary fat compared to those who were supplemented with 3 g of fat (59).

Studies suggest that a higher amount of fat is probably required for optimal absorption in adults. The plasma response to β-carotene has been reported to be significantly greater in those who consumed a high fat diet (18-24 g at breakfast and 45 g at lunch) compared to a low fat (0 g at breakfast and 6 g at lunch) diet (60). Another study in adults reported a 4-fold increase in serum β-carotene when consumed with a high fat meal (51mg β-carotene and 200 g fat) compared to consumption without fat (61). A more recent study conducted among adult volunteers in the Netherlands showed no difference in α- and β-carotene concentration between high (36 g/d) and low (3 g/d) fat supplementation although the high fat group had significantly higher plasma lutein concentration (62). While fat supplementation appears to improve carotenoids absorption and vitamin A status in children, and the limited data have findings on adults suggest the same, no such data are available on pregnant and lactating women. It is therefore important to determine whether increasing fat intake may have beneficial effects on the vitamin A status of pregnant and lactating women in populations with low dietary fat intake who largely depend on plant sources for their vitamin A intake.

*If an increase in dietary fat in women with low habitual dietary fat intake improves provitamin A bioavailabiltiy, it would be predicted that an improvement in maternal and infant vitamin A status would result. If such an independent effect of fat intake on the vitamin A status of population is established, this would have relevant programmatic implications in the prevention and amelioration of vitamin A deficiency (**Chapter 2**).*

DIETARY ASSESSMENT OF RISK OF VITAMIN A DEFICIENCY

Assessment of vitamin A deficiency is essential to identify and characterize the magnitude of the problem, for evaluating the impact of interventions, and for monitoring the situation over time. Most commonly used methods for assessing vitamin A deficiency include clinical, histological, and biochemical assays, all of which have their strengths and limitations (64). Moreover, most are not suitable for large-scale population based studies. Therefore, it has been of increasing interest to identify an inexpensive, rapid and practical tool to establish vitamin A deficiency in vulnerable populations. It is difficult to assess the true vitamin A status, which is best reflected in liver stores, where more than 90% of vitamin A is stored under normal conditions (31). All other assessment methods, therefore, evaluate the relative status. Dietary assessments, on the other hand, are often considered to serve as a tool for measuring the proxy indicators of vitamin A status (dietary intakes), and are more easily and less expensively implemented in developing country situations. The main theoretical basis for the dietary assessment of risk of vitamin A deficiency is that prolonged low vitamin A intake leads to a state of vitamin A deficiency.

General dietary assessment, not focused solely on vitamin A containing foods, poorly predicts vitamin A status (Ref). In 1989, the International Vitamin A Consultative Group (IVACG) proposed a semiquantitative dietary assessment method called the Simplified Dietary Assessment (SDA), which focuses only on the intake of one nutrient, vitamin A, and which can be quickly developed, administered, and evaluated in the field situation (65). The main purpose of the tool is to identify population groups with inadequate dietary intake of vitamin A and therefore at risk of vitamin A deficiency. There are two major components to the guidelines: a food frequency questionnaire (FFQ) and a 24-hour history of vitamin A food intake. Both FFQ and 24-h history can independently classify groups at risk of vitamin A deficiency. However, the guidelines recommend using FFQ results if there is an inconsistency in results derived from the two methods. This method is primarily designed to identify populations of children at risk of vitamin A deficiency and has been used in several studies and shown significant potential in identifying groups of young children at risk of vitamin A deficiency (66-68). However, the instrument can be easily modified and applied to other vulnerable groups such as pregnant and lactating women to assess the risk of vitamin A deficiency.

*Therefore, a dietary assessment method that can be successfully used and proved to be a valid field tool for assessing risk of vitamin A deficiency in pregnant and lactating women will complement other assessment methods, and may also serve as a practical, noninvasive, rapid and alternative tool for assessing the risk of VAD in these vulnerable groups (**Chapter 3**).*

MATERNAL NUTRITION DURING PREGNANCY AND LACTATION

Maternal nutritional status and dietary intakes are significant predictors of birth weight (9;69). Infants birth weight is the single most important predictor of mortality and morbidity during infancy and childhood, with low birth weight being associated with increased risk (69;70). Recent studies also suggest that low birth weight has long term adverse health consequences in later life (71). Although low birth weight may be due to short gestational duration or intrauterine growth retardation (IUGR), the later type is more common in developing countries (73). Current estimates indicate that nearly 15% of infants weigh less than 2500 g at birth, however, this percentage is disproportionately high in some developing countries. For example, in Bangladesh approximately 50% of all infants are born with low birth weight and the incidence of IUGR exceeds 39.4% (74;75).

Maternal nutritional factors including low maternal height, low prepregnancy weight, and low pregnancy weight gain are highly correlated with risk of low birth weight, although their relative contribution varies between developed and developing countries (69). Low dietary energy intake during pregnancy and low pregnancy weight gain commonly observed in developing countries are widely implicated as one of the major causes of low birth weight in poor countries (9;69). Pregnancy weight gain in women in developing countries is often less than half that observed in Western developed countries and a substantial proportion of women even lose weight during pregnancy (10). This not only increase a risk of bad a pregnancy outcome but also has the adverse implications for maternal nutrition. However, because of the paucity of dietary energy intake data, it is difficult to define the critical energy balance during pregnancy in developing countries.

Maternal energy requirement during lactation is relatively higher than in any other period because of the additional energy needed for milk production. It is estimated that women on average produce about 700 ml of breast milk during the first six months of lactation, which requires ~600 kcal daily additional energy, assuming an energy density of 0.70 kcal/g of milk and a conversion efficiency of

80% (44;76). Therefore, maternal nutrition during lactation and dietary intake during lactation may have important implications for maternal health as well as growth of their fetus and breastfed infants. Women in high income countries gain nearly 2 kg of fat mass during pregnancy which serves as energy depot to support accelerated growth of the fetus during late pregnancy and as an additional energy source during lactation (9;77). In contrast, there is no evidence that the vast majority of women in developing countries accrue fat during pregnancy (10;78). The estimation of energy requirements for lactation is based on assumption that fat mobilization from accrued fat developed during pregnancy would occur (44). It has been observed that women in high income communities meet this extra energy demand by increasing energy intake, mobilization of fat store, and reduced physical activity (77). In developing countries, where energy from fat mobilization is an unlikely possibility, increased energy intake might be the main mechanism to meet the full energy cost of lactation. Therefore, failure to meet the total energy cost of lactation through increased dietary energy intake would be predictable of and may result in depletion of maternal stores.

Therefore, an assessment of the maternal dietary energy intake during pregnancy and lactation, and pattern of pregnancy weight gain and its relationship with birth weight would provide valuable information about the rural women's response to the energy stress during these two critical periods. The results might be useful for selecting possible interventions for maintaining adequate maternal nutritional status of women during pregnancy and lactation in this population ***(Chapter 4)****.*

INFANT FEEDING PRACTICES AND GROWTH

Good infant feeding practices during the first six months of infancy essential because this is the period of rapid somatic and organ growth and it also coincides with the period when exclusive is breast feeding is recommended by the international agencies. Furthermore, it has been shown that growth deficits during infancy persist into later stages of life (79). Recent evidence also suggest that birth weight as well as postnatal growth may have long term consequences for metabolism, development and chronic diseases in later life (71)

Infant feeding practices should provide sufficient amount of food of adequate quality to satisfy nutrient requirements. Whether that objective is achieved or not is reflected in the growth performance of the infants. Growth of infants also reflects lactation performance of mothers, particularly when infants are exclusively breastfed. Breastfeeding is considered to be a natural and nutritionally complete source for infants up to 4-6 months of age. However, the appropriate age of introduction of complementary foods and duration of exclusive breast-feeding is still under debate. Recent evidence shows that exclusive breastfeeding is sufficient to support nutrient demand of full-term normal birth weight infants up to 6 months of age (80). Evidence is also available to suggest that infants between the age of 4 and 6 months self-regulate their total energy intake, and consume less breast milk when other foods are introduced (81). These findings imply that the introduction of complementary foods before six months of age has no growth advantage, and such foods might even have a negative effect by increasing the risks for diarrhoeal morbidity (82). Even in low birth weight infants, who are considered to be unable to suck breastmilk adequately introduction of complementary food earlier than six months does not show any growth advantage compared to those infants who were exclusively breastfed (83).

Infant feeding practices have certain cultural and social determinants. A study in India has shown that less educated mothers were more likely to exclusively breastfeed than more educated women (84). They also reported that infants' age and weight were significant predictors of exclusive breastfeeding, and low birth weight was an independent significant predictor of non-exclusive breastfeeding. A more recent study in rural Bangladesh has shown that only 15% of the infants were exclusively breastfed up to five months of age (85). This study also reported that exclusive breastfeeding was higher among those who knew about the benefits of breastfeeding and were delivered by medically trained professional. However, the authors did not relate that feeding practices with any infant outcomes.

From these reports and others, it is evident that infant feeding practices vary widely among different communities; in low income communities complementary foods are often introduced to a large majority of the infants earlier than recommended. Often such foods are contaminated, and therefore increase the incidence of diarrhoeal morbidity. A study in Peru reported that diarrhoea prevalence in infants younger than six months was double or more among those infants given liquid or solid foods before six months compared to those exclusively breastfed (82).

Growth is again a modifying factor for infant feeding. Adequacy of growth is generally judged through comparison with the National Centre for Health Statistics (NCHS)/WHO reference population (Hamil et al 1977), which often used as the standard in many populations. There is a subtle difference in growth patterns of infants who are fed according current WHO feeding guidelines and that of the NCHS reference population, which do not represent infants of the same socioeconomic, enviromental, and feeding background (WHO working Group on Infant Growth 1995). Breastfed infant seem to grow faster between birth and 3 mo and then the rate falls compared to the NCHS reference (Dewey et al. 1995). This phenomenon in growth of breastfed infant may result in a misdiagnosis of growth failure and inappropriate feeding recommendations, which in certain cases may be harmful. Therefore, data have been generated from breastfed infants form afluent countries to show the optimal physiologic potential of growth of infants.

It is important to look into the infant growth pattern in relation to feeding practices and growth performance during the first six months of life in communities where nearly half of the infants are born with low birth weight and maternal malnutrition is widespread. ***(Chapter 5).***

DIARRHOEAL ILLNESSES AND GROWTH DURING CHILDHOOD

Bangladesh has the highest rate of low birth weight as well as the highest prevalence of childhood malnutrition (14). It has been reported that malnourished infants and children have a greater incidence (Tomkins et al. 1981) and longer duration of diarrhoea (12). The annual incidence in preschool aged children in some communities often exceeds three episodes per child per year (11). Diarrhoeal illnesses continue to be a major cause of morbidity and mortality, its role in childhood malnutrition is debated. Several studies have demonstrated negative effects on growth and others showed little or no effect (86). One possible explanation for the discrepant observations is the heterogeneity of diarrhoeal illnesses, which is often ignored (87).

An episode of diarrheoa is commonly defined as three or more loose stools or any number of stools with blood. However, diarrhoea can also be defined clinically and/or etiologically. On the basis of clinical classification, diarrhoeal illnesses can be broadly classified as dysentery and non-dysentrery (watery). The presence of blood in stool distinguishes dysentery from other types of diarrhoea. However, etiologic classification requires expertise and costly laboratory resources.

Dysentery is noted for its greater impact on mortality and negative effect on nutritional status of children. Although dysentery accounts for only 10 percent of all diarrhoeal episodes in under five children, about 30% of diarrheal deaths are attributable to dysentery (88). Dysentery is also more likely to be detrimental to child nutrition than other types of diarrhoea because of relatively longer duration of episode (12;89), associated fever (90), damage to the intestinal structure and function (90;91), and greater endogenous protein loss(92). It is important from a public health point of view to know whether they understand the nutritional consequences of diarrhoea. Because mothers or caregivers they are the most important group that will be finally targeted for intervention. Data from rural communities have shown clinical types of diarrhoea are locally categorized and treatment is mainly guided by the type of diarrhoea.

Therefore, if the clinical classification of diarrhoeal illnesses is observed to be associated with growth of the young children, additional nutritional messages should be developed to reduce the detrimental effects of the most offending types of diarhoea and practical preventive measure can be suggested ***(Chapter 6).***

OUTLINE OF THE THESIS

The research described in this thesis was conducted between November 1995 and April 1997 in 16 rural villages in Matlab Thana (Sub-district) under Chandpur District, Bangladesh where maternal malnutrition and vitamin A deficiency (especially in children) are known to be widely prevalent.

Chapter 2 describes the effect of daily dietary fat supplementation during late pregnancy and throughout the first six months of lactation on vitamin A status of pregnant and lactating women and their breast fed infants.

Chapter 3 presents the results of a vitamin A-focused dietary assessment for identifying pregnant and lactating women at risk of vitamin A deficiency using the principle described in guidelines for Simplified Dietary Assessment (SDA) by the International Vitamin A Consultative Group.

Chapter 4 reports on maternal energy stress during pregnancy and lactation among rural Bangladeshi women and its implications for maternal nutritional status and pregnancy outcome.

Chapter 5 presents the findings of infant feeding practices and growth performance during the first six months

Chapter 6 demonstrates the association between diarrhoea and growth of children younger than five years.

Chapter 7 discusses important findings of the research described in this thesis and the potential policy implications for mothers, breastfed infants, and young children. Conclusions were drawn based on our findings and recommendations have been made on future research in the related fields.

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2

Effect of dietary fat supplementation during late pregnancy and throughout the first six month of lactation on maternal and infant vitamin A status in rural Bangladesh.

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ABSTRACT

Background: Fat intake is extremely low in most communities with vitamin A deficiency. However, its role in vitamin A status of pregnant and lactating women is poorly understood.

Objective: The aim was to examine the effect of supplementing women with fat from mid/late pregnancy until 6 mo postpartum on their vitamin A status and that of their infants.

Subjects and Methods: Women at 5-7 mo pregnancy were supplemented daily with 20 ml soybean oil (n=248) or no supplement (n=251). Dietary fat intake was assessed by 24-hr dietary recall. Maternal plasma retinol, β-carotene and lutein concentrations were measured at entry, and 1, 3 and 6 mo postpartum, and those of infants' at 6 mo postpartum. Breast milk retinol concentration was measured at 1, 3, and 6 mo postpartum.

Results: Compared to values during pregnancy, the difference in plasma retinol concentration at 3 mo postpartum was significant in supplemented than that in the controls (+0.04 vs -0.07 μmol/L, $p < 0.05$ respectively). Plasma β-carotene and lutein concentrations declined in both groups during postpartum but the decline was significantly less in the supplemented than control women at 1 and 3 mo postpartum. Breast milk retinol concentration was also significantly greater in the supplemented group at 3 mo postpartum than in the controls [0.68±0.35 vs 0.55±0.34 μmol/L (mean±SD), $p < 0.03$]. Infants' plasma retinol, β-carotene and lutein concentrations did not differ between groups.

Conclusions: Fat supplementation during pregnancy and lactation in populations with very low dietary fat intake has beneficial effects on vitamin A status during early lactation.

INTRODUCTION

Vitamin A deficiency (VAD) among pregnant and lactating women is a serious public health problem in many developing countries (1-3) because of implications for maternal health and survival (4). Maternal vitamin A status also has potential implications for the vitamin A status of their fetus and breastfed infants (5). Although the etiology of VAD is complex and often multifactorial, a common ecological association exists between VAD and dietary pattern that consists of very high proportion of vitamin A intake from the plant sources as provitamin A carotenoids in populations with high prevalence of vitamin A deficiency (6).

Conventional approaches to combat deficiency include periodic supplementation with vitamin A, fortification of foods, and a food-based approach increased consumption of dark green leafy vegetables. Among the intervention strategies, the dietary approach with an emphasis on increased consumption of provitamin A carotenoid-containing foods has been advocated as preferable and sustainable strategy to eliminate vitamin A deficiency (7;8). However, the effectiveness of this approach has recently been questioned as the bioavailability of provitamin A plant carotenoids has been shown to be lower than it is currently considered (9;10;10;11;11;12;12). Bioavailability of provitamin A carotenoids is further constrained by other dietary factors, most notably dietary fat intake which is characteristically low in populations where vitamin A deficiency is highly prevalent (13-15). While preformed vitamin A can be absorbed substantially independent of fat due to its polar characteristics (16;17), dietary fat is essential for optimal absorption and utilization of provitamin A carotenoids,

Studies to date investigating the relationship between dietary fat and vitamin A status have been limited to efficacy investigations in highly controlled settings with relatively small number of subjects. Studies in children showed that fat supplementation enhances absorption of beta-carotene and improves vitamin A status (15). A recent study in Indonesia demonstrated significant improvement in vitamin A status in vitamin A deficient children supplemented with carotenoid foods and high fat (18 g/d) compared to those supplemented with only 3 g/d. Adults showed a significantly greater plasma β-carotene concentration in those who received a high fat supplementation (>60 g/d) than those consuming a low fat (<8/d) diet. Although fat supplementation appears to improve carotenoids absorption and/or vitamin A status in children, population based data are lacking and to our knowledge are nonexistent in pregnant and lactating women.

We therefore conducted a controlled intervention trial in a poor rural community in Bangladesh in which women in mid/late pregnancy and throughout the first six months of lactation received either a daily supplement of 18 g fat or no supplement. Data were collected on indicators of vitamin A status of women and their infants.

SUBJECTS AND METHODS

Study area and subjects

This community-based controlled trial was conducted from November 1995 to October 1997 in 16 villages in Matlab Thana (Sub-district) of Chandpur District, Bangladesh. The area is located approximately 55 km south east of the national capital of Dhaka and is considered to be a typical of rural and riverine delta areas of Bangladesh (18). The usual diet in rural Bangladesh is known to be monotonous as well as low in fat (14). Rice is the main staple that is usually eaten with green leafy vegetables and a small amount of fish. Consumption of meat and other animal products very seldom.

Sixteen villages were grouped into two clusters of eight each, separated by a distance of about 2 km and a series of canals. One of the two clusters was randomly selected for dietary intervention. Study subjects were healthy pregnant women, identified by the last menstrual period without any known chronic illness. A survey was conducted to identify all currently eligible women and to prepare a list of all married women of child-bearing age, including those in early pregnancy. A field team consisting of a health assistant, community health worker (CHW), dietary interviewer (a CHW with graduate level education specially trained for dietary interviews), and a logistical assistant (porter) visited each eligible subject at home , and explained the characteristics of the study. Informed written consent was obtained from each woman before enrollment into the study. A detailed interview on socioeconomic, demographic and household characteristics was performed. Maternal body weight, height, mid-upper arm circumference (MUAC) were measured by trained and experienced field worker following procedures suggested by Gibson (1990). Body mass index (BMI; kg/m^2) was calculated. Data on food intake were estimated by 24-hr dietary recall and maternal blood specimens were collected at baseline.

During the study period, 341 and 335 subjects were recruited from the intervention and control villages, respectively. From the total sample there were 137 subjects who were lost to follow up, including 3 false pregnancies, 5 miscarriages, 6 too early delivery (delivered less than one month after enrollment), 14 still births, 7 twin births, 23 neonatal deaths, 30 out migrations, and 48 refusals. Data of women who delivered singleton babies available during the postpartum period were included in the analysis. In total, 326 women in the intervention and 315 women in the control villages respectively delivered live singleton infants. However, 248 from the intervention 251 from the control group had complete baseline data. Missing data at baseline were mainly biochemical ones which could not be obtained due to scanty amount or precipitation of serum samples.

Subjects in the intervention villages were supplied with soybean oil as a daily supplement and women in the control villages were not given any dietary supplement and maintained their usual diet. Twenty four hour dietary recalls and maternal blood and breast milk samples were scheduled at 1, 3 and 6 months postpartum. A single blood specimen from the infants was collected between 6 and 7 months of age.

Dietary fat supplementation

Each woman in the intervention villages was supplied with a weekly ration of 140 ml soybean oil and was asked to consume 20 ml (~18 g fat), 10 ml of oil twice a day with their main meals (usually afternoon and night meals) as supplement. The CHW delivered the oil in a plastic bottle to each study women at home. A graduated and clearly marked (at 10 ml) plastic dispenser was also provided to dose the amount of oil. Women were given several options to consume the supplement that include mixing of the oil with their foods, drinking during the meal or frying their portion of the meal with the oil. However, pre-testing revealed that drinking during the meal was the most preferred choice of supplement intake followed by mixing the oil directly with their meal portion. The study was therefore designed to supplement the diet in this fashion rather than instructing women to add oil during household food preparation to ensure a measured "dose" of oil for more accurate quantification of supplement intake. The total period of supplementation was 8-10 months depending on women's gestational age at enrollment.

Motivational efforts were continued throughout the study period to maximize high compliance to the intervention and to avoid any replacement of the usual diet by the supplement. Each subject was trained to maintain daily record of oil intake using an easily understandable record keeping sheet supplied each time the oil was delivered. The CHWs collected the maternal intake records during their next weekly home visits and supplied the subject with another bottle of oil for the ensuing week. They also interviewed the subject, obtained information about her attitude and compliance to the oil during the previous one week, and provided written reports to the study office.

Food and nutrient intake

Fat intake from all sources was estimated by 24-hour dietary recall administered four times during the study period following standard guidelines (19). A survey was conducted to obtain representative recipes for multi-ingredient foods consumed in the community. All locally used household measures (plates, cup, spoons etc.) were standardized for respective food items during the initial phase of the study. Dietary intake during the last 24-hour was then estimated by using standardized household measures (serving units). All dietary interviews were conducted at home. To avoid possible reporting bias related to supplement intake, the interviewers who conducted the 24-hour recall interviews were not involved in the distribution of oil or collection of other data. Dietary interviewers were frequently rotated between intervention and control villages. Special instructions were given to the dietary interviewers to obtain specific information about intake of the dietary fat supplement during the last 24-hour.

Nutrient intake data were calculated using a computerized food composition table for Bangladeshi foods, which used nutrient data from regional food composition databases (20;21). The computerized database was created and updated using softwares VBSedit and KOMEET (Wageningen University, the Netherlands). The estimate of fat intake derived from all sources, including the supplemental fat, captured by 24-hour recall was recorded as the subjects' total fat intake.

Biochemical analysis

Blood samples of 0.5 ml were collected in lithium heparinized microtainers either by finger prick or ante-cubital venipuncture, immediately put on ice and shielded from light in a cold carrier, and transported to the field laboratory within 3-6 hours of collection. Plasma was then separated by centrifugation (3000 rpm for 5 minutes) and transferred to labeled screw-top, amber-coloured cryovials and stored at -20 C for 2-4 weeks in the field laboratory before being transferred to the central laboratory in Dhaka for storage at -70C until analysis 2-3 months later.

Simultaneous determination of serum retinol, lutein, and beta-carotene concentrations were done by HPLC in the Nutritional Biochemistry Lab of ICDDR,B (22). Briefly, an aliquot of plasma was deproteinized with ethanol containing internal standard (IS) and extracted in hexane. Tocol was used as the internal standard for retinol and beta-apo-8'-carotinal as the internal standard for carotenoids. The extracted layer was evaporated to dryness under nitrogen stream and reconstituted with mobile phase and was analyzed for retinol and carotenoids using a reverse phase, isocratic HPLC system equipped with Millenium software 2010 (Waters). The system consisted of 510 monopump (Waters), Waters 490E multiwavelength detector is set 320 nm for retinol and carotenoids at 450 lamda using YMC-ODS-AL, size: S-5 μm 120 Å column of 250x4.6 mm inner diameter (YMC Co. Japan). The mobile phase was composed of acetonitrile 87%, tetrahydrofuran 10%, methanol 3%, triethylamine 0.1%. The chromatogram was run for 15 min at flow rate of mobile phase at 1.5 ml/min. Retinol and carotenoids were quantified by determining the peak areas in HPLC chromatograms against standards curves. The lower limit of detection for retinol and carotenoids was 10 μg/L. Within-run coefficients of variation for retinol, lutein, β-carotene were 1.0%, 2.0%, and 1.4%, respectively. Between-run coefficients of variation for retinol, lutein, β-carotene were 2-4%.

A random breast milk sample was collected by a nurse or a trained interviewer according to method of Rice et al. (23). Breastmilk (5 ml) was collected in a clean plastic container by manual expression by the mother between 0900 and 1530 h from the breast that was not used for feeding the infant during the previous one hour or longer. All samples were collected in the field and transported within 1-2 hour of collection to the field laboratory on ice and shielded from light in a cold box. Upon arrival to the field laboratory, breast milk samples were gently swirled 15-30 seconds to ensure thorough mixing and aliquots placed into amber colored, labeled cryovials for storage at -20 °C for 2-4 weeks before transported to central laboratory in Dhaka for storage at -70 °C until analysis 3-4 mo later. Standard procedures were followed in collecting and handling breastmilk samples ((24)) and the HPLC method was used for determining breastmilk retinol concentration (25).

Data Analysis and Statistics

Numeric variables were examined for their distributions outliers and extreme values were identified and excluded from the analyses. Such values did not exceed 3-4% of the observations. Results are presented as mean and standard deviation for the normally distributed variables or median and interquartile range for the non-normally distributed variables. Differences between groups were examined by student's t-test for statistical significance. Changes in each of the outcome variables from baseline (pregnancy) to different follow-up periods were calculated for each subject by subtraction. Paired t-test was used to compare within group differences from baseline to the respective time period. Independent sample t-tests were used to compare changes at respective study periods between groups. Breast milk retinol concentrations were compared between intervention and control group by independent sample t-test of means. In this case, the change was not examined or tested because the intervention had been continuing for certain period before the first sample of breast milk was collected. Infants' mean plasma retinol, β-carotene and lutein concentrations were compared between groups by independent sample t-test. A p-value of <0.05 was considered statistically significant.

RESULTS

Baseline characteristics of 248 supplemented and 251 control women are presented in **Table 1**. Socioeconomic status, history of nightblindness and nutritional status were comparable between groups. Dietary intakes of carbohydrate, protein, and total energy were significantly higher in the control group than in the intervention group. During pregnancy, dietary fat intake was 6-8 energy%. The intervention group had a slightly higher fat intake compared to that of the control group and the difference reached statistical significance. Both groups had comparable but low mean plasma retinol concentrations and high prevalence of low plasma retinol. Both β-carotene and lutein concentrations were significantly higher in the control than in the intervention group at baseline.

Table 1. Characteristics of the study subjects at baseline (pregnancy).

	Oil supplemented N = 248	Control n = 251
Maternal age (y[1])	27 ± 6*	26 ± 5
Gestational age (wk)	24 ± 3	25 ± 3
Parity	2.3 ± 1.6	2.1 ± 1.5
Maternal illiteracy (%)	40	41
History of nightblindness (%)	3.2	2.4
Income (x,000 Taka)[3]	35 (20 - 49)	30 (20 - 49)
Anthopometric assessment		
Weight (kg)	45.2 ± 5.5	45.1 ± 5.4
Height (cm)	150.3 ± 4.9	149.4 ± 5.3
MUAC (mm)	227 ± 18	227 ± 18
BMI (wt kg/m^2)	20.0 ± 2.1	20.2 ± 1.9
Dietary intake		
Carbohydrate (g/d)	298 ± 87	339 ± 91***
Fat (g/d)	12 ± 9	10 ± 8*
Protein (g/d)	43 ± 21	48 ± 19**
Energy (kcal/d)	1378 ± 367	1527 ± 389**
β-carotene μ g/d	852 ± 1029	990 ± 1275
Plasma concentrations		
Retinol (μmol/L)	0.83 ± 0.27	0.85 ± 0.27
Retinol <.70 μmol/L (%)	34	29
β-carotene μmol/L	0.16 ± 0.07	0.17 ± 0.07*
Lutein μmol/L	0.64 ± 0.35	0.72 ± 0.33**

[1] Mean ± SD, [2] Percentages, [3]Median (25^{th}-75^{th} percentile) 1 US$ = 48 Taka
*,** Significantly different from the other group: *p < 0.05, **p < 0.01

As expected, the mean fat intake from all sources (including supplement) was significantly higher in the intervention group at one, three and six months postpartum than that of the control group (**Table 2**). Fat intake in the control group increased slightly by the 6 month postpartum measurement but it was not significantly different from earlier intakes. Supplementation with 18 g oil resulted in a doubling of fat in the intervention group compared to their baseline intake.

Table 2. Fat intake during postpartum.

		Intervention		Control
	n	Mean ± SD g/d	n	Mean ± SD g/d
One month PP	125	21.1 ± 12.5[1]	140	9.5 ± 6.9
Three month PP	114	23.1 ± 12.8[1]	97	9.3 ± 6.2
Six month PP	164	24.6 ± 11.2[1]	166	13.8 ± 9.6

[1]Significantly higher than the control group ($p < 0.001$).

Compared to the pregnancy (baseline) level, plasma retinol concentrations increased in the intervention women in all three measurements periods during the postpartum period (9%, 5%, and 0.4% at 1, 3, and 6 months postpartum, respectively), however the change at one month postpartum reached statistical significance (**Figure 1**). In the control group, compared to the pregnancy level, a small (~5%), but not statistically significant, increase in plasma retinol was observed at one month postpartum whereas the mean changes at three and six months postpartum indicated a decrease in plasma retinol concentration compared to pregnancy. The decline in plasma retinol in the control group compared to that of the pregnancy level (-8%) at three months postpartum was statistically significant. When the changes in plasma retinol concentrations at different stages during postpartum were compared between intervention and control groups, the change at three months postpartum in the intervention group was significantly different from that in the control group. The prevalence of vitamin of vitamin A deficiency did not differ significantly between groups although in the supplemented group it tended to be lower at 1 and 3 months postpartum (**Table 3**).

During the postpartum months, plasma β-carotene concentration declined compared to the pregnancy level in both intervention and control groups (**Figure 1**). The decline in the intervention group was not statistically significant except at 6 month postpartum when the highest decline in β-carotene concentration (-16%) was observed. In contrast, the control group experienced a substantial decline ranging from -23% to -28% of baseline value and was statistically significant at all three measurements. When the changes (declines) between groups were compared, the decline was significantly less in the intervention group at one and three month postpartum while that at six month was also less in the intervention group but did not reach statistical significance.

Table 3. Prevalence of vitamin A deficiency during postpartum months

Postpartum month	Group (n)	Plasma retinol		Breastmilk retinol	
		<0.70 μmol/L	<1.05 μmol/L	<0.70 μmol/L	<1.05 μmol/L
1 month	Oil supplemented (189)	23	63	40	68
	Control (221)	30	68	43	71
3 month	Oil supplemented (167)	28	68	63	84
	Control (143)	37	75	69	91
6 month	Oil supplemented (245)	31	75	48	73
	Control (240)	37	74	49	74

Plasma lutein concentration also declined during the postpartum measurements and followed the same trend as that observed in β-carotene (**Figure 1**). However, compared to the pregnancy level, within group change (decline) in lutein concentrations during postpartum period was significant in all three measurements in both study groups. When the changes in lutein were compared between intervention and control groups, the decline was significantly less in the supplemented group, and the supplemented women comparatively maintained a significantly higher level of lutein at one and three month postpartum but not at six month postpartum.

Breastmilk mean retinol concentrations were higher in the intervention group than that in the control group at one and three month postpartum (0.06 and 0.13 μmol/L respectively) and the difference was statistically significant at three month postpartum (**Figure 2**). However, by 6 months postpartum there was no statistically significant difference in breast milk retinol concentration between intervention and control groups.

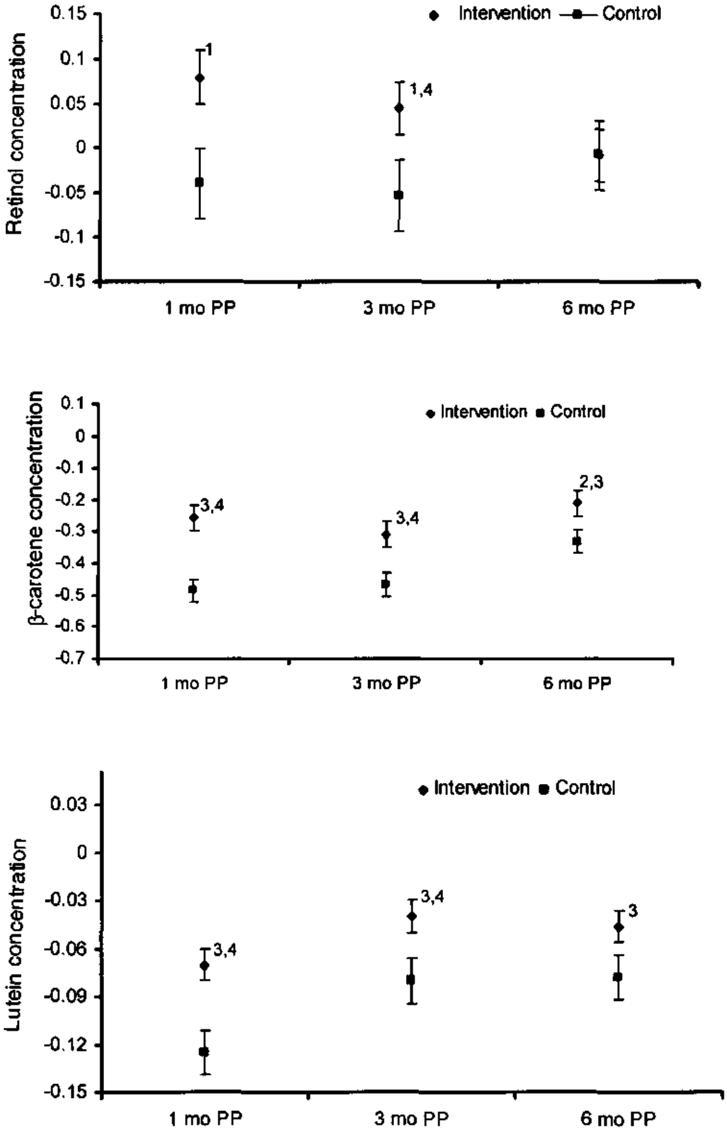

Figure 1. Mean changes in plasma (μmol/L) from baseline (pregnancy) to 1, 3, and 6 month postpartum.

[1,2,3] Significantly different from zero: [1]$p<0.05$, [2]$p<0.01$, [3]$p<0.001$

[4]Significantly different from the control group: $p<0.05$

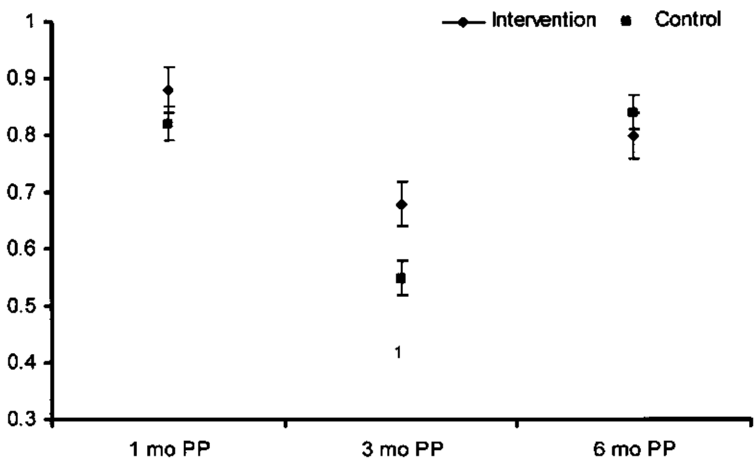

Figure 2. **Mean breastmilk retinol concentrations during first six month of lactation**

[1]Significantly different from the control group: p<0.03

No significant differences were observed in plasma retinol, β-carotene or lutein concentrations between infants born to supplemented mothers and those born to control mothers (**Table 4**).

Table 4. Infants' plasma retinol, β-carotene, and lutein concentrations at six month.

		n	Mean ± SD μmol/L
Retinol	Supplemented	54	0.50 ± 0.15
	Control	66	0.48 ± 0.17
β-carotene	Supplemented	15	0.10 ± 0.01
	Control	19	0.10 ± 0.01
Lutein	Supplemented	54	0.28 ± 0.17
	Control	66	0.29 ± 0.17

DISCUSSION

The aim of this study was to examine the effect of dietary fat supplementation on vitamin A status of pregnant and lactating women and their infants in a population where vitamin A deficiency is prevalent and the habitual dietary fat intake is low. The results of this study show that in a population of women with very low dietary fat intake, increasing dietary fat through supplementation had significant positive effect on maternal vitamin A status during early lactation. This was indicated by improvement of blood retinol and carotenoids concentrations as well as improvement in breastmilk retinol concentrations in the supplemented group.

Several factors that have potential implications for interpretation of our results need further elaboration. We aimed to increase fat intake by 18 g per day in the intervention group but the actual increase was less in many individual women. This was not unexpected since administration of the supplement was ultimately controlled by the study subjects themselves and not by one of the study team. Further, consumption of oil in this fashion, that is in a medicinal dosing, is not a normal dietary practice. Nonetheless, supplementation resulted in a doubling or more of fat intake in our population.

The supplemented group increased plasma retinol concentrations at 1, 3, and 6 month postpartum measurements (9, 5 and 4 % above the baseline level) while an increase in the control group was observed only at the one month postpartum measurement (5%) and a slightly declining tendency was noted at three and six months postpartum (-8 and -1%). The change compared to the baseline level at one month postpartum in the intervention was statistically significant, suggesting that dietary fat had a positive effect on plasma retinol concentration of the supplemented women. Both groups showed lower plasma β-carotene and lutein concentrations during postpartum however, relatively less so in the supplemented group indicate that provitamin A bioavailability was probably higher in the oil supplemented women. These findings are consistent with earlier reports that fat supplementation in deficient population has beneficial effects on retinol and carotenoids concentrations (15;26;27).

In our study population plant sources are the major contributors of dietary vitamin A, with over 80% of vitamin A contributed by plant sources (unpublished). Therefore, improvement in biochemical parameters in the dietary fat supplemented group compared to the control group can be attributed to improved bioavailability of the provitamin A carotenoids and their subsequent conversion. These results are consistent with earlier observations in children in which dietary fat enhanced bioavailability of vitamin A and carotenoids (26;28;15). Although only a small improvement in vitamin A and carotenoids concentrations in the intervention was observed in this study, the absolute level of vitamin A was very low in both groups, and even lower than those reported from other developing countries (29). This may be due to overall lower dietary intake of provitamin A by Bangladeshi women (30) and possibly low intake of other essential nutrients related to vitamin A metabolism such as dietary protein and zinc in particular (31). Becuase the dietary source of vitamin A in this population is of plant origin, the absolute intake rarely meets the Recommended Daily Allowance (RDA) during most of the year even when the traditional conversion factors ($1/6^{th}$ for β-carotene and $1/12^{th}$ for other carotenoids) are used (30). Actual vitamin A content in diet in this population would be much lower if recently recommended conversion factors are applied (9;32). A recent study in Indonesia reported a poor plasma response in terms of retinol concentration with of provitamin A caroteoids obtained from green leafy vegetable, and this has raised concern whether the strategy to promote vegetable consumption would have any significant role in ameliorating vitamin A deficiency (12). However, this study was not designed to evaluate the effect of increase in dietary fat on vitamin A and carotenoids status.

Our women in the supplemented group had relatively higher concentrations of breastmilk retinol at one and three months postpartum compared to the control group, although both groups had mean concentrations less than the adequate level (<1.05 μmol/L, the cut-off for adequacy of breastmilk retinol concentrationl). The increase in breastmilk retinol concentration in the supplemented group translates into 8 –18 μg daily additional preformed retinol delivery in their infants (assuming a breast milk intake of 700 ml/day), an amount equivalent to 4-10% of the basal requirements for the infants up to the age of six months (6). The supplemented mothers had comparatively better vitamin A status during early lactation, a critically important period for the newborns (29;33;34). Any improvement in early lactation would be expected to have significant impact in developing countries because exclusive breast feeding is greatest during this period. A study in rural Bangladesh showed that breast milk is the only source of vitamin A during infancy (30). This study also found that infants between 7-12 months of age who were just weaned had a daily intake of 3 RE/day of vitamin A and concluded that infants in Bangladesh have virtually no other source of vitamin A than breastmilk during their first year of life.

Our findings are consistent with other studies in developing countries where breastmilk retinol level has been found to be either deficient or at marginal range (29;35). Of interest, the breast milk retinol found in our rural population was lower than that of a similarly disadvantaged urban population in Bangladesh (36). The urban population may have greater access to foods of animal origin and might have higher dietary fat intake than in this rural community.

We have observed a substantial fluctuation in breastmilk retinol concentrations. These fluctuations in breastmilk retinol concentration have also been reported by others (37). It is also known that breastmilk retinol concentration is highly dependent on breast milk fat concentration (38). Furthermore, fat is the most variable macronutrient in human milk and reflects maternal diet (39).

There was no difference observed in infants' plasma retinol, β-carotene or lutein concentrations. Possible reason might be the infants' vitamin A status was measured at after six months of age when the difference in maternal vitamin A status between supplemented and control mothers did not exist. It might also be that the benefit to the infants of the intervention mothers, if had occurred in early infancy, might not have been large enough to maintain a sustained higher concentration up to six months of age. Although the intervention mothers had a better breastmilk retinol concentration during early lactation, it is notable that in most of the women in both groups retinol concentration was always less than 1.05 μmol/L, the concentration considered to be just enough to meet the basal requirement of the infant but not enough to build good vitamin A stores (5).

In summary, we conclude that a small amount of dietary fat supplementation during pregnancy and lactation has a beneficial effect on maternal vitamin A status during lactation in populations where plant sources are the major contributors to dietary vitamin A intake and fat intake is extremely low. However, it is notable that the mean plasma retinol concentrations in this population were far below that of the developed countries (40-42) and even lower than those reported from other developing countries (35;43;44). Therefore, it is highly probable that this population probably requires multiple interventions to improve vitamin A substantially, including increased consumption of vitamin A containing-foods in addition to the promotion of increased dietary fat intake.

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3

Validity of IVACG Simplified Dietary Assessment (SDA) in identifying pregnant and lactating women at risk for vitamin A deficiency in rural Bangladesh.

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ABSTRACT

Background. The Simplified Dietary Assessment (SDA) proposed by IVACG has been successfully used in identifying populations of children at risk for vitamin A deficiency (VAD). However, its validity in other vulnerable groups has not been defined.

Objective. Assess the validity of the vitamin A focused dietary assessment to identify pregnant and lactating women at risk for vitamin A deficiency (VAD) in rural Bangladesh.

Methods. The IVACG modified SDA questionnaire was administered and plasma retinol concentration measured at 5-7 mo of pregnancy (n=264), and at 1 (n=200), 3 (n =128) and 6 mo (n=212) postpartum (PP). Vitamin A intake was estimated as Consumption Index (CI) and Usual Pattern of Food (UPF) scores (1 score ≅ 120 μg RE) from dietary intakes in the last 24-h and frequency of consumption in the preceding one month respectively. Based on derived scores, women were classified into *high* (CI<5/UPF<120), *moderate* (CI 5-7/UPF120-210), and *low* (CI>7/UPF>210) risk categories. Vitamin A deficiency was defined as plasma retinol concentration <0.70 μmol/L. Women were screened for VAD using different CI/UPF scores cut-off values.

Results. The mean of CI scores indicated the women to be at *high* risk for VAD during pregnancy and at 1 mo PP and at *moderate* risk at 3 and 6 mo PP while UPF scores indicated *moderate* risk throughout but *low* risk at six mo PP. CI and UPF scores classified 56-73% and 24-33% women respectively at high risk for VAD. The prevalence of VAD ranged between 33% and 42% at various stages of the study. Both sensitivity and specificity were widely variable by both CI and UPF criteria but the false positive rate was consistent at 60% or higher.

Conclusion. The SDA can be a used as an additional tool to characterize risk for VA deficiency in populations of pregnant and lactating women. Not unexpectedly, there is an unacceptably high possibility for misclassification of individuals.

Key words. Simplified Dietary Assessment, Vitamin A status, pregnant and lactating women, validity, Bangladesh.

INTRODUCTION

Vitamin A deficiency (VAD) is widespread among young children in many developing countries and continues to be a major public health problem (1). Subclinical vitamin A deficiency is associated with significantly increased risk of morbidity and mortality (2-4). More recent data indicates that VAD is also common in women of reproductive age (5).(6) Supplementation with vitamin A or β-carotene in a deficient population during pregnancy was reported to reduce maternal mortality by nearly a half although they did observe any effect on morbidity (7). Because of the important role of vitamin A in human health and survival, it has been of increasing urgency to develop an inexpensive, rapid and practical field tool for assessment and monitoring of the risk for VAD among vulnerable populations.

Commonly employed assessment methods are based on clinical and biochemical measures that have the relative advantages and disadvantages, however are often not appropriate or feasible for large-scale population based evaluations (8). In contrast, dietary methods are promoted as rapid, non-invasive, relatively inexpensive, and suitable for large-scale population assessment. The International Vitamin A Consultative Group (IVACG) has earlier proposed a Simplified Dietary Assessment (SDA) method to identify populations at risk for vitamin A deficiency in areas where dietary intake and behaviour is not clearly known (9). Although this method was designed to identify groups of children at risk for vitamin A deficiency, the principles described in the guidelines for development of questionnaires might also be applicable in other vulnerable groups. Intake of vitamin A estimated by SDA method has been reported to be consistent with other more detailed and complex dietary methods (10;11). A study in young children in Thailand also indicated that the SDA has potential to identify groups at risk for VAD when compared with biochemical indicators of vitamin A status (12). However, information on the validity of the SDA is non-existent for pregnant and lactating women. The SDA, if established to be a valid field tool in these vulnerable groups, might complement other assessment methods and perhaps serve as an independent, noninvasive and complementary or alternative method for assessing risk of VAD.

In this study, we examined the validity of the SDA as field tool to assess risk for VAD in pregnant and lactating women.

SUBJECTS AND METHODS

Subjects and study design

The study was conducted in eight villages in Matlab, a rural sub-district of Chandpur District in south eastern Bangladesh, between October 1995 and April 1997. A baseline survey was conducted to identify all pregnant women in the selected villages and was continued during the study period to identify pregnant women. Those at 5-7 month of pregnancy and without any known chronic illness were eligible for participation into the study. A field team consisting of a local Community Health Worker (CHW), one Health Assistant or a nurse, one dietary interviewer and a logistical assistant (porter) visited each eligible woman at home. All members of the field team were trained on administering the questionnaires and other data collection procedures. The characteristics of the study were explained to each woman and informed written consent was obtained before enrollment. A detailed interview was conducted on socioeconomic, demographic and household characteristics, and history of nightblindness, and responses were recorded on a precoded questionnaire. In total, 335 women were recruited during the study period. During the first visit, a Simplified Dietary Assessment (SDA) questionnaire (see below) was administered, and a venous blood sample was collected. Weight, height, and MUAC were also measured following standard procedures (13) and body mass index (BMI) was calculated from weight and height data (kg /m^2). SDA data and blood samples were additionally collected at one, three and six months postpartum.

Of the 335 initially recruited, 315 delivered live singleton babies. Twenty-two women refused continued participation after delivery and eight were omitted due to their infants' death. Only subjects who delivered singletons and with results of plasma retinol and simplified dietary assessment (SDA) during the respective data collection period were included in the analysis. At baseline, complete data were available for 264 women (**Table 1**). The major reasons for incomplete data at baseline were insufficient plasma sample and/or precipitated plasma samples that could not be analyzed on HPLC. At baseline, the mean age of the women studied was 26 years and they were enrolled at a mean of 25 weeks of gestation. Most households had current vegetable producing home gardens. During the postpartum period, only individuals with both plasma retinol values and SDA scores were included in the analysis. Therefore, complete data were available for 200 women at one month postpartum, 128 at three months postpartum and 212 at six months postpartum. Missing data during the postpartum periods, particularly at one and three months postpartum, occurred primarily because women could not be visited on schedule due to socio-political unrest prevailing in Bangladesh including the study area, during the scheduled period. However, baseline socio-economic, demographic and biochemical characteristics of subjects who were included in the analysis did not differ significantly between those not included at different stages of the study.

Development and Application of Simplified Dietary Assessment (SDA)

Since the original IVACG guidelines for developing questionnaires to estimate vitamin A intake were intended for young children (9), modifications were necessary to develop a questionnaire applicable to women of child bearing age. The main modification was done in categorizing foods according to their vitamin A content in small serving size. For example, in case of children in any small serving of a food contains >250 μg RE then it is categorized as *high* in case of pregnant and lactating women >600 μg RE was required for the food to be categorized as *high* vitamin A containing food. Details are described in section 4 below.

Development of the Simplified Dietary Assessment (SDA) Questionnaire involved the following steps:

1. All sources of vitamin A for the study population were catalogued through market and household surveys, agriculture information, and individual interviews. Foods containing less than 10 RE per 100 g were not included in the list. Vegetables or other foods that were not marketed commercially but consumed by the local population (mainly wild leafy vegetables) were also listed. The result of this food survey was a comprehensive list of the potential key contributors to vitamin A intake in that community.
2. Information on methods of food preparation and recipes for multi-ingredient foods was collected by interviews of women from the study area (both participants and non-participants), and also of the Community Health Workers (CHWs) who belonged to the same community.
3. Portion sizes (small, medium and large) of all available foods in the list prepared in the first step were determined by direct observation. A randomly selected group of 37 study participants was also asked to prepare those foods at home. All ingredients used in preparation of particular a food were measured before cooking and the total amount of cooked food was measured to determine raw equivalent per unit portion of the cooked food. Each participant in the cooking demonstration sessions was asked to serve what they consider a small serving of that particular food for herself with her commonly used household measure. The average amount of the food contained in a small serving was calculated from all the small servings and was considered as the representative small portion size for that particular food for women in that community. Medium and large servings were not measured but assumed to be twice and four times the small serving, respectively.
4. The retinol equivalent (RE) of vitamin A activity for the small usual serving size of each food was calculated from the Food Composition Table appropriate for Bangladeshi foods ((14;15). Conversion factors of 6 and 12 μg for 1 μg RE were *used for betacarotene and other provitamin A carotenoids respectively* as suggested in the SDA guidelines. In the original questionnaire as designed for children, a food was classified as a *high*, *moderate* or *low* vitamin A (VA) *containing food if* the small serving size contained >250 μg, 50-250 μg and <50 μg RE respectively, which is about 14%, 14-71% or >71% respectively of their RDA assuming that RDA for the preschool children is 350 RE. Based on this concept, *and considering the RDA for pregnant women* ((16) *in this study*, a proportional adjustment was made in classifying foods. Foods were graded '*high VA*',

'moderate VA', or 'low VA' if the small serving of those foods contained >600, 600-120, or <120 RE respectively. Although the RDA is higher for the lactating women than that of pregnant mothers, separate classifications for pregnancy and lactation were not made to minimize complexity.

5. Based on the information collected through the above steps, a precoded Simplified Dietary Assessment questionnaire was developed which contained classification of foods, serving size and frequency of consumption. The questionnaire had two parts: one for recording the intake of VA containing foods during the last 24 hours, and the other part for recording frequency of intake over the last one month (daily, weekly or monthly) as a measure of more usual intake. Before actual data collection, the questionnaire was pretested and necessary modifications were made for clear understanding. Trained dietary interviewers administered the questionnaire and were supervised by senior field staff.
6. From the food consumption data, the Consumption Index (CI) was derived by converting all servings consumed during the last 24-hr into their small serving equivalent of low VA score. For such conversion, multiplication factors of "2" and "4" for medium and large serving sizes respectively, and "3" and "5" for vitamin A ranking of moderate and high respectively were used. This procedure resulted in a score of 5 for a small serving of high vitamin A score food which is equivalent to an intake of 600 RE (5x120). A total daily CI score of 7 would be equivalent to 840 RE, approximately the RDA for the study subjects, and an intake at that level is assumed to maintain normal store without depletion. In contrast, a CI score of less than 5 is likely to result in depletion of body store of vitamin A and place the subject at high risk for VAD. Based on this concept, CI risk categories are created as '*high risk*' (CI score <5), '*moderate risk*' (CI score 5-7) and '*low risk*' (CI score >7).
7. The Usual Pattern of Food (UPF) consumption score was calculated based on frequency of consumption of VA containing foods over the last one month (daily, weekly or monthly). According to the SDA guidelines, daily consumption of one small serving of *high* VA containing food would give a score of 150 (5x30 d) and a score of 60 (5x3 days/week x 4) for weekly consumption (assuming average 3 days/week), and a score of 15 (5 x 3) for monthly (average 3 days/month) consumption respectively. The score is then down graded for moderate and low VA score foods as $3/5^{th}$ and $1/5^{th}$ respectively. Adding the scores contributed by all the foods reported derives the total score of each individual. The risk categories by UPF score are then created based on the same concept as CI risk categories: '*low risk*', if the score is >210 (equivalent to daily CI of >7x30), '*moderate risk*' (120-210) and '*high risk*' (<120) respectively.

Plasma retinol concentration

Plasma retinol concentration was measured during pregnancy (enrollment), and at one, three and six month postpartum. Blood specimen of 0.5 ml was collected in lithium heparinized microtainers either by finger prick or antecubital venepuncture. Samples were collected at home except the last round when it was collected in the clinic. Blood was immediately put on ice in a cold carrier and transported to the field laboratory within 3-6 hours of collection. In the laboratory, plasma was separated by centrifugation (3000 rpm for 5 minutes) and transferred to labeled screw-top amber colour cryovials and stored at –20 ^{O}C for 2-4 weeks in the field laboratory before transfer to the central laboratory in Dhaka (transportation time 2-3 h) for storage at –70 ^{O}C and subsequent analysis. Simultaneous determination was done of serum retinol, lutein and beta-carotene by HPLC in the Nutritional Biochemistry Lab of ICDDR,B (17). Briefly, an aliquot of plasma was deproteinized with ethanol containing internal standard (IS) and extracted in hexane. Tocol was used as the internal standard for retinol and beta-apo-8'-carotinal as the internal standard for carotenoids. The extracted layer was evaporated to dryness under nitrogen stream and reconstituted with mobile phase and was analyzed for retinol and carotenoids using a reverse phase, isocratic HPLC system equipped with Millenium software 2010 (Waters). The system consisted of 510 monopump (Waters), Waters 490E multiwavelength detector is set 320 nm for retinol and carotenoids at 450 lamda using YMC-ODS-AL, size: S-5 μm 120 Å column of 250x4.6 mm inner diameter (YMC Co. Japan). The mobile phase was composed of acetonitrile 87%, tetrahydrofuran 10%, methanol 3%, triethylamine 0.1%. The chromatogram was run for 15 min at flow rate of mobile phase at 1.5 ml/min. Retinol and carotenoids were quantified by determining the peak areas in HPLC chromatograms against standards curves. The lower limit of detection for retinol and carotenoids was 10 μg/L. Within-run coefficients of variation for retinol, lutein, β-carotene were 1.0%, 2.0%, and 1.4%, respectively. Between-run coefficients of variation for retinol, lutein, β-carotene were 2-4%.

Data analyses and statistics

Distributions of all variables were examined and extreme values were identified and excluded from the analysis, however, such values did not exceed 4% of the observations. Results are presented as mean and standard deviation for normally distributed variables and as median and interquartile range for non-normally distributed ones. Linear relationship of CI and UPF scores with plasma retinol concentration was examined by Spearman rank correlation. Subjects were classified into different risk categories for vitamin A deficiency using cut-off values of SDA scores suggested in the SDA Guidelines. Relationships between different SDA risk categories and plasma retinol concentrations were compared by one-way ANOVA and that between SDA risk categories and vitamin A deficiency (plasma retinol <0.70 μmol/L) was examined by chi-square test. Sensitivity, specificity and predictive values were calculated for SDA using plasma retinol cut-off <0.70 μmol/L for vitamin A deficiency as the gold standard. All analyses were performed using SPSS 7.5 for Windows.

RESULTS

Dietary intake of vitamin A as estimated by Consumption Index (CI) and Usual Pattern of Food (UPF) Consumption scores obtained through the SDA questionnaire are presented in **Table 2**. The mean value of CI scores indicated that a majority of women were at *high* risk during pregnancy and one month postpartum, and at *moderate* risk for vitamin A deficiency (VAD) at three and six months postpartum. The UPF scores indicated a *moderate* risk for VAD throughout the study period except at six months postpartum when the risk for VAD was *low*. Women had low mean plasma concentration (**Table 2**). The mean value of plasma retinol concentration was slightly higher at one month postpartum compared to other periods but the difference was not statistically significant. The prevalence of VAD ranged between 30 and 38 % during the study period. While over 70% women had inadequate vitamin A status (plasma retinol <1.05 μmol/L).

CI scores had low but significant correlation with plasma retinol concentration during pregnancy, and 1 and 3 months postpartum ($r=0.122$, $p<0.05$; $r=0.194$ $p<0.05$; and $p=0.274$, $p < 0.05$ respectively) but had no correlation at six months postpartum. UPF scores had significant correlation with plasma retinol concentration at one and six months postpartum ($r=0.141$, $p<0.05$ and $r=0.162$, $p<0.05$ respectively).

Risk categorization for vitamin A deficiency by CI and UPF scores differed significantly (Table 3). CI scores classified higher number of women (56 - 73%) at '*high*' risk for VAD than UPF scores, which classified 24 to 36% percent of women at *high* risk (Table 3). The mean plasma retinol concentration was significantly lower among *high* risk women by CI scores at pregnancy and one month postpartum but not at 3 or 6 months postpartum. Also the prevalence of VAD were significantly higher during the corresponding periods among the *high* risk group. While high risk women by UPF scores had significantly lower mean retinol concentration at one month postpartum only. However, the prevalence of vitamin A deficiency did not differ significantly by UPF risk categories at any time during the study.

The sensitivity of SDA CI scores in identifying women at risk for vitamin A deficiency was *relatively* greater during pregnancy and one month postpartum than during 3 and 6 months postpartum (Table 4). During pregnancy and one month postpartum, 81 to 90% of women with vitamin A deficiency were identified by CI scores cut-off <5 (<~600 μg RE/d) or <=7 (<~840 μg RE/d) respectively. However, the specificity was much lower and ranged between 23 and 36% during the same period. At 3 and 6 months postpartum, CI scores with similar cut-off values correctly identified 48 to 67% women with VAD while only 27 to 43% of women without VAD were correctly identified. The UPF score showed poorer sensitivity but higher specificity at corresponding risk level. Between 25 and 45% of women with VAD were identified when UPF cut-off value <120 was used but the specificity ranged between 66 and 78%. The sensitivity was substantially increased when the cut-off values was raised to <=210 when 62 – 73% women with VAD were correctly identified but the specificity decreased to as low as 26% at 1 mo postpartum and 50% at 6 months postpartum. The false positive rate was high and consistent with both CI and UPF criteria and was at 60% or higher during any assessment period.

Table 1. Characteristics of the study subjects at baseline (n = 264).

Maternal age (years)	26 ± 5[1]
Gestational age (weeks)	25 ± 3
Parity	2.1 ± 1.5
Maternal illiteracy (%)	44
Night blindness (%)	2
Household annual income (Taka)[2]	30,000(20,000 – 48,000)[3]
Family size	5.6 ± 2.8
Landless households (%)	42
Household own <1/2 acre land (%)	97
Households have homegarden (%)	83
Anthopometric assessment	
Weight (kg)	45.2 ± 5.4
Height (cm)	150 ± 5
MUAC (mm)	227 ± 18
BMI (Wt_{kg}/Ht_m^2)	20 ± 2

[1] Mean ± SD, [2]1 US$ = ~Taka 45, [3]Median (25th-75th percentile)

Table 2. Vitamin A status, and dietary intake of vitamin A assessed by Simplified Dietary Assessment.

	Pregnancy n = 264	1 mo. postpartum n = 200	3 mo. postpartum n = 128	6 mo. postpartum n = 212
Plasma retinol				
μmol/L (Mean ± SD)	0.84 ± 0.28	0.90 ± 0.37	0.83 ± 0.33	0.83 ± 0.31
<0.35 μmol/L (%)	3	6	4	5
<0.70 μmol/L (%)	30	30	38	37
<1.05 μmol/L (%)	74	70	76	74
Dietary Intake				
CI Score (Mean ± SD)	4.1 ± 5.9	3.3 ± 5.4	5.1 ± 5.7	5.9 ± 6.6
Risk for VAD	High	High	Moderate	Moderate
UPF Score (Mean ± SD)	199 ± 129	165 ± 98	189 ± 96	222 ± 136
Risk for VAD	Moderate	Moderate	Moderate	Low

Table 3. Plasma retinol concentrations and prevalence of vitamin A deficiency by SDA risk categories.

	Pregnacy N=264			One month PP N=200			Thre months PP N=128			Six months PP N=212		
UPF Risk category	n (%)	Retinol Mean ± SD	Retinol %<0.70	n (%)	Retinol Mean ± SD	Retinol %<0.70	n(%)	Retinol Mean ±SD	Retinol %<0.70	n (%)	Retinol Mean ± SD	Retinol %<0.70
High (<120)	87(33)	0.86 ± 0.30	39	72(36)	0.81 ± 0.32	38	32(25)	0.78 ± 0.30	37	50(24)	0.75 ± 0.28	42
Moderate (120-210)	82(31)	0.84 ± 0.28	33	76(38)	0.96 ± 0.36	22	49(38)	0.79 ± 0.32	41	65(31)	0.83 ± 0.32	43
Low (>210)	95(36)	0.84 ± 0.26	30	52(26)	0.93 ± 0.41	31	47(38)	0.92 ± 0.33	36	97(45)	0.87 ± 0.31	31
p value*		NS1	NS		0.02	0.048		N.S	NS		0.067	NS
CI Risk category												
High (<5)	182 (69)	0.82 ± 0.29	36	147(73)	0.86±0.37	36	74(58)	0.80±0.30	39	118(56)	0.85±0.31	32
Moderate (5-7)	25 (9)	0.90 ± 0.28	28	14(7)	1.10±0.27	0	13(10)	0.80±0.32	31	25(12)	0.83± 0.29	32
Low (>7)	57 (22)	0.92 ± 0.25	14	39(20)	1.04±0.36	18	41(32)	0.90±0.37	38	69(32)	0.79±0.32	48
p value		0.02	0.008		0.01	0.004		NS	NS		NS	NS

*One way ANOVA/χ^2 test

[1]NS – Statistically not significant

Table 4. Sensitivity, specificity and predictive values of Consumption Index (CI) and Usual Pattern of Food (UPF) as compared to vitamin A deficiency assessed by plasma retinol concentration <0.70 μmol/L during pregnancy and postpartum (PP).

	Cut-off point	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Pregnancy (n=264)	CI <5	81*	36	36	82
	CI <=7	90	27	35	86
One mo PP (n=200)	CI <5	88	33	36	87
	CI <=7	88	23	33	82
Three mo PP (n=128)	CI <5	59	43	39	63
	CI <=7	67	32	38	61
Six mo PP (n=212)	CI <5	48	40	32	56
	CI <=7	58	27	32	52
Pregnancy (n=264)	UPF <120	31	66	29	69
	UPF =210	65	36	31	71
One mo PP (n=200)	UPF <120	45	68	38	74
	UPF =210	73	26	30	69
Three mo PP (n=128)	UPF <120	25	75	38	62
	UPF =210	65	38	40	64
Six mo PP (n=212)	UPF <120	27	78	42	64
	UPF =210	62	50	43	69

*Numbers are percentages

DISCUSSION

This study applied the IVACG's Simplified Dietary Assessment (SDA) method, modified for application in pregnant and lactating women to estimate vitamin A intake and to assess risk for vitamin A deficiency in a rural area of Bangladesh. Vitamin A intake assessed by the SDA method indicated a high or moderate risk for VAD which was consistent with the low plasma retinol concentrations and high prevalence of vitamin A deficiency. There was poor correlation between SDA scores and plasma retinol concentrations, and high rate of misclassification individual women with regard to their vitamin A status indicate poor utility of the SDA method in identifying individual women for specific dietary intervention. This was not unexpected because the SDA was designed to assess populations, not individuals, at risk for poor VA intake and VAD.

Women were at *high* risk for VAD during pregnancy and one month postpartum and at *moderate* risk at three and six months postpartum by CI scores criteria, while during the corresponding periods the UPF scores classified the same women at moderate and low risk respectively. It appears, therefore that CI scores classified women relatively at greater risk than UPF scores. We also observed that the proportion of women at *high* or *moderate* risk was greater by CI than UPF scores and that was due to differences in dietary information captured by CI and UPF scores. Since the CI score is derived from last 24 hour intake that reflects women's current intake, any woman not consuming vitamin A containing foods on that particular day was naturally classified as *high* risk. In fact, over 40% women did not report any vitamin A food intake during the last 24 hour. On the other hand, UPF scores reflects intake over longer period and women were therefore unlikely to report no intake of vitamin A food during the period under question. Furthermore, CI scores are derived from both frequency and amount of vitamin A containing foods consumption while UPF scores require only the frequency of consumption and therefore less vulnerable to misreporting. Such misreporting is more likely to happen in case of foods of low social value such as green leafy vegetables. However, both CI and UPF scores classified women as group at risk for VAD in that community. The estimates of vitamin A intake by SDA methods for the groups were consistent with their low mean plasma retinol concentration and high prevalence of vitamin A deficiency.

It is essential to determine whether inadequate vitamin A intake as estimated by the SDA related to biochemical signs of vitamin A deficiency. Data from both individual pregnant and individual lactating women showed poor linear association between SDA scores and plasma retinol concentrations although it occasionally reached statistical significance. Dietary intake estimated by SDA, therefore, has very poor explanatory power to predict plasma retinol, which contradicts the basic principle for risk categorization that assumes dietary intake would determine the risk for vitamin A deficiency. This was evident when the mean plasma retinol concentrations and the prevalence of vitamin A deficiency were compared among different risk categories, which showed an inconsistent pattern (**Table 3**).

Several factors including methodological constraints of the SDA might explain the low correlation between SDA scores and plasma retinol concentrations. The SDA estimates vitamin A intake by a weighing factor "score" and does not measure the actual intake by physical units such as μg retinol equivalent (RE). As the SDA scores are derived through several steps of data reductions, they are likely to be error prone. Therefore, different subjects with the same score may have different amounts of actual vitamin A intake in terms of RE. Such potential is higher for certain foods assigned with moderate score vitamin A foods, which range between 120 – 600 μg

RE. Therefore, scores derived from foods close to the lower or upper spectrum of this group may lead to erroneous estimation of vitamin A intake. Again SDA scores do not differentiate sources of food, animal or plant, contributing to the scores. Therefore, plasma response to same SDA scores derived from different food sources is likely to be different due to their differences in bioavailability (18). This indicates the conversion factors used in SDA method might not be appropriate. The SDA vitamin A focused method does not take account of other nutrient intakes. Low dietary fat is one of the well recognized limiting factor for provitamin A carotenoid bioavailability in poor communities and supplementation has been shown to have beneficial effect (19;20). A recent study, which examined the validity of a food frequency method as field tool for assessing vitamin A deficiency reported strong correlation between dietary protein intake (among all dietary components) and community prevalence of low serum retinol (21). Other potential variables include maternal infections which may cause abnormal catabolism and excretion of vitamin A (22;23).

The main purpose of the SDA is to identify populations with inadequate intake of vitamin A and therefore at risk for vitamin A deficiency for dietary advice or intervention. It is therefore important to assess how well SDA categorize individuals at risk for vitamin A deficiency compared to biochemical criteria. With CI scores risk cut-off, identification of vitamin A deficient subjects varied over time. During pregnancy and one month postpartum between 80 and 90% subjects with vitamin A deficiency were identified and that at three and six months postpartum ranged between 48 and 67%. It is important to note that the shifting of CI cut-off value from <5 (*high risk*) to <=7 (*moderate risk*) did not have much effect on the sensitivity or specificity. This was due to large number of women had scores less than 5 and only a few women had scores between 5 and 7. UPF scores showed poorer sensitivity but better specificity at corresponding cut-off values. However, as opposed to CI scores, change in cut-off values in UPF scores had substantial effect on the sensitivity and specificity. This was due to more uniform distribution of subjects in different UPF risk categories. Notably nearly 60% or more women who had inadequate vitamin A intake identified by SDA had no vitamin A deficiency by biochemical criteria. These results indicate that SDA might identify substantial number of subjects with VAD when applied for assessing the magnitude of the problem (prevalence) but with low positive predictive value. On the other hand, if applied for evaluation of intervention when high specificity is required, SDA would not be optimally efficient in identifying those who do not have vitamin A deficiency. The results of this study suggest that dietary intake estimated by SDA is consistent with the population vitamin A status and CI scores provide relatively more accurate estimates of vitamin A intake than UPF scores. Although it may be used as an additional tool to assess the population intake, at individual level, there is high possibility of misclassification and therefore it would not be useful for identification of household with individual vitamin A deficient subject for dietary intervention.

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4

Energy stress during pregnancy and lactation: consequences for maternal nutrition in rural Bangladesh

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Abstract

Objective: To assess the relationship of energy stress during pregnancy and lactation to maternal body stores in marginally nourished rural Bangladeshi women.

Subjects and Methods: Two hundred fifty-two women were followed from 5-7 mo of pregnancy until 6 months postpartum. Energy intake was estimated during pregnancy and at 1, 3, and 6 month(s) postpartum using 24-h dietary recall. Body weight was measured on enrollment, another 1-2 time(s) during pregnancy, and at 1, 3, and 6 month(s) postpartum. The weekly pregnancy weight velocity and postpartum weight changes were determined. Weight and length of the infants were measured at birth and at approximately 1, 3, and 6 months of age.

Results: Maternal energy intake at 5 – 7 months of gestation was 1464±416 kcal/d (Mean±SD). Women gained a mean of 200 g/wk or a total of 4 kg during the second half of pregnancy. An analysis of maternal weight showed no evidence of accrual of fat stores during pregnancy. Dietary energy during lactation exceeded intake during pregnancy by 248-354 kcal/d. Mothers lost an average of 1 kg of weight during the first 6 months of lactation. The mean (±SD) birth weight was 2.55± 0.38 kg, and the prevalence of low birth weight (<2500 g) was 48%. Infants exhibited a small amount of catch-up growth only during the first 3 months but overall growth during the first 6 months of life did not change from their relative status at birth.

Conclusions: These rural Bangladeshi women failed to compensate for the energy stress during pregnancy and lactation. Growth of these primarily breastfed infants raises concern about the adequacy of lactation in this community that occurs at the cost of substantial depletion of maternal energy stores.

Keywords: Energy intake, pregnancy weight gain, postpartum weight, birth weight, infant growth, rural Bangladesh.

INTRODUCTION

Maternal energy balance during pregnancy, a complex and widely variable physiological process (1), is recognized as an important determinant of pregnancy outcome (2). Women should be in positive energy balance during pregnancy to support the energy demands of pregnancy and to build adequate fat reserves for additional energy requirements of late pregnancy and lactation (3). Major determinants of energy balance are energy intake and energy expenditure, with the degree of positive energy balance during pregnancy reflected in pregnancy weight gain. Although data from high income countries are abundant, very little is known about dietary energy during pregnancy and pregnancy weight gain of women in poor developing countries.

In healthy Western populations, weight at four weeks postpartum closely approximates weight at mid-pregnancy, and the weight in excess of pre-pregnant weight primarily represents fat mass accrued during the first half of pregnancy (4). This amount of fat accrual corresponds to about 2 kg in high income countries (3;5). Pregnancy weight gain in developing countries is about a half or less compared to that in developed countries, and there are indications that women often lose weight during the course of pregnancy (6-8).

Maternal energy requirements are greatest during lactation because of additional requirements (~600 kcal) for milk production (9). Energy demands of lactation are usually met through various strategies including increased energy intake, mobilization of fat from maternal reserves developed during pregnancy, and energy sparing through reduced physical activity and/or possible energy-sparing adaptations (10;11). However, in poor subsistence developing communities such options are not often available or met.

The quantity and quality of breast milk from different populations with diverse socioeconomic and nutritional status have been shown to be comparable (12). Therefore, the energy cost of lactation is presumed to be similar across well- and poorly-nourished women (13). However, as women in developing countries more often enter lactation without additional fat stores, they are likely to be at risk for adverse nutritional consequences unless the extra energy demand is met through enhanced dietary intake. While adequacy of lactation is difficult to measure, breastfed infant growth provides an indirect measure of adequacy during the early months. Evidence from poor developing countries suggest maternal lactation can support adequate infant growth during the first six months of age (14) although the consequences for maternal nutrition is not clearly known.

In this study we examined the extent to which energy stress during pregnancy and lactation affects maternal energy stores by analyzing maternal weight during pregnancy and weight changes during lactation in relation to their dietary energy intake.

SUBJECTS AND METHODS

Subjects and study design

This study was conducted between November 1995 and October 1997 in eight villages in Matlab, a rural sub-district in Chandpur district typical of the rural and riverine delta of Bangladesh (15). Pregnant women at 5-7 months of gestation without apparent chronic illness were eligible. Trained Community Health Workers

(CHWs) identified eligible subjects and new pregnancies. Written informed consent was obtained from each woman before enrollment.

An interview was conducted on socioeconomic, demographic and household characteristics, and obstetric history, and responses were recorded on precoded questionnaires. Weight, height and mid upper arm circumference were measured. Maternal weight was again measured one to two time(s) during pregnancy, and at 1, 3 and 6 month(s) postpartum. Maternal dietary intake was assessed by 24-hour dietary recall at entry, and at 1, 3 and 6 months postpartum. Weight and length were measured soon after birth (>80% within 72 hours after birth) and at about 1, 3 and 6 month of age.

In total, 335 women were enrolled during the study period of which 315 women delivered live singleton babies (1 false pregnancy, 3 miscarriages, 2 twin deliveries, 10 still births, and 4 unknown). Out of these 315 mothers, 252 had complete baseline anthropometric and dietary data, and had infant anthropometric data at birth (19 migrated out, 22 refused to continue and 22 had no maternal/infant anthropometric measurements). Characteristics of these 252 women are presented in **Table 1**. Baseline characteristics of the 83 women excluded were comparable with those included in the analysis.

Maternal Anthropometry

Anthropometric measurements were taken by trained workers following standard procedures (16). Weight of the women was measured by a portable battery-operated SECA electronic scale (Model 770) with a digital display and accurate to nearest 100 g. The scales were calibrated daily before use. Maternal height to nearest 0.1 cm was measured using a locally made wooden height stick fixed to a flat wooden platform and a movable head plate. Mid upper arm circumference (MUAC) was measured to the nearest one mm at the mid-point between the shoulder and elbow on the bare left arm with a non-elastic MUAC tape.

Body mass index (BMI: kg/m^2) was calculated from weight and height data. Weekly pregnancy weight gain was calculated for each mother by subtracting the weight at entry from the last weight measured in pregnancy and by dividing the difference by the number of weeks between the measurements. Pregnancy weights of all the mothers were plotted against gestational age and weight gain during pregnancy for the total group was also estimated using the slope of linear regression of maternal weight on gestational age. For each individual mother, monthly weight changes during the postpartum period were calculated by subtracting the weight measured at six month postpartum from that at one month postpartum. The pattern of maternal weight change during postpartum was examined using all the weight measurements available from all the mothers by regression of postpartum weights on the stage of postpartum.

Infant Anthropometry

Weights of the infants were measured using a portable paediatric SECA beam balance (accurate to10 g). The scale was calibrated daily by senior field staff, and checked carefully before each measurement. Infants were weighed nude during hot weather or light clothes on during cold weather, and in the latter situation, the weight subtracting the weight of the clothes was recorded as the infant weight. Weight measured within 72 hours or sooner after birth was recorded as the birth weight because of no significant difference in birth weight and weight measured within the first three days of birth (17). Infants' length was measured using a locally constructed wooden length board and measured to the nearest 0.1 cm. Weight and length were compared with the NCHS reference and converted to age and sex specific Z-scores using the ANTHRO software (18).

Maternal Energy Intake

Energy intake was estimated by the 24-hour dietary recall method following standardized procedures (16). Nutrient intakes were calculated using a computerized food composition table for Bangladeshi foods that was developed using nutrient data from the regional food composition databases (19;20). The computerized database was updated and supplemented for this study with the nutrient composition of multi-ingredient foods commonly consumed in the Matlab community. All commonly used local household measures were standardized (ml or g/unit) for respective food items during the initial phase of the study. Nutrient intake data were calculated using computer package KOMEET, developed at the Wageningen University, Wageningen, the Netherlands.

Data Analysis and Statistics

Results are presented as mean and standard deviation except where otherwise indicated. Group means were compared by Student's t-test or one way Analysis of Variance (ANOVA) when more than two group means were compared. Within subject repeated measure Analysis of Variance (ANOVA) was used to examine if there was any significant within subject difference in energy intakes during pregnancy and postpartum measurements. If the F statistic was significant in ANOVA, Bonferroni test, which takes account of multiple comparisons, was used post hoc to identify the individual means that were significantly different from one another. A p-value <0.05 was considered statistically significant. All data analyses were performed using SPSS PC version 7.5 for Windows.

RESULTS

During late pregnancy mean±SD daily energy intake was 1464±416 kcal/d or 33±10 kcal/kg/d (**Table 2**). Postpartum energy intake increased significantly as compared to women's intake during late pregnancy. The difference in mean (±SD) daily energy intake between late pregnancy and postpartum period ranged between 248±590 and 354±555 kcal/d (9±14 and 12±14 kcal/kg/d). Mean daily energy intake was significantly greater at three months postpartum than that at one or six months postpartum.

Pregnancy weight gain during later half of pregnancy was 0.20 kg/wk ($p < 0.05$) as estimated from the regression slope (**Figure 1**). As most women were measured more than once during pregnancy, the data points are not truly independent. However, measurements on the same women were well balanced throughout the pregnancy period. This rate of weight gain means that women gained an estimated 4-kg during the second half of the pregnancy in that population (i.e. 44.3 kg, and 48.3 kg at 20 and 40 weeks of gestation respectively). This estimated weight gain corresponded well with pregnancy weight gain (0.20 kg/wk, $p<0.001$) that was calculated for individual women (**Table 1**).

During the postpartum period, mothers' weight tended to decline. The regression slope derived from plotting maternal weight on postpartum week indicated a weekly weight loss of 46 g although it did not reach statistical significance (**Figure 2**). Maternal weights were clustered around 5, 15 and 28 weeks, however, each woman contributed maximum once in each of those clusters of measurements. According to the obtained regression line, maternal weight was estimated to be 41.8 kg and 40.8 kg at one and six months respectively, indicating a weight loss of 1 kg between 1 and 6 months postpartum. Weight of each individual mother measured between 1 and 2 months and again between 6 and 7 months postpartum also showed a mean weight loss of ~1 kg ($p<0.005$), consistent with that estimated from the regression line.

The mean weight of the infants at birth was 2.55±0.38 kg and 48% had low birth weight (<2.50 kg). At birth the infants were relatively shorter and thinner compared to the NCHS reference population (**Figure 3**). There was a slight but significant increase in length-for-age Z-scores (LAZ) at one month of age compared to that at birth, at which their "catch-up" in length plateaued approximately at -1.4 SD. Infants performed relatively better by weight for length Z-scores (WLZ) which was significantly higher at 1 and 3 month compared to that at birth. At six month WLZ declined slightly and tended to be lower than that of three months and fell below the reference median. Although always remained far below the reference median, infants' weight for age Z-scores (WAZ) were significantly higher at 1 and 3 month of age as compared to that at birth but did not sustain the catch-up in weight gain after then.

Table 1. Characteristics of the mothers at 5-7 months of pregnancy (n = 252).

Age (y)	26 ± 6
Gestational age (wk)	25 ± 3
Education (y)[1]	3 ± 3
Parity	2.2 ± 1.5
Family size	5.7 ± 2.8
Income[2](Taka)	36,000 (22,000 - 48,000)
Weight (kg)	45.2 ± 5.4
Height (cm)	149.5 ± 5.3
MUAC (mm)	227± 18
BMI[3] (kg/m^2)	20.2 ± 1.9
Pregnancy weight gain (g/wk)[4]	200 ± 120
Postpartum weight loss (kg)[5]	1.07 ± 2.20

All values are Mean ±SD except where noted.
[1]Number of years attended at school
[2]Household annual income in Taka (1 US$ = 43.5 Taka): Median (25th and 75th percentiles)
[3]BMI calculated based on weight measured at 5-7 mo pregnancy.
[4]Pregnancy weight gain calculated from weight change in individual women ($p<0.001$)
[5]Weight loss calculated from change in weight in individual women between one and six month postpartum ($p<0.001$).

Table 2. Maternal energy intake during pregnancy and lactation

	Energy intake kcal/d	Energy intake kcal/kg/day
Pregnancy	1464 ± 416 (252)	33 ± 10 (252) [1]
1 mo postpartum	1732 ± 398 (189) [1]	42 ± 11 (158)
3 mo postpartum	1800 ± 421 (145) [1,2]	45 ± 12 (120) [2]
6 mo postpartum	1713 ± 479 (199) [1]	42 ± 13 (136)

Values are Mean ± SD (n)
[1]Significantly higher than pregnancy intakes ($p < 0.01$).
[2] Significantly different from 1 and 6 mo postpartum ($p < 0.05$).

FIGURE 1. MATERNAL WEIGHT DURING PREGNANCY

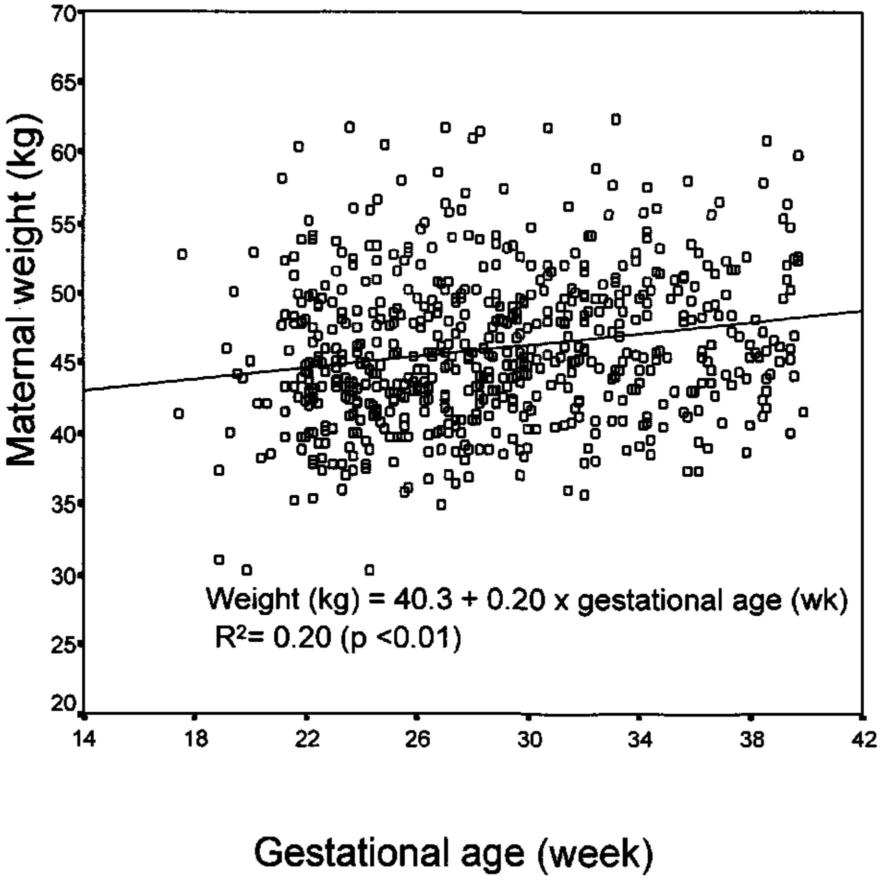


FIGURE 2. MATERNAL WEIGHT DURING POSTPARTUM

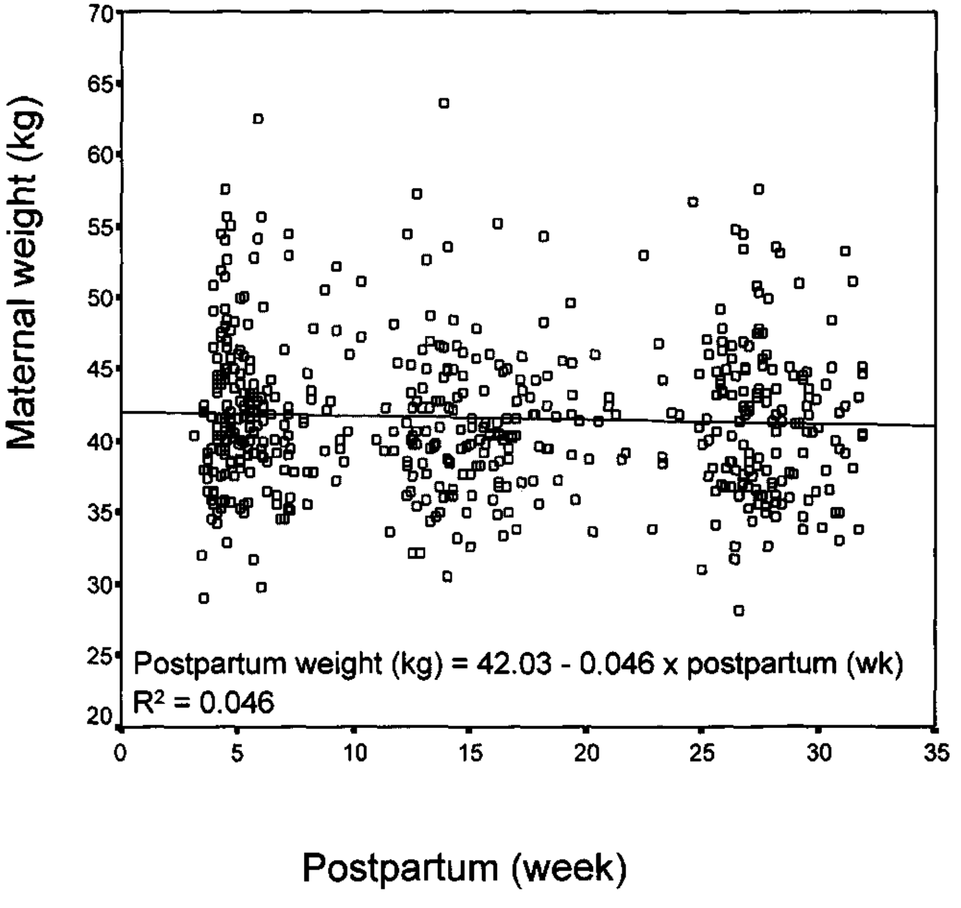
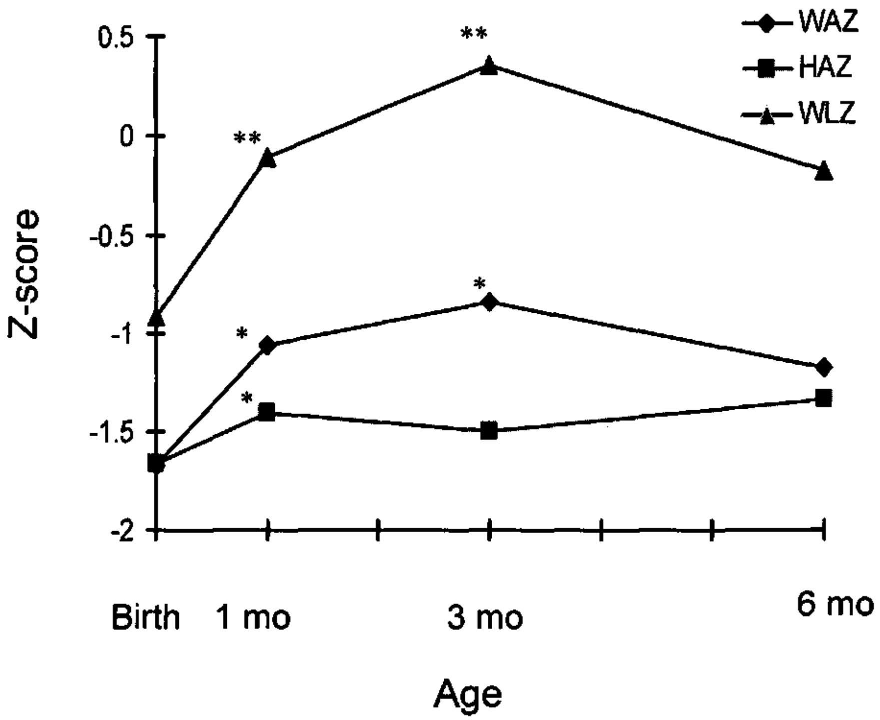


FIGURE 3. INFANT GROWTH PATTERN (0-6 MONTHS)



WAZ = Weight for age Z-score, HAZ = Height for age Z-score,
WLZ = Weight for length Z-score

Compared to Z-score at birth: *p<0.05, **p<0.01

DISCUSSION

The main aim of this study was to examine the response of women in rural Bangladesh to the energy stress of pregnancy and lactation and its effect on maternal energy stores (body mass) and infant growth. This study clearly demonstrates that poorly-nourished rural women fail to compensate for the energy stress during critical periods of energy demand and that is reflected by low pregnancy weight gain as well as postpartum weight loss. This low maternal energy intake and low pregnancy weight gain is also consistent with the very low mean birth weight and high prevalence of low birth weight. Of interest and potentially significant programmatic relevance, the growth performance of the infants who were primarily breast fed up to 6 months of age showed only an inadequate catch-up growth, which was not sustained beyond 3 months. Since there was no indication of increased maternal fat stores during pregnancy, a decline in maternal body weight during lactation indicates a compromised maternal ability to deal with energy stress during lactation in that community.

Maternal energy intake during 5-7 months of pregnancy was only 1464 kcal/d. This amount of energy intake may be considered extremely low for these women who mostly work on their foot most of the day. Studies have shown that energy intake during pregnancy either remain unchanged or increases only slightly in late pregnancy (5;21). Although low, the energy intake observed in this study can be considered as usual intake by pregnant women in rural Bangladesh and is consistent with previous reports (22). Underreporting of dietary energy intake is often a problem in dietary assessment (23). However, the low energy intake observed in these poor non-obese women is unlikely to represent underreporting because dietary data suggest that their dietary pattern consists of limited number food items, fairly fixed meal pattern (2-3 meals per day), virtual lack of snack foods or drinks and no tradition or facilities for the women to eat out.

A key question is whether or not the observed energy intake in these women is adequate, but that should be considered in relation to their energy requirement. However, a major and direct outcome of energy balance during pregnancy is the pregnancy weight gain, which was measured in this study. These women gained about 4 kg weight during the second half of pregnancy that is less than half the weight gain in corresponding period in well-nourished Western women (3-5). This low pregnancy weight gain is a clear indication of gross inadequacy of dietary energy intake during pregnancy.

In well-nourished Western mothers weights at mid-pregnancy are on the same level as that at one month postpartum (4). Weight at these two periods represent prepregnancy weight plus additional weight mainly attributable to fat mass laid down during early pregnancy and retained after delivery. Recent review has shown that the amount of gain in fat mass is about 2- 2.5 kg in high-income Western countries (24). The extrapolated weight at mid-pregnancy was estimated to be 44.3 and that at one month postpartum was 41.8 kg in these study women that is a difference of –2.5 kg between weight at mid pregnancy and one month postpartum. This lower weight at one month postpartum compared to that at mid-pregnancy suggests that mothers in this study did not possibly accrue any fat during the first half of pregnancy just representing their approximate prepregnancy weight.

The women in our study increased their energy intakes quite substantially (248 – 354 kcal/d) during lactation compared to their intakes during 5-7 months of pregnancy. Such increases are expected because of higher energy demand during lactation to support the energy cost of breast milk, and the amounts are comparable with that observed in other populations (10;11). We also observed a significantly higher energy intake at three months postpartum compared to one and six months which coincides with peak lactation (24). However, that increase in energy intake is only about half the estimated extra energy required for lactation (9). This means if other sources of energy are not available women would undergo a negative energy balance. Our observation is supported by recent study in a small number of malnourished Bangladeshi women, which reported a negative energy balance at 3, and 10 month of lactation (25).

Breast milk has been shown to be adequate for growth during the first six months of age even in poor communities (14). Therefore breastfed infant growth serves as a proxy indicator of adequacy of breastmilk production by the nursing mother. In this community prolonged breastfeeding is common and breast milk serves as an exclusive or predominant source of nutrients for infants up to six months of age (26). Infants in this study were born relatively shorter and thinner compared to the reference population and had low mean birth weight. They showed only limited catch-up in both linear and ponderal growth but that was not sustained beyond three months which showed a growth pattern almost similar to breastfed cohort of WHO, although these infants growth curve followed a lower level compared to reference as well as WHO breastfed cohort (27). This pattern of infant growth raises concern about the adequacy of lactation in malnourished women who live on very low dietary energy as observed in this study. The other likely explanation for poor growth during the first six months may the limited potential of growth low birth weight infants who were mostly due to intrauterine growth retardation. However, it is clearly evident from maternal pregnancy weight gain that there are few possibilities that mothers enter lactation with any additional energy reserve. Again loss of 1 kg body weight in these mothers during lactation suggests substantial depletion of maternal stores. More importantly, the mothers come out of the whole process of harsh nutritional condition in a very poor nutritional status, which is likely to have greater implications for subsequent pregnancies particularly if the next pregnancy occurs soon.

From these findings we conclude that mothers in this poor rural community fail to deal with energy stress during pregnancy and lactation resulting in substantial weight loss, an undesirable nutritional consequence especially for the mothers. Although basic data on energy expenditure are not available for this population, it is clear that to protect maternal stores a substantial increase in energy intake and a desirable reduction in physical activity or an optimal combination of both should be achieved during these periods. Further studies should be undertaken to quantify health and nutritional consequences both for mothers and newborns in situations with critical energy balances. The situation of pregnant Bangladeshi women is really critical which needs immediate intervention to protect maternal nutrition and to prevent extremely high occurrence of low birth weight in this population.

Acknowledgements

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Infant feeding practices, morbidity and growth during the first six months of life in rural Bangladesh

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ABSTRACT

Background: Current infant feeding recommendations suggest that exclusive breastfeeding is adequate for the infant and should be continued up to six months of age. In both normal and low birth weight infants, there is no growth advantage of introducing complementary foods earlier than that age.

Objective: The objective of this study was to examine a) the breastfeeding pattern of infants, b) type of complementary foods and their frequency of feeding, and c) whether there was any association between feeding practices with growth and morbidity during the first six months.

Setting: Low-income rural community in Bangladesh

Subjects and methods: Data on feeding practices, morbidity and growth were collected from 324 singleton infants from birth to 6 mo. Infants were classified either as exclusively breast-fed (EBF) if they received only breast-milk except occasional sips of plain water or non-exclusively breastfed (NEBF) otherwise. Daily intake data were summarized for each infant at three and six months. Growth parameters were compared between groups at 1, 3, and 6 mo, and morbidity data at 6 mo.

Results: All the infants were breastfed at 6 mo although 30% at 3 mo, and 63% at 6 mo also received some kinds of local complementary foods. Diarrhoea, upper- and lower respiratory infections were comparable between groups. There were no significant differences in weight for length or length for age between groups at any point in time except at 6 mo when length for age Z-score of NEBF infants tended to slightly higher than that of the EBF infants. However, EBF growth pattern was exactly similar to that of WHO breastfed reference infants.

Conclusion: These findings confirm earlier observations that there is no growth advantage of introducing complementary foods earlier than 6 mo. The similarity in growth patterns of EBF infants with that of WHO breastfed reference infants indicate that our infants might have shown their physiologically optimal growth potential although their growth trajectories followed a lower level.

INTRODUCTION

Current recommendations suggest that infants should be exclusively breastfed until 6 months and then complementary foods should be gradually introduced while continuing breastfeeding (1). Breastfed infants follow a unique growth pattern with an initial higher rate of growth followed by a decline after the first 3-4 months of age when compared with the international reference (2;3). This difference in growth between the NCHS reference population and that of the breastfed infants are mainly attributable to the differences in the feeding practices. Therefore, it is suggested that the relative negative deviance in growth in breast-fed infants from the NCHS reference population between 3 and 6 months should not be considered as growth faltering.

Data on infant growth in poor communities have demonstrated that breast milk is adequate for growth up to 6 months of age and no additional growth benefit can be expected by earlier introduction of even hygienically prepared complementary foods (4). Similar results were observed in small for gestational age infants who are often considered to be unable to suck adequate amount of breastmilk (5). In Bangladesh, poor infant growth and maternal perception of inadequate breast milk enhance introduction of complementary foods even before six months (6). Although hygienically prepared local complementary foods have been shown to have beneficial effect on growth in infants 5-6 months old. While breastfeeding is traditionally very high and continue beyond two years in most mothers (7) and breastmilk serve as almost exclusive source of nutrients(8), data are scanty whether infant feeding practices are consistent with current feeding recommendations and their growth pattern during the first six months of life.

The objective of this study was to examine the breastfeeding pattern, and the type and frequency of feeding of complementary foods and to assess the morbidity and growth in relation to breastfeeding pattern.

SUBJECTS AND METHODS

Study site and sample

Infant data feeding, morbidity and anthropometric measurements were taken from our oil intervention study. This study was conducted from November 1995 to October 1997 in 16 villages in Matlab Thana of Chandpur District, the principal field site the ICDDR,B: Centre for Health and Population Research in Bangladesh. The study was designed to look at the effect of daily 18 g soybean oil (as fat) supplementation during late pregnancy and throughout the first six months of lactation on vitamin A status of mother and infants. In total, 676 (341 intervention and 335 control) mothers were recruited. Of them, 610 mothers delivered live singleton babies. There was no significant difference in birth weight, length or any other parameters of size at birth between intervention and control groups. Singleton infants, who were measured within 72 hours after birth and weighed more than 1500 g, and who had feeding, morbidity and growth data were included in this study. Since there was no difference in infant characteristics at birth between groups, data were pooled from both groups. Characteristics of 324 eligible mother-infant pairs are presented in **Table 1**.

Infant Anthropometry

Weight was measured with a portable SECA paediatric beam balance accurate to the nearest 10 g. The scale was calibrated daily by senior field staff and checked carefully before each measurement. Weight measured within 72 hours after birth was considered as the birth weight since the changes often observed in weight during that period in developing countries is practically negligible (Arefeen et al.2000;(9). The infant's recumbent length was measured to the nearest 0.1 cm using a locally constructed wooden length board. Although measurements were scheduled monthly, those taken at birth, and 1, 3 and 6 months of age were used in this study. All anthropometric measurements were taken by trained workers using standardized and validated techniques (Gibson 1990; Lohman et al. 1988). Weight less than 2500 g at birth was considered as low birth weight (LBW). Infants' weight and length measurements were compared with those of the NCHS reference population (10) and converted to age and sex standardized Z-scores using the software ANTHRO (11). The Z-scores of WHO breastfed infants pooled data set (1) were also plotted with that of the study infants to compare the growth of infants with different feeding pattern.

Infant feeding

Daily feeding practices were recorded birth to six months by maternal weekly recall. Trained Community Health Workers (CHWs) conducted the interviews by home visit. Information on breastfeeding and complementary foods and frequency of feeding was collected using a pre-tested and validated questionnaire. Before the final list of foods was prepared for the questionnaire, name of all complementary foods were first identified by a cross-sectional survey of 100 infants in the study area (who were not included in the study). Additional information on local complementary foods was also gathered from the local public health workers in the study area. Although only 4-5 foods were reported to be the most common and frequently given, ultimately, 12 foods were considered to be included in the list. During weekly home visit CHW asked each mother about breastfeeding frequency and whether she gave any of the 12 foods on the list. If any food other than breastmilk was given during the preceding one week then the date and frequency of feeding were recorded. Feeding data were summarized at 3 months and at 6 months for each infant to illustrate the pattern of feeding.

Definitions of infant feeding

From previous report it is known that nearly all children in this community are breastfed, and intensive and prolonged breastfeeding is common (7;8). In classification of breastfeeding status of infants it is worthwhile to consider several other traditional practices such as giving a few drops of honey at birth, sips of water at birth and sometimes during very hot and humid seasons. In this study such practice was not considered as additional infant feeding or nutritional supplement. Therefore, we defined *exclusively breastfed* (EBF) infants as those who received only breastmilk as source of fluid, energy and nutrient and no other nutrient or non-nutrient foods except occasional sips of water or medicinal agent. Our definition *exclusive breastfeeding* conforms to *almost exclusive breastfeeding* by the WHO Expert Committee on Complementary Feeding. However, considering frequency and amount of water given we considered that would not have any consequence on the outcome. Infants were defined *non-exclusively breastfed* (NEBF) when they received any nutrient containing food irrespective of nutrient density and at any frequency in addition to breastmilk. We did not categorize any infant as non-breastfed or even partially breastfed because almost all infants were receiving breastmilk regularly as the principal source of nutrient during the first six months of age.

NEBF infants were further categorized based on the frequency of consumption. Food items which were given at least once every day was defined as "regular", those given at least once for 1-6 days a week were defined as "irregular", and foods with frequencies less than "irregular" category was considered as "occasional" complementary foods.

Morbidity

The CHWs collected infants' morbidity data by maternal 7-day recall during routine weekly home visit using a precoded questionnaire. Major morbidity symptoms related to diarrhoea, upper and lower respiratory tract illnesses included in the questionnaire. Women were specifically asked whether their infants suffered any of the listed symptoms during the last one week (since the interviewer's last visit). Then the date of onset and duration were recorded. If an infant had been found to have any morbidity symptoms during her previous visit then the current status with regard to presence or absence of symptoms was recorded. Morbidity symptoms were recoded to define different illness episodes and their respective duration. Diarrhoea was defined as three or more loose stool in last 24 hours or any number of stools with blood with it. An episode of upper respiratory infection (URI) was defined as mucoid or mucopurulent nasal discharge with or without cough but without any chest indrawing or respiratory distress that lasted for more than one day. Lower respiratory infection was defined when there were concurrent presence of cough, difficulty in breathing and chest indrawing with or without fever. Fever was also recorded as a separate morbidity symptom.

Data Analysis and Statistics

All data analyses were carried out using the statistical package SPSS version 7.5 for Windows. Since both exclusive and non-exclusive breast-fed groups were comparable with respect to gender distribution, birth weight and the prevalence of low birth weight, different outcome variables were compared between groups without stratification by those variables. Also no analysis by gender was considered because evidence from poor communities suggests boys and girls grow similarly within the first six months of infancy (12). Results are mainly presented as mean and the standard deviation or as numbers and percentages. For categorical variables frequency distributions were compared by Chi-square test and for the continuous variables two sided Student's t-tests were used. Any P value <0.05 was considered statistically significant.

RESULTS.

Maternal and infant characteristics at birth are presented in **Table 1**. Seventy percent of the infants were exclusively breastfed at three months and that dropped to 37% at six months of age. Both exclusive and non-exclusively breastfed infants had comparable maternal nutritional status, and infants' gender, birth weight and proportion of low birth weight distributions. However, those who received were non-exclusively breastfed at 3 months, the mothers of those infants were slightly older, more educated, and came of relatively higher income households. There were no such differences in maternal characteristics observed at six months between groups.

Infant feeding pattern

Table 2. shows food intake pattern among non-exclusively breastfed infants. Notably all the non-exclusively breastfed were receiving breast regularly in addition to other foods. Among the 12 complementary foods, which were on the questionnaire, only seven foods reported to have been given during that period. It may be noted that the percentage of children receiving different foods are not mutually exclusive which means a non-exclusively breastfed infant might have received several different foods at varying frequencies. Within the first three months, the most frequently given foods were *suji* (a thin porridge with or without milk), *luta* (thick porridge), and cow's milk and other milk (mainly powdered milk). While these were also the major foods given between three and six months of age, rice was reported as another frequently given food. Although *Khichuri* (a semisolid food mainly prepared with rice, lentil and some oil) often promoted as an ideal complementary foods in Bangladesh, surprisingly almost none of the infants were given *khichuri* by the age of six months.

Morbidity

Most common morbidity was reported to be upper respiratory illness followed by fever in those study infants. However, there was no significant difference in morbidity observed between exclusive and non-exclusive infants **(Table 3)**.

Table 1. Characteristics of the study subjects

	At 3 months		**At 6 months**	
Maternal	**EBF[a]**	**NEBF[b]**	**EBF**	**NEBF**
	n(%)=227(70)	n(%)=97 (30)	n(%)120(37)	n(%)=204(63)
Age	26.1±5.7	27.5±5.8[c]	26.3±5.6	26.6±6.0
Education	2.7±2.9	3.5±3.5*	2.4±2.7	3.2±3.3
Parity	2.2±1.5	2.5±1.7	2.1±1.4	2.5±1.7
Height (cm)	148.5±11	148±16	148±14	149±12
MUAC (mm)	228±17	226±25	227±17	227±21
Family member	5.4±2.6	6.0±26	5.4±2.7	5.7±2.5
Household income (Taka* x ,000)	39±41	53±60*	36±29	47±56
Infant				
Sex (%male)	48	55	47	52
Birth weight	2536±345	2516±411	2534±345	2527±378
Birth length	46.7±2.0	46.9±2.1	46.6±2.1	46.8±1.9
Low birth weight (%)	43	51	46	45

Mean±SD

[1]EBF – Exclusively breastfed

[2]NEBF – Non-exclusively breastfed

[3]1 NLG = 24 Taka

Figure 1. Weight for length Z-scores

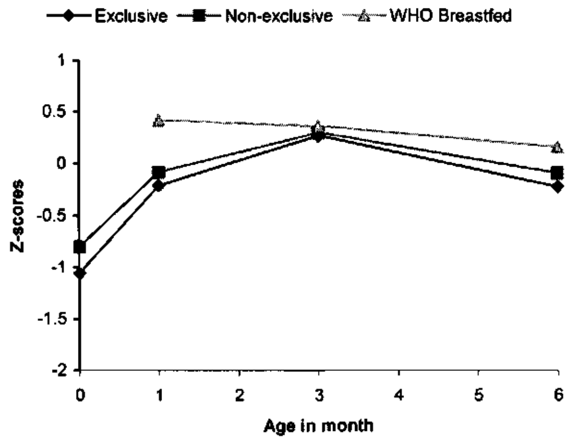
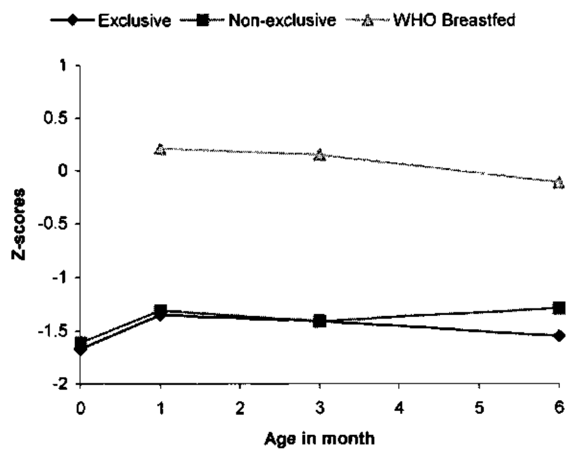


Figure 2. Length for age Z-scores



DISCUSSION

Our results confirm that there is no significant growth advantage of introducing complementary foods in infants earlier than six months. Interestingly, EBF infant growth followed a similar pattern as that of the WHO breastfed infants although they started at a lower level and maintained that level throughout. These pattern of growth is also consistent with the growth pattern of infants from urban slum population in Bangladesh (13). Our findings also confirm previous observations in this community that breastfeeding is universally practiced in rural Bangladesh and all infants are breastfed regularly up to the age of six months (7). Although about 1/3rd at 3 months and 2/3rd at 6 months also receives some kinds of complementary foods, growth and morbidity did not differ between groups suggesting no benefit of giving any foods other than breast milk before that age (5).

About 30 % of our study infants were reported to have received complementary foods at 3 months of age but the frequency of feeding of those foods indicate a very negligible contribution in most of the infants' diet. Therefore, all nutrient intake can virtually be attributable to breast milk in our study infants. At six months, infants receiving some kinds of extra foods reached 63%. In that case also the amount and frequency suggest that actually breastmilk was still the major nutrient source. Earlier report in the same area suggest breastmilk is almost the exclusive source of nutrients in this age group although they might receive additional foods (8). Data from other studies also suggest that introduction of complementary foods simply replaces breastmilk intake as the infants self regulate their intake (4)

This pattern of growth observed in these infants was consistent with that of WHO breast-fed pooled data set which also showed relative decline in the rate of weight gain after the first three months of age (1). WHO breastfed infants started at a higher level than the NCHS reference median and remained above the median until about 4 months of age than showed a relative decline. Infants in this study who had a poor nutritional status at birth never attained weight at the reference level. Although data from some poor communities showed adequate catch-up during the first few months of infancy achieved the same level as the NCHS reference median (12).

Infants from higher socioeconomic status in Brazil had a higher rate of weight and length gain than NCHS reference until 3 months before their growth faltered (2). In this study both normal and low birth weight infants showed a similar rate of weight gain throughout the first half of infancy. However, it is known that low birth weight infants fail to achieve the same level of growth even in high-income communities (14). Our study groups were a mixture of normal and low birth weight infants but their distribution was similar across feeding groups. Our aim was to illustrate growth of these infants as a group based feeding pattern because that is the proportion of low birth weight expected in the normal rural communities in Bangladesh.

Although not significant, length for age Z-score tended to be higher in the NEBF infants at six months. Interestingly EBF infants showed exactly the same growth pattern as that of the WHO breastfed infants. This finding suggest that breastfed infant behave similarly with respect to growth wherever the starting point is. Growth reference is often used as the standard, if so, these rural Bangladeshi infants have severe growth deficit which warrants immediate intervention. However, it may be noted that the big gap between WHO breastfed cohort or the NCHS reference population and our infants was mainly attributable to the deficit at birth.

From these findings we conclude that growth of breastfed infants follow the same pattern irrespective of their starting point (at birth). These rural Bangladeshi infants have probably shown their unique growth pattern and that might be their optimal physiological growth potential as they were born small at birth.

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Association between clinical type of diarrhoea and growth of children younger than 5 year old in rural Bangladesh

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ABSTRACT.

Background. The role of diarrhoea in the aetiology of growth retardation in young children remains controversial. To evaluate this, a population-based, longitudinal study of young children aged 6 to 48 months was conducted in Matlab, a rural area of Bangladesh, between May 1988 and April 1989.

Methods. Data obtained from 584 children were examined by one-year (n=412) and three-month (n=1220) growth periods. Each growth period was analyzed based on clinical types of diarrhoea, namely, non-diarrhoea, non-dysentery diarrhoea (diarrhoea without blood), and dysentery (diarrhoea with blood). Weight and height gains were compared among the study groups initially by one-way analysis of variance followed by multivariate analysis adjusting for potential confounding variables.

Results. Compared to non-diarrhoea and non-dysentery diarrhoea, dysentery was associated with significantly lower annual weight gain [1866 g ($p < 0.01$) and 1550 g ($p < 0.05$) vs 1350 g respectively] and height gain [6.51 cm and 5.87 cm vs 5.27 cm ($p<0.01$) respectively]. Both 3-mo dysentery and non-dysentery intervals were significantly associated with less weight gain compared to non-diarrhoea intervals [490 g and 522 g vs 637 g ($p<0.05$) respectively], and .dysentery intervals were also associated with significantly poorer height gain compared to other intervals [2.19 cm vs 2.42 cm ($p<0.05$) and 2.46 cm ($p<0.01$) respectively].

Conclusions. The growth of young children is strongly influenced by the clinical type of diarrhoea, and that the impact is dependent on the proportion of dysentery episodes in the total diarrhoeal burden.

Key Words Bangladesh, children, diarrhoea, clinical type, dysentery, weight gain, height gain

INTRODUCTION

Diarrhoeal illnesses are well recognized as a major cause of morbidity and mortality in young children in many developing countries (1-3), however, their role in causing childhood malnutrition is controversial (4-8). Community-based prospective studies in developing countries have consistently demonstrated a significant negative association between diarrhoea and short-term (1-4 months) weight gain but the conclusions on short-term height gain are less consistent (7,9-14). The negative effects of diarrhoea on linear growth velocity during longer intervals (□6 months) were documented in some studies (12,15,16), while a little or no such effects have been reported from several other studies (13,17,18).

One possible explanation for the discrepant observations is the heterogeneity of diarrhoeal illnesses (19). Most studies that examined the association between diarrhoea and growth considered diarrhoea as a single disease entity, and were not designed to identify potential differences due to different types of diarrhoeal illnesses. Studies that looked at the effects of diarrhoea on growth by type of illness have suggested that certain etiologic (*Shigella* spp) and clinical types (dysentery) are associated with significant growth retardation (4,12,20). While these studies were constrained by not accounting for the effects of other important confounding variables, such as socioeconomic factors, the findings, nevertheless, suggest variations in nutritional outcome by type of diarrhoea. Confirmation of this heterogeneity is of public health significance in understanding the impact of diarrhoea on the growth of children, and subsequently, for the design of appropriate interventions.

Findings that contribute to this controversy include: lack of consistent evidence of sustained negative effect by type of diarrhoea (4,8); failure to demonstrate a significant improvement of nutritional status following reduction in diarrhoeal incidence (6,21); and less relative impact of diarrhoea on growth compared to other illnesses or low dietary energy intake (7). Consequently, a careful reappraisal of the association with proper recognition of the heterogenic nature of diarrhrea is necessary to better define the nutritional impact and public health significance of diarrhoea in the community.

The main objective of this study was to examine the effects of diarrhoea by clinical types on both short- and long-term growth of children younger than 5 years in rural Bangladesh while controlling for the effects of some important potential confounding variables.

SUBJECTS AND METHODS

This study was conducted in three villages at the Matlab Field Station of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) where the Centre has maintained a diarrhoea treatment hospital and research activities since 1963. Details of the study villages and selection criteria have been reported elsewhere (22,23). Briefly, children aged between six and 48 months, and permanently residing in the three selected villages were eligible for enrollment in the study. An informed written consent was obtained from the mother of each participating child before enrollment into the study. In total, 584 children were enrolled in the study.

At baseline, sociodemographic information including parental education, land ownership, family income, water sources, latrine types, and other household characteristics were collected by trained health workers. Trained Community Health Workers (CHWs), who were females with minimum eight-grade education and permanent resident of the study villages, collected morbidity data by visiting each child every fourth day at home. The mother or the regular caretaker of the study child was asked a standard series of questions about her child's daily stool frequency, consistency and presence or absence of blood in stool on the day of the visit and preceding three days by recall. An episode of diarrhoea was defined as three or more loose, liquid or watery stools, or any number of stools with blood in 24 hours. Three diarrhoea-free days were required to separate the consecutive episodes. Diarrhoea was further classified as dysentery if stool contained blood in any day during the episode.

Anthropometric measurements were taken by trained research assistants at the time of enrollment and then monthly, following standard procedures (24). Each child was weighed nude or with light clothes to the nearest 0.1 kg with a Salter-type spring scale. The scale was calibrated daily against known weight before use. Recumbent length of all children below the age of 36 months and the standing heights of children aged 36 months or older were measured to the nearest 0.1 cm using a locally constructed length board or height stick. Two trained and experienced workers took each measurement independently, and the mean of the two measurements recorded as the actual value. As a part of the quality assurance weight and height of the 10% of the study children were measured on the following day. There was no significant difference between the first and the repeat measurement.

Data Analysis and Statistics

Different observation periods were chosen to examine the short- and long-term effects of diarrhoea on growth. Observations with complete diarrhoea surveillance data and anthropometric measurements both at the beginning and at the end of the respective observation periods were included in the analysis.

The short-term effects of diarrhoea on growth were examined in three-month child intervals. Each child's three calendar-month observation period constituted one child interval. Although multiple observations (child intervals) were available from one individual child, each child interval was assumed to be an independent observation (25). The number of observations (n) in the analysis refers to child intervals (three-month), and not children. However, the effects of diarrhoea on long-term growth were examined over one year period, and the number of observations (n) in that case referred to the number of children included in the analysis.

Each observation (3/12 months period) was classified by clinical type namely: non-diarrhoea (no diarrhoea during the period), non-dysentery (diarhroea without blood) or dysentery (diarrhoea with blood) type of diarrhoea. Percent time with diarrhoea was calculated for each observation, using the number of days with diarrhoea divided by the actual number of days observed during the respective analytic periods. This percentage was adjusted for 90 days in the case of child intervals and 365 days in the case of a one-year observation periods.

Nutritional status was assessed at the beginning of each observation period by comparing the weight and height of the study children with the medians of the NCHS reference population of same age and sex (26). Weight and height gains during the respective analysis periods were the main outcome variables and were determined by the difference between the last and the first measurements during the respective analysis periods and these figures were also adjusted for 90 days in the case of child intervals and for 365 days for a one-year observation period.

Spearman rank correlation was used for assessing the linear relationship between the independent and dependent variables. Mean weight and height gains were compared initially by one-way analysis of variance, and Duncan's Multiple Range (DMR) test was used for examining any statistically significant difference in unadjusted means. All analyses were followed by a multivariate analysis controlling for age, sex, parental education, household income, land ownership, baseline nutritional status and percent time with diarrhoea using the General Linear Model (GLM) in SAS ver 6.04 [27]. In the multivariate models, independent variables with high correlation (Spearman correlation coefficient ≥0.7) were not fitted simultaneously. However, the variable more closely related to the hypothesis was always retained in the models. Variables that were significant at < 0.1 level on univariate analysis were included in the multivariate model. Also the variables that were not significant at < 0.1 level but considered a priori to be confounders were also examined by fitting them into the multivariate model. Two-way interaction between diarrhoea variables and other independent variables was assessed for any effects on the outcome variables. Non-significant interaction terms were excluded from the final model.

RESULTS

Of the 584 children enrolled, 512 were followed for one year, and the remaining 72 were followed for part of the year. Complete data were available for 1220 three-month child intervals and 412 one-year observations. Of the 1220 three-months child intervals, 184 children contributed four intervals each (184x4), 352 children contributed 3 intervals each 352x3), 412 two each (412x2) and another 512 children contributed one interval each (512x1). The main baseline characteristics of the 412 study children who had complete one-year data are presented in **Table 1**. The children were two and a half years old at enrollment and were of poor nutritional status as indicated by their mean anthropometric indices and very high prevalence of underweight, stunting, and wasting. The overall mean incidence of diarrhoea was 4.5 episodes per child per year (median, 4 episodes/child/yr; range, 0-13 episodes/child/yr). Dysentery accounted for 15% of all diarrhoeal episodes. About 44 percent of the study children had at least one episode of dysentery, and one-half had at least one episode of non-dysenteric type of diarrhoea over the year. Only 5% of the children did not experience any diarrhoea during the study period. On average, children spent about 10 percent of the time with diarrhoea. However, over a quarter of the children had diarrhoea for more than 10 percent of the time another quarter of the children suffered between 5 and 10 percent of their time with diarrhoea during the year.

The mean annual weight and height gains were 1558 g and 5.8 cm. Both rate of weight and height gain significantly decreased with age (β=-15 g/mo., $p<0.001$; and β=-0.11 cm/mo., $p<0.01$ respectively). There was a significant positive correlation between height and weight gain ($r=0.5$, $p<0.001$).

None of the socioeconomic variables, all of which were considered as possible confounding variables, was found to have a significant association with annual or quarterly weight or height gain on univariate analysis. However, land ownership approached statistical significance. Children from the households that owned land had a higher mean annual weight gain compared to those from the landless families (1578 g vs. 1398 g, $p<0.08$).

Table 1. Characteristics of the study samples (n=412) in Matlab, Bangladesh

Variable	Value
Age (mo)[1]	24.5 ± 12.3
Nutritional status[1]	
Weight for Age Z score (WAZ)	-2.68 ± 0.94
Height for Age Z score (HAZ)	-2.59 ± 1.13
Weight for height Z score (WHZ)	-1.50 ± 0.81
Underweight (WAZ <-2Z) (%)	77
Stunted (HAZ <-2Z)(%)	71
Wasted (WHZ <-2Z) (%)	28
Maternal schooling (%)	
Never attended school	74
Attended 1-5 yr	22
Household annual income(Taka)[2]	
<12,000	26
12,000-22,000	50
Landless households (%)	11
Diarrhoeal morbidity	
Episodes/child/yr	4.5
Days with diarrhoea/child/yr	37
Clinical type of diarrhoea (%)	
None	5
Nondysentery	50
Dysentery[3]	44
Annual weight gain (g)[1]	1558± 655
Annual height gain (cm)[1]	5.83± 1.47

[1]Mean±SD
[2]1US$ = 40 Taka (Bangladesh currency)
[3]Suffered from at least one episode of dysentery

Clinical Type of Diarrhoea and Short-term Weight Gain

The mean weight gain during the child intervals varied significantly by clinical type of diarrhoea (**Table 2**). Significantly greater weight gain was observed during nondiarrhoeal intervals compared to both nondysentery and dysentery intervals. Although weight gain was less during the dysentery intervals compared to nondysentery intervals, the difference was not statistically significant (**Table 2**). When the analysis was adjusted for age, sex, baseline nutritional status, parental education, household income, land ownership and percent time with diarrhoea, the association among the adjusted means remained nearly the same (**Table 2**).

Clinical Type Of Diarrhoea And Short-term Height Gain

Comparison of the unadjusted mean height gain during the intervals by clinical type of diarrhoea showed no statistically significant difference, although there was reduced height gain during dysentery intervals compared to the two other groups (**Table 2**). After adjusting for possible confounding variables, the type of diarrhoea was found to have a significant main effect. Comparison of adjusted means showed that the intervals with dysentery were associated with significantly lower mean height gain compared to both nondiarrhoea and nondysentery diarrhoea intervals (**Table 2**).

Clinical Type of Diarrhoea and Annual Weight and Height Gain

The results of one-way ANOVA showed that the mean annual weight gain varied significantly by clinical type of diarrhoea (**Table 3**). In the unadjusted analysis, Although the unadjusted mean annual weight gain was the lowest among children with dysentery and the highest among nondiarrhoeal children, differences did not reach statistical significance on Duncan's Multiple Range (DMR) test. On the other hand, the adjusted mean weight gain by the non-diarrhoea children was significantly greater than that of nondysentery or dysentery children. Between diarrhoeal children, the annual weight gain in the dysentery group was significantly lower than in the nondysentery group (**Table 3**). The model explained 13% of variations in annual weight gain.

The adjusted annual height gain varied significantly by clinical type of diarrhoea (**Table 3**). Comparison of the adjusted means revealed that children who suffered dysentery had a significantly reduced annual height gain compared to nondiarrhoea and non-dysenteric groups (**Table 3**).

Interactions were examined between diarrhoea and other independent variables on their effect on the outcome variables in all analyses. However, none of the interaction terms between clinical type of diarrhoea, and age, sex, nutritional status or socioeconomic factors reached statistical significance.

Table 2. Weight and height gains during three-month child interval by type of diarrhoea

Type of diarrhoea	n[1]	Weight gain		Height gain	
		Unadjusted Mean (SEM)[3] in g	Adjusted[2] Mean (SEM) in g	Unadjusted Mean (SEM) in cm	Adjusted Mean (SEM) in cm
None	378	549[4] (22)	637[5] (32)	1.89 (1.06)	2.46[5] (1.09)
Nondysentery	627	468 (18)	522[4] (27)	2.06 (1.03)	2.42[4] (1.03)
Dysentery	215	420 (32)	490 (38)	1.72 (1.05)	2.19 (1.05)

[1]n represents the number of child intervals

[2]Adjusted for age, sex, parental education, household income, land ownership, percent time with diarrhoea and baseline nutritional status

[3]SEM - standard error of mean

[4]Significantly different from two other means ($p<0.05$)

[5]Significantly different from dysentery ($p<0.01$)

Table 3. Annual weight and height gains among the 412 children by type of diarrhoea

Type of diarrhoea	N[1]	Weight gain		Height gain	
		Unadjusted Mean (SEM)[3] in g	Adjusted[2] Mean (SEM) in g	Unadjusted Mean (SEM) in cm	Adjusted Mean (SEM) in cm
None	19	1705 (223)	1866 (158)	5.72 (1.04)	6.51 (1.08)
Nondysentery	210	1617 (43)	1550[4] (66)	6.09 (1.02)	5.87 (1.03)
Dysentery	183	1476 (48)	1350[5] (67)	5.55 (1.03)	5.27[5] (1.04)

[1]n represents the number of children

[2]Adjusted for age, sex, parental education, household income, land ownership, percent time with diarrhoea and baseline nutritional statu

[3]SEM - standard error of mean

[4]Significantly different from non-diarrhoea group ($p<0.05$)

[5]Significantly different from two other means ($p<0.0$

DISCUSSION

Results of this study provide strong evidence that the effects of diarrhoea on growth in both short- and long-terms are associated with the clinical type of illness. Dysentery had the most deleterious consequences on both ponderal and linear growth although other type of diarrhoea showed a similar but relatively less strong negative association with growth. About half a kilogram less annual weight gain, and one and a quarter of a centimeter less height gain was associated with dysentery in under five children in this population. However, several limitations of this study need to be addressed before interpreting the results.

One of the major limitations includes, no control for other co-morbidities such as acute respiratory tract infections (ARI), malaria etc. that were however, assumed to be equally distributed among the study groups. Also energy and other nutrients intake was not controlled for. Lack of adequate power for certain comparisons rendered some important differences statistically non-significant. Furthermore, there were some unavoidable misclassifications in categorizing children or intervals by clinical type of diarrhoea as one single episode of dysentery during the observation period characterized the observation as dysentery. The negative effects of dysentery perhaps would have been amplified if this misclassification could have been avoided. In contrast, data collected using frequent surveillance (every 4th day) minimized the possibility of recall bias (28). Seasonal variations in diarrhoeal incidence and growth did not bias our findings since data for an entire year were analyzed.

The observed negative effect of dysentery on both short- and long-term growth has strong biological plausibility. Dysenteric episodes last relatively longer (4,29), often associated with fever (30), damage the intestinal structure and impair function (31), and is associated with greater endogenous protein loss (protein losing enteropathy) than other types of diarrhoea (32). This endogenous protein loss might have greater biologic significance, and be more detrimental to growth in children in developing countries where protein intake is generally low (33). Further, the high catabolic response to infection may also result in inadequacy among children with an apparently adequate intake (34).

Our results support the findings of earlier study (20) which observed no significant difference in the overall incidence of diarrhoea between two groups of children with different rates of growth but a higher overall incidence of dysentery among the children with poorer growth rates. The aetiology based study (12) also suggested that diarrhoeal illnesses caused by *Shigella* spp. had a longer-lasting negative effect on growth while those caused by enterotoxigenic *Escherichia coli,* commonly associated with watery diarrhoea, had no significant effect on long-term growth. This indicates that agents causing invasive diarrhoea and which normally manifest as dysentery have a substantial nutritional cost. Dysentery has been found to have a longer-lasting negative effect (evident up to six months after the episode) on growth of the young children in a community-based study (4).

The results of our study suggest careful interpretation of the findings of certain studies that showed reduction in diarrhoeal incidence had no effect on growth of children (6,21). Apart from lack of adequate power to show a significant difference in some of the comparisons, these studies did not mention which type of diarrhoea was most amenable to their intervention. Another recent study reported no significant effect of diarrhoea on child growth based on the comparison of growth trends between frequent (>9 episodes in 18 months) and infrequent (<4 episodes in 18 months) sufferers of diarrhoea in a poor community (8). However, the results of that study were not controlled for the type of diarrhoea or for the duration of illness, both of which have great potential to confound the outcomes.

The controversy surrounding the relative importance of diarrhoea and inadequate dietary energy intake as causes of child malnutrition in developing countries also needs careful reappraisal. Those who believe that inadequate energy intake is the major cause of poor growth based their conclusion on indirect evidence (4-8). However, in one study the relative effects of diarrhoea and dietary energy intake on monthly weight gain were quantified (7). In that study, diarrhoea was used as a single disease entity although the same authors' earlier study indicated that diarrhoeal effects on growth vary by aetiologic type (12). It may be noted that the inadequacy of intake is assessed relative to Recommended Daily Allowance (RDA), which is not necessarily the minimum requirement for optimal growth (35). Rather, RDA is a general recommendation for intake by the population based on intake data from healthy populations. A recent study, based on precise estimate of energy expenditure, suggested that the RDA for energy might be an overestimate of the actual requirement (36). More recent studies suggested a positive association between protein intake and growth, despite normal or low calorie intake (37,38). However, it may be possible that higher energy intake is associated with higher intake of proteins, vitamins and minerals (39). Conversely, very low energy consumption may be associated with compromised intake of other nutrients (40). Therefore, seemingly inadequate energy intakes frequently observed in developing countries might not be responsible for growth faltering although both low energy intake and malnutrition may co-exist.

The findings of this study clearly suggest that the proportion of dysenteric episodes among the total diarrhoeal burden is probably the most important determinant of the impact of diarrhoea on growth of young children. Although low energy intake is common among children who suffer from diarrhoea in developing countries, the literature on evidence to suggest that only improved dietary energy intake will result in better growth is limited. Therefore, to achieve the nutritional goal of diarrhoea control programs, in addition to other general measures, more specific interventions aimed at reducing the incidence of dysentery, and the development of vaccines against common organisms causing dysentery should be emphasized.

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7

General Discussion

In this thesis, five important nutritional issues in rural Bangladesh that have particular relevance to maternal, infant and child nutrition have been reported. A special emphasis was given to examining practical relationship between dietary fat supplementation and vitamin A status of mothers and their infants. The following topics have been investigated: (1) maternal vitamin A status during pregnancy and lactation, and the effect of an intervention with dietary fat on vitamin A status of women and their breastfed infants; (2) validity and utility of a VA-focused dietary assessment method to identify pregnant and lactating women at risk for vitamin A deficiency; (3) response of rural women to energy stress during pregnancy and lactation and its consequences for maternal nutrition; (4) infant feeding practices and growth performance during the first six months of age; and (5) effect of diarrhoeal illnesses on growth of children younger than 5 years of age in rural Bangladesh.

In this chapter, the major findings of the studies described in the thesis will be discussed in relation to their public health significance, and implications for the policy. The major conclusions and recommendations will be presented at the end of the chapter.

Maternal nutrition in rural Bangladesh

Vitamin A status during pregnancy and lactation

Women in this study received about 80% of their dietary vitamin A through plant sources with only a negligible contribution from animal sources (*Chapter 3*). Recent reports indicate that such an intake pattern increases the risk for vitamin A deficiency due to poor bioavailability of provitamin A carotenoids (1;2). Consistent with this dietary intake, women in this study were found to have a poor vitamin A status as indicated by their low plasma retinol concentration, high prevalence of subclinical vitamin A deficiency (plasma retinol < 0.70 µmol/L), and a high prevalence of night blindness by history (3%). Nearly one third of the pregnant women had subclinical vitamin A deficiency, and 77% had plasma retinol concentration <1.05 µmol/L. Subclinical vitamin A deficiency during lactation was prevalent at a similar magnitude as observed during pregnancy, and 69-86% of lactating women had breast milk retinol concentration <1.05 µmol/L. These findings confirm that vitamin A deficiency is widely prevalent in these rural Bangladeshi pregnant and lactating women and the magnitude identifies VAD as a significant public health problem (3).

Poor vitamin A status may increase the risk of maternal mortality and morbidity (4;5). Another major implication of this poor maternal vitamin A status is related to the vitamin A status of their fetus and breastfed infants, which is poor because breastmilk is the exclusive source of VA for rural Bangladeshi infants and in vitamin A deficient mothers it is insufficient (6).

Dietary fat intake during pregnancy and lactation

Our results show that women in this rural community have very low dietary fat (10-12 g/d or 6-8 energy%) intake (*Chapter2*). These data also showed a beneficial effect of dietary fat supplementation on women's vitamin A status during early lactation although the magnitude of the effect was limited. Dietary fat might be a critical constraint for overall poor vitamin A status observed in this population as it co-exists with provitamin A carotenoids as the major dietary source of vitamin A. With fat intervention the total fat intake in these women could be raised to 10-15 energy%. Low dietary fat intake may not only impede absorption and assimilation of vitamin A and provitamin A carotenoids, it has implications for other fat soluble vitamins such as D, E, and K and essential fatty acids (7). Therefore poor status or deficiency of other fat-dependent nutrients may also exist in this population.

In this population animal food consumption is very low and edible oil used in cooking is almost the exclusive source for dietary fat. Improving dietary fat intake might therefore require an overall increase in availability as well as affordability of oil for the poor rural population. Availability again depends on domestic production and import. Data from National Food Balance Sheet show only 10 g per capita edible oil available daily for Bangladeshi population (8). Because edible oil is relatively expensive among food items for poor rural population, consideration might need to be given to subsidizing oilseed production, import and distribution in order to improve dietary fat intake on the population level.

Effect of fat supplementation on maternal vitamin A status

Women supplemented with dietary fat during pregnancy and lactation showed a positive change in their plasma retinol concentration by about 5-7% from their baseline concentration (pregnancy). Whereas non-supplemented control women showed no such change except a small non-significant one at 1 month postpartum. The mean breastmilk retinol concentration in supplemented women showed a difference of 13 μmol/L at 3 months postpartum. Although small this is important because infants in rural Bangladesh receive very negligible amount of vitamin A from other sources (6). Overall, the size of the effect indicates that dietary fat alone may only have a very limited role although optimal amount of dietary fat required for adequate absorption of vitamin A and provitamin A carotenoids still need to be quantified. Another limiting factor for the small effect might be the dependency of this population on plant sources for its dietary vitamin A intake. Therefore, to bring a significant change in the vitamin A status of this population needs multiple strategies including increasing dietary fat consumption.

Dietary energy intake during pregnancy and lactation

Study women had daily energy intake of about 1500 kcal during pregnancy. This amount is critically low considering their lifestyle which involves working on foot for most of the day. However, the judgment about the energy adequacy is difficult because of paucity of energy expenditure data on this population. However, the pattern of pregnancy weight gain strongly indicates that women had poor energy balance. Women in this study gained only 4 kg weight during the last 20 weeks of pregnancy. This situation is indicative of poor pregnancy outcome.

Our data showed that lactating women in rural Bangladesh increase their daily energy intake by about 300 kcal/d on top of their intake during pregnancy although the absolute amount was still relatively low (*Chapter 4*). The increase in energy intake can be considered quite substantial and comparable with the increase observed in healthy Western mothers (9). Since there is little or no possibility of stored fat mobilization, increase in energy intake is apparently the basic mechanism to meet the extra energy required for lactation Our data also showed that women lose 1 kg of body weight during the first six months of lactation, which is consistent with the pattern of their energy intake during lactation. This means that maternal depletion continues through lactation in rural Bangladeshi women, and is predictive of poorer nutritional status at the end of pregnancy and lactation cycle. This gradual depletion of maternal stores may have carried over effect for the next pregnancy outcomes particularly if the next one is too early.

Effective prevention of such deterioration in maternal nutritional status needs better understanding of the mechanisms involved in the process such as energy balance during pregnancy and lactation in that population. As an immediate strategy interventions aimed at increasing awareness among pregnant and lactating women, and the family members about the special nutritional needs of the pregnant and lactating women should be considered. If possible food supplementation might be considered as short term response for protecting maternal nutritional status during these energy demanding periods in most vulnerable groups.

Infant and child nutrition in rural Bangladesh

Nutritional status at birth

Infants in this study were born with a mean birth weight of only 2.55 kg, and 48% of those had low birth weight (<2.50 kg). These findings are consistent with the reported national estimate that nearly 50% of Bangladeshi infants are born with low birth weight (10). Over 80% of these infants in our study were born after 37 week of gestation, which indicates most of these low birth weight infants suffered intrauterine growth retardation (IUGR). These findings support the conclusions of previous review of birth weight data from developed and developing countries that most of the low birth weight infant in developing countries are mainly due to IUGR (11). At birth these Infants were also shorter and thinner than the NCHS reference population. Birth weight being considered as the single most important predictor of infant mortality and morbidity (12), it can be anticipated that most of these infants in this community would be highly vulnerable to morbidity and mortality during their infancy and later in childhood. This high percentage of low birth weight is probably one of the major reasons for high infant mortality rate in Bangladesh which is still 77 per thousand live births (13). This small size at birth, suggestive of undernutrition during fetal life, may have long term consequences for health and survival due their increased risk of hypertension, diabetes and coronary heart disease in their later life (14;15). This means that the high prevalence of low birth weight not only have an immediate risk of poor survival and growth but also might have great health and economic consequences for the individuals and for the community at large.

Infant feeding and growth from birth to six months of age

Breastfeeding is regular and intensive in this population although a substantial proportion of infants (30% at 3 month and 63% at 6 months) receive some kind of extra foods in addition to breastmilk. However, there was no difference in morbidity and growth outcomes between exclusive and non-exclusive breastfed infant. Findings in this population also support that complementary feeding before the age of six months has no extra benefit (16). It is known that when complementary foods are given before six months it replaces breastmilk intake because infants seem to self-regulate their intake (17).

Linear growth of these study infants as measured by length for age followed a relatively lower but followed a parallel growth pattern as the WHO breastfed reference infants. This similarity of growth pattern has important implication for interpreting their growth. It may be noted that these infants were born of relatively shorter mothers compared to healthy and taller Western mothers. Since maternal height seems to have significant positive association with infant length (18), it might also be possible that these infants in our study might have shown their normal and optimal physiologic growth potential. The deficit they have compared to the NCHS reference or to the WHO Breastfed cohort is mainly incurred during intrauterine life. Therefore, growth during infancy may be a continuation of that growth pattern. It may also be possible that comparatively lower linear growth in these infants reflects some environmental constraints such as possible inadequacy of important micronutrients in breast milk, which might have restricted growth of these infants, and may need potential interventions for improvement. It might also be a reflection of altered metabolic and physiologic functions in these infants, which might have resulted as a consequence of undernutrition during fetal life. But these issues need further investigations.

Growth reference is frequently being considered as the standard therefore it should reflect the normal growth of the infants who follow the current feeding recommendations. Data from different populations indicate that the current reference is inappropriate for comparing growth of the breastfed infants (19). If the growth of these infants is compared with that of WHO breastfed reference infants, the gap even becomes wider at birth and first three months (20) although the pattern shows a close similarity. The difference in growth i.e. poorer weight therefore, is mainly attributable to the intrauterine rather than postnatal factors.

Vitamin A status of infants at six months.

Vitamin A status of the infants in this study was poor irrespective of maternal supplementation with dietary fat although there was improvement in maternal vitamin A status during early lactation (**Chapter 2**). At birth infant vitamin A stores in the liver is low (21). Breastmilk is probably the major or exclusive source of vitamin A particularly in poor communities, as the one we studied, up to infancy and beyond (6). It is also notable that maternal breastmilk retinol concentration clearly indicates that the estimated vitamin A supply from mother to infant would still be less than the recommended daily allowance (RDA) for the infants even when the milk output is considered at its normal limit (22). This might be another reason that infants' status was marginal in both groups. These findings clearly indicate that infants are at risk for vitamin A deficiency in early infancy in that community and warrants early intervention with vitamin A.

Diarrhoea and growth in young children

Children less than five years old in this community suffer, on average, 4.5 diarrhoeal episodes annually and about 15% of all diarrhoeal episodes are due to dysentery (bloody diarrhoea). Children who suffered dysentery experienced significantly lower weight and height gain in both short (3 mo) and longer term (1 yr) growth periods. While other type of diarrhoea also has detrimental effect on child growth, their negative effect is relatively weaker than dysentery. This negative effect of dysentery on growth of already malnourished children may worsen their nutritional status.

Malnutrition again increases the risk of diarrhoea, therefore, the cycle is likely to continue in this community unless effective interventions are implemented (23)

CONCLUSIONS AND RECOMMENDATIONS

1. Increasing dietary fat intake has a beneficial effect in populations with low fat intake and who depend on plant sources for their dietary vitamin A intake. However, the size of the effect indicates that an increase in dietary fat intake alone has a very limited role. Therefore, strategies to ameliorate vitamin A deficiency in pregnant and lactating women in rural Bangladesh would require long-term multidisciplinary approach. However, supplementation with low dose vitamin A may be considered as an immediate strategy to improve the situation in pregnant and lactating women. As the evidence of improving vitamin A status through increased consumption of dark green leafy vegetables is doubtful alternative approaches must be considered. Food fortification with vitamin A may be considered as one of the possible long term interventions as vitamin A deficiency is so widespread in Bangladesh.
2. Women in rural Bangladesh are in critical energy balance during pregnancy and lactation. The high prevalence of low birth weight and the poor postnatal growth might be the outcomes of poor maternal nutrition during these two critical periods. However, there is a paucity of energy expenditure data on this population limits the assessment of adequacy of dietary intake. Therefore, assessment of energy balance of these rural women should receive a research priority.
3. Dietary assessment to identify pregnant or lactating women at risk of vitamin A deficiency should only be used at group level when necessary. At individual level the misclassification rate is unacceptably high from the public health point of view. Both at group and individual level 24-hour recall of vitamin A food provide more reliable estimate and has stronger association with biochemical indicator of vitamin A than food frequency questionnaire (FFQ). This contradicts with the "Guidelines for developing a Simplified Dietary Assessment (SDA) Questionnaire" which suggest the results of the FFQ part should be used when the results of the two methods differ. The SDA methods are prone to several sources of error which include no consideration for wide variation in vitamin A content of individual foods within food category, equal weight for each retinol equivalent derived from animal or plant sources, and approximation in portion size estimates. Dietary assessment of vitamin A intake may be improved by using only 24-hour recall of vitamin A intake, single portion size for each

particular age group and more quantitative estimate of vitamin A intake in retinol equivalent.

4. Breast-feeding is universal in Bangladesh during the first six months of infancy. However, a substantial proportion of infants is given additional fluid or traditional local complementary foods in small quantities earlier than six months. Whether exclusively or non-exclusively breastfed growth was comparable up to the age of 6 months. Notably, infant growth followed the same pattern as that of WHO breastfed reference infants although they could not make up the initial growth deficit they had at birth. Further studies are needed to confirm whether the growth pattern observed in these infants might be their optimal physiologic growth potential as these infants were born relatively smaller in size at birth. On the other hand, whether maternal lactation is adequate in terms of quantity and quality also needs further investigation. Nevertheless, our findings support that of others that complementary fooding has no advantage if introduced before six months. Therefore, policy should be directed towards promotion and protection exclusive breastfeeding up to the first six months of infancy.
5. Diarrhoeal illnesses still exists as a significant public health problem among young children in rural Bangladesh. The dysentery type of diarrhoea causes most detrimental effect of diarrhoea. Prevention of dysentery through improving sanitation and/or developing effective vaccine against most common dysentery causing organisms such as *Shigella spp* may substantially improve both linear and ponderal growth in children less than five years old.

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Summary

The prevalence of maternal and child malnutrition in Bangladesh is one of the highest in the world. It is estimated that 50% of women of childbearing age suffer from chronic energy deficiency (BMI<18.5) and nearly half of infants are born with a low birth weight (<2.50 kg). About 60% of the preschool children are stunted. Protein energy malnutrition and deficiency of micronutrients often coexist in pregnant and lactating women, in breastfed infants and in young children. Vitamin A deficiency is widespread and exists as a significant public health problem in pregnant and lactating women in Bangladesh, although clinical vitamin A deficiency (xerophthalmia) the situation has improved in preschool children in recent years. In this population, the contribution of preformed retinol, available in foods of animal origin, is insignificant because of very low consumption of such foods. Major sources of dietary vitamin A are the provitamin A carotenoids from plant sources. These foods, are now, known to have poor bioavailability and are unlikely to improve vitamin A status. Moreover, the fat content of the diet in Bangladeshi population is extremely low (5-7 g/d per adult) which is another important constraint for the bioavailability of provitamin A carotenoids. The consequences of maternal malnutrition are enormous and include a higher risk of maternal mortality and morbidity, and increased risks of impaired growth and development of their fetus which may result in premature delivery and/or low birth weight infants. Those infants who survive through infancy often suffer from further growth impairment due to environmental conditions predisposing them to diarrhoea.

Research described in this thesis focused on: a) vitamin A deficiency in pregnant and lactating women and a possible role of dietary fat supplementation on vitamin A status of women and their breast fed infants in rural Bangladesh; b) use of a specific dietary assessment method as a field tool to identify pregnant and lactating women at risk of vitamin A deficiency; c) maternal energy intake and an assessment of maternal energy stress during pregnancy and lactation; d) infants' growth performance in relation to breastfeeding practice; and e) the effect of diarrhoea on growth of young children. All studies were performed in a poor rural community in Bangladesh.

In *Chapter 2* data on the effect of 18 g of dietary fat (soybean oil) supplementation during mid/late pregnancy and throughout the first six months of lactation on vitamin A status of mothers and infants have been presented. In this population daily dietary fat intake was found to be extremely low (5-7 energy%). Oil supplementation (18 g/d), however, doubled the daily fat intake in the supplemented women. Compared to the baseline (pregnancy) level, plasma retinol concentrations increased significantly at 1 and 3 months postpartum (by 0.07±0.35 and 0.04±0.37

μmol/L, $p<0.05$ respectively). In the control women, retinol concentration at 1 month postpartum was slightly higher than the pregnancy level but the difference did not reach statistical significance; while the values at 3 and 6 months postpartum were in fact lower than that during pregnancy. When compared between supplemented and control, the mean change in plasma retinol concentration (from zero) was significantly greater in the oil supplemented group at one month postpartum. Both β-carotene and lutein concentrations showed a decline during all postpartum measurements compared to the values during pregnancy, but oil supplemented women experienced significantly less decline that the control women.

Dietary assessment to identify pregnant and lactating women at risk for vitamin A deficiency was performed using 24-hour recall of dietary intake and food frequency method following the guidelines for developing questionnaire for Simplified Dietary Assessment suggested by IVACG (*Chapter 3*). Modifications in the method were made to adapt the questionnaire for adult pregnant and lactating women. At individual level, dietary assessment showed poor sensitivity and specificity. However, the findings were consistent with the biochemical indicators of the groups. Both at individual and group level, 24-hour recall of vitamin A containing food showed stronger association with biochemical indicators of vitamin A than did a longer-term food frequency questionnaire.

Data on maternal energy intake during pregnancy and lactation, pregnancy weight gain and weight changes during lactation are presented in. Energy intake during pregnancy was low (~1500 kcal/d) and this was consistent with only 4 kg of weight gain during the last 20 weeks of pregnancy. The mean birth weight was 2.55 kg, and 48% infants were born with low birth weight (<2.50 kg). During lactation energy intake of these women increased by ~300 kcal/d above their pregnancy level. Women lost about 1 kg body weight during the first six months of lactation.

During the first six months all infants in our study were breastfed. However, a substantial proportion of infants also received some kinds of local complementary foods even at three months of age (30%). By six months of age about 63% of the infants were non-exclusively breastfed. Whether exclusive or non-exclusively breastfed, infants were regularly receiving breastmilk. There was no significant difference in morbidity and growth between groups at any point in time except at 6 mo when linear growth of the non-exclusively breastfed infants tended to be slightly higher than that of the exclusively breastfed group. However, the exclusively breastfed infants showed a similar growth pattern as the WHO breastfed reference infants. However, both groups had length-for-age Z-scores less than -1.25 SD of the NCHS reference median.

Data from our study showed that children less than five years old suffer 4.5 diarhhoeal episodes per child per year and about 15% of all episodes are due to dysentery (diarrhoea with blood in stool). Dysentery was negatively associated with short-term (3 mo) weight gain and long-term (1 yr) weight and height gain. Other types of diarrhoea also had some detrimental effect on child growth but those effects were less stronger than those related to dysentery.

From these findings it can be concluded that dietary fat supplementation during pregnancy and lactation has a beneficial effect on vitamin A status during early lactation in populations with low fat intake. However, assessment of dose response the dose response and of the optimal amount of fat intake required for adequate vitamin A status needs further investigation. If dietary assessment is considered to assess the risk of vitamin A deficiency in a population, the 24-recall of vitamin A containing foods should be chosen instead of the often-recommended food frequency questionnaire method. However, at the individual level dietary assessment has a high possibility of misclassification. Maternal energy intake during pregnancy and lactation is extremely low in rural Bangladesh and our findings suggest that mothers pass through a process of depletion during these two periods. However, the gap in energy balance cannot be quantified unless energy expenditure data are available for this population. Breastfeeding is universal in this rural Bangladeshi community, nevertheless, a substantial proportion of infants receive complementary foods earlier than six or even earlier than three months of age. The absence of a significant difference in growth between exclusive and non-exclusively breastfed infants indicated that complementary feeding earlier than six months has no growth advantage nor a disadvantage in these infants. More importantly, the similarity growth pattern between the exclusively breastfed infants of this study and that of the WHO breastfed reference infants suggests that this growth pattern might be the physiologically optimal for infants in this community who born with a relatively lower birth weight. There is no evidence of extra benefit from complementary feeding at an earlier age than 6 mo therefore, should not be promoted in this community before that age. Diarhroeal illnesses still continue to be a significant public health problem and a substantial contributor to childhood malnutrition in rural Bangladesh. However, the detrimental effect of diarrhoea on growth depends on the proportion of dysentery among the diarrhoeal episodes. In diarrhoea control programs, specific preventive measures should be included for the prevention of dysentery, if possible through vaccination against common dysentery pathogens.

Samenvatting

Ondervoeding bij moeder en kind komt in Bangladesh op grote schaal voor. Men schat dat 50% van de vrouwen in de vruchtbare leeftijd lijdt aan een chronisch energietekort (Body Mass Index: BMI<18.5) en dat bijna de helft van alle kinderen wordt geboren met een laag geboortegewicht (Low Birth Weight: LBW<2.50 kg). Ongeveer 60% van de peuters hebben een groeiachterstand (Eng: stunted). Het betreft echter niet alleen een energietekort Zowel bij zwangeren en lacterenden als bij borstgevoede kinderen en bij peuters bestaan tekorten aan energie, eiwit en microvoedingsstoffen naast elkaar. Zo is vitamine A tekort wijdverspreid in Bangladesh en vormt daar een belangrijk volksgezondheidsprobleem. Aangezien de consumptie van dierlijke produkten beperkt is, is de inneming van reeds gevormd retinol beperkt. De belangrijkste bronnen van vitamine A zijn de provitamine A carotenoïden uit plantaardige produkten. Het is bekend dat deze carotenoïden veelal een beperkte biobeschikbaarheid hebben (afhankelijk van het type voedingsmiddel) en zij zullen dus maar in beperkte mate de vitamine A status kunnen beïnvloeden. Bovendien is de vetinneming in deze bevolking erg laag (5-7 energy%) wat de biobeschikbaarheid van carotenoïden ook nadelig beinvloedt. De gevolgen van maternale ondervoeding zijn enorm en betekenen een hogere morbiditeit en mortaliteit bij de vrouw en een toegenomen kans op een verstoorde groei en ontwikkeling van de foetus wat kan leiden tot een premature bevalling en/of een laag geboortegewicht. De kinderen die het eerste levensjaar doorkomen laten ook daarna vaak groeivertraging zien, mede alsgevolg van diarree.

Het in dit proefschrift beschreven onderzoek richt zich op: (a) vitamine A tekort bij zwangere en lacterende vrouwen en op mogelijke effecten van suppletie met voedingsvet op de vitamine A status van moeder en kind; (b) het gebruik van een in het veld bruikbare voedselconsumptie methode voor het identificeren van vrouwen met een verhoogd risico op een vitamine A tekort; (c) de energie-inneming van de vrouw en de vaststelling van de energetische stress tijdens zwangerschap en lactatie; (d) de groei van de zuigeling in relatie tot de borstvoedingspraktijk; en (e) het effect van diarree op de groei van jonge kinderen. De studies werden uitgevoerd in een arme gemeenschap in ruraal Bangladesh.

De dagelijkse vetinneming vanaf het midden van de zwangerschap tot 6 maanden lactatie werd verdubbeld door dagelijkse suppletie met 18 gram vet (sojaolie). Vergeleken met de plasma retinol concentraties in het midden van de zwangerschap waren de waarden bij 1 en 3 maanden postpartum significant toegenomen (met 0.07 en

0.04 µmol/L, respectievelijk). In de controle groep was de retinol concentratie bij 1 maand postpartum licht verhoogd ten opzichte van de zwangere waarde maar het verschil was niet significant en bij 3 en 6 maanden postpartum waren de waarden zelfs lager dan tijdens zwangerschap. Als de gesuppleerde en controle groep met elkaar vergeleken worden dan is de gemiddelde verandering in plasma retinol concentratie bij een maand postpartum in de olie-gesuppleerde groep significant groter. Zowel β-caroteen als luteïne concentraties lieten een verlaging zien bij alle postpartum metingen vergeleken met de zwangere waarden, maar de gesuppleerde vrouwen hadden een duidelijk kleinere daling dan de controle vrouwen.

De International Vitamin A Consultative Group (IVACG) heeft een vereenvoudigde voedselconsumptie methode (Simplified Dietary Assessment: SDA) voorgesteld om groepen met een verhoogd risico op vitamine A tekort te kunnen identificeren. De methode werd geschikt gemaakt voor vrouwen (zie Hoofdstuk 3). Zowel op individueel niveau als op groepsniveau bleek de 24-uurs recall van vitamine A bevattende voedingsmiddelen (in SDA methode) valider dan de voedselfrequentie vragenlijst.

De energie-inneming tijdens de zwangerschap was laag (1500 kcal/dag) en dit was consistent met de geringe gewichtstoeneming van 4 kg over de tweede helft van de zwangerschap van (zie Hoofdstuk 4). Het gemiddelde geboortegewicht bedroeg 2.55 kg, en 48% van de kinderen had een laag geboortegewicht. Tijdens de lactatie nam de energie-inneming toe met ~300 kcal/dag indien vergeleken met de zwangerschapsinneming. De moeders verloren 1 kg lichaamsgewicht gedurende de eerste 6 maanden van lactatie.

Gedurende de eerste zes levensmaanden werden alle kinderen in de studie borstgevoed. Bij 3 maanden kreeg 30% bijvoedsel (Eng: complementary foods), bij 6 maanden was dit 63%. Er waren geen duidelijke verschillen in morbiditeit en groei tussen de uitsluitend borstgevoede kinderen en de kinderen die ook bijvoedsel kregen, behalve op de leeftijd van 6 maanden waar de kinderen met bijvoedsel een iets betere lengtegroei lieten zien. Aan de andere kant hadden de uitsluitend borstgevoede kinderen een soortgelijk groeipatroon als de WHO-referentie voor borstgevoede kinderen. Beide groepen hadden een lengte-voor-leeftijd Z-score van < -1.25 (bij vergelijking met de NCHS-referentie).

Onze resultaten laten zien dat kinderen onder de vijf jaar gemiddeld 4.5 diarree episodes per jaar hebben. Ongeveer 15% van alle diarree episodes werd veroorzaakt door dysenterie (diarree met bloed in ontlasting). Het aanwezig zijn van dysenterie was negatief geassocieerd met gewichtstoeneming en lengtegroei. Andere vormen van diarree hadden ook nadelige effecten op groei, maar niet zo uitgesproken als dysenterie.

We concluderen dat in een bevolking met een lage vetinneming vetsuppletie tijdens zwangerschap en lactatie een gunstig effect kan hebben op de vitamine A status De optimale hoeveelheid voor een adequate vitamine A status moet nog nader onderzocht worden. De energieinneming tijdens zwangerschap en lactatie is zeer laag en onze resultaten suggereren dat de moeders met verdere depletie van hun reserves te maken hebben. Borstvoeden is algemene praktijk in deze rurale Bangladeshi gemeenschap. Toch krijgt een groot deel van de kinderen bijvoedsel vóór de leeftijd van 6 maanden, een deel zelfs vóór de leeftijd van 3 maanden. Het afwezig zijn van een verschil in groei tussen uitsluitend borstgevoede kinderen en kinderen met ook bijvoedsel suggereert dat het geven van bijvoeding jonger dan 6 maanden geen extra voor- of nadelen lijkt op te leveren. Het feit dat de borstgevoede kinderen hetzelfde groeipatroon lieten zien als de WHO-referentie voor borstgevoede kinderen, suggereert dat het waargenomen groeipatroon normaal en wellicht optimaal is. Diarree is nog steeds een belangrijke volksgezondheidsprobleem in Bangladesh. Het nadelige effect van diarree-episodes op de groei hangt sterk af van de mate waarin dysenterie voorkomt. Maatregelen ter voorkoming van dysenterie verdienen daarom aandacht.

সারমর্ম

পৃথিবীর যে সকল দেশগুলোতে মা ও শিশু চরম পুষ্টিহীনতায় ভোগে তার মধ্যে বাংলাদেশ অন্যতম। বাংলাদেশের প্রায় ৫০% সন্তান জন্মদানে সক্ষম মা দীর্ঘ মেয়াদী পুষ্টিহীনতায় ভোগছেন (BMI <১৮.৫) এবং জন্মের সময় প্রায় অর্ধেক শিশু স্বল্প ওজন (<২.৫ কেজি) নিয়ে জন্মগ্রহণ করে। তা ছাড়া বাংলাদেশের শতকরা ৬০ ভাগ ৫ বছরের কম বয়সী শিশু তুলনামূলকভাবে খর্বকায় (stunted)। পুষ্টিহীনতা এবং micronutrient এর অভাব গর্ভবতী ও বুকের দুধ খাওয়াচ্ছে এমন মায়েদের, দুগ্ধপোষ্য শিশু এবং অন্যান্য ছোট শিশুদের মধ্যেই বেশী বিরাজমান। বাংলাদেশে গর্ভবতী ও বুকের দুধ খাওয়াচ্ছে এমন মায়েদের মধ্যে ভিটামিন-এ'র অভাব খুবই ব্যাপক। তবে ছোট শিশুদের ক্লিনিক্যাল ভিটামিন-এ অপুষ্টির হার ইদানিং বেশ হ্রাস পেয়েছে। বাংলাদেশে খাদ্যে ভিটামিন-এ এর উৎস মূলত উদ্ভিদ জাতীয় খাবার। কিন্তু বর্তমানকালের পরীক্ষা-নিরীক্ষা থেকে জানা গেছে যে, এই উদ্ভিদ জাতীয় উৎস থেকে প্রাপ্ত ভিটামিন-এ জাতীয় পদার্থ শরীরে পর্যাপ্ত পরিমাণে ভিটামিন-এ'তে রূপান্তরিত হয়না। এর ফলে এসব খাদ্য দিয়ে ভিটামিন-এ জনিত অপুষ্টি দূর অসম্ভব বলে ইদানিং ধারণা করা হচ্ছে। তা ছাড়া বাংলাদেশী সাধারণ খাবারে স্নেহ জাতীয় পদার্থ খুব কম থাকে (মোট ক্যালরীর মাত্র ৫-৭ শতাংশ) বলে বিশেষ করে উদ্ভিদ জাতীয় ভিটামিন-এ'র কার্যকারিতা ব্যহত হয় কারণ স্নেহ জাতীয় পদার্থ এসকল ভিটামিন-এ জাতীয় পদার্থকে শরীরে প্রবেশে সাহায্য করে থাকে। ভিটামিন-এ জনিত পুষ্টিহীনতা মায়ের মৃত্যু এবং মায়েদের রোগের হার বৃদ্ধির সাথে জড়িত রয়েছে বলেও ধারণা করা হয়। তাছাড়াও মায়ের পুষ্টিহীনতা শিশুদের মধ্যে স্বল্প ওজন নিয়ে জন্মগ্রহণের হার ও অকাল জন্মের হার বৃদ্ধির সঙ্গে যোগ রয়েছে বলে মনে করা হয়। বাংলাদেশে শিশুর শারীরিক বৃদ্ধি জন্ম পরবর্তীকালে বিভিন্ন পরিবেশগত কারণে বিশেষ করে ডায়রিয়া জনিত রোগে ব্যাহত হয়।

এই থিসিসে নিম্নোক্ত বিষয়গুলোর উপর আলোকপাত করা হয়েছে এবং গবেষলব্ধ ফলাফল বর্ণনা করা হয়েছেঃ-

১. গর্ভবতী ও বুকের দুধ খাওয়চ্ছে এমন মায়েদের মধ্যে খাদ্যে স্নেহ জাতীয় পদার্থের পরিমান দৈনিক ১৮ গ্রাম বাড়ালে মায়েদের ও তাদের দুগ্ধপোষ্য শিশুদের ভিটামিন-এ জনিত অবস্থার উপর কি প্রভাব পড়ে;

মধ্যে), এবং দীর্ঘকালীন (১ বছর সময়ের মধ্যে) ওজন ও দৈর্ঘ্য বৃদ্ধির নেতিবাচক সম্পর্ক পরিলক্ষিত হয়। অন্যান্য উদরাময় জনিত রোগেরও এ ধরণের পুষ্টির উপর বিরূপ ফলাফল পরিলক্ষিত হয়, তবে রক্ত আমাশয়ের ক্ষেত্রে এই পরিবর্তন লক্ষ্যনীয়ভাবে বেশী।

উপসংহারে বলা যায় যে গর্ভকালীন ও প্রসব পরবর্তী কালে খাদ্যে স্নেহ জাতীয় পদার্থের সংযোজন, প্রসব পরবর্তীকালের প্রথম দিকে রক্তে ভিটামিন-এ পুষ্টির উন্নতি ঘটায়। কিন্তু ঠিক কি পরিমাণ স্নেহ সংযোজন করলে পরিমিত পরিবর্তন আশা করা যায় তা পরবর্তী গবেষণার বিষয় হতে পারে। ভিটামিন-এ জনিত পুষ্টিহীনতার ঝুঁকি রয়েছে এমন জনগোষ্ঠি নির্বাচনের ক্ষেত্রে গত ২৪ ঘন্টায় গ্রহণকৃত খাদ্য তালিকা দীর্ঘ মেয়াদী খাদ্যাভ্যাসের পরিমাপের চেয়ে বেশী উপযোগী কিন্তু ব্যক্তি পর্যায়ে এ পদ্ধতি ব্যবহার করে সঠিক ফলাফল পাওয়া যায়না। গর্ভকালীন ও প্রসব পরবর্তীকালে বাংলাদেশের মায়েদের খাদ্য থেকে শক্তি গ্রহণের পরিমান খুব বেশী বৃদ্ধি পায়না। এর ফলে এসব মায়েদের খাদ্যে ক্যালরী কম জনিত অপুষ্টির সম্ভাবনা বাড়িয়ে দেয় এবং মায়েদের ওজন কমে যেতে দেখা যায়। এক্ষেত্রে খাদ্যে সঠিক ক্যালরী ঘাটতি নির্ধারণের জন্যে দিনের বিভিন্ন কার্যকলাপে ব্যবহৃত শক্তির হিসাবের তথ্য বাহির করা প্রয়োজন। বাংলাদেশের ছোট শিশুদের মধ্যে বেশ উচ্চ হারে বুকের দুধ খাওয়ার প্রবণতা লক্ষ্য করা যায়। কিন্তু এর মধ্যে বেশীরভাগ শিশুকে ৬ মাস বয়সের আগেই অন্যান্য খাবার দেয়া হয়। শুধু বুকের দুধ নির্ভর শিশু এবং অন্যান্য শিশুর বেড়ে উঠার হার প্রমাণ করে যে ৬ মাসের আগে বুকের দুধ ছাড়া অন্যান্য খাবার খাওয়ানোর সাথে শিশুর বৃদ্ধির তেমন লক্ষ্যনীয় সম্পর্ক নেই; বরং শুধু বুকের দুধ নির্ভর শিশুরা কম ওজন নিয়ে জন্ম নিলেও তাদের বৃদ্ধির ধরণ WHO সমীক্ষায় গৃহীত শিশুদের মত যা এই শিশুদের বৃদ্ধিকে এ দেশীয় প্রেক্ষিতে স্বাভাবিক বলে ধরে নেয়া যায়। আমাদের শিশুদের ওজন ও দৈর্ঘের এই ব্যবধান মূলতঃ মাতৃ গর্ভকালীন অপুষ্টির-ই পরিণতি। এক্ষেত্রে ৬ মাস বয়সের আগে মাঠ পর্যায়ে অন্যান্য খাবার খাওয়ানোর পরামর্শ দেয়া যুক্তিযুক্ত নয়। উদরাময় জনিত রোগ বাংলাদেশে শিশুদের পুষ্টিহীনতার আরেকটি উল্লেখযোগ্য কারণ। সকল প্রকার ডায়রিয়া জনিত রোগের মধ্যে রক্ত আমাশয় শিশুর বৃদ্ধিতে অধিক নেতিবাচকভাবে প্রভাব ফেলে। সুতরাং ডায়রিয়া নিবারণ প্রোগ্রামে রক্ত আমাশয় নিবারণের ব্যবস্থা থাকা বিশেষ প্রয়োজন যা শিশু পুষ্টিতে বিশেষ অবদান রাখতে পারে।

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About the author

Dewan Shamsul Alam was born on 26 August 1957 in Brahmanbaria, Bangladesh. He passed Secondary School Certificate (SSC) Examination from Sarail Annada High School, Brahmanbaria in 1972 and Higher Secondary Certificate ((HSC) Examination from Brahmanbaria College, Brahmanbaria in 1975. He obtained Bachelor of Medicine and Bachelor of Surgery (MBBS) degree from Sir Salimullah Medical College, Dhaka University, Dhaka, Bangladesh in 1982. Soon after completion of his one year of in-service-training as Medical Officer in Bangladesh Health Services, he accepted a job offer in Nigeria as Resident Medical Officer where he worked from March 1983 until October 1984. He joined ICDDR,B: Centre for Health and Population Research in early 1985. In 1991, he received training award from Australian Development Assistance Bureau (ADAB) for undertaking Master of Community Nutrition (MCN) course in Nutrition Program, University of Queensland, Brisbane, Australia. After completion of the coursework of MCN program, he joined a Research Masters program in the same department in late 1992 and obtained the degree of Master of Medical Science (M Med Sc) in 1996. He carried out the research presented in this thesis between late-1995 and mid-1997 within the framework of collaboration between ICDDR,B and Division of Human Nutrition & Epidemiology, Wageningen Univerisity, The Netherlands. He received one of the finalists' award from the Society for International Nutriton Research (SINR), USA for his research in 1999. Currently he is involved in a number of research projects in ICDDR,B as Principal Investigator and/or Co-Investigator. He is a member of the research team awarded research grants from UNICEF and NIH for nutrition intervention studies aimed at improving maternal nutrition and reducing the incidence of low birth weight in rural Bangladesh. As Principal Investigator of the project aimed to investigate the association between size at birth, and childhood blood pressure and specific metabolic and hormonal paratmeters, he received research award from the World Bank grant to the Nutrition Centre of Excellence in ICDDR,B in 2000.

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