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Maternal Micronutrient Deficiencies in Early Pregnancy and Infant Nutritional Status in Urban South India

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ACADEMIC DISSERTATION
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3) TM Samuel, T Thomas, S Bhat, AV Kurpad (2012) Are infants born in baby-friendly hospitals being exclusively breastfed until 6 months of age? **Eur J Clin Nutr** 66(4), 459-65. DOI: 10.1038/ejcn.2011.179.

4) Tinu Mary Samuel, Tinku Thomas, Prashanth Thankachan, Swarnarekha Bhat, Suvi M Virtanen, Anura V Kurpad (2012) Breast milk zinc transfer and early postnatal growth among urban South Indian term infants using measures of breast milk volume and breast milk zinc concentrations. **Matern Child Nutr** June 27. DOI: 10.1111/j.1740-8709.2012.00421x.

ABBREVIATIONS

ACC/SCN	Administrative Committee on Coordination/Sub-committee on Nutrition
ANOVA	Analysis of variance
RMANOVA	Repeated measures analysis of variance
BFH	Baby-friendly hospitals
BFHI	Baby-friendly hospital Initiative
BMI	Body mass index
CI	Confidence interval
DCT	Divalent cation transporter
DMT	Divalent metal transporter
DOPA	Dihydroxyphenylalanine
DRI	Dietary reference intake
FAO	Food and Agriculture Organization
FFQ	Food frequency questionnaire
HAZ	Height for age Z score
Hb	Haemoglobin
Hcy	Homocysteine
HCZ	Head circumference for age Z score
Holo TC	Holotranscobalamine
IAEA	International Atomic Energy Agency

IZiNCG	International Zinc Nutrition Consultative Group
LBW	Low birth weight
MCV	Mean corpuscular volume
MMA	Methylmalonic acid
MUAC	Mid upper arm circumference
NADPH	Nicotinamide adenine dinucleotide phosphate oxidase
NTD	Neural tube defects
PR	Prevalence ratio
RDA	Recommended dietary allowances
RR	Relative risk
SAM	S-adenosyl methionine
SAH	S-adenosyl homocysteine
SGA	Small for gestational age
TBW	Total body water
UNU	United Nations University
UNCF	United Nations Children's Fund
UNICEF	United Nations International Children's Education Fund
WAZ	Weight for age Z score
WHZ	Weight for height Z score
WHO	World Health Organization

ABSTRACT

Pregnancy is a critical period in the life cycle with increased metabolic and physiological demands. The nutritional status of the mother has intergenerational effects, not only affecting her pregnancy and birth outcomes, but also impacting the growth and development of her child. Micronutrient deficiencies during the critical window period of the first 1000 days, starting from the mother's pregnancy through her child's second birthday, can have long lasting implications for growth and development of the child.

This thesis explores the maternal nutritional status in urban South India by evaluating the prevalence and modifiable risk factors of two specific micronutrient deficiencies highly rampant in India namely anaemia & vitamin B_{12} deficiency. This thesis also explores a key issue pertaining to infant feeding practices (compliance to exclusive breastfeeding in baby friendly hospitals (BFH) in infants less than 6 months of age), as well as deficiency of zinc in breastfed infants by assessing the zinc transfer through breast milk and infant zinc status. Research questions of this thesis were addressed in two different populations. The maternal cohort consisted of pregnant women (n = 366) in early pregnancy (≤ 14 weeks of gestation) from urban South India belonging to a low socioeconomic status (cross-sectional study), while the infant cohort consisted of term infants, followed from birth to 6 months of age, and their mothers from urban South India belonging to diverse socioeconomic status (prospective observational study).

In the maternal cohort we observed a high prevalence of anaemia (30.3%) and microcytic anaemia (20.2%). Low plasma vitamin B_{12} concentration was observed in 51.1% of the pregnant women, while 42.4% had impaired vitamin B_{12} status. Elevated methylmalonic acid (MMA> 0.26 µmol/l), elevated homocysteine (Hcy >10 µmol/L) and low erythrocyte folate (<283 nmol/L) was observed among 75.8%, 43.3% and 22.2% of women, respectively. Although mean intake of several macro- and micronutrients were well below the recommended daily allowances (RDA) for both pregnant anaemic and non-anaemic women, intake of iron absorption inhibitors such as manganese, phosphorus and zinc were high in anemic pregnant women. The median (25^{th}, 75^{th} percentile) dietary intake of vitamin B_{12} was 1.25 (0.86, 1.96) µg/day. Energy-adjusted dietary vitamin B_{12} intake significantly correlated with plasma B_{12} concentration (r = 0.164, P = 0.002). Among the pregnant women, who had a low intake of iron and several other nutrients, higher intake of calcium and phosphorus (dietary components known to inhibit iron absorption), were independently associated with a

higher risk of anaemia, relative risk (RR), (95% CI): 1.79 (1.16-2.76), and 1.96 (1.31-2.96), respectively. While lower maternal body weight was associated with higher vitamin B_{12} concentration prevalence ratio (PR) (95% CI): 0.57 (0.39, 0.84)), the important predictors of impaired vitamin B_{12} status were non- consumption of yoghurt (PR (95% CI): 1.63 (1.03, 2.58)), non-consumption of fish (PR (95% CI): 1.32 (1.01, 1.71)) and primiparity (PR (95% CI): 1.41 (1.05, 1.90)).

Among South Indian term infants and their mothers we observed poor compliance to exclusive breastfeeding and early introduction of complementary foods despite being born in a BFH, where counselling for breastfeeding is the norm (complementary foods introduced as early as 1 month among 44% of the infants, only 14% remained as exclusively breastfed by month 6). Intake of breast milk significantly declined from 166 to 87 ml/kg/day and non breast milk water intake significantly increased from 23 to 51 ml/kg/day from month 1to 6 ($P<0.01$). The primary reason for the early introduction of complementary foods was a crying infant, perceived by her to be due to insufficient breast milk. Breast milk zinc intakes were also low owing to low volumes of breast milk intake, despite breast milk zinc concentrations being in the normal range. Breast milk zinc concentration and intake significantly declined from months 1 to 6 ($P<0.001$ for both). Mean infant serum zinc concentration at months 3 and 6 were 93.0 ± 27.1 and 99.6 ± 30.1 µg/dL, respectively.

Collectively, the results presented in this thesis points towards the sub-optimal nutritional status of mothers and infants from urban South India and calls for the immediate attention of policy makers to formulate action oriented strategies that would improve the maternal and child nutritional status as a primary step towards achievement of the Millennium Development Goals. Primary efforts towards improving the micronutrient status of pregnant women should focus on a food based strategy, aiming to improve the overall quality of the diet, and increasing the intake of specific foods that are high in nutrients critical to this stage such as iron, folate and vitamin B_{12}. In addition, promotion of breastfeeding and thereby increasing the volumes of milk produced should be the first important step towards improving breast milk intake and zinc intake among infants.

1: INTRODUCTION

Pregnancy is a critical period in the lifecycle during which additional nutrients are required to meet the metabolic and physiological demands as well as the increased requirements of the growing foetus and placenta (Broughton 2007). Nutritional status before conception and intake of macro and micronutrients during pregnancy are both of utmost importance since they are known to affect pregnancy and birth outcomes (Forsum 2003, Cuco et al 2006, Villar et al 2003). Good maternal nutrition not only affects the mother in terms of morbidity but also impacts the growth and development of her child and has been linked to the 'foetal origins' of adult diseases implying that the foetal programming for the adult diseases takes place in *utero* (Barker 1998). This concept of Developmental origins of Health and Disease proposes that maternal characteristic before and during pregnancy influence foetal survival, growth, size and body composition as well as the functioning of various systems. The quality of nutrition in the first 1,000 days starting from the mother's pregnancy through her child's second birthday is a critical window when a child's brain and body are developing rapidly and good nutrition is essential to lay the foundation for a healthy and productive future. If children do not get the right nutrients during this period, the damage is often irreversible and it then presents an inter generational cycle of ill health by increasing the risk of perinatal, infant, and child morbidity and mortality, poor long term physical growth, cognitive development and future learning capacity, school and work performance, and ultimately reproductive and lactation outcomes (State of worlds mothers 2012).

Undernutrition is an underlying cause of death, and it leaves millions more with lifelong physical and mental impairments. In India, the burden of reproductive and child health nutrition is greater than any other country, with 1.8 million deaths among children under 5 years and 68,000 deaths among mothers, and 52 million children who are stunted in the year 2008 (Paul et al., 2011). Although the Millennium Development Goals call for a reduction in child mortality by two-thirds, reduction in maternal mortality ratio by three-quarters and universal access to reproductive health as key targets that need to be achieved by the year 2015, the pace of improvement has been slow and falls short of the National and Millenium Development Goal targets. According to the National Family Health Survey 3 carried out in 2005-06, 58% of Indian pregnant women are anaemic, 8.3 million babies are born low birth weight, 50% children die of malnutrition, 46% children are underweight, 25.5

million children are stunted, 13 million children are wasted and only 46% of the children under the age of 6 months are exclusively breastfed (National Family Health Survey 2005-2006). These statistics are alarming and warrant immediate action. In India, the prevalence of low birth weight (LBW) and small-for-gestational age (SGA) have been on the rise and the majority of LBW infants in India are a result of intrauterine growth restriction (ACC/SCN Administrative committee on Coordination/Sub-committee on Nutrition 2000). Babies born small in size in India were ascribed to the small size and 'chronic under nutrition' of Indian mothers (Gopalan 1994). Despite routine antenatal practices followed in urban settings; there are gaps in understanding the mechanisms that would help in reducing the burden of these outcomes.

While macronutrient deficiencies (both energy and protein) are known to affect fetal growth and maternal health, there is increasing awareness that micronutrient deficiencies also have important implications for foetal growth and outcome (de Onis et al., 1998). Concurrent deficiencies of micronutrients are well documented among young pregnant women and young children, especially in low- and moderate income countries (Black 2003, Allen and Peerson 2009, Christian et al., 2010), and are a result of poor- quality diets, high fertility rates, repeated pregnancies, short interpregnancy intervals, increased physiological needs, as well as inadequate health systems with poor capacity, poverty and inequities, and socio-cultural factors such as early marriage and adolescent pregnancies and some traditional dietary practices. Although iron deficiency is highly common there is often at least one other deficiency that co-exists (Allen 2009). Pregnant women in India have coexisting deficiencies of zinc, iron, folate, and vitamin A along with a deficit in the intake of energy, protein, and fats (Kapil 2009). Equally, child malnutrition remains to be a significant challenge in developing countries undermining the survival, growth and development of children (Khan and Bhutta 2010, Harrison et al., 2010), and has been associated with concomitant micronutrient deficiencies of vitamin A, iron, zinc and iodine. Maternal and child under nutrition has been associated with almost 35% of all deaths in children under the age of 5 years worldwide, 178 million stunted children and an additional 19 million children with severe acute malnutrition or wasting, based on estimates from 2008 for low and middle income countries from Asia, Africa and Latin America (Black et al., 2008). The National Family Health Survey reports state that in India eight in ten children 6 to 35 months of age

are anemic and for children 6 to 59 months, 26% have mild, 40% have moderate, and 3% have severe anemia (National Family Health Survey 2005-2006).

While there is a high concurrent prevalence of two, three or four micronutrient deficiencies such as zinc, iron, magnesium and folic acid, among pregnant Indian women due to the poor dietary intake of food and low frequency of consumption of food groups rich in micronutrients (Pathak et al., 2004), the rates are alarming specifically for anaemia. India contributes to about 80% of the maternal deaths due to anaemia in South Asia (Ezzati et al., 2002). Anaemia in early pregnancy is associated with a 50% greater risk of inadequate weight gain for gestation (Scholl and Hediger 1994), and mild anaemia increases the risk of preterm delivery by 10-40% (Scanlon et al., 2000). The National Family Health Survey reports the prevalence of anaemia among pregnant women in India to be 59% (National Family Health Survey 2005-2006). Despite the ongoing National Anaemia prophylaxis programme of iron and folic acid supplementation to pregnant women, two large national surveys (National Family Health Survey 2005-2006, District Level Household Survey 2002-2004) have indicated that there has been little change in the prevalence of anaemia and the adverse consequences associated with it.

Deficiency of vitamin B_{12} is also considered to be highly prevalent in India and the metabolic signs of vitamin B_{12} deficiency have been reported in 75% of adult men and women from urban areas of West India (Refsum et al., 2001a). Vitamin B_{12} deficiency during pregnancy may play a key role in elevating plasma homocysteine (Hcy) levels (Waterland and Michels 2007), and is associated with increased risk for several adverse outcomes like neural tube defects (NTD) (Ray et al., 2007, Molloy et al., 2009), intrauterine growth retardation (IUGR) (Muthayya et al., 2006) and is of concern since the vitamin B_{12} status of the mother correlates directly with that of the foetus (Obeid et al., 2000). In addition to the increased requirements during pregnancy, chronic low intakes of dietary vitamin B_{12} (Allen 2009) and malabsorption due to intestinal infections (Casterline et al., 1997) may lead to a negative vitamin B_{12} balance and depletion of tissue stores, leading to a functional deficiency in pregnancy. This may be more pronounced in the Indian sub-continent where vegetarianism is commonly practiced. Collectively, both anaemia and vitamin B_{12} deficiencies are highly prevalent among pregnant women in developing countries like India, and are also associated with poor pregnancy outcomes (Allen 1997, Ray et al., 2007, Molloy et al., 2009, Muthayya et al., 2006), and it therefore important to identify the causative factors for these.

The effects of maternal micronutrient deficiencies not only span in one generation, but also across generations. While addressing maternal micronutrient deficiencies in pregnancy is critical, it is equally important to focus on good quality nutrition for the infant and the child. In this regards, cost effective strategies needs to developed to reduce under five mortality in children. For example, exclusive breastfeeding up to 6 months of age and continued breastfeeding up to 12 months has been ranked the most effective child survival intervention for preventing under-five mortality (Jones et al., 2003). However, globally less than 36% of the infants are exclusively breastfed for at least 6 months (Gupta et al., 2012) and in India only 46% of the infants under 6 months and 58% of infants under 4 months of age are exclusively breastfed (Patel et al., 2010). Towards an effort in improvement in exclusive breastfeeding rates, an Indian study has shown that in baby-friendly hospitals (BFH) where mothers were counselled by the lactation support staff to exclusively breastfeed for 6 months, breastfeeding was initiated early, prelacteal feeds were less common and the intake of supplementary feeds like milk and fluids during the hospital stay was significantly lower (Breastfeeding Promotion Network of India, 2000). Nevertheless, it has not been studied whether these practices have been observed by the mothers even after return to their homes as the compliance to exclusive breastfeeding has not been previously documented across the country using actual measures of volumes of breast milk and non-breast milk water intake. In addition to poor breastfeeding practices, zinc deficiency remains a problem of public health significance and is responsible for approximately 4% of the worldwide morbidity and mortality burdens of young children (Black et al., 2008), and is an important cause of stunting, increases infectious disease morbidity and mortality due to diarrhoea and pneumonia (Hambidge 1997, Hambidge and Krebs 2003). For term infants with normal birth weight, zinc requirements are generally assumed to be met by exclusive breastfeeding due to high bioavailability of zinc in human milk (Krebs et al., 1996). However, at 5 to 6 months of age, infants may become marginally zinc deficient due to the physiological decline in BM zinc concentration, making the infants vulnerable to sub-optimal zinc intake and thereby impaired growth (Krebs 1999; Walravens et al., 1992). In addition, a decreased intake of zinc from breast milk due to lower volumes of breast milk intake, attributed to poor breastfeeding practices, may predispose infants to an elevated risk of zinc deficiency even before 6 months. There is a paucity of data on zinc intake in Indian breastfed infants less than 6 months of age based on measured volumes of breast milk intake and breast milk zinc concentrations.

2. REVIEW OF LITERATURE

2.1 PREGNANCY AND MATERNAL MICRONUTRIENT DEFICIENCIES

Pregnancy is a period of increased metabolic demands with physiological changes as well as increase in requirements of a growing foetus (Broughton 2007). Numerous anatomic, biochemical, and physiologic changes occur during pregnancy to maintain a healthy environment for the growing foetus such as increase in plasma volume by 45 to 50% by 34 weeks of gestation, increase in red cell mass by 33%, increase in cardiac output by 40%, respiratory changes to support maternal and foetal requirements for oxygen, increase in glomerular filteration rate by 50%, increase in basal metabolic rate and changes in gastrointestinal tract to support the increased demand for nutrients (Turner 2006). Many of these changes begin in the early weeks of pregnancy, and together they regulate maternal metabolism, promote foetal growth, and prepare the mother for birth, and lactation. Daily requirements for many micronutrients during pregnancy are higher to meet the physiologic changes and increased nutritional needs of pregnancy.

Adequate maternal micronutrient status is critical during pregnancy and lactation. Pregnant women in developing countries are recognized to be at risk of multiple micronutrient deficiencies, such as iron, folic acid, iodine, zinc, vitamins A and D, riboflavin, B_6 and B_{12}, with the likelihood of adverse effects on the mother and pregnancy outcomes (Black et al., 2008). Concurrent deficiencies of micronutrients are well documented among young pregnant women and young children, in low- and moderate income countries (Black 2003, Allen and Pearson 2009, Christian et al., 2010), and are due to a poor quality diet, inadequate intake of animal source foods, increased requirement for nutrients, high amounts of phytates and polyphenols in the diet that limit the absorption of nutrients, intestinal parasites and infections that may alter the metabolism of multiple micronutrients (Allen 2005). Other contributing factors are high fertility rates, repeated pregnancies, and short inter- pregnancy intervals, increased physiological needs, as well as inadequate health systems with poor capacity, poverty and inequities, and socio cultural factors such as early marriage and adolescent pregnancies and some traditional dietary practices (Shrimpton et al., 2009). **Table** 1 elaborates the causes of micronutrient deficiencies.

Table 1: Causes of micronutrient deficiencies

Causative factors in micronutrient deficiencies
Primary causative factor: low dietary intake of a micronutrients **Secondary causative factors:** • Genetic factors, mutant genes (e.g., acrodermatitis enteropathica and Menkes disease), polymorphisms (e.g., mutations in the methylenetetrahydrofolate Reductase gene). • Nutritional interactions, dietary binding factors (e.g., fiber and phytate), micronutrient-micronutrient interactions (e.g., zinc-copper, iron manganese, cadmium-zinc and zinc-vitamin A interactions). • Physiological stressors. • Disease-associated changes in micronutrient metabolism (e.g. diabetes and hypertension-induced changes in mineral metabolism). • Drugs or other chemicals and toxicants. • Antimetabolites (e.g., dicumarol). • Metal chelation (e.g., decreased absorption and increased excretion). • Decreased gut absorption and/or increased kidney loss (secondary to tissue damage). Toxicant-induced changes in tissue pools (secondary to inflammatory or acute phase response). **Other contributing factors**: • High fertility rate, repeated pregnancies, and short inter- pregnancy intervals. • Early/adolescent marriage. • Poor health care system. • Poverty and poor access to food. • Traditional dietary practices. • Increased physiological requirements.

Source: Keen CL, Clegg MS, Hanna LA, Lanoue L, Rogers JM, Daston GP, Oteiza P, Uriu-Adams JY. The plausibility of micronutrient deficiencies being a significant contributing factor to the occurrence of pregnancy complications. J Nutr. 2003 May;133 (5 Suppl 2):1597S-1605S.

In this context, it is important to recognize the continuum of maternal micronutrient status from the peri-conception period through lactation, and of foetal and infant dependency on adequate maternal status through this time. Therefore the challenges of poor nutrition are not restricted to pregnancy alone but continue throughout the life cycle. **Figure** 1 illustrates the intergenerational effects of poor nutrition. Poor nutrition starts in utero and not only extends in to adolescent and adult life but also spans across generations. Under nutrition that occurs during childhood, adolescence and pregnancy has an additive negative impact on infant birth weight. LBW infants with IUGR are more likely to be

underweight or stunted in early life, and also at higher risk of dying in the neonatal period or later infancy. Infants are less likely to catch up on growth as well and thereby suffer from a variety of developmental deficits. Understanding the intergenerational nature of growth failure, therefore becomes important (ACC/SCN 2000).

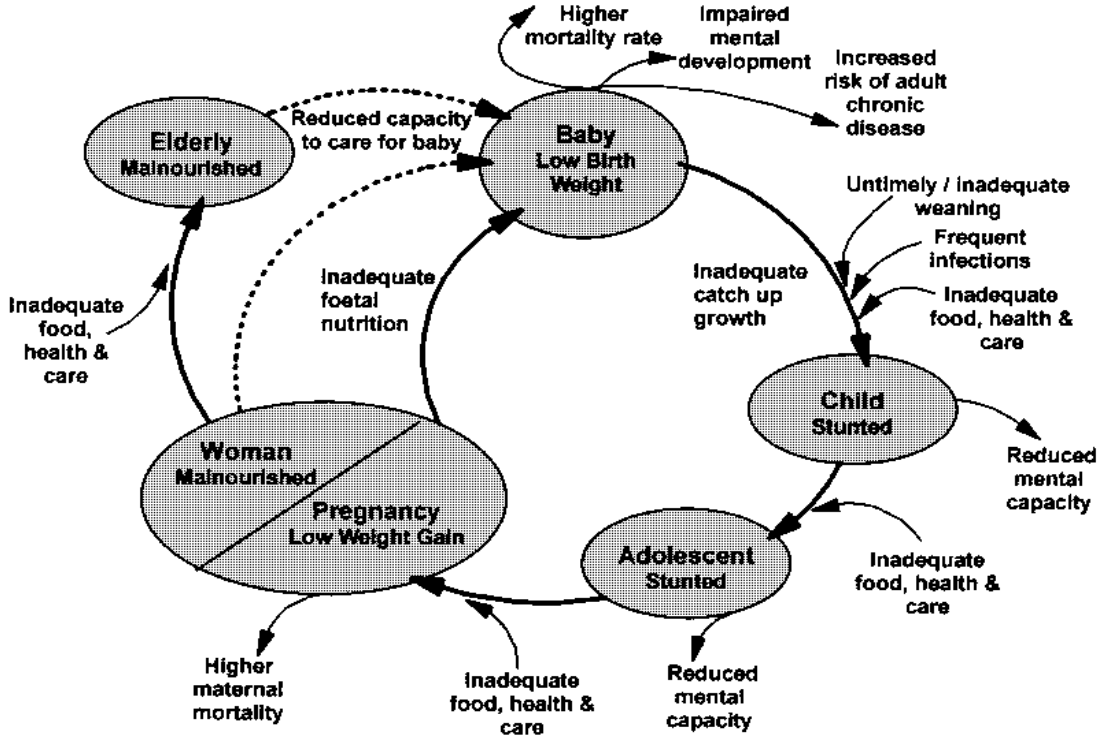

Figure 1: Intergenerational effects of poor nutrition

Source: Darnton-Hill I, Nishida C, James WP. A life course approach to diet, nutrition and the prevention of chronic diseases. Pub Health Nutr. 2004 Feb;7 (1A):101-21.

The consequences of being born undernourished also extend in to adulthood. Epidemiological evidence from both developed and developing countries suggest a link between foetal under nutrition and increased risk of various adult chronic diseases (Barker 1998). Birth weight has been inversely related to the development of adult disease such as hypertension, diabetes, and heart disease ((Huxley et al., 2000, Harder et al., 2007). In adults with type 2 diabetes, those with the LBW, and who are obese as adults appear to have a decrease in the number of functioning β-cells, which causes a decrease in insulin production in adulthood (Philips et al., 1994). There is also evidence that the time of the nutrition insult may have different effects on adult disease risk. For example, it is thought that a fetal growth

trajectory is established early in pregnancy. If nutrients and energy delivered to the foetus are decreased in early pregnancy, the foetus may adapt to a lower energy requirement. This will prevent increased growth later in pregnancy despite an increase in energy and nutrient intake later. The timing of the nutrition insult may be important in determining the long-term health of the infant even when birth weight is normal. Early life nutritional exposures, combined with changes in lifestyle in adult life, can result in increased risk of chronic diseases.

Although much of the focus on the developmental origins of disease has been on birth size, growth in postnatal life and the availability of energy and protein during these critical developmental periods, micronutrient deficiencies may also play an important role in foetal growth and development. Micronutrient status in foetal and early life may alter metabolism, vasculature, and organ growth and function, leading to increased risk of cardio metabolic disorders, adiposity, altered kidney function, and, ultimately, to type 2 diabetes and cardiovascular diseases (Christian and Stewart 2010). A number of hypotheses and pathways have been suggested through which maternal micronutrient deficiencies may lead to developmental impairments in the foetus. **Figure 2** illustrates how maternal micronutrient deficiencies may affect the development of chronic diseases in the offspring. Maternal micronutrient is known to influence hormonal regulatory pathways in the developing foetus and neonate such as zinc or iron deficiency, causing a reduction in the activity of insulin like growth factor 1 and thereby inhibiting foetal growth. Alterations in DNA methylation patterns due to deficiencies in cofactors involved in one one carbon metabolism such as folate and vitamin B_{12} is known to result in heavier adults with higher percent body fat, increased insulin resistance, and elevated blood pressure. In addition, micronutrient restriction may cause defects in developing organs, with severe micronutrient deficiencies likely to have teratogenic effects on the developing foetus and moderate nutritional deficiencies or excesses causing more subtle damage due to reduced tissue oxygenation as a result of anemia, increased oxidative stress or impaired organ development (Christian and Stewart 2010).

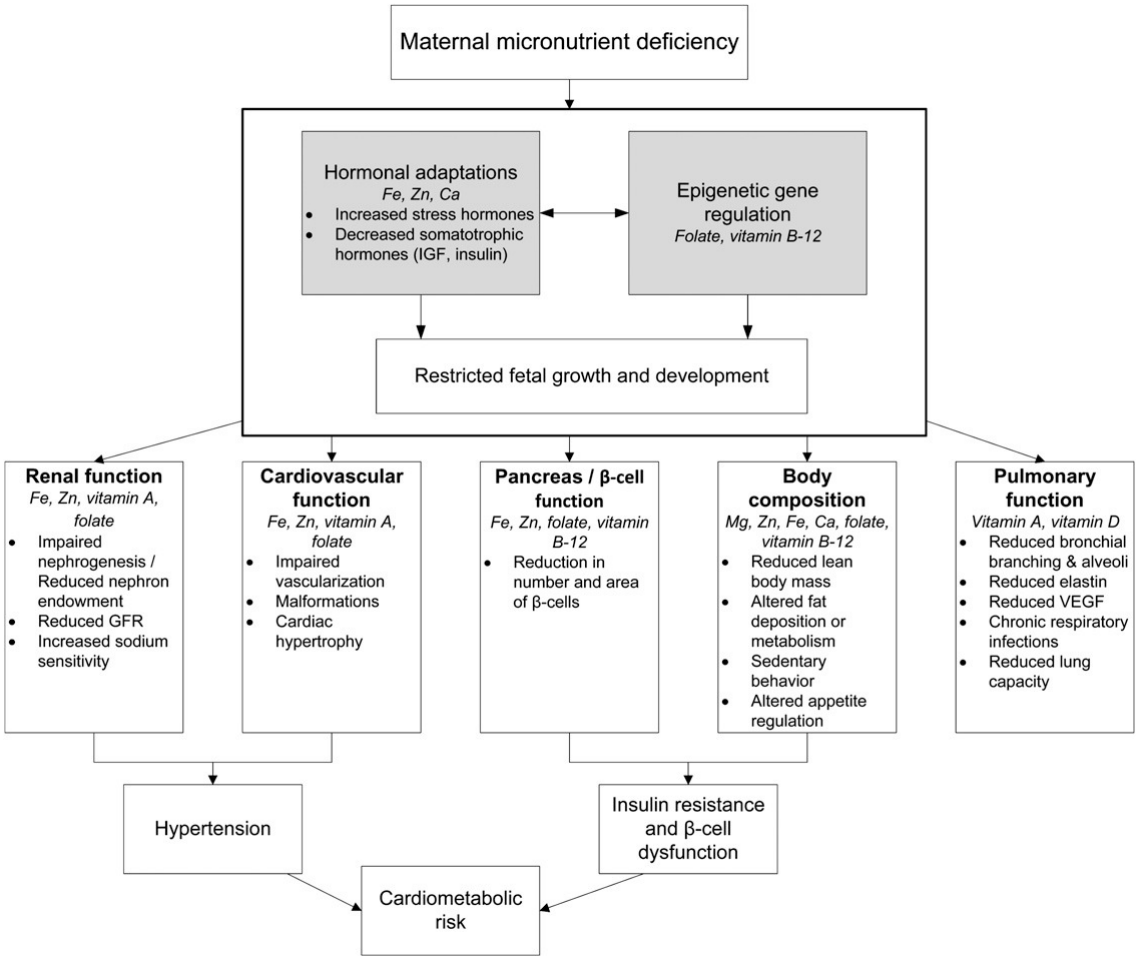

Figure 2: Pathways through which maternal diet and micronutrient status may affect the development of cardiometabolic diseases in the offspring

GFR: Glomerular filtration rate

Source: Christian P, Stewart CP. Maternal micronutrient deficiency, foetal development, and the risk of chronic disease. J Nutr. 2010; 140:437-45. Epub 2010 Jan 13.

Poor maternal nutritional status has been related to adverse birth outcomes; the association between maternal nutrition and birth outcome being complex and influenced by many biologic, socioeconomic, and demographic factors, which vary widely in different populations (Villar et al., 2003). Women of low pre-pregnancy weight, height and body mass index (BMI) and with low pregnancy weight gain are at increased risk of having infants with LBW, suggesting that energy balance is an important determinant of birth outcomes. Evidence from systematic reviews of randomized controlled trials on the effectiveness of nutritional interventions aimed at reducing IUGR has demonstrated the beneficial effects of macronutrient (protein/energy) supplementation, with an overall odds ratio of 0.77 (95% CI 0.58, 1.01) for reducing IUGR (De Onis et al., 1998). Supplementation was associated with

increases in maternal weight gain and mean birth weight, and a decrease in the number of LBW babies of borderline significance. In the Gambia, pregnant women given a high-energy groundnut snack showed better weight gain and a lower incidence of LBW than mothers not receiving the supplement (Ceesay et al., 1997). However, data on the effects of maternal malnutrition on birth outcomes in 797 mothers in rural India found no relation between maternal energy and protein intake, but instead demonstrated a stronger relationship with dietary intake of micronutrient-rich foods (Rao et al., 2001).

Inadequate stores or intake of micronutrients can have adverse effects on the mother, such as anemia, hypertension, and complications of labour and even death (Ramakrishnan et al., 1999). Furthermore, the foetus can be affected, resulting in stillbirth, pre-term delivery, intrauterine growth retardation, congenital malformations, reduced immunocompetance and abnormal organ development. Although iron-deficiency anemia is recognized as an important risk factor for maternal and perinatal mortality globally (Stoltzfuz 2003), the contribution of other micronutrient deficiencies to adverse outcomes of pregnancy is less clearly understood. However, evidence suggests that micronutrients such as vitamin B_{12} and folic acid (De Bonist 2008) vitamin D (Kovacks 2008) and selenium (Kupka et al., 2008) may also be important for maternal, infant and child outcomes. Since multiple micronutrient deficiencies often co-exist among pregnant women in developing countries, and there may be numerous confounding factors in the association between micronutrient status and birth outcomes, observational studies often do not provide the best understanding. The best causal evidence for micronutrients and adverse outcomes of pregnancy comes from randomized controlled trials. However, these have not provided unequivocal evidence of an association between micronutrient intakes and pregnancy outcomes such as birth weight, IUGR, preterm delivery and pregnancy-induced hypertension due to methodological variations across these studies with respect to study population, sample size, study design and the fact that these were conducted in women not at high risk for poor micronutrient intakes (Ramakrishnan et al., 1999). In an extensive review of randomized controlled trials, cross sectional and prospective case control studies focussing on the relation between micronutrient intakes and pregnancy, it was concluded that supplementation with zinc, calcium and magnesium showed improved pregnancy outcomes in developed countries and vitamin A supplements may be associated with reduced maternal mortality and increased birth weight. However, evidence demonstrating the role of folic acid and iron supplementation in reducing

adverse pregnancy outcomes, other than neural tube defects and increasing hemoglobin was limited. Equally, vitamin C deficiency may have a role in the etiology of preterm delivery and severe maternal iodine deficiency results in mental retardation and cretinism, but evidence is weak in the case of marginal iodine deficiency (Ramakrishnan et al., 1999).

Multiple micronutrient supplementations have also shown promising results. In a meta-analysis of 13 published trials, supplementation with multiple micronutrients, compared with iron and folate supplements only, was associated with a 17%–19% reduction in the risk of LBW; and a 54 g (95% confidence interval 36 g–72 g) increase in mean birth weight was demonstrated (Shah and Ohlsson 2009). In a recent systematic review of trials comparing the effect of maternal multiple micronutrient supplementation with that of iron and folic acid supplementation on pregnancy outcomes in developing countries, maternal micronutrient supplementation was shown to reduce the risk of LBW, but had no overall effect on perinatal mortality (Kawai et al., 2011).

2.1.1 Prevalence and aetiology of anaemia in pregnancy

Anaemia is a global public health problem affecting people at all stages of life cycle in both developing and developed countries, however, it is more prevalent among pregnant women and young children compared to adult men and women as well as elderly. The WHO has defined anaemia in pregnancy as blood haemoglobin (Hb) concentration less than 11 g/dLin the first half of pregnancy, and less than 10.5 g/dL in the second half of pregnancy. WHO has further divided anaemia in pregnancy into: mild anaemia (Hb 10-10.9g/dL), moderate anaemia (Hb 7.0- 9.9g/dL) and severe anaemia (Hb< 7g/dL) (WHO 2004b). Blood Hb concentration is the most reliable indicator of anaemia at the population level, is relatively easy and inexpensive, and is frequently used as a proxy indicator of iron deficiency. However, anaemia can be caused by factors other than iron deficiency. In addition, in populations where the prevalence of inherited hemoglobinopathies is high, the mean level of Hb concentration may be lowered.

Anaemia is the result of a wide variety of often coexisting causes. Studies conducted in India suggested that micronutrient deficiencies (iron, folate and vitamin B_{12}) are the primary cause of anaemia in pregnant women from India (Yajnik et al., 2008), and affects

50% of the pregnant women (Seshadri 2001). Dietary intake surveys among pregnant women show low intake of several key micronutrients like vitamin A and B_{12}, folate, riboflavin, and copper (National Nutrition Monitoring Bureau 2003). The relative contribution of each of these factors to anaemia in pregnancy often varies by geographical location, season and dietary intake (Vanderjagt et al., 2007). Among the other causes of anaemia, heavy blood loss as a result of menstruation, or parasite infections such as hookworms, ascaris, and Schistosomiasis can lower blood Hb concentrations. Acute and chronic infections, including malaria, cancer, tuberculosis, and human immunodeficiency virus can also lower blood Hb concentrations. The presence of other micronutrient deficiencies, including vitamins A and B_{12}, folate, riboflavin, and copper can increase the risk of anaemia. Furthermore, the impact of hemoglobinopathies on anaemia prevalence needs to be considered within some populations (WHO 2008). Knowledge of the different causes is important to form the basis for intervention strategies to control anaemia (Van den Broek et al., 1998). The WHO also suggests taking up more studies on prevalence and aetiological factors to identify the definite cause for control of anaemia (WHO 1993).

Micronutrient deficiencies among pregnant women are widespread in low-income countries (Jiang et al., 2005). Diets of pregnant women in the poor income groups are deficient in micronutrients, as well as energy (Rao et al., 2009, Anderson et al., 2003). Pregnant women of undernourished population groups have coexisting deficiencies of zinc, iron, folate, and vitamin A along with a deficit in the intake of energy, protein, and fats (Kapil 2009). In a community based cross sectional survey conducted among pregnant women with pregnancy duration greater than 28 weeks in six villages of Haryana, India there was a high concurrent prevalence of two, three or four micronutrient deficiencies, possibly due to the poor dietary intake of food and low frequency of consumption of food groups rich in micronutrients (Pathak et al., 2004). Even among urban slums the prevalence of micronutrient deficiencies amongst pregnant women of was high (Kapil et al., 1999). More specifically, anaemia (Hemoglobin < 11g/dL in pregnancy) is a public health problem in India. In a survey done across 16 districts from 11 states in India, it was observed that 85% of pregnant women were anaemic (hemoglobin < 11 g/dL); while 13% had severe anaemia (hemoglobin < 70 g/L), and 60% had moderate anaemia (hemoglobin > or = 70 to 100 g/L) (Toteja et al., 2006).

Table 2 summarizes the prevalence and risk factors for anaemia as observed in prominent studies from India as well as other developing countries. Globally, by World Health Organization (WHO) criteria, 52% of pregnant women from undeveloped or developing countries are anaemic compared with 20% from industrialized nations (WHO 2001). African countries such as Malawi, Nigeria, Ghana, Sudan and Senegal all report a high prevalence of anaemia among pregnant women in the second and third trimester of pregnancy ranging from 28 to 80 % (Verhoeff et al., 1999, Oboro et al., 2002, Engmann et al., 2008, Abdelrahim et al., 2009, Seck et al., 2010). Significant risk factors for anaemia among these women were high intake of iron absorption inhibitors (Seck and Jackson 2010), Iron deficiency, malaria parasitaemia, Mid Upper Arm Circumference (MUAC) < 23 cm (Engmann et al., 2008, Verhoeff et al., 1999), primigravidity, booking in late pregnancy and wet season (Oboro et al., 2002). Prevalence of anaemia in South Asian countries is among the highest in the world. Reports from Asian countries such as China, Bangladesh and Pakistan also suggest that anaemia is highly prevalent among the pregnant women, and is associated with risk factors such as increased consumption of iron absorption inhibitors like tea (Baig Ansari et al., 2008), not consuming adequate amounts of animal foods, poultry and dark green leafy vegetables and fruits (Ahmed et al., 2003, Baig Ansari et al., 2008) and low education and pregnancy Induced hypertension (Zhang et al., 2009). WHO has estimated that even among the South Asian countries, India has the highest prevalence of anaemia (Ezzati et al,. 2002). The National Family Health Survey reports the prevalence of anaemia among pregnant women in India to be 59% (National Family Health Survey 2005-2006). A survey among pregnant women from 16 districts of 11 states in India showed that 85% of pregnant women were anaemic (Hb < 11 g/dL) (Toteja et al., 2006). In general, the prevalence ranges from 48 to 85% (Sharma et al., 2003, Toteja et al., 2006, Agarwal et al., 2006, Bharati et al., 2008, Noronha et al., 2010), and the prominent risk factors were decreased consumption of meat (Sharma et al., 2003) and low educational level and poor socioeconomic status (Bharati et al., 2008a, Noronha et al., 2010). Despite the ongoing national anaemia prophylaxis programme of iron and folic acid supplementation to pregnant women, two large national surveys (National Family Health Survey 2005-2006, District Level Household Survey 2002-2004) have indicated that there has been little change in the prevalence of anaemia and the adverse consequences associated with it.

Table 2: Prevalence and risk factors for anaemia[1] among pregnant women from India and other developing countries during early and late pregnancy [2].

Study population (reference)	Anaemia prevalence, pregnancy trimester	Significant finding
India		
Pregnant women from Delhi (n = 1150) (Sharma et al., 2003).	Mild (90%), severe (5%), 2nd and 3rd trimester	High prevalence of anaemia due to low frequency of meat eating.
Pregnant women and adolescent girls from 11 states (n = 6923) (Toteja et al., 2006).	Mild (85%), moderate (60%) severe (13%) (Across all trimesters) .	High prevalence among pregnant women.
Pregnant women from 7 states (n = 1148) (Agarwal et al., 2006).	Any (84%), mild (23%), moderate (51%), severe (9%).	Anaemia is highly prevalent
Pregnant (n = 5619) and non pregnant rural and urban women (n = 72, 660) (Bharati et al., 2008).	Any anaemia among pregnant women (48%), Any anaemia in non pregnant women (50%) all trimesters	High prevalence of anaemia among non pregnant and pregnant women Level of education and standard of living of households were important determinants of degree of anaemia.
Pregnant women from 3 towns of Udipi district of Karnataka (n = 1077) (Noronha et al., 2010).	Anaemia (50%), <14 wks of gestation	High prevalence of anaemia was strongly associated with low socioeconomic status
Asian countries other than India		
Pregnant women from poor urban areas of Bangladesh (n = 383) (Ahmed et al., 2003).	Any anaemia during second trimester (20 to 30 wks of gestation) (40%).	Anaemia is a highly significant problem in Bangladesh. Intake of meat, dark green leafy vegetables, fruits and serum vitamin A levels were inversely and independently associated with anaemia
Pregnant women from Pakistan (n = 1369) (Baig Ansari et al., 2008).	Any (91%), mild (75%), moderate (15%) (20 to 26 wks of gestation).	Drinking more than 3 cups of tea daily before pregnancy, consumption of clay or dirt during pregnancy, and never consuming eggs or consuming eggs less than twice a week during pregnancy were directly associated with anaemia
Pregnant women from Eastern China (n = 16466) (Zhang et al., 2009).	Overall anaemia (Hb < 100 g/l (33%) First trimester (11%) Second trimester (20%) Third trimester (26%)	High prevalence of anaemia Risk factors for anaemia included maternal education below junior high school farming occupation, mild and and severe pregnancy induced hypertension

African countries		
Pregnant women from rural Malawi (n = 801) (Verhoeff et al., 1999).	Moderately severe anaemia (< 8 g/dL) (30%) Iron deficiency (43%), ≤ 16 wks to ≥ 35 wks	High prevalence of moderately severe anaemia and iron deficiency. Iron deficiency and malaria parasitaemia associated with increased risk for moderately severe anaemia in primigravidae, while iron deficiency and Mid upper arm circumference < 23 cm in second gravidae, and iron deficiency)in third gravidae
Pregnant women from rural and semi urban states of South Nigeria(n = 779) (Oboro et al., 2002)	Overall (56%) Severe anaemia (7%), Across all trimesters	Independent risk factors for anaemia were primigravidity, booking in late pregnancy and wet season
Pregnant women from Accra, Ghana (n = 428), ≤ 24 weeks (n = 181) and ≥ 36 weeks of gestation (n = 247) (Engmann et al., 2008).	≤ 24 weeks (42%) ≥ 36 weeks (28%), Ranging from ≤ 24 wks and ≥ 36 wks of gestation.	Risk factors for anaemia were iron deficiency and malaria parasitaemia.
Pregnant women from Eastern Sudan (n = 279) (Abdelrahim et al., 2009).	Anaemia (80%) Iron deficiency (14%) Iron deficiency anaemia (11%), Across all trimesters	High prevalence of anaemia among pregnant women from Eastern Sudan.
Pregnant women from urban Senegal (n = 480) (Seck and Jackson 2010)	Anaemia (39%), Second trimester	Anaemia attributed to high intake of iron absorption inhibitors. Mean haemoglobin and serum ferritin levels were significantly higher and erythrocyte protoporphyrin levels were lower in women who consumed iron inhibitors less frequently.

[1] Definition of anaemia used in these studies is based on WHO/UNICEF/UNU 2001 guidelines wherever cut off values are not specifically mentioned: Mild anaemia: Hb 100 to 109 g/l, moderate anaemia: Hb: 70 to 99 g/l, severe anaemia: < 70 g/l

[2] Inclusion/Exclusion of studies: Studies pertaining to the prevalence and risk factors for anaemia among pregnant women across all trimesters in India, other developing countries as well as developed countries were included.

2.1.2 Consequences of anaemia in pregnancy

Anaemia is highly prevalent among pregnant women in developing countries and is associated with poor pregnancy outcomes (Allen 1997). Severe anaemia is associated with maternal morbidity and mortality in developing countries (Koblinsky 1995, Schwartz and Thurnau 1995), since anemic mothers are more likely to die from postpartum hemorrhage (Stoltzfuz et al., 2003), and postpartum hemorrhage is the leading cause of maternal mortality (Khan et al., 2006). The Global Burden of Disease project estimated 18% of maternal mortality due to low Hb in pregnancy (WHO 2009). Anaemia, specifically iron deficiency anaemia, early in pregnancy has adverse consequences on pregnancy outcome, including inadequate weight gain in pregnancy, LBW and preterm birth (Klebanoff et al., 1991; Scholl and Hediger 1994; Scanlon et al., 2000; Rasmussen 2001). A systematic review of the literature containing associations between maternal anemia and SGA outcomes showed that moderate to severe, but not mild, maternal anemia had an association with SGA outcomes (Kozuki et al., 2012). There is strong evidence for an association between maternal Hb concentration and birth weight as well as between maternal Hb concentration and preterm birth, however it is unknown as to how much of this association is attributable to iron-deficiency anemia in particular (Rasmussen et al 2001). Rates of LBW and preterm birth (but not SGA) were related to early pregnancy Hb concentration in a U-shaped manner (Zhou et al., 1998). In addition to poor pregnancy outcomes, fatigue, pallor, light-headedness, tachycardia, dyspnea, poor exercise tolerance, and suboptimal work performance have all been reported in pregnant women with iron deficiency (US Preventive task force 1993, Murray-Kolb and Beard 2009, Reveiz et al., 2007, Kalaivani 2009). As an intervention strategy to reduce the burden of anaemia, multi micronutrient supplementation may be more effective in improving anaemia and other gestational and neonatal outcomes in specific populations in comparison to supplementation with individual micronutrients (Christian et al., 2003, Haider and Bhutta 2006, Fawzi et al., 2007).

2.1.3 Iron deficiency anaemia

Globally, the most significant cause of anaemia is iron deficiency (WHO 2001). It has been estimated that 75% of anaemia is due to iron deficiency followed by folate and vitamin B_{12} deficiencies (Allen and Casterline-Sabel 2000). Iron depletion, development of iron deficiency and eventually anemia occur in stages. In the initial stage of iron depletion, storage

iron or serum ferritin levels are reduced. However, there is no overt effect on erythropoiesis and blood hemoglobin levels are usually normal. The second stage is the iron deficient erythropoesis in which there is a shortage of iron available to the erythroid precursors in the bone marrow for Hb synthesis. This stage may be characterized by low transferrin saturation levels and elevations in free erythrocyte protoporphyrin in addition to reduced serum ferritin levels. When iron stores are depleted and insufficient iron is available for erythropoesis, Hb synthesis in erythrocyte precursors becomes impaired, and the hematologic signs of iron deficiency anemia appear. Iron deficiency may exist with or without anemia. Anemia is the end stage of iron deficiency and is characterised by a decrease in blood Hb concentration and hematocrit.

2.1.4 Digestion, absorption, transport, storage and uptake of iron

Figure 3 shows the digestion, absorption, enterocyte use and storage of iron in the human body. Heme iron needs to be hydrolyzed from the globin portion of hemoglobin or myoglobin before absorption. Proteases in the stomach and the small intestine helps in the release of heme iron from globin. Heme iron is absorbed across the brush border of the enterocyte by heme carrier protein 1 found in the proximal small intestine. Within the mucosal cell, the absorbed heme porphyrin ring is hydrolysed by heme oxygenase into inorganic ferrous iron and protoporphyrin. The released iron then gets associated with proteins such as mobilferrin and can be used by the intestinal mucosal cell or used by other body tissues, following transport out of the enterocyte.

Hydrochloric acid and proteases in the stomach help in the release of non heme iron from food components. In the stomach iron is mainly present in the ferric form, some of which may be reduced to the ferrous form. Once the iron moves in to the small intestine, ferric iron mixes with alkaline juice secreted by the pancreas to produce ferric hydroxide. Ferrireductases such as ferric/cupric duodenal cytochrome b reductase present at the brush border of the enterocyte helps to reduce ferric iron in to ferrous state. Ascorbate helps reduce iron non enzymatically. Ferrous iron is absorbed across the brush border membrane into the intestinal mucosal cell by binding to Divalent Cation Transporter 1 (DCT 1) located in the intestinal cell brush border membrane. DCT 1 transports not only iron but to a lesser extent minerals such as zinc, copper, manganese, nickel and lead. Synthesis of DCT 1 increases when iron stores are low. Absorption of ferric iron is facilitated by chelation of iron

with chelators that help solubilise the ferric iron. A membrane protein called integrin facilitates the ferric iron absorption across the brush border membrane of the enterocyte.

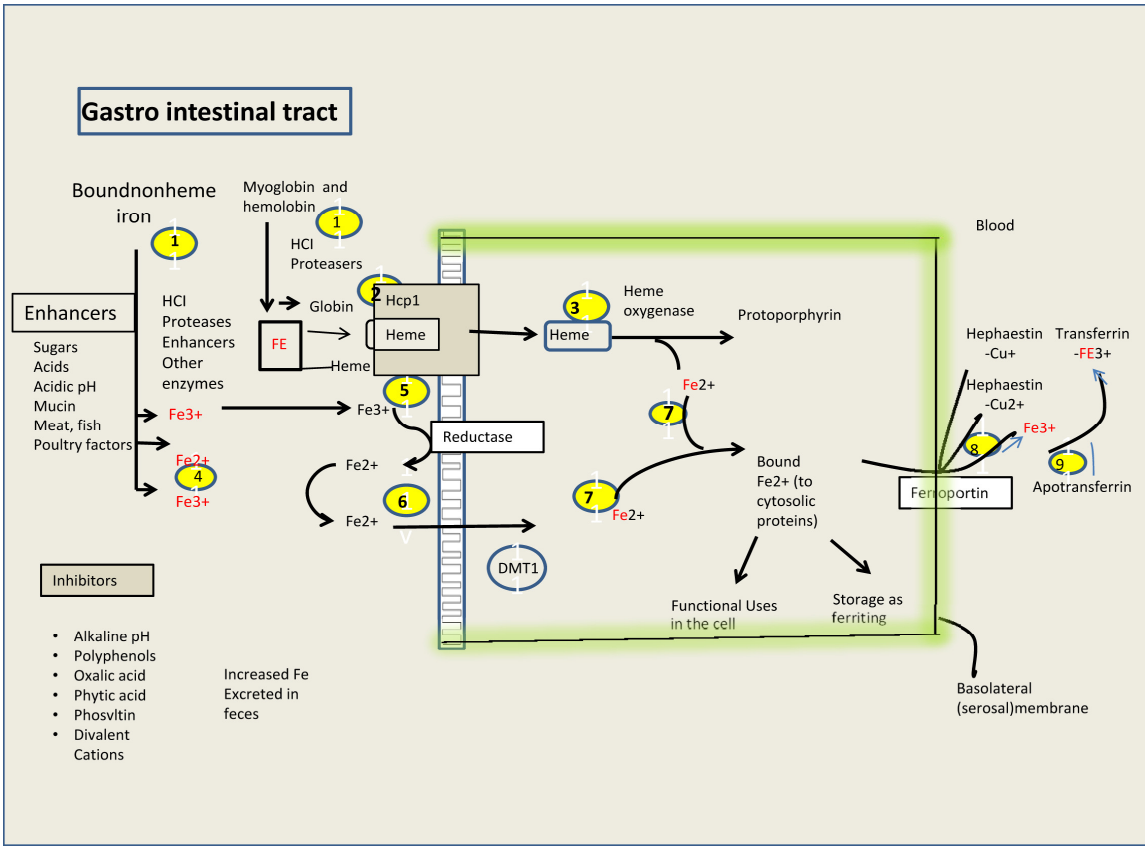

Figure 3: Iron digestion, absorption, enterocyte use and transport

Figure legend:

HCL: hydrochloric acid, Fe: Iron Fe^{2+}: ferrous iron, Fe^{3+}: ferric iron, DMT 1: Divalent Metal Transporter 1

1. Iron is released from food bound components. Hydrochloric acid in the stomach reduces Fe^{3+} to $Fe^{2+.}$
2. Free heme is absorbed by heme carrier protein 1(located in proximal small intestine).
3. Within the enterocyte, heme is catabolised by heme oxygenase to protoporphyrin and Fe^{2+}.
4. In the small intestine non heme iron may react with one or more inhibitors, which promotes fecal excretion of iron.
5. Cytochrome b reductase 1 reduces Fe^{3+} to $Fe^{2+.}$
6. DMT 1 carries Fe^{2+} across the brush border membrane into the cytosol of the enterocyte.
7. Fe^{2+} may bind to polyrC binding protein for transport in to cytosol, iron may also be used within the cell or stores as a part of ferritin.

8. Ferroportin transports iron across the basolateral membrane. Iron transport is coupled with its oxidation to Fe^{3+} by hephaestin.
9. Fe^{3+} attaches to transferrin for transport in blood.

Hepcidin is a regulator of iron absorption that is released from the liver when body iron stores are adequate or high. Once released from the liver, hepcidin travels in the blood targeting enterocytes and macrophages, and hepcidin's interaction promotes the internalization and degradation of the protein ferroportin. With the hepcidin induced loss of ferroportin from the cell membranes, iron cannot be transported out of the enterocyte or out of the macrophage and thus cannot get into the blood for use by other tissues, leading to increased enterocyte and macrophage iron concentration. In addition to ferroportin, a histocompatibility class 1 like protein is present at the enterocyte basolateral membrane that interacts with transferrin receptors to mediate transferrin bound iron uptake across the basolateral membrane and in to the enterocyte from the plasma. When body iron is high, uptake of iron from the plasma and into the intestinal cells is increased. Increased iron in enterocytes diminishes synthesis of proteins involved in iron absorption such as DCT 1. Therefore iron absorption is diminished when stores are high. On the contrary, when iron stores or levels of hepcidin are low or when there is low uptake of iron from the plasma into the enterocyte, there is continued synthesis of DCT 1 and continued ferroportin expression in the membranes. Iron is then transported out of the enterocyte and macrophages into the blood to be used by the body.

Ferric iron is transported in the blood attached to the protein transferrin. Hephaestin in the intestinal cells and ceruloplasmin found throughout the body catalyse the oxidation of ferrous iron to its ferric form so that it can bind to transferrin in the plasma. Binding of iron by protein serves as a protective mechanism against the generation of harmful free radicals as well as to prevent bacteria from using iron for their own growth during an infection. Transferrin transports iron throughout the body and delivers both new and recycled iron to tissues either for use or storage.

Iron not needed for functional capacity is stored in the liver, bone marrow and spleen. Transferrin delivers iron to the liver which stores about 60 % of the body's iron. The remaining 40 per cent is found within the cells of the mononuclear phagocyte system within the liver spleen and bone marrow. Most of the iron stored in these cells is derived from

phagocytosis of red blood cells and subsequent degradation of the hemoglobin within the cell. Ferritin is the primary storage form of iron and consists of apoferritin in which iron atoms are deposited (Advanced Nutrition and Human Metabolism 2012).

2.1.5 Functions of iron

Iron is an important component of iron containing heme proteins such as haemoglobin, myoglobin and cytochromes. Oxygen bound to an iron containing porphyrin ring, either as part of haemoglobin or myoglobin, facilitates oxygen diffusion in to tissues. In tissue iron deficiency, the concentration of myoglobin in the muscle is reduced drastically, thereby reducing the rate of diffusion of oxygen from erythrocytes to mitochondria. Cytochromes act as electron carriers, and contain heme as the active site with the iron containing porphyrin ring functioning to reduce ferric iron to ferrous iron. In addition, iron is an important part of iron sulphur enzymes such as flavoproteins and hemeflavoproteins. (Food and Nutrition Board & Institute of Medicine 2001). Myeloperoxidase is a heme-containing peroxidase enzyme that binds superoxide anion radicals or hydrogen peroxide and thereby protects the cell from oxidative injury. Iron also forms an important part of metalloenzymes which are involved in the synthesis of tyrosine, dihydroxyphenylalanine (DOPA) (precursor of dopamine) and 5-hydroxy-tryptophan (precursor of serotonin) (Wood and Ronnenberg 2006).

2.1.6 Causes of iron deficiency anaemia

Iron deficiency anaemia usually arises when physiological requirements cannot be met by iron absorption from diet. The main risk factors for Iron deficiency anemia include a low intake of iron, poor absorption of iron from diets high in phytate or phenolic compounds, and specific stages in life with increased demands like growth and pregnancy (WHO 2008). In populations consuming plant-based diets with little or no meat, dietary iron bioavailability is often low (Zimmermann et al., 2005). Meat has 30–70% of iron as heme iron and 15–35% of it is absorbed, while in plant-based diets in developing countries most dietary iron is in the form of non-heme iron, and its absorption is often less than 10% (Food and Nutrition Board/Institute of Medicine (Food and Nutrition Board/Institute of Medicine 2001). The absorption of non-heme iron is increased by meat and ascorbic acid, while dietary factors that reduce the bioavailability of non-heme iron are polyphenols (eg, tea, coffee, and spices such

as cinnamon) (Hurrell et al., 1999), phytates (whole grains, legumes) (Hurrell et al., 2003), and calcium (dairy products) (Hallberg et al., 1991, 1993). Phosphorus in the form of phytates and manganese are also known to inhibit iron absorption (Monsen & Cook 1976). Pregnancy also raises the requirement for iron due to increased red cell mass and growth of the foetal-placental unit, putting the woman at a greater risk for Iron deficiency anaemia, although during pregnancy, the absorption of iron increases. Absorption efficiency for iron in the first trimester is similar to that of non pregnant females; however in the second and third trimesters, the efficiency is increased to 25% due to the increased demand for iron as part of the physiological regulation of iron flux. Increased blood loss from gastrointestinal parasites such as hookworm and infections due to helicobacter pylori also aggravates dietary deficiencies in many developing countries (Food and Nutrition Board/Institute of Medicine 2001).

2.1.7 Consequences of iron deficiency anaemia

Deficiency of iron in pregnancy increases the risk of severe anaemia and increased maternal morbidity and mortality (Khan et al., 2006). Iron deficiency in pregnancy, is also associated with maternal and infant complications such as LBW, prematurity, perinatal mortality, increased risk of maternal infections and lowered tolerance to blood loss and infection (Murray Kolb and Beard 2009). In addition to anaemia, deficiency of iron may be associated with neuropsychological consequences such as quality of life and cognition, including memory and learning (Murray Kolb 2011). Iron supplementation in iron depleted young women has shown improvements in fatigue resistance, exercise performance and muscle function (Brutsaert et al., 2003, Brownlie et al., 2004, Hinton and Sinclair 2007). In infants and young children is associated with delayed mental, motor, and emotional maturation (Beard 2008).

2.1.8 Function of vitamin B_{12} (cobalamin) in the human body

Vitamin B_{12} is an important micronutrient required for the human body that has important functions in DNA replication, in the synthesis of red blood cells and in maintaining the myelin sheath that surrounds nerve cells. It functions as an essential coenzyme for two enzymatic reactions in the human body:

The first is the methionine synthase reaction wherein methionine synthase catalyzes the methylation of homocysteine to methionine in the cytoplasm. This reaction is important for a number of methyl-transfer reactions and is involved in nucleotide synthesis. This reaction occurs in two steps. In the first step vitamin B_{12} binds to methionine synthase and picks up a methyl group from 5-methyl tetrahydrofolate to form methylcobalamin bound to tetrahydrofolate and methionine synthase. In the next step, methionine synthase releases the methyl group from the bound methylcobalamin for transfer to Hcy, producing methionine and cobalamin. Cobalamin is easily oxidised, and on its oxidation, methionine synthase becomes inactive. Methionine synthase is reactivated by Nicotinamide Adenine Dinucleotide Phosphate (NADPH) dependent flavoenzyme called methionine synthase reductase. Polymorphisms in this reductase are linked to increased risk for NTD in people with sub-optimal vitamin B_{12} status, as well as those with mutations in 5,10-methylene tetrahydrofolate reductase. Since formation of 5,10-methyl tetrahydrofolate is irreversible, vitamin B_{12} deficiency traps body folate in the 5 methyl form which is inactive. This is known as the "methyl folate trap hypothesis" (Advanced Nutrition and Human Metabolism (2012).

The second reaction is the methylmalonyl-CoA mutase reactions wherein methylmalonyl-CoA mutase catalyzes the conversion of methylmalonyl-CoA to succinyl-CoA in the mitochondrion. The methylmalonyl-CoA mutase reactions are involved in digestion of different organic compounds, including branched amino acids and odd-chain fatty acids (Nielson et al., 2012).

2.1.9 Digestion, Absorption and metabolism of vitamin B_{12}

Ingested vitamin B_{12} must be released from the proteins or the polypeptides to which they are linked in food through the action of the gastric proteolytic enzyme pepsin and hydrochrloric acid in the stomach. Vitamin B_{12} then binds to an R protein in the saliva and gastric juice, prior to or just after the vitamins release from food proteins in the stomach and duodenum, which protects it from bacterial use. In the alkaline environment of the small intestine, R

protein is hydrolyzed by pancreatic proteases, and free vitamin B_{12} is released. Once released from the R protein, vitamin B_{12} binds to intrinsic factor that is a glycoprotein synthesized by gastric parietal cells.

The vitamin B_{12}-intrinsic factor complex travels from the duodenum to the ileum, where it interacts with a protein receptor called cubilin. Cubilin interacts with another protein that facilitates cubilin's attachment to ileal cell plasma membrane. Binding of vitamin B_{12}-intrinsic factor complex to the receptor triggers active endocytotic internalization. Vitamin B_{12} gets absorbed throughout the ileum, especially in the distal third. Within the enterocyte the vitamin B_{12} gets released from the intrinsic factor complex. Vitamin B_{12} then binds to the protein transcobalamin 2 for transport in portal blood.

Several conditions can interact with the absorption process, such as destruction of gastric parietal cells causing an absence of intrinsic factor that results in vitamin B_{12} malabsorption (pernicious anaemia), inability to release food bound vitamin B_{12} so that it may bind to intrinsic factor especially in elderly (food bound cobalamin malabsorption) and pancreatic insufficiency resulting in an acidic intestinal pH that impairs the release of vitamin B_{12} from R protein.

Intrinsic factor mediated absorption of vitamin B_{12} gets saturated at 1.5 to 2 µg of B_{12}/meal. Approximately, 1 to 3% of B_{12} may be absorbed by passive diffusion, when pharmacological doses of B_{12} are ingested. Absorption of B_{12} is 50%, and with increased intake the efficiency of absorption decreases.

Vitamin B_{12} is excreted in the bile. However, it can bind to intrinsic factor in the small intestine and be reabsorbed in to the ileum. This is known as enterohepatic circulation and provides 3 to 8 µg of B_{12} per day. Once absorbed, vitamin B_{12} appears in circulation in 3 to 4 hours. In the blood methylcobalamin constitutes 80% and adenosylcobalamin upto 20% of the total vitamin B_{12}. The other forms of vitamin B_{12} are hydroxocobalamin and cyanocobalamin.

In the blood, vitamin B_{12} circulates bound to transporter proteins such as transcobalamin (mainly transcobalamin 2) and haptocorrin like proteins (transcobalamin 1 and 3). Transcobalamin 2 transports 20 to 30% of the vitamin B_{12}. Much of the vitamin B_{12} is then transferred from transcobalamin 2 to 1, which functions as a circulating storage form and transports up to 80% of vitamin B_{12}. Uptake of vitamin B_{12} into tissues is receptor dependent. The transcobalamin vitamin B_{12} complex binds the transcobalamin cell receptors

and enters the cell by endocytosis with subsequent fusion to lysosomes that provide for proteolytic degradation of transcobalamin 2, and release of vitamin within the cell cytosol.

Vitamin B_{12} can be stored in the body for long periods of time, and it may take 3 to 5 years for a deficiency to develop. Approximately 2 to 4 mg of vitamin B_{12} is stored in the liver, while small amounts may also be found in muscles, brain, heart, spleen, bone and kidney or circulating in the blood as transcobalamins. The primary storage form of vitamin B_{12} in the liver, red blood cells, kidney and brain is adenosylcobalamin, while methylcobalamin is the main form found in the blood (Advanced Nutrition and Human Metabolism 2012).

2.1.10 Dietary sources of vitamin B_{12}

The main sources of vitamin B_{12} for humans in a non-vegetarian diet are meat (2–5 μg/100 g), fish (2–8 μg/100g), milk (1.5 μg/100 ml), cheese (1–2 μg/100 g) and eggs (2 μg/100g). The bioavailability of vitamin B_{12} in healthy humans from fish meat, sheep meat, and chicken meat averaged 42%, 56%–89%, and 61%–66%, respectively. Vitamin B_{12} in eggs seems to be poorly absorbed (<9%) relative to other animal food products (Watanabe 2007). The absorption efficiency of B_{12} from liver reportedly was low because of its high B_{12} content (Institute Of Medicine 1998). Since the main sources of B_{12} are meat, fish and poultry, there has been less concern about B_{12} deficiency among vegetarians who consume some animal-based products; however people who follow a vegan diet may be at an increased risk of vitamin B_{12} deficiency (Herbert 1994).

2.1.11 Vitamin B_{12} deficiency: biochemical measures for diagnosis

Vitamin B_{12} deficiency has been defined in several ways, depending mainly on the population studied and on the particular assay kits used (Snow 1999, Zittoun and Zittoun 1999, Klee 2001). Owing to the lack of a precise ‘gold standard’ for the diagnosis of cobalamin deficiency, test results show varying sensitivities and specificities. Vitamin B_{12} deficiency is defined as plasma vitamin B_{12} levels < 150 pmol/L. Elevated MMA is defined as MMA levels > 0.26 μmol/L (Refsum et al., 2001b). Elevated Hcy is defined as Hcy levels > 10.0 μmol/L. Low erythrocyte folate concentration is defined as < 283 nmol/L (Refsum et al., 2004).

Although measurement of the total vitamin B_{12} concentration in plasma is the usual method for assessing vitamin B_{12} status, neurological and hematological symptoms of deficiency can occur in individuals with plasma vitamin B_{12} concentrations in the low-normal range (Karnaze and Carmel 1990), and equally individuals with low vitamin B_{12} concentrations can remain symptom-free. Typically in pregnancy, plasma vitamin B_{12} concentration may not be a reliable indicator of vitamin B_{12} status since there is a gradual, physiologically normal decline in the plasma concentration of vitamin B_{12} during an uncomplicated pregnancy (Koebnick et al., 2002, Milman et al., 2006) due to hemodilution, hormonal changes, alterations in the concentration of vitamin B_{12} binding proteins, or active transport of vitamin B_{12} across the placenta (Obeid et al., 2006). The serum MMA level has been recognized as a sensitive marker of functional vitamin B_{12} deficiency although not fully specific for vitamin B_{12} deficiency. It is in addition expensive, and also increases in renal insufficiency. An elevated Hcy level, which precedes clinical signs of vitamin B_{12} deficiency, is also an early marker and displays the metabolic consequence of vitamin B_{12} deficiency. Hcy, is however highly nonspecific and is increased in folate and vitamin B_6 deficiency and renal insufficiency.

Recently, holotranscobalamine (Holo TC), the metabolically active cobalamin fraction, has garnered much attention as the earliest diagnostic marker of vitamin B_{12} deficiency. Holotranscobalamin is a more sensitive indicator of vitamin B_{12} status than the total serum vitamin B_{12} level or the serum concentration of MMA and Hcy, both of which are elevated in vitamin B_{12} deficiency (Hermann et al., 2003, Obeid and Hermann 2007). Holo TC is not an indicator of recent dietary intake but rather a marker of long-term vitamin B_{12} status (Hvas et al., 2005). In addition, in a longitudinal study of healthy pregnant women from 18 weeks gestation, it was observed that although serum vitamin B_{12} levels decreased over the course of the pregnancy, holoTC levels remained constant in women with an adequate intake of vitamin B_{12} (Morkbakk et al 2007). Measurement of holo TC and MMA provides a better index of cobalamin status than the measurement of total vitamin B_{12}. Holotranscobalamin is the most sensitive marker, followed by MMA. The use of holo TC and MMA enables us to differentiate between storage depletion and functional vitamin B_{12} deficiency (Herrmann et al., 2003).

2.1.12 Vitamin B_{12} deficiency: prevalence in India and other developing and developed countries

Asian Indians have long been identified to be at increased risk for vitamin B_{12} deficiency (Gammon et al., 2012). In a cross-sectional study of adult residents of Pune (Maharashtra state), the incidence of vitamin B_{12} deficiency (serum concentration < 150 pmol/L) was nearly 50% in healthy individuals with a high prevalence of vegetarianism and hyperhomocysteinemia was found in 77%, in whom less than 5% had both folate deficiency and anaemia (Refsum et al., 2001a). **Table 3** summarizes the studies assessing the vitamin B_{12} concentrations as well as other indicators of B_{12} deficiency such as MMA and Hcy among pregnant women in India**.** In general, most of these studies report low concentrations of vitamin B_{12} and approximately 43 to 80 % of the pregnant women had plasma vitamin B_{12} concentration < 150 pmol/l in the second and third trimester of pregnancy. It is noteworthy that most of these studies have been conducted in the second and third trimester of pregnancy (Yajnik et al., 2006, Pathak et al., 2004, Katre et al., 2010, Veena et al., 2010, Sucharita et al., 2012). Only one study has reported serum B_{12} concentrations early in pregnancy (12 weeks of gestation), and report a low value of 171 pmol/L (Muthayya et al., 2006). All these studies point towards the fact that vitamin B_{12} deficiency is rampant in India among pregnant women. Not only are B_{12} concentrations in plasma/serum low, but elevated MMA and Hcy (indicators of biochemical deficiency) is also observed among these women, confirming that the sub-clinical deficiency needs to be addressed. For instance, 94% and 90% of the pregnant women had MMA > 0.26 µmol/l in the second and third trimester, respectively (Yajnik et al., 2006), while hyperhomocysteinemia was observed in 3 to 29% of the pregnant women in later pregnancy (Veena et al., 2010, Katre et al., 2010, Yajnik et al., 2006).

Table 3: Prevalence of vitamin B_{12} and folate deficiency among pregnant women from India and other countries during early and late pregnancy [1].

Study design and population (reference)	Biochemical measures, timing	Prevalence of B_{12} deficiency
India		
Prospective observational study of rural pregnant women from Pune (n = 700) (Yajnik et al., 2006)	Serum folate, vitamin B_{12}, homocysteine (Hcy)[2]and methylmalonic acid (MMA)[3] at 18 and 28 wks of gestation.	At 18 wks of gestation: 60% had plasma B_{12} <150 pmol/l, 94% and 28% had MMA > 0.26 µmol/l and Hcy > 10 µmol/l. At 28 wks of gestation: 71% had plasma B_{12} < 150 pmol/l, while 90% and 33% had MMA > 0.26 µmol/l and Hcy> 10 µmol/l.
Prospective observational study of pregnant women and their infants from urban Bangalore (n = 478) (Muthayya et al., 2006)	Plasma B_{12} at 12 wks of gestation.	Mean serum B_{12} concentration was 171 pmol/l. Prevalence of B_{12} deficiency not mentioned
Pregnant women from rural Haryana (n = 283) (Pathak et al., 2007)	Serum folate and vitamin B_{12} across any trimester of pregnancy	Low folate (<6.8 nmol/l) and low vitamin B_{12} (<147 pmol/l) in 26% and 74% , respectively.
Pregnant women from rural farming community (n = 86) and from a lower middle-class urban community (n = 77) in Pune (Katre et al., 2010)	Plasma vitamin B_{12}, folate, and total homocysteine at 17, 28, and 34 wks gestation	At 17 wks plasma B_{12} < 150 pmol/l was observed in 80% of the rural women and 64% of the urban women, while Hcy > 10 µmol/l was observed in 28% of the rural women and 26% of the urban women. In women receiving no vitamin B_{12} supplementation (n=17) plasma vitamin B_{12} and folate did not change from 17 to 34 wks gestation, but Hcy increased. Hcy concentrations at 34 wks gestation in women receiving only folic acid (mean 8.4 µmol/L) were comparable to the unsupplemented group (9.7). However, women who received a total dose of > 1000 µg of Vitamin B_{12} up to 34 wks (n=42, all with folic acid) had lower concentrations of Hcy in comparison to the unsupplemented group and those receiving only folic acid (6.7).

Maternal concentrations of plasma vitamin B_{12}, folate and Hcy analysed in stored plasma samples of their mothers during their pregnancy (< 32 wks of gestation) (n = 536) from Mysore (Veena et al., 2010)	Maternal folate, plasma B_{12} and Hcy measured at 30 wks of gestation	At 30 wks of gestation, 4% had low folate concentrations (<7 nmol/L), 43% had low vitamin B_{12} concentrations (<150 pmol/L), and 3% had hyperhomocysteinemia (>10 µmol/L).
Healthy children between 3 and 8 years of age and blood sample of their mothers that had been stored (n = 79) from Bangalore (Sucharita et al., 2012)	Cardiac autonomic nervous activity in children born to mothers with low vitamin B_{12} status in the first trimester.	The median maternal first trimester vitamin B_{12} status was 114 pmol/l. Children born to mothers with a lower vitamin B_{12} status had a reduced cardiac sympathetic activity.
Other developing countries		
Pregnant women from Kathmandu, Nepal (n = 382) (Bondevik et al., 2001)	Serum Hcy and MMA in the first antenatal visit of pregnant women	Elevated Hcy (>7.5 µmol/l) was found in 68% , while 61% had elevated MMA (>0.26 µmol/l). Low cobalamin values (<150pmol/l) were observed in 49%, while only 7% had low serum folate values (< 4.5nmol/l).
pregnant women in Nigeria (n = 98) (Vanderjagt et al., 2009)	Serum vitamin B12 and MMA 3rdtrimester of pregnancy	Low serum vitamin B_{12} concentration (< 148 pmol/l) was observed in 12% and high serum MMA level (>0.27 µmol/l) in 18%. Using a combination of low serum vitamin B_{12} and elevated MMA concentrations, subclinical vitamin B_{12} deficiency was observed in 8.1% of the pregnant women.
Pregnant women from rural Bangladesh (n = 740) (Lindstorm et al., 2011)	Plasma concentrations of folate and vitamin B_{12} at 14 wks of pregnancy	Vitamin B_{12} deficiency (< 150 pmol/l) was observed in 46% while 18% were folate deficient (< 6.8 nmol/l).
Developed countries		
Healthy pregnant women from Germany (n = 39) (Koebnick et al., 2002)	Serum vitamin B_{12}, assessed in wks 9–12, 20–22, and 36–38 of gestation.	Significant decrease in vitamin B_{12} concentration during the course of pregnancy. Serum vitamin B_{12} decreased from257 pmol/l in the first trimester to 178 pmol/l in the third trimester. 35% of the participants had serum vitamin B_{12} concentration <150 pmol/l.
Pregnant women from urban Gran Caracas and semi rural areas surrounding it in Venezuela (n = 1289) (Garcia Casal et al., 2005)	Concentrations of serum folic acid and vitamin B_{12} across any trimester in pregnancy	The prevalence of folic acid (<6.8 nmol/l) and vitamin B_{12} (<147 pmol/l) deficiencies was 36% and 61%, respectively.

Healthy pregnant Danish Caucasian women (n = 406) (Milman et al., 2006)	Plasma cobalamin, MMA, Hcy measured at 18, 32 and 39 wks of gestation	Prevalence of vitamin B_{12} deficiency (< 150 pmol/l) increased from 15% at 18 wks to 43% at 39 wks while prevalence of high MMA (> 0.28 µmol/l) increased from 3% at 18 wks to 8% at 39 wks of gestation.
Healthy pregnant women from Spain (n = 92) (Murphy et al., 2007)	Plasma cobalamin, MMA, and holotranscobalamin (holoTC)[4] concentrations determined at preconception, 8, 20, and 32 wks of pregnancy, at labour, and in the cord.	Cobalamin, holoTC and MMA decreased from preconception to wk 20 of pregnancy. [geometric mean percentiles 10, 90 (P_{10}, P_{90})] at preconception: Cobalamin: 293 (155, 535) pmol/L; holoTC: 63 (38, 98) pmol/L; MMA: 0.12 (0.09, 0.17) μmol/L] At week 20: [cobalamin: 230 (123, 432) pmol/L; holoTC: 48 (34,78) pmol/L; MMA: 0.11 (0.08, 0.15) μmol/L $P < 0.001$]. Plasma cobalamin and holoTC remained lower at week 32 as well, plasma cobalamin: 198 (107, 339) pmol/l; holoTC: 45 (26,82) pmol/l.

[1] Inclusion/Exclusion of studies: Studies pertaining to vitamin B_{12} status and prevalence of vitamin B_{12} deficiency among pregnant women across all trimesters from India, other developing countries as well as developed countries were included.
[2] Hcy: Homocysteine
[3] MMA: Methylmalonic acid
[4] holoTC: Holotranscobalamin

2.1.13 Causes of vitamin B_{12} deficiency in pregnancy

Low cobalamin status may occur among pregnant women, especially during late pregnancy. Typically, in an uncomplicated pregnancy, plasma vitamin B_{12} concentration declines over the course of pregnancy (Koebnick et al., 2002, Milman et al., 2006). This decline is usually due to hemodilution, hormonal changes, alterations in the concentration of vitamin B_{12} binding proteins, or active transport of vitamin B_{12} across the placenta (Obeid et al., 2006). The serum cortisol concentrations were an important variable for explaining the variance in cobalamin concentrations at the 34th week of pregnancy. Cortisol, progesterone and prolactin were associated with decreasing folate concentrations during pregnancy (Bruinse and Van Den Berg 1995).

Inadequate dietary intake is one of the main causes of vitamin B_{12} deficiency (Allen 2009). Several studies have shown biochemical evidence of vitamin B_{12} deficiency based on elevated plasma Hcy and MMA in vegans and ovo-lacto vegetarians, indicating that the risk of vitamin B_{12} deficiency in these groups has been underestimated (Hermann et al., 2003, Misra et al., 2002, Hung et al., 2002, Hermann et al., 2001, Herbert

1994, Barr et al., 2000, Donaldson 2000). In one of these studies, the degree of vitamin B_{12} deficiency was related to the degree of animal product restriction, resulting in the highest risk for vegans (Hermann et al., 2003). In a study conducted by Yajnik et al on 441 vegetarians and non-vegetarian middle aged men from rural, urban and slum areas, 67% of all recruited men had low cobalamin concentration (<150 pmol/L), and 58% had hyperhomocysteinemia (>15 µmol/L). Vegetarians still had a 4.4 times higher risk of low cobalamin concentrations and a 3.0 times higher risk of hyperhomocysteinemia than their non-vegetarian counterparts (Yajnik et al., 2006).

Lower intakes are associated with a higher prevalence of deficient and marginal serum B_{12} concentrations and strong correlations were found in all studies that measured both vitamin B_{12} intake and serum vitamin B_{12} (Mc lean et al., 2007, Siekmann et al., 2003, Taneja et al., 2007, Refsum et al., 2001a, Davey et al., 2003, Hermann et al., 2003, Koebnick et al., 2004, Allen 2008). In addition, folate and vitamin B_{12} intakes are difficult to estimate accurately, because nutrient composition data are unreliable for these nutrients and because little is known about factors affecting their bioavailability. There are limited evidence about the bioavailability of vitamin B_{12} in lacto ovo vegetarian and non vegetarian diets and the proportion of the vitamin B_{12} ingested either through diet or supplements that get converted to analogues. The RDA for pregnant women is set at 2.6 µg/d. This would ensure absorption of 1 µg/d because the average absorption from food is about 50% (WHO/FAO 2004a). The absorption of vitamin B_{12} from food is a complex process (Hermann and Giesel 2002). A low intake of vitamin B_{12} from food or intestinal malabsorption could lead to a negative balance and finally to functional deficiency when tissue stores of vitamin B_{12} are depleted.

Other causes of B_{12} deficiency include lack of intrinsic factor, poor absorption and other intestinal factors (malabsorption) and rare genetic disorders (Braunwald et al., 2001, Snow 1999). In addition to the afore-mentioned factors, Giardiasis caused due to Giardia lamblia is a common protozoan intestinal infection observed in certain populations and may cause vitamin B_{12} deficiency due to decreased absorption of vitamin B_{12}(Cordingley and Crawford 1986) even when carriage is asymptomatic. Infection with Helicobacter pylori has also been suggested as an important agent in the aetiology of vitamin B_{12} deficiency and pernicious anemia (Perez Perez 1997). Several studies on the relationship between Helicobacter pylori, gastritis and vitamin B_{12} deficiency have been conducted (Gumurdulu et al., 2003, Kaptan et al., 2000). The high prevalence of Helicobacter pylori infections in

developing countries increases the scope of finding vitamin B_{12} deficiency in certain members of the population.

2.1.14 Consequences of vitamin B_{12} deficiency in pregnancy

Deficiency of Vitamin B_{12} can cause megaloblastic anaemia and neurological disease owing to its role in normal DNA and RNA and myelin synthesis (Carmel et al., 2003). Vitamin B_{12} is an essential nutrient particularly during pregnancy when it is necessary for normal foetal development, with the requirement for vitamin B_{12} being higher during pregnancy and lactation owing to transfer to the foetus and the infant (Mackey and Picciano 1999). Vitamin B_{12} deficiency causes an elevation in plasma Hcy levels in pregnancy and has implications for adverse pregnancy outcomes including LBW (Vollset et al. 2000). There is strong evidence that plasma Hcy is a predictor of folate and vitamin B_{12} status (de Bonist et al., 2008). Methionine synthase is an enzyme which catalyzes the methylation of Hcy to methionine using vitamin B_{12} as a cofactor and methyltetrahydrofolate as a substrate (Finkelstein 1990). Formation of methionine through this pathway is an important component of the one-carbon metabolism for synthesis of phospholipids, proteins, myelin, catecholamines, DNA and RNA. A deficiency of either vitamin B_{12} and/or folic acid is likely to affect this pathway, resulting in an elevation of plasma Hcy with a relatively low methionine. Strong associations have been reported between elevated plasma Hcy and adverse pregnancy outcomes such as recurrent spontaneous abortion, intra-uterine death, abruption placenta, NTD and pre-eclampsia possibly due to disturbed methionine metabolism (Steegers-Theunissen et al., 2004, Kirke et al., 1993, Kim et al., 2012). Elevated Hcy has also been associated with increased risk for preterm births among Chinese women (Ronnenberg et al., 2002) and SGA infants among Indian women (Yajnik et al., 2005). Associations between low B_{12} concentrations and increased risk for IUGR have also been reported among South Indian urban infants (Muthayya et al., 2006). However, additional studies are needed to elucidate the role of vitamin B_{12} together with Hcy in the prevention of adverse outcomes, particularly IUGR. In addition, low serum vitamin B_{12} concentrations can also lead to consequences for the mother such as macrocytic anemia, neurological complications, and cognitive disabilities (Savage and Lindenbaum 1995). B_{12} deficiency is also an independent risk factor for NTD (Vanderjagt et al 2004), and therefore folate supplements in the absence of vitamin B_{12} administration may offer minimal protection against birth defects.

2.1 INFANT NUTRITIONAL STATUS

2.2.1 Infancy and childhood: the 1000 days critical window period

In addition to pregnancy, the period of infancy and childhood are important stages where there are unique needs for growth, developmental changes in organ function and body composition and maintenance needs. Good quality nutrition is important and critical during the first 1000 days of a child's life starting from being in the mother's womb through his/her second birthday. Pregnancy and infancy therefore become the most important periods for development of the brain and body as well as to lay the foundation for a healthy and productive future. Mothers and babies need good nutrition to lay the foundation for the child's future cognitive, motor and social skills, school success and productivity. Children with restricted brain development in early life are at risk for later neurological problems, poor school achievement, early school dropout, low skilled employment and poor care of their own children, thus contributing to the intergenerational transmission of poverty (Alive and thrive 2012). **Figure 4** elucidates the factors affecting child nutrition. Immediate causes of malnutrition is often poor intake and poor health due to recurrent infection, while the intermediate causes may be poor access to food, health services, child care and sanitation. Underlying causes are often more complicated and needs to be tackled at a governmental or

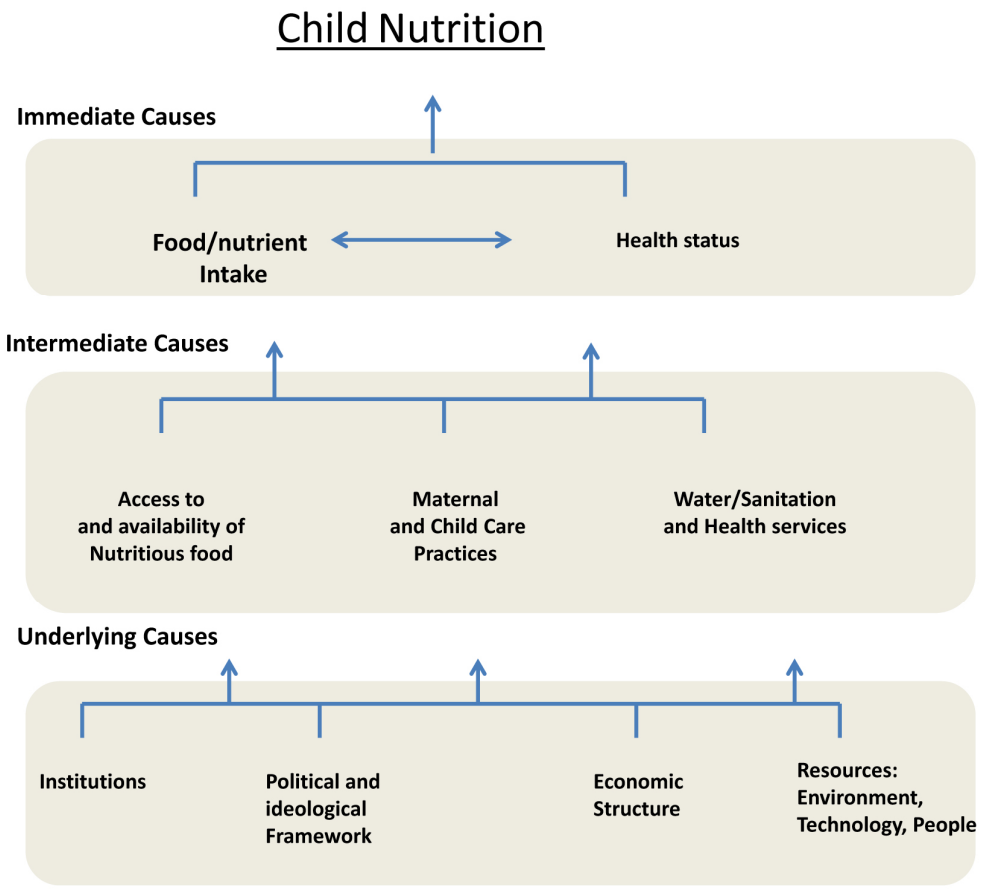

Figure 4: Determinants of child nutrition

Source: World Bank, Moving towards consensus: a global action plan for scaling up nutrition investments, Global Action Plan presentation draft 2011; Save the children (a life free from hunger (London 2012)

Malnutrition during this period is an underlying cause of death for 2.6 million children each year, 1.7 million stunted children and 2 to 3% losses in Gross Domestic Produce annually (State of the World's Mothers 2012). Worldwide, 20 million babies are born with LBW each year. Many of these babies are born pre term, are SGA age or suffer from IUGR. Even babies who are born with a normal weight may still have been malnourished in the womb if the mother's diet was poor. Others become malnourished in infancy due to disease, inadequate breastfeeding or lack of nutritious food. Malnutrition weakens young children's immune systems and leaves them vulnerable to death from common illnesses such as pneumonia, diarrhea and malaria (State of the world's mothers 2012).

2.2.2 Interventions to tackle child malnutrition

The Lancet medical journal published in 2008 a five part series on nutrition coming up with a group of 13 cost effective direct nutrition interventions, which if scaled up to reach every mother and child in the 36 countries that are home to 90% of malnourished children, could prevent approximately 25% of child deaths and cause substantial reductions in childhood illnesses and stunting (http://www.thelancet.com/series/maternal-and-child-undernutrition). In 2010, experts from the Scaling Up Nutrition movement recommended a revised group of 13 programmatically feasible, evidence-based direct nutrition interventions (Horton et al., 2010). **Table 4** illustrates the evidence based interventions developed by scaling up nutrition to combat under nutrition.

Table 4: Evidence based interventions to combat under nutrition developed by Scaling Up Nutrition

Evidence based interventions to combat under nutrition
Promoting good nutritional practices
❑ Breastfeeding
❑ Complementary feeding for infants after the age of six months
❑ Improved hygiene practices including hand washing
Increasing intake of vitamins and minerals: Provision of micronutrients for young children and their mothers:
❑ Periodic Vitamin A supplements
❑ Therapeutic zinc supplements for diarrhoea management
❑ Multiple micronutrient powders
❑ De-worming drugs for children (to reduce losses of nutrients)
❑ Iron-folic acid supplements for pregnant women to prevent and treat anaemia
❑ Iodized oil capsules where iodized salt is unavailable
Provision of micronutrients through food fortification for all:
❑ Salt iodization
❑ Iron fortification of staple foods
Therapeutic feeding for malnourished children with special foods:
❑ Prevention or treatment for moderate under nutrition
❑ Treatment of severe under nutrition (“severe acute malnutrition”) with ready-to-use therapeutic foods.

Source: Horton S, Shekher M, Mc Donald C, Mahal A, Brooks JK. Scaling up nutrition. What would it cost? World Bank publications (2010)

Save the Children has developed 6 key nutrition solutions that have the greatest potential to save nearly 1.3 million children's lives in the first 1,000 days and beyond without massive investments in health infrastructure. Three of the 6 solutions – iron, vitamin A and zinc capsules cost as much as 1 to 2 US Dollar per person, per year, while the other three solutions that is breastfeeding, complementary feeding and good hygiene and behaviour-change solutions could be delivered through community nutrition programs at a cost of $7.50 per child (Horton et al., 2010). **Figure 5** summarises the different interventions at key stages of the lifecycle that is pregnancy, lactation, infancy and childhood. If effectively scaled up these interventions are likely to improve maternal and child nutrition and reduce the severity of childhood illness and under five mortality.

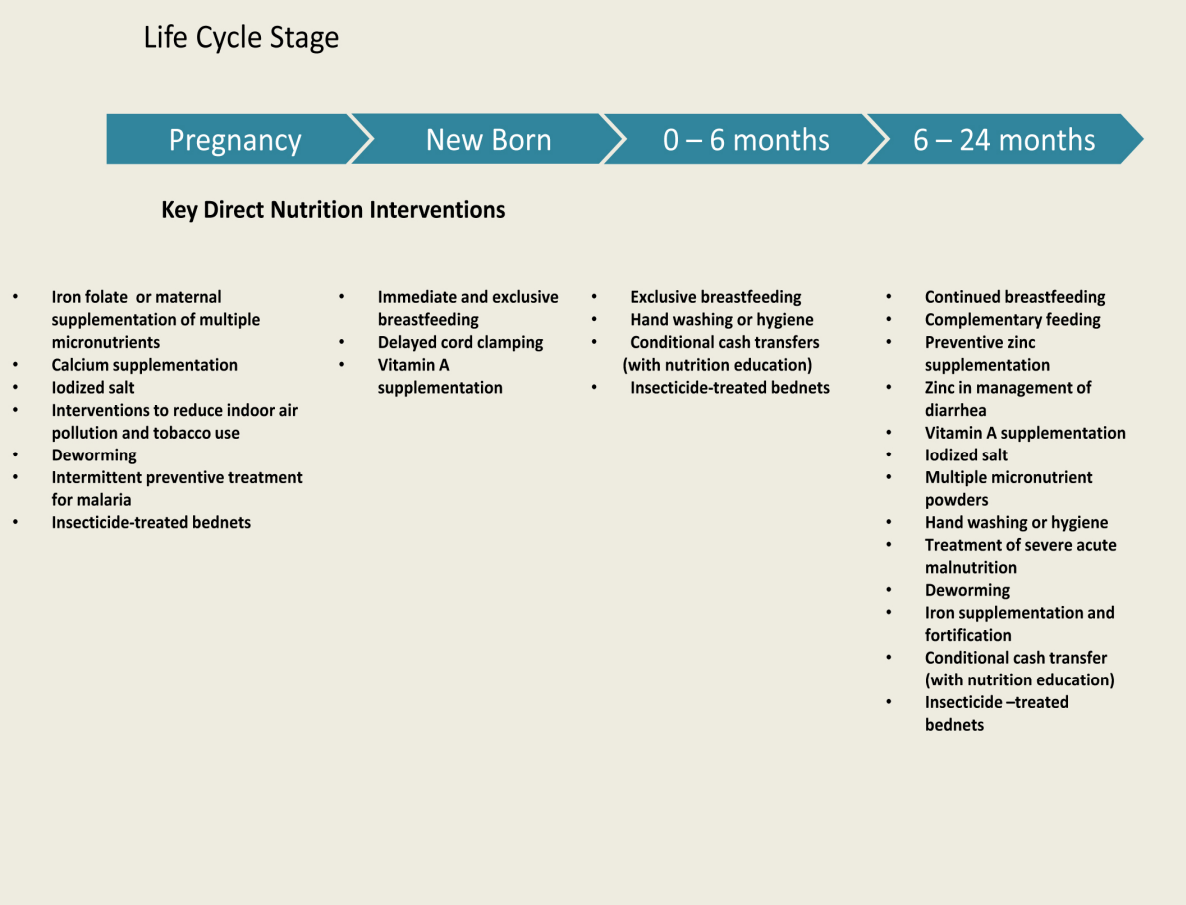

Figure 5: Interventions to improve maternal and child nutrition

Source: State of the Worlds mothers report 2012

2.2.3 Advantages of breastfeeding for the infant and the mother

Breast milk is considered the normative standard for infant feeding and nutrition owing to its several benefits for the infant and for the mother (American Academy of Pediatrics 2012) (**Table 5).** The recommendation by WHO that exclusive breastfeeding should be continued until 6 months of age and that nutritionally adequate and safe complementary foods should be introduced in conjunction with continued breastfeeding thereafter (WHO/UNICEF 2003), is supported by Krammer and Kakuma's systematic review of the benefits of exclusive breastfeeding in promoting healthy growth and development of the infant and health of the mother (Krammer and Kakuma 2002). More recently, a systematic review by the same authors in the Cochrane database reconfirm that infants who are exclusively breastfed for 6 months experience less morbidity from gastrointestinal infection than those who are partially breastfed as of 3 or 4 months, exclusive breastfeeding for 6 months or longer does not lead to any deficits in growth among infants from either developing or developed countries and mothers of exclusively breastfed infants have more prolonged lactational amenorrhea (Krammer and Kakuma 2012).

Table 5: Possible health benefits of breastfeeding for infant and the mother

For the mother
1) Decreased postpartum blood loss and rapid involution of the uterus.
2) Increased child spacing secondary to lactational amenorrhea.
3) Increased depression and substabce abuse in mothers who did not breastfeed.
4) In mothers without a history of gestational diabetes, breastfeeding duration was associated with a decreased risk of type 2 diabetes mellitus.
5) Reduced risk of breast and ovarian cancer.
For the infant
1) Reduces risk of hospitalization for lower respiratory tract infections by 72% (breastfeeding > 4 months)
2) Reduces incidence of otitis media by 50% (breastfeeding > 3 months)
3) Reduces incidence of non specific gastrointestinal tract infections by 64% (breastfeeding for any duration)
4) Reduces incidence of necrotizing enterocolitis in preterm infants by 58% (breastfeeding for any duration)
5) Reduces risk for sudden infant death syndrome by 36% (breastfeeding for any duration)
6) Prevents 13% of childhood mortality in 42 developing countries in which 90% of the world's child deaths occur (exclusive breastfeeding for 6 months and weaning after 1 year)
7) Reduces incidence of clinical asthma, atopic dermatitis and eczema by 27% in low risk

population and upto 42% in infants with positive family history (exclusive breastfeeding upto 3 to 4 months)

8) Reduces the risk of developing celiac disease by 52% in infants who were breastfed at the time of gluten exposure.
9) Reduces the risk for childhood inflammatory bowel disorders by 31% (breastfeeding for any duration).
10) Reduces the risk for adolescent and adult obesity by 15 to 30% (breastfeeding for any duration).
11) Reduces the incidence of type 1 diabetes by 30% (exclusive breastfeeding for atleast 3 months).
12) Reduces the risk of acute lymphocytic leukemia by 20% and acute myeloid leukemia by 15% (infants breastfed for 6 months or longer)
13) Higher intelligence scores and higher teaching ratings in infants exclusively breastfed for 3 months or longer.

Source: American Academy of Pediatrics. Breastfeeding and the Use of Human Milk. Policy statement Pediatrics 2012; 129(3):e827-e841.

2.2.4 Exclusive breastfeeding: prevalence and barriers

Undernutrition is responsible, directly or indirectly, for 60% of the 10.9 million deaths annually among children under five. More than two-thirds of these deaths are associated with inappropriate feeding practices during the first year of life, and they are the most serious threat to attaining health and preventing malnutrition in this age group (WHO/UNICEF 2003). Exclusive breastfeeding upto 6 months of age and continued breastfeeding up to 12 months has been ranked the most effective child survival intervention for preventing under-five mortality (Jones et al., 2003). However globally, the rates of exclusive breastfeeding in infants less than 6 months of age, is only 36% (Gupta et al., 2012). Even the situation in India is no better, with reports from the National Family Health Survey India indicating that only 46% of the infants under 6 months and 58% of infants under 4 months of age are exclusively breastfed based on questionnaires (Patel et al., 2010). Demographic and health surveys carried out in Bangladesh and Nepal as well, report the prevalence of exclusive breastfeeding among infants under 6 months of age to be as low as 43% and 53%, respectively (Mihrshahi et al,. 2010; Pandey et al., 2010).

In many societies infants are predominantly breastfed, that is to say they receive water, tea and juices (Labbok & Krasovec 1990) in addition to receiving breast milk. In India, data has shown that complementary feeding begins early, for example in North India water mixed with honey and herbs, boiled water, tea, and animal milk were commonly used pre-lacteal feeds and 47 % of the women were not aware of the benefits of breastfeeding (Mahmood et al., 2012).. There is evidence that the early introduction of complementary

foods reduces levels of breast milk intake (Cohen et al., 1994, Heinig 2004), and others have found an association between the introduction of infant formulas and early termination of breastfeeding (WHO 1998b). It is also thought that even the introduction of non-nutritious liquids decreases breast milk production (Sachdev et al., 1991), but the precise extent to which breast milk intake is replaced by other liquids, milk or complementary foods remains a matter of some uncertainty. Understanding the barriers to exclusive breastfeeding is important to design community oriented approaches that could target lactating women to improve rates of exclusive breastfeeding (**table 6**).

Table 6: Barriers to exclusive breastfeeding

Sociodemographic	Biomedical	Psychosocial
Return to work after birth	Cesarean delivery	Lack of breastfeeding education
Living in an urban area	Early introduction of solid foods	Low maternal confidence
Low income	Introduction of infant formula for non medical reasons	Low maternal self efficacy and optimism
Low education	Limited parental education	Maternal anxiety
Single marital status	Maternal obesity	Maternal depression
Younger maternal age	Maternal tobacco use	Negative maternal attitudes about breastfeeding
	No prior breastfeeding experience	
	Introduction of pacifiers	
	Primiparity	

Source: Whalen B and Cramton R (2010) Overcoming barriers to breastfeeding continuation and exclusivity. Curr Opin Pediatr 22:655–663

2.2.5 The baby friendly hospital initiative: towards improving rates of exclusive breastfeeding

The baby-friendly hospital initiative (BFHI) and the 10 Steps to Successful Breastfeeding has been proposed by United Nations Children's Fund (UNICEF), and has shown to increase breastfeeding duration and prevalence in different settings (WHO 1998a, Krammer et al., 2001, Dulon et al., 2003).

The ten steps to successful breastfeeding in the BFHI are:

1. Have a written breastfeeding policy that is routinely communicated to all health care staff.
2. Train all health care staff in skills necessary to implement this policy.
3. Inform all pregnant women about the benefits and management of breastfeeding.
4. Help mothers' initiate breastfeeding within a half-hour of birth.
5. Show mothers how to breastfeed and how to maintain lactation even if they should be separated from their infants.
6. Give newborn infants no food or drink other than breast milk, unless medically indicated.
7. Practice rooming-in – allow mothers and infants to remain together – 24 hours a day.
8. Encourage breastfeeding on demand.
9. Give no artificial teats or pacifiers (also called dummies or soothers) to breastfeeding infants.
10. Foster the establishment of breastfeeding support groups and refer mothers to them on discharge from the hospital or clinic.
(UNCF/WHO 1992)

There has been a statistically significant annual increase in rates of breastfeeding among infants under 2 and 6 months in 14 developing countries with the initiation of BFHI (Abrahams and Labbok 2009). An Indian study has shown that when breastfeeding was initiated early, prelacteal feeds were less common and the intake of supplementary feeds like milk and fluids during the hospital stay was significantly lower in BFH (Breastfeeding Promotion Network of India 2000). However, the actual volumes of breast milk and non breast milk water intakes (indicating liquids or solids introduced other than breast milk) of infants born in these settings have not been studied at home, to confirm if these practices were continued by mothers.

2.2.6 Methods of assessing breast milk intake of infants

Measurement of breast milk volume is an important method for assessing nutritional intake in infants, however it is difficult to obtain accurate measurements of breast milk intake since the quantity consumed cannot be directly observed. There are several methods for measuring breast milk intake in such as infant or maternal test-weighing, maternal breast milk

expression and questionnaire method. However these approaches do not reflect habitual infant intakes due to their interference with physiological or behavioral aspects of lactation and are not precise and accurate (Da Costa et al., 2010). .The test weighing method measures the intake of breast milk by weighing the infants before and after each feed. Briefly, in this method, mothers are requested to breastfeed day and night, according to their usual feeding pattern or as demanded by the child. Breast milk is offered ad libitum, and the amount consumed is measured by test-weighing before and after feeding. The feed-related weight changes are adjusted for child-specific insensible losses, by multiplying the total number of minutes of feeding and the body weight with the child's mean insensible losses and the amount of insensible losses are added to the feed-related weight changes. The duration of each episode of breastfeeding is measured and the frequencies of feeding and total duration per 24 h are recorded (Islam et al., 2008). However, this technique is time consuming, the procedure can disturb the normal feeding pattern (Savenije et al., 2006), and cannot be applied to field or large group studies (Da Costa et al., 2010). In many settings, infants are nursed frequently, on demand, including during the night, which results in practical limitations to the use of test weighing. The practical problems associated with test weighing can be overcome by using the stable isotope technique.

2.2.7 Dose-to-mother deuterium dilution method

The dose-to-mother deuterium dilution technique was first described by Andy Coward and co-workers in 1982 (Coward 1982). The introduction of isotope tracer methods to measure breast milk intakes was a significant achievement in this area and was pioneered by Coward and colleagues. (Coward et al., 1982). The dose-to-mother deuterium dilution method uses a two compartment model to assess the breast milk intake and intake of water from sources other than breast milk (**Figure 6**). In the two compartment model, the mother's total body water (TBW) is the first compartment and the baby's TBW is the second compartment. Intake of breast milk and water from sources other than breast milk can be assessed over a period of 14 days by fitting the deuterium enrichment data to a model for water turnover in the mother and the baby. Breast milk intake by the baby is calculated from the flow of water from the mother to the baby. The baby's total water intake includes water from the oxidation of breast milk proteins, lipids and carbohydrates, and water from sources other than breast milk. Allowance is made for the baby's growth during the two week study duration (IAEA 2010). The technique also allows the baby's intake of water from sources other than breast

milk and the mother's body composition to be estimated (Coward et al., 1982, Haisma et al., 2003). A more detailed report on the methodology of sample collection and analyses is presented in the results section.

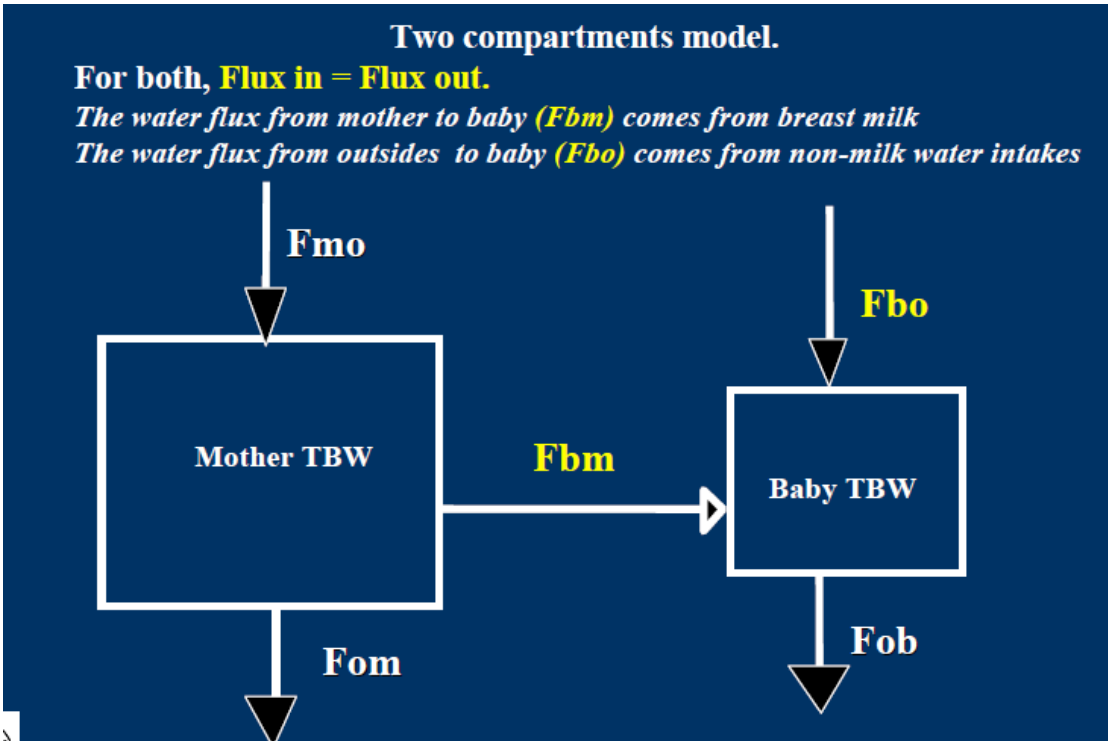

Figure 6: Two compartment model for transfer of human milk from mother to the infant
Source: IAEA 2010

Figure 6 represents a two compartment model, the mother's body water is the first compartment and the baby's body water is the second compartment. These two compartments are connected by the flow of milk from the mother to the baby. In a steady state model, the total water input is equal to the total water output. The first letter after the F indicates where the flow goes to, and the second letter indicates where the flow is from.

TBW: Total body water (This term refers to the total water content of the body, which makes up 70–75% of body weight at birth, but decreases to 50–60% of body weight in lean adults and less than 40% in obese adults. Fat free mass is approximately 73.2% water in adults. Measuring TBW establishes the amount of Fat free mass. Fat mass is calculated as the difference between Fat free mass and body weight. TBW includes both intracellular fluid and extracellular fluid.
Fmo: Water flux from outside to mother (water consumed by the mother)
Fbm: Water flux from mother to baby (human milk intake by the baby)
Fbo: Water flux from outside to baby (water intake by the baby from sources other than human milk)
Fob: Water flux from baby to outside (water lost from the baby's body in urine, faeces, sweat, saliva and breath).
Fom: Water from mother to outside (water lost by the mother from her body in urine, faeces, sweat and breath.

The two compartment model is based on several assumptions such as 1) The body water pool in both the mother and the baby is a single compartment in each individual. 2) The deuterium dose equilibrates rapidly and uniformly throughout the body water pool of the mother and her baby. 3) The size of the body water pool in the mother is constant. The baby's body water pool is assumed to change linearly with time due to growth. 4) All water regardless of the route of exit is labelled with deuterium in proportion to deuterium in the body water pool. 5) Deuterium leaves the system only as water 6) Water intake by the baby is only by ingestion. Where these assumptions do not hold true, an adjustment is included in the calculations (IAEA 2010).

The dose-to-mother deuterium dilution method has several advantages over the conventional test weighing technique where infants are weighed before and after each feed to assess the breast milk intake. The test weighing technique is time consuming and the procedure can disturb the normal feeding pattern of an infant (Savenige et al., 2006). Dose-to-mother deuterium dilution method overcomes the practical problems associated with the test weighing method. This technique has been used to address various research questions such as evaluating the efficacy of counselling and education programmes on infant feeding practices (Albernaz et al., 2003, Moore et al., 2007); evaluating the association between the intake of BM by breastfed infants and maternal body composition (Ettyang et al., 2003); evaluating community nutrition programmes for lactating women (Cisse et al., 2002a); evaluating the effect of the introduction of complementary foods on human milk intake by breastfed babies (Galpin et al., 2007); and quantifying nutrient flux or transfer of toxic elements from mother to baby (Cisse et al., 2002b, Sian et al., 2002).

The specific advantages of this method are

- It is non invasive.
- Does not depend on maternal ability to recall the time she breastfed her infant.
- Does not interfere with the normal feeding process.
- Allows estimating the baby's intake of water from sources other than breast milk (Coward et al., 1982, Butte et al., 1988, Haisma et al., 2003).
- May be used to evaluate the efficacy of counselling and education programmes on infant feeding practices, to study association between breast milk intake and maternal body composition, to evaluate community nutrition programmes for lactating women

and to evaluate effect of introduction of complementary foods on breast milk intake in infants (Albernaz et al., 2003, Moore et al., 2007, Ettyang et al., 2005, Cisse et al., 2002a, Galpin et al., 2007).

- To determine whether nutrient intake from breast milk would allow children of different ages to meet their energy and micronutrient requirements and to estimate the amount of energy and nutrients that should be contributed by complementary foods. It is recommended that nutrient requirements from complementary foods be estimated as the difference between young children's estimated total nutrient needs and the amounts transferred in breast milk to children of different ages (Brown et al., 1998a) and currently this is a very good tool to quantify breast milk intake volume.

2.2.8 Breast milk intake of infants

Table 7 summarizes the breast milk intake of infants assessed using the 24 h test weighment method as well as the "dose to mother" deuterium dilution method. The breast milk intake reported among infants aged 9 to 11 months from Bangladesh and 5 months of age from Burkino Faso using the test weighment method is higher than what we report using the isotopic method (Coulibaly et al., 2004, Islam et al., 2008). In these studies, contrasting results have been observed with respect to the displacement of breast milk with intake of other fluids/solid foods that were measured by weighment of the solid or measuring the volume of the fluid. Studies have been done using the isotopic methods to get a precise and accurate estimate of the breast milk intake, as well as water from sources other than breast milk. The breast milk intake of infants from Brazil and Bangladesh at 4 months of age was higher than the intakes of infants that we report from India (Haisma et al., 2003, Albernaz et al., 2003, Moore et al., 2007). In one of the studies the intake of water from supplements was significantly lower in the exclusive breastfeeding group in comparison to the partially breastfed group as well as predominantly breastfed group (Haisma et al., 2003). In yet another study, frequent home visits by the lactation counseling team for imparting breastfeeding education was successful in preventing breastfeeding from being terminated beyond 4 months, as well as in reducing the intensity of weaning in comparison to those who did not receive such counseling, indicating that providing education to the mother can impact the decision to continue breastfeeding (Albernaz et al., 2003). In a pooled analysis of data of breast milk intake, in infants aged 0-24 mo from 12 countries across 5 continents, the overall

mean breast milk intake was estimated to be 780 ml/d. Age-specific estimates indicated that intake increased over the first 3-4 mo and remained above 800ml/d until 6-7 mo (Da Costa et al., 2010). However in these analyses, India was not considered since there is no data on breast milk intake of Indian infants using the "dose to mother" deuterium dilution method.

Table 7: Breast milk (BM) and non breast milk (NBM) water intakes of infants reported using the test weighment and "dose to mother" deuterium dilution method [1]

Study design and study population	**Reported breast milk (BM) [2] and non breast milk (NBM) [3] water intake in the study**	**Principal finding of the study**
Test weighment method		
Cross sectional study on Infants at 5 mo from rural and urban Burkino Faso (n = 97 and 69, respectively) (Coulibaly et al., 2004)	Daily BM intake was 776 ml/d for urban and 835 ml/d for rural infants. Median extra fluid intake was 79 and 122 ml/d, respectively.	BM intake and nutritional status of infants were not affected by the consumption of extra fluid or food.
Intervention study on healthy breastfed children 8–11 mo of age from Bangladesh (n = 18) (Islam et al., 2008).	The mean BM intake of the infants was 828 ml/d.	BM intake decreased slightly but progressively, with greater energy density and feeding frequency of Complementary foods (CF) [4]. The energy density and feeding frequency of CF affect infants' total daily energy intake and BM consumption.
"Dose to mother" deuterium dilution method		
Cross sectional community based study on infants aged 4 mo recruited at birth from urban Pelotas, Southern Brazil (n = 70, divided in to 3 groups EBF (35), PBF (16), PartBF (19) for analyses, based on questionnaire assessment) (Haisma et al., 2003).	Adjusted mean BM intakes in Exclusively breast fed (EBF) [5] group was 806 ml/d while in Predominantly breastfed (PBF) [6] group it was 778 ml/d and in the Partially breastfed (PartBF)[7] group it was 603 ml/d. Water from supplements was 10 ml/day (EBF), 134 ml/day (PBF) and 395 ml/day (PartBF).	BM intake was not significantly different in the EBF and PBF group, however intakes were significantly higher in EBF group compared to PartBF group (P = 0.004) Intakes of water from supplements was significantly lower in the EBF group compared to PBF and PartBF (P = 0.005; and P< 0.001, respectively)
Blinded intervention trial on	BM intake of infants in the	Mothers in the control group were

infants from urban Pelotas in Brazil assessed for breast milk intake at 4 mo of age (n = 188). Intervention group (n = 94) received home based counseling by lactation support team in addition to hospital counseling, while control group (n = 94) attended pediatric clinics but did not receive specific lactation counseling (Albernaz et al., 2003).	intervention and control group was similar (761 vs. 723 ml/d) as were NBM water intake (107 vs. 195 ml/d)	almost twice as likely to stop breastfeeding by 4 mo as those in the Intervention group. Velocity of weaning was twice as high in the control group. Lactation counselling reduced early weaning, but breast milk intake at 4 mo was not affected.
Lactating mothers and their infants aged 2 to 4 months from Pastoral communities of Pokot, Kenya (n = 10) (Ettyang et al., 2005).	BM intake was 555 ml/d at 2 to 4 mo.	Infant weight and weight/age Z score were 4.956 kg and -1.750, respectively. BM intake was low and may not be enough to support adequate growth.
Cross sectional study on mother infant pairs from rural Bangladesh studied at infant age of 14.3 wks (n = 98) (Moore et al., 2007).	75 of the 98 subjects reported EBF. Mean BM intake was 884 ml/d in the group that was EBF while it was 791 ml/d in the group reported as nonexclusively breastfed (P = 0.0267). Intakes of NBM water were 40 and 166 ml/d (P< 0.0001), respectively.	Objective cross-validation using deuterium dilution data showed good accuracy in reporting of feeding practices, although apparent misreporting was widely present in both groups. Deuterium dilution technique can be used at the group level however, it is not adequate to distinguish between feeding practices in individual infants.
A pooled analysis of data points of human milk intake, was undertaken in infants aged 0-24 mo from 12 countries across 5 continents (n = 1115) (Da costa et al., 2010).	The overall mean human milk intake was 780 ml/d, and the age-specific estimates indicated that intake increased over the first 3-4 mo and remained above 800ml/d until 6-7 mo.	Human milk intake remains above 800 ml/d until 6-7 mo of age among infants in the pooled analyses.

[1] Inclusion/Exclusion of studies: Studies pertaining to Breast milk intake in infants using test weighment and "dose to mother" deuterium dilution method were identified and included from India as well as other countries.

[2] BM: Breast Milk, [3] Non breast milk, [4] CF: Complementary Foods, [5] EBF: Exclusive Breastfeeding, [6] PBF: Predominantly Breastfed, [7] PartBF: Partially Breastfed.

2.2.9 Functions of zinc in human health

In the human body zinc has 3 main functions: catalytic, structural and regulatory. Zinc is involved in the catalysis by the enzymes that control cell processes such as DNA synthesis, normal growth and brain development, behavioural response, reproduction, foetal development, membrane stability, bone formation, and wound healing. It also has a structural and functional role in proteins involved in DNA replication and reverse transcription and is critical for the function of a number of metalloproteins. In addition, zinc regulates both enzymatic activity and the stability of the proteins, as an activator or as an inhibitor and modulates cellular signal transduction processes (Chasapis et al., 2012)

Zinc and morbidity related to diarrhea

The role of zinc in reducing the risk for diarrhea has been documented: two meta analyses, including 15 and 24 studies each, showed a 14% and 20% reduction in the incidence of diarrhea with zinc supplementation, respectively (Agarwal et al., 2007, Brown et al., 2009a). Another systematic review of efficacy and effectiveness studies to estimate the effect of zinc for the treatment of diarrhoea on diarrhoea mortality and subsequent pneumonia mortality, showed zinc to be an effective therapy that could decrease diarrhoea morbidity and mortality and could be scaled -up in low-income countries (Walker and Black 2010). In a more recent meta analyses, zinc supplementation was found to have a 9% reduction in incidence of diarrhoea, a 19% reduction in the prevalence of diarrhoea and 28% reduction in the occurrence of multiple diarrheal episodes, although the evidence was inconclusive due to large heterogeneity in studies evaluated (Patel et al., 2011). The Cochrane database systematic review has shown that in areas where the prevalence of zinc deficiency or moderate malnutrition is high, and among children aged 6 months or more, zinc supplementation may be beneficial. However, a similar benefit has not been documented in infants below 6 months of age (Lazzaeini and Ronfani 2012).

Zinc and morbidity related to respiratory diseases

Supplementation of zinc has shown a 15% reduction in acute lower respiratory tract infections among children 1 to 49 months, and this reduction was more significant among children who had a greater degree of stunting initially. The factors that were associated with reduction of risk of acute lower respiratory tract infections were the initial height-for-age z-

score and the quality of diagnosis for acute lower respiratory tract infections (Brown et al., 2009a).

Zinc and morbidity related to malaria

There is insufficient evidence to draw definitive conclusions on the effect of zinc supplementation in reducing the risk for malaria primarily due to very few studies which have been conducted and also the inability to attribute the effect of supplementation on reduction of malaria risk to zinc alone, considering that vitamin A was also supplemented in this study along with zinc. Nevertheless, the weight of the evidence suggests that zinc may reduce the incidence of malaria especially that of more severe cases that result in clinic attendance (Brown et al., 2009a).

Zinc and mortality

The role of zinc in reducing child mortality is evident in that, if programs were conducted with greater than 90% coverage to prevent zinc deficiency then child mortality would reduce by 5% globally (Jones et al., 2003). Pooled data from trials in Nepal (Tielsch et al., 2006), Tanzania (Sazawal et al., 2006) and India (Bhandari et al., 2007) have shown that zinc supplementation reduced mortality of children 12 months of age or older by approximately 18%, but had no effect on younger children. However, when iron and folic acid were provided in addition to zinc, the impact of zinc among older children was no longer evident. In studies from Brazil (Lira et al., 1998) and India (Sazawal et al., 2001) on LBW and SGA infants, there was a 52% to 68% lower mortality rates among children who received zinc. Additionally, studies from Nepal in infants with birth weight < 2000g have shown that infants receiving zinc had half the mortality risk as against those who were not supplemented. Collectively, these studies indicate that providing preventive zinc supplementation in settings where there is an elevated risk of zinc deficiency would reduce mortality among children greater than 1 year of age and possibly among LBW infants (Brown et al., 2009a).

Zinc and immunity

The importance of zinc for the optimal functioning of innate and acquired immunity is known, and deficiency of zinc may lead to impaired immune function (Tapiero and Tew 2003). Deficiency of zinc deficiency is known to depresses lymphocyte proliferation, interleukin-2 and interferon-γ production, causes imbalance in type 1 and type 2 helper cells

and depress and antibody responses to T-cell dependent antigens (Maggini et al., 2010). Zinc levels are also known to modulate the function of monocytes, macrophages, and neutrophils polymorphs and the release of reactive free radicals from phagocytes (Ibs and Rink 2003). Mothers supplemented during their pregnancy with zinc, in addition to iron and folic acid, had infants who had significantly fewer episodes of diarrhoea and improved immunity with 16% higher interleukin-6 production during the first 6 months of life (Wieringa et al., 2010)

Zinc and growth

Zinc is an essential nutrient needed to maintain the normal structure and function of multiple enzymes, including those that are involved in transcription and translation of genetic material andin cell division (Cousins 1996). The effect of zinc supplementation in improving growth and circulating growth factors in growth-retarded children has been demonstrated in several studies (Nakamura et al. 1993, Cavan et al. 1993, Friel et al. 1993, Castillo Duran et al. 1994, 1995, Nishi 1997; Ninh et al. 1996; Friis et al. 1997, Brown 1998b; Kaji et al. 1998). In a quantitative review of 33 studies assessing the effect of zinc supplementation on children's growth, zinc supplementation was shown to produce a small, but significant, positive impact on the weight gain and linear growth in children with low weight-for-age or height-for-age, the effect being moderately large among stunted children (weighted mean effect sizes expressed in SD units were 0.350 (95% CI: 0.189, 0.511) for height, 0.309 (95% CI: 0.178, 0.439) for weight) (Brown et al., 2002). In a more recent meta analyses, zinc supplementation among children aged < 1 month to 134 months produced a small, but highly statistically significant, positive impact on their height-for-age Z score (HAZ) (mean effect size (expressed in SD units) of 0.170 (95% CI, 0.075 to 0.264)), weight-for-age Z score (WAZ) (mean effect size of 0.119 (95% CI, 0.048 to 0.190)), and a marginal effect on weight-for-height Z score (WHZ) (mean effect size was 0.062 (95% CI, 0.000 to 0.123)), although the studies on growth velocity had a significant heterogeneity (Brown et al., 2009a). Not only has linear or ponderal growth been affected, but zinc supplementation also confers a beneficial effect on the rate of head growth, as demonstrated in rural Nepali infants and children aged 4-17 months, where zinc treatment was associated with 0.11 (95% CI: 0.05 to 0.17) decrease in the rate of decline in head circumference Z score across visits as compared to the group that was not supplemented (Surkan et al., 2012).

2.2.10 Sources of dietary zinc

The major sources of dietary zinc are lean red meat, whole-grain cereals, pulses, and legumes and provide approximately 25-50 mg of zinc/kg raw weight of the food. Processed cereals, polished rice, and lean meat have a moderate zinc content (10-25 mg/kg), while fish, roots and tubers, green leafy vegetables, and fruits provide only modest amounts of zinc <10 mg/kg (Johnson et al., 1993). Animal-source foods, especially meats, including organ meats, not only contain the highest concentrations of zinc but provide zinc in a bioavailable form (Brown et al., 2004). The utilization and absorption of zinc would depend on the presence of promoters and inhibitors in the diet. Competitive interactions between zinc and other ions with similar physicochemical properties, such as iron and copper, can affect the uptake and intestinal absorption of zinc, when consumed in high doses in supplements. Phytates are known to bind zinc and inhibit its absorption, and plant foods such as grains and legumes with favourable zinc concentrations, also have the highest phytate concentrations (Food and Nutrition Board/Institute of Medicine 2001).

2.2.11 Maternal zinc intake and breast milk zinc concentration

Long term controlled supplementation trials of zinc have shown conflicting results with respect to increases in breast milk zinc concentrations (Krebs et al. 1995, Moser Veillon and Reynolds 1990). In populations with adequate zinc intake, breast milk zinc concentration is resistant to increases in maternal zinc intake. For example, in well nourished populations there has been no correlation between maternal dietary zinc intake and breast milk zinc concentration; although these studies have to be viewed with caution owing to limitations of study design and sample size (Krebs 1999). Among lactating Mexican-American women from Texas less than 3 months postpartum, there was no statistically significant correlation observed between dietary intake and breast milk concentration of zinc, despite a significantly lower intake of dietary zinc in comparison to percent recommended intake of zinc as stated by DRI (Hannan et al., 2009). Equally, in Chinese lactating women at 2 months postpartum, despite marginal zinc intake of 7.6 mg zinc/day, they were able to secrete 2 mg zinc/day in to their breast milk, possibly due to adaptive responses in zinc secretion into breast milk (Sian et al., 2002). Breast milk zinc concentration has a wide variability; and does not depend on maternal zinc intake, but is affected by factors such as parity, stage of lactation, and type

(fore or hind milk) of feeding (Dorea 1993). To the contrary, in populations with chronically low zinc intakes, lower mean breast milk zinc concentration has been observed, nevertheless there have been no randomised controlled prospective intervention trials in such groups to confirm this (Krebs 1998). Breast milk zinc concentration among women in developing countries tend to be lower than those of well-nourished women from the United States at comparable times postpartum (Krebs 1998). The observations from the developing countries where women have chronically low intake, may be suggestive that there could be a dietary zinc threshold below which maternal homeostatic adjustments may not be able to maintain breast milk zinc concentration (King 2002). Overall, these inconsistent findings are suggestive that maternal zinc intake is not a major determinant of breast milk zinc concentration (Brown et al., 2009b). There may be physiologic changes associated with lactation that alters both intestinal zinc absorption and endogenous excretion. In addition, hormonal signals probably play a role; however, the hormones involved and their target tissues are unknown. There could also be an up-regulation of metal transcription factor 1, which induces the expression of genes for zinc transporter proteins that facilitate zinc transport across the basolateral membrane of the mucosal cell (Cousins 2000). Recent literature has shed light on the following: 1) Breast milk zinc concentration declines about 75% over the lactation period irrespective of maternal zinc intake 2) The efficiency of zinc absorption increases during lactation and this is further enhanced with low zinc diets (≤8 mg/day) or when maternal zinc status is marginal. 3) Endogenous faecal zinc excretion also declines among lactating women with low zinc intakes (Donangelo and King 2012). All together, there is limited evidence suggesting that breast milk zinc concentration is reduced with low dietary zinc intakes. In addition, the effect of low maternal zinc intakes on zinc kinetics and the regulation of mammary gland zinc uptake and secretion is not fully understood (Donangelo and King 2012)

Breast milk zinc concentration of lactating women in India and other countries has been illustrated in **Figure 7.** As shown in the figure, at 1 month postpartum, the breast milk zinc concentration was higher among lactating women from Guatemala (Dhonuksha Rutten et al., 2005) in comparison to those from Bangladesh (Simmer et al., 1990, Islam and Brown 2010). A study from India (Orun et al., 2012) has shown a very low concentration of zinc in breast milk at 2 months postpartum in comparison to those observed in Iran (Khaghani et al., 2010) and Korea (Han et al., 2011). In these studies from India, Iran and

Bangladesh, breast milk zinc content was not related to any of the maternal factors such as maternal age, weight, education, occupation, parity, birth weight, gestational age or feeding type. Studies from Guatemala (Dhonuksha Rutten et al., 2005), Bangladesh (Islam and Brown et al., 2010) and Iran (Khaghani et al., 2010) have all shown a consistent decrease in breast milk zinc concentration over the postpartum period. Breast milk zinc concentration are high in the early weeks of lactation and decline sharply over time (Krebs et al., 1995, 1999) and age at postpartum is the only determining factor for breast milk zinc concentration (Brown et al., 2009b). In spite of increasing volumes of milk consumed by the infant, the total zinc intake falls as a function of the rate of decrease in the milk metal concentration (Dorea et al., 2012).

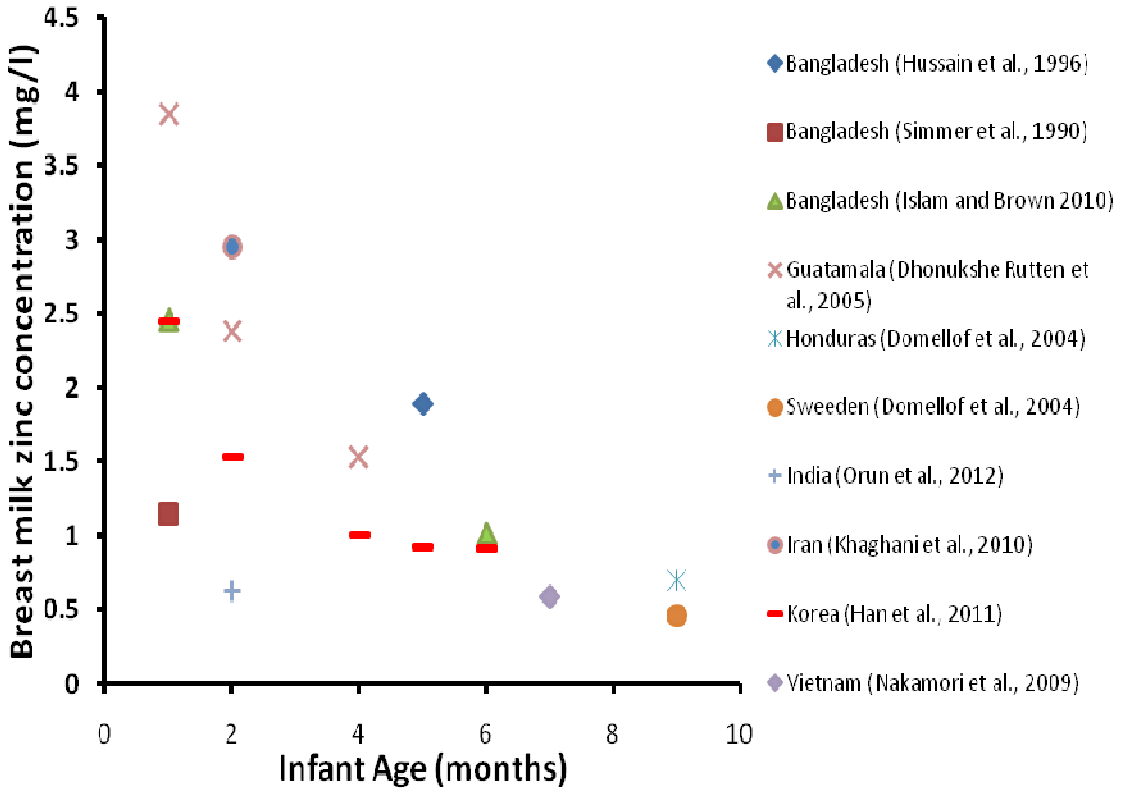

Figure 7: Breast milk zinc concentration in lactating mothers from studies across India and other countries

Figure legend:
Breast milk zinc concentrations across all studies have been converted to mg/l
Prominent studies from India and other countries in the past decade have been included

For studies where breast milk zinc concentrations have been reported over a range of months, the values have been indicated for the average month. For example, 1) Bangladesh (Simmer et al., 1990), breast milk zinc content reported for 3 to 6 months (in graph breast milk zinc concentration reported for 4.5 months) 2) Vietnam (Nakamori et al., 2009) breast milk zinc content reported for 6 to 8 months (in graph BM zinc concentration reported for 4.5 months).

2.2.12 Absorption and intake of zinc through breast milk

Studies using stable isotope methodology and based on current estimates of zinc requirement the mean fractional absorption of zinc has been estimated to be approximately 0.50. Breast milk alone is considered adequate for exclusively breastfed term infants for at least 3 months and possibly until 6 months, depending on the availability of zinc accumulated by the foetus during gestation for metabolism (Brown et al., 2009b). In contrast to this, the fractional absorption by young infants consuming cow milk based formula has been found to be only half that of human milk (Krebs 1999).

Inadequate dietary intake of absorbable zinc is one of the major causes of zinc deficiency (Hess et al., 2009). The risk of zinc deficiency is considered to be of public health concern when the prevalence of inadequate intakes is greater than 25%, in which case an intervention to increase dietary zinc intakes is recommended (de bonist et al. 2007). The young infant has a relatively high zinc requirement to support the very rapid growth of early infancy. With the assumptions of urinary and sweat zinc losses to be 20 µg/kg/day, endogenous faecal zinc losses to be 50 µg/kg/day and zinc required for new tissue accretion to be 20 µg/g weight gain or 30 µg/g lean tissue gain (Butte et al., 2002), the total requirement of zinc for infants (boys and girls) would be 0.94, 0.86 and 0.79 mg/day at 1, 3 and 6 months, respectively.

Breast milk is an important source of zinc for infants and young children, and promotion of breastfeeding is an important measure to support adequate zinc nutrition of young children (Brown et al., 2009b). Prolonged breastfeeding and low complementary foods intake has been associated with higher zinc concentrations in breast milk among Honduran women, despite lower maternal plasma zinc concentrations, and this may be an important factor in the prevention of zinc deficiency among breastfed infants in developing countries (Domelloff et al., 2004). For term infants with normal birth weight, zinc requirements are generally assumed to be met by exclusive breastfeeding due to high bioavailability of zinc in breast milk (Krebs et al., 1996). However, at 5 to 6 months of age, infants may become marginally zinc deficient due to the physiological decline in breast milk zinc concentrations,

despite increases in volume of breast milk consumed, making the infants vulnerable to sub-optimal zinc intakes and thereby impaired growth (Krebs et al., 1994, Krebs 1999, Walravens et al., 1992). In addition, SGA and LBW infants may be at increased risk for low intakes of breast milk zinc (Krebs and Westcott 2002). Even with very efficient absorption, high bioavailability of zinc from breast milk and conservation of endogenous losses, factorial estimates predict that net absorption is only marginally adequate by 6 months of age (Krebs 1999).

Breast milk provides an excellent source of highly bio available zinc and meets the needs of the healthy young exclusively breastfed infants for the first several months of life. However zinc intake from breast milk alone may become limiting by around 6 months of age. The older infant clearly becomes dependent on non-breast milk sources of zinc, i.e., from complementary foods. Zinc has been classified by WHO as a ‘‘problem’’ nutrient for which requirements cannot be met without supplementation or fortification of complementary foods (Dewey and Brown 2003). Introduction of animal products or zinc supplementation may be important to meet the older infant's zinc requirements (Krebs and Westcott 2002). Among exclusively breastfed infants from Denver, who were randomly assigned to receive commercially available pureed meats, iron-and-zinc-fortified infant cereal, or whole-grain iron-only-fortified infant cereal as the first and primary complementary foods from 5 to 10 months of age, the mean (± standard error of mean) total absorbed zinc amounts were 0.80 ± 0.08, 0.71 ± 0.09, and 0.52 ± 0.05 mg/d for each of the groups, respectively. Zinc from breast milk contributed < 25% of total absorbed zinc for all groups. The authors concluded that the requirements for older breastfed-only infants are difficult to be met without the regular consumption of either meats or zinc-fortified foods (Krebs et al., 2012). Novel technologies such as bio fortification of grains with zinc and lowering the phytate content of plant products could go a long way in optimising infant zinc nutrition beyond 6 months (Nestel et al., 2006, Mazariegos et al., 2006).

2.2.13 Zinc status and deficiency among breastfed infants

The term breastfed infant usually has an excellent status at birth due to the combination of hepatic stores and the superior bioavailability of human milk (Lombeck and Fuchs 1994, Hemalatha et al., 1997). However, zinc status can become marginally adequate in the second half of the first year of life (Krebs 1999). Studies have shown formula fed infants to have lower plasma zinc concentration in comparison to the breastfed ones (Michaelson et al., 1994,

Lombeck and Fuchs 1994, Hemalatha et al., 1997). Reports from breastfed infants in India have shown higher plasma and leukocyte zinc concentration in the early months of life compared to formula fed infants. Plasma zinc concentration was lowest at 4 to 6 months and improved to normal concentration by 9 months, following weaning (Hemalatha et al., 1997).

Zinc deficiency in infancy and early childhood is an important cause of stunting, increases infectious disease morbidity and mortality due to diarrhoea and pneumonia, and therefore is of public health concern especially in developing countries (Hambidge and Krebs 2003). Deficiency of zinc in term breastfed infants has been attributed to low maternal breast milk zinc concentrations (Prasad et al., 1996). In the weaning period higher prevalence of zinc deficiency may be primarily due to low intake of zinc from animal sources and high dietary phytate content (that limits the bioavailability of zinc), and inadequate food intake (Shrimpton 1993). Zinc deficiency is responsible for approximately 4% of the worldwide morbidity and mortality burdens of young children (Black et al., 2008). Mild-to-moderate zinc deficiency due to inadequate dietary intake is prevalent in all parts of the world. The suggested lower cut-offs for serum zinc concentrations of young children are 9.9 μmol/l (65 μg/dl), if they are examined in the morning while non fasting, and 8.7 μmol/l (57 μg/dl), if studied in the afternoon. In a community based cross-sectional study to study the prevalence of zinc deficiency in children aged 6-60 months of age from five states across the North and South of India, the overall prevalence of zinc deficiency was 44%, indicating that there is a high prevalence of zinc deficiency among children belonging to low Socio-economic Index in India (Kapil and Jain 2011). In a cohort of infant-mother pairs (220 LBW infants and 119 normal birth weight infants and their mothers) from Delhi, zinc deficiency was present in 51% of LBW and 42.4% of normal birth weight infants at birth, and in 79% of LBW and 67% of normal birth weight infants between 2 and 10 months of age, indicating that zinc status was poor in infants irrespective of birth weight (Agarwal et al., 2012a).

. Zinc status is often difficult to assess because the effective regulation of zinc homeostasis buffers the functional response to dietary deficiency and excess (Lowe et al., 2009). To obtain the best estimate of the risk of zinc deficiency in a population and to identify subgroups with elevated risk, the recommended biochemical indicator by WHO/United Nations Children Fund (UNCF)/IAEA/International Zinc Nutrition Consultation Group (IZiNCG) is the prevalence of serum zinc concentration less than the age/sex/time of day-specific cut offs (improvement in zinc status recommended where

prevalence is < 20%), for dietary indicators, the risk is considered elevated when the prevalence of inadequate intakes of zinc > 25%, and for growth indicators the risk is elevated when the prevalence of low HAZ ≥ 20% (de Bonist et al., 2007). Serum zinc concentration can be considered a useful biomarker of a population's risk of zinc deficiency and response to zinc interventions, although it may not be a reliable indicator of individual zinc status (Hess et al., 2007). Serum zinc is also not an ideal indicator of zinc status because infection can lower serum and muscle break down during weight loss can liberate zinc into circulation and increase serum zinc concentration (IZiNCG 2007). Although plasma/serum zinc concentration has been widely used to assess the nutritional status, zinc concentration may respond to metabolic conditions unrelated to zinc status and are insensitive to changes in dietary zinc (King 1990).

2.3 MATERNAL AND CHILD NUTRITION AND HEALTH IN INDIA

In India malnutrition has been called 'The Silent Emergency'. The proportion of under-nutrition among children and women in India is one of the highest in the world. India is committed to halving the prevalence of underweight children by 2015 as one of the key indicators of progress towards the Millennium Development Goals. Despite the unprecedented economic growth, improvements in childhood nutritional status in India over the last decade have been slow. The analysis of the situation of children and women in India gets complicated due to widespread disparities that exist between and within states, districts and sub-district levels. There are also inequalities that persist among different subgroups of the population, notably women and girls, Scheduled Castes, Scheduled Tribes. National Family Health Survey reports have shown high rates of maternal under-nutrition measured by low BMI, anaemia as well as sub-optimal infant and young child feeding practices (National Family Health Survey 2005-2006). **Table 8** illustrates the salient features of the National Family Health Survey survey pertaining to maternal and child nutrition.

Table 8: Salient features of the National Family Health survey 2005-2006 (maternal and child nutrition)

Maternal nutrition
• One in three women 15 – 49 years of age has a body mass index (BMI) below 18.5 indicating severe nutritional deficiency and under-nutrition • Among pregnant women, 58% are anemic • **Child nutrition**Malnutrition is the major underlying cause of 50% of deaths among children • 8.3 million babies are born low birth weight (less than 2.500 grams). • Nearly half (46%) of children under 3 years of age, or 31 million children are underweight (low weight for age). • 25.5 million, or 1 in 3, children under 3 years of age are stunted (low height for age) as a result of poor nutrition • 13 million, or 1 in 5, children below 3 years of age are wasted (low weight for height) • Eight in ten children 6 – 35 months of age are Anemic. For children 6-59 months, 26% have mild, 40% have moderate, and 3% have severe anemia • Only 23% of children below 3 years of age in India initiated breastfeeding within one hour of birth as recommended by WHO • Less than half (46%) of children under 6 months of age are exclusively breastfed • Only half (56%) of children aged 6 – 9 months are provided with the recommended semi-solid complementary foods and breast milk • Only one in four children aged 12 – 35 months receive the six monthly Vitamin A supplement • Only 1 in 5 children 6 – 23 months of age receive the recommended appropriate feeding

Source: National Family Health Survey 3 2005 – 06, India: Volume I, & National Fact Sheet, International Institute for Population Sciences, Mumbai, September 2007

2.4 QUALITY OF MATERNAL HEALTH CARE IN INDIA

The specific interventions that can reduce the elevated risk of morbidity and mortality due to complications before, during and after birth are antenatal care, skilled attendance at birth, emergency obstetric care, post-partum care, contraception and family planning delivered across a continuum of care (Progress for Children: A Report Card on Maternal Mortality 2008). Approximately 270 million women are in child bearing age based on 2011 census in India (Census of India 2011). The antenatal care services and skilled delivery care remain low and about 59% of women have had no postnatal check up at all (National Family

Health Survey 2005-2006). The contraceptive prevalence rate is only about 55%, and the unmet need on contraception is 21% (District Level Household Survey 2002-2004). About 13% of girls in the age-group 15-19 have begun childbearing (either have had a live birth or pregnant with the first child) (National Family Health Survey 3 2005-2006). The poor status of health of child-bearing adolescents, coupled with physiological immaturity elevates the risk of maternal and perinatal deaths. However, the quality of maternal care is lacking as seen below from relevant indicators

Situation of Indian maternity health care system:

Indian adolescent girl's profile: They constitute 20% of the population. The mean age of marriage has increased over the years and is 20.6 years. Among the 27% married adolescents, 43% are married less than 18 years, of which 13% are mothers. The adolescent birth rate is 45/1000 live births. Approximately 6000 teenage mothers die every year (State of the world's mothers 2012, census 2011, National Family Health Survey 2005-2006).

Antenatal care indicators in India: Based on the Coverage Evaluation Survey in 2009, 90% of the pregnant women attend one antenatal survey during pregnancy, while only 27% attend four antenatal visits. Iron folic acid supplementation was received for greater than 90 days by only 31% of the pregnant women (Chaterjee and Paily 2011)

Status of Delivery and postnatal care in India: The coverage evaluation survey reports 79% of the pregnant women report to have skilled birth attendant at birth and 76% report to have institutional delivery. There is however no data available on postnatal care within 2 hours of birth (Chaterjee and Paily 2011).

Maternal mortality ration in India: Although the maternal mortality ratio has decreased from 522.7 per hundred thousand live births in 1990, to 186.5 in 2011, we are way behind the Millenium Development Goal target of 109. In 2011, there were 50, 648 maternal deaths and 1.3 million infant deaths (Lozano et al., 2011).

3. AIMS OF THE STUDY

The purpose of this thesis was to gain insight into some of the key areas related to maternal and infant nutrition in India. From the maternal nutrition point of view we wanted to study the prevalence and socio demographic and dietary correlates of two public health concerns in India, mainly anaemia and vitamin B_{12} deficiency, both of which are associated with micronutrient deficiencies. From the infant nutrition point of view we wanted to assess compliance to exclusive breastfeeding in infants, which has the greatest potential to save under five deaths with maximum universal coverage. Additionally, we aimed to assess breast milk zinc intake and serum zinc level among infants, considering that zinc deficiency in infants is a public health concern in India.

THE SPECIFIC OBJECTIVES OF THE STUDY:

1. To study the prevalence and correlates of anaemia among pregnant urban South Indian women in early pregnancy (**Paper 1**).
2. To evaluate the vitamin B_{12} status of South Indian women in early pregnancy and its relationship with sociodemographic, anthropometry and dietary intake **(Paper 2).**
3. To assess compliance to exclusive breastfeeding until 6 months of age among healthy term infants born in BFH (**Paper 3**).
4. To assess the breast milk zinc transfer and serum zinc levels among healthy term urban South Indian infants using measures of breast milk volume and breast milk zinc concentrations. (**Paper 4**).

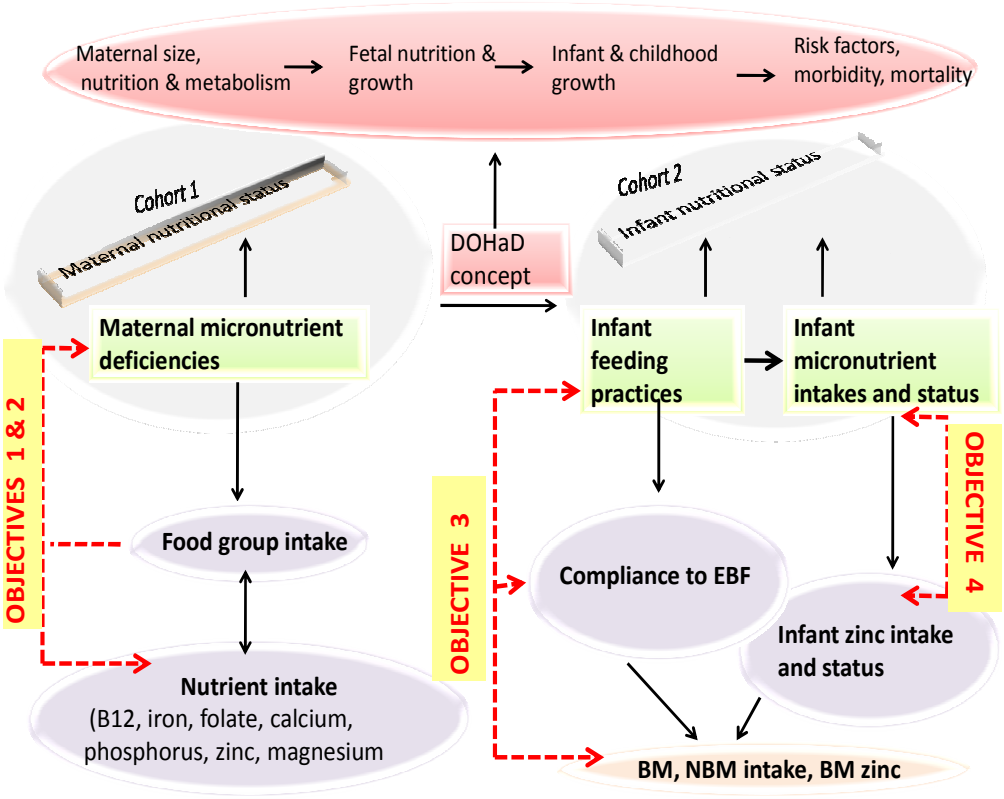

Figure 8: Framework of the thesis
DOHaD: Development origins of Health and Disease
BM: Breast milk, NBM: Non breast milk
EBF: Exclusive breastfeeding

Explanation of the framework

Figure 8 represents the overall framework of this thesis. The relation between maternal and infant nutritional status has been highlighted using the development origins of health and disease hypothesis (Barker 1998). This concept suggests that maternal nutritional status impact the foetal growth and nutrition which in turn influences the growth and development of the child and determines his/her risk for morbidity and mortality at all stages in life. The risk progressively accumulates throughout the life course and the disease manifest in later life. The growth and development of the child is dependent on maternal nutritional status and the first 1000 days is a critical period for both the mother and child. At the maternal level this thesis focuses on the prevalence and modifiable risk factors for micronutrient deficiencies

such as anaemia and vitamin B_{12} deficiency in early pregnancy (≤ 14 weeks gestation) among 366 pregnant urban South Indian women. The dotted lines (objectives 1 & 2) represent these. From the infant nutrition point, this thesis focuses on critical infant feeding practices such as compliance to exclusive breastfeeding until 6 months of age among healthy term infants born in a BFH, and in addition, assesses an important micronutrient deficiency in infants that is zinc deficiency. The zinc intake and status of breastfed infants using measures of breast milk intake, non breast milk water intake and breast milk zinc is assessed. The dotted lines (Objectives 3 & 4) represent these.

4: STUDY METHODS

4.1. Subject recruitment and eligibility criteria

Papers 1 and 2

All pregnant women aged ≥18 and ≤40 years, ≤14 weeks of gestation and registered for antenatal screening at Hosahalli Referral hospital at Bangalore, Karnataka, India were invited to participate in the study. Hosahalli referral hospital is a government maternity center that primarily caters to the needs of the women from poor socioeconomic status. At the antenatal clinic between December 2008 and November 2010, 1376 women were contacted. Of these 958 women were excluded for the following reasons: 836 women planned to deliver outside Bangalore at their maternal home town; 67 women wanted to terminate the pregnancy; four women were < 18 years; seven women had a history of hypertension; four women had a previous caesarean section and pregnancy was not confirmed among 40 women. Of the remaining 418 women who were considered eligible, 52 women declined to participate, leaving 366 women who consented. Women with multiple gestation, those with a clinical diagnosis of chronic illness (diabetes mellitus, hypertension, heart disease or thyroid disease), those tested positive for Hepatitis B surface antigen, Human Immunodeficiency Virus or syphilis, those who anticipated moving out of the city before delivery, those who were already consuming vitamin B_{12} supplements, or those were treated for infertility were excluded.

Papers 3 and 4

Pregnant women aged 18–40 years, who planned to exclusively breastfeed their infants until 6 months of age, and who were in the last trimester of pregnancy, were invited to participate in the study. These women were recruited from St. Johns Medical College and Hospital, Bangalore, Karnataka which is a 1200 bed tertiary care hospital catering to the needs of patients from diverse socio economic groups. 58 mother-infant pairs were recruited. During the study, 8 mother-infant pairs were lost to follow up such that 50 mothers and their infants completed the study successfully. Of these eight mother-infant pairs who were lost to follow up, five of them relocated from Bangalore to elsewhere, while another three expressed their disinterest to continue. In India, it is a common practice for mothers to go to their maternal homes for delivery and subsequently in 3 to 4 months they get back to their husband's house.

Women with multiple pregnancies and any illness were excluded. Only term infants born to these pregnant women were eligible to participate.

4.2. Characteristics of the study subjects

Research questions of the present study are addressed in two different populations:

- **Cross sectional study in early pregnancy:** Pregnant women in early pregnancy ≤ 14 weeks of gestation from urban Bangalore (Hosahalli Referral Hospital, **Figure 9**) belonging to a low socioeconomic status (n = 366). The mean (SD) age of the pregnant women was 22.6 (3.7) years and the mean (SD) gestational age was 11.2 (2.4) weeks**.** Of the pregnant women 95.8% were at least high school graduates. A majority of them (83.8%) were unemployed. Primiparous pregnant women made up 64.5% of the cohort. The mean weight and height of the pregnant women were 47.8 (8.1) kg and 153.0 (5.6) cm respectively. 31.3% of the pregnant women in the study cohort had a BMI < 18.5 kg/m^2. None of the pregnant women were consuming iron or folate supplements on study entry (**Papers 1 and 2**).

Figure 9: Hosahalli Referral Maternity Health Care centre, Bangalore

Hosahalli referral hospital is a government maternity center that primarily caters to the needs of the women from poor socioeconomic status and renders Maternal and child health care services in order to reduce maternal and child mortality and morbidity in Bangalore, India. This Hospital has approximately 200 deliveries in a month.

- **Prospective longitudinal observational study in term infants < 6 months of age:** Term infants under 6 months of age born to mothers from urban Bangalore (St. Johns Hospital and Medical college, **Figure 10**) belonging to the diverse socioeconomic status (n = 50 mother-infant pairs). The study mothers were on average 23.0 ± 2.9 years old, and approximately 76% of them were Primiparous. The median family income was 9000 Indian Rupees, indicating that the mothers were not from a low socioeconomic status. The mean birth weight of the infants was 2.7 ± 0.5 kg and the mean length at birth was 49.6 ± 1.7 cm. The mean gestational age at birth was 39.1 ± 0.9 weeks. Nearly half (48%) of the infants born in the study had birth weight less than the 10^{th} percentile for the gestational age at birth. 58% of the infants in the cohort were male (**Papers 3 and 4**).

Figure 10: St. Johns Medical College and Hospital, Bangalore

St. Johns Medical College and Hospital, Bangalore, Karnataka is a 1200 bed tertiary care hospital catering to the needs of patients from diverse socio economic groups. This hospital was established in 1975, and has 24 full-fledged departments to provide specialty and super specialty services. The hospital has a daily average of 1379 outpatients registered and a daily average of 128 admissions.

4.3. Ethical considerations

The pregnant women recruited from Hosahalli Referral Hospital were part of an ongoing randomized controlled trial of vitamin B_{12} supplementation in pregnancy to be followed up until 2 years of their infant age (NCT00641862). The institutional review boards at St John's

Medical College Hospital and Harvard School of Public Health (collaborator in the study) approved all study procedures, and approvals were taken from the government level since it was a government maternity health care centre. The study procedure was explained to each and every subject along with the family members present, in their local language by trained research assistants. The risks (blood collection) and the benefits (advice by nutritionists, measurement of anthropometry and diet) were explained. Sufficient time was given to allow them to decide whether or not to participate in the study. In the event of consent, written informed consent was obtained from each participant at enrolment. For participants who were illiterate, thumb impressions were obtained.

Similarly for the infant study at St. Johns Research Institute, approvals were taken by the institutional review boards at St John's Medical College Hospital. The study procedure was explained to the mother of the infant by trained research assistants. The risks (blood, saliva, breast milk collection) and the benefits (advice by nutritionists, measurement of anthropometry and diet) were explained, and time was given for them to express their decision to continue or not. If agreed to consent, written informed consent was obtained from each mother at enrolment. In either of the studies, pregnant women/mothers had all freedom of choice to discontinue in case of disinterest.

4.4. Sociodemographic data and gestational age at recruitment (Papers 1, 2, 3 and 4)

Information on age, education, parity, occupation, income and obstetric history was obtained from the mother using a close ended questionnaire. Gestational age (in weeks) at enrolment was calculated from the reported first day of the last menstrual period. Gestational age at birth was calculated using an ultrasonography report.

4.5. Maternal anthropometry (Papers 1, 2, 3 and 4)

A digital balance (Salter's 9016, Kent, UK) was used to record the weights of all mothers to the nearest 100 g. Weight was measured without shoes, jackets or cardigans, heavy jewellery, loose change or keys. They were asked to stand with their feet together in the centre and their heels against the back edge of the scale. They were asked to keep their arms hanging loosely and head facing forward. Measurements of height were made using a stadiometer to the nearest 0.1 cm (**Figure 11**). They were asked to remove their shoes and stand with their feet flat on the centre of the base plate, back straight, feets together, heels against the rod, eyes

straight and Frankfurt plane in a horizontal position (WHO 1995). Maternal BMI was calculated as weight in kg by the square of height in meters (kg/m^2).

Figure 11: Measurement of maternal weight and height by trained research assistants.

4.6. Infant anthropometry (Papers 3 and 4)

WHO guidelines were used for measuring and weighing the infant (WHO job aids. Measuring and weighing a child). The nude weight of the infant was measured using a portable paediatric weighing scale (accurate to 10 g, Salters, Kent, UK), within 48 hours of birth. Weights at the following months were measured on the days fixed for appointment when each child would attain 1, 3 and 6 months of age. Infant length was measured using an infantometer (locally constructed) to the nearest 0.1 cm. The child was made to lay straight on his/her back with head against the fixed headboard compressing the hair. The child's legs were held with one hand, and the footboard was moved with the other, and gentle pressure was applied to the knees to straighten the legs. The head was positioned such that an imaginary vertical line from the ear canal to the lower border of the eye socket was

perpendicular to the board. Head circumference was measured at a level passing from supraorbital protuberance anteriorly and occipital protuberance posteriorly using a non-elastic accurately scaled standard tape to the nearest 0.1 cm. All anthropometric measurements within 48 hours of birth and at month 1, 3 and 6 of infant age were done by a trained research assistant in duplicates and the mean was recorded. Infant weight-for-height (WHZ), height for age (HAZ), weight for age (WAZ) and head circumference for age (HCZ) were interpreted using the Z score classification system (WHO 2006).

4.7. Maternal dietary data (Papers 1, 2 and 4)

A pre-tested interviewer-administered Food Frequency Questionnaire (FFQ) was used to assess the habitual dietary intake for the 3 months preceding the date of the subject's enrolment into the study. Standard measures were placed before the respondent to quantify the portion size of each food item when administering the questionnaire (**Figure 12**). This questionnaire was adapted from a questionnaire developed for the urban population residing in South India (Bharati et al., 2008b) and has a food list of 127 items, derived from a food database developed from studies at St John's Medical College. In these experiments done previously at St. Johns Medical College, raw food items required for each recipe were entered and the nutrient and food group values were obtained for the cooked weight of that recipe. To obtain the cooked weight of that recipe a recipe collection and standardization process was followed as detailed. A database of recipes for the foods included in the FFQs was obtained separately from the urban and the rural groups. People who provided the recipes were individuals who routinely cooked these recipes in their households. The food items were cooked in the metabolic kitchen at the research facility according to the recipes provided, or in the houses of individuals who provided the recipes. Research assistants and the individuals preparing the recipes were given standard instructions for preparation of the recipes. A weighing scale (sensitive to 2g) was used to weigh all the ingredients. Weights were obtained for the edible portion of the foods used in the recipe. The Indian Food Composition Tables were used to estimate the nutrient content of the raw ingredients reported in the recipes (Gopalan 1996). For nutrients unavailable in the Indian food composition database such as vitamin E and fatty acids and for the raw foods that were not listed in the food composition database, the United States Department of Agriculture nutrient database in the public domain was used (United States Department of Agriculture, Agriculture Research service; http://www.nal.usda.gov/fnic/foodcomp/search/). Nutrient content for cooked recipes was

obtained by applying a conversion factor accounting for the weight/ volume change on cooking. The same recipes were also used to estimate the contribution of the ingredients to various food groups such as cereals, pulses, vegetables, fruits, salt, sugar, milk products, eggs or meats. Calculation sheets were developed to convert individual reporting of the foods to obtain daily intakes of the nutrients and food groups using the nutrient database. The daily nutrient or food group intake was calculated by multiplying the intake recomputed for a day with the serving size and the nutrient or food group content per portion of the food item. The nutrients and food groups were estimated for all the foods listed in the FFQ and summed to obtain the total nutrient or food group intake per day for an individual. Energy-adjusted nutrient intakes were calculated by the residual method (Willet 1998). Data on the amount of food groups consumed were calculated as the total grams of the food groups consumed per day. In the absence of information about the micronutrient losses during food preparation and cooking for Indian foods, losses during food preparation were not taken into account while developing the nutrient database.

Figure 12: Administration of dietary data using standard cups and measures

4.8. Infant dietary intake (Papers 3 and 4)

A single 24 hour dietary recall from the mother, of the infant's solid or liquid intake (if any) on a typical day of the week during the deuterium study was captured at months 1, 3 and 6 of infant age. Infant intake of tonics and vitamin/mineral supplements was also recorded.

Breast milk and non-breast milk water intake

Breast milk and non breast milk water intake was measured using the "dose-to-the mother" deuterium-oxide turnover technique at infant age of 1, 3 and 6 months (IAEA, 2010). A baseline sample of 2 ml of saliva from the mother and infant were collected on day 0, after which the mother received an oral dose of 30 g deuterium labelled water. The dose was measured to the nearest 0.01 g. Saliva samples from the mother (days 1, 2, 13 and 14) and from the infant (days 1, 2, 3, 4, 13 and 14) were then collected over a period of 14 days. Adsorbant sorbettes (Sallimetrics, UK) were used to collect saliva samples (2 ml), which were centrifuged at 3500 rpm, and the supernatant was stored at -20^{0}C for subsequent analysis of their ^{2}H enrichment, using Fourier Transformed Infrared Spectrophotometry at St. Johns Research Institute by trained biochemists (IAEA, 2010). The breast milk and non breast milk water intake by isotopic method was not available for 2 mother-infants pairs at month 3 and one mother-infant pair at month 6. The weight of the infant was recorded on day

0 and day 14. Intake of breast milk and water from non breast milk sources was calculated by fitting the isotopic enrichment data to a mathematical model for water turnover in the mother-infant pair and the transfer of water from mother to the baby, based on assumptions as described earlier (IAEA, 2010). An infant was considered to be exclusively breastfed by the deuterium dilution method if the non breast milk water intake was 0 ml/d, and by the 24 hour dietary recall method, if the mother reported that she was not feeding her infant any solid/liquid food or water other than breast milk.

4.9. Infant morbidity data (Papers 3 and 4)

Maternal and infant morbidity symptom data (for diarrhea, dysentery, upper respiratory illness and fever) were collected by maternal recall at months 1, 3 and 6. The proportion of infants with any particular illness was calculated.

4.10. Haematology data (Paper 1 and 2)

Blood was drawn from pregnant women after an overnight fast by venipuncture and collected in both ethylene diaminetetraacetate and plain vacutainers (BD Franklin Lakes, NJ, USA). Hb and complete blood count were measured on an automated Coulter counter by a trained technician at St. Johns Research Institute, Bangalore (ABX Pentra 60C+, Horriba Ltd, Kyoto, Japan) (**Figure 13**). Anaemia was defined as Hb<11.0 g/dL and severe anaemia as Hb<7.0 g/dL (UNCF/WHO/UNU 2001).Microcytosis was defined as Mean corpuscular volume MCV < 80 fL (Rempher and Little 2004) while microcytic anaemia was defined as anaemia with MCV < 80 fL. Hematocrit was considered to be low at values < 36% (UNCF/WHO/UNU 2001) while the reference range for red cell distribution width was considered to be 11.5% to 14.5% (Rempher and Little 2004).

Figure 13: Collection of blood sample and analyses for Hb and complete blood count on an automated coulter

4.11. Biochemical and microbiological data (Papers 2,3 and 4)

Vitamin B_{12}, MMA and Hcy

Analyses of plasma vitamin B_{12}, MMA and Hcy were done by trained biochemists at St. Johns Research Institute, Bangalore. Vitamin B_{12} was measured by the electrochemiluminescence method (Elecsys 2010, Roche Diagnostics Mannheim, USA). The combined measurement of Hcy and MMA was performed by gas chromatography-mass spectrometry method (Varian 3800, Palo Alto, CA, USA) (Shobha et al., 2011).The intra- and inter-day assay coefficients of variation for vitamin B_{12} were 0.54 and 2.44 respectively. The inter-day assay coefficient of variation for MMA and Hcy was 5.57 and 5.04 respectively while the intra-day assay coefficient of variation was 6.92 and 5.60 respectively. Low vitamin B_{12}concentraion was defined as plasma vitamin B_{12} concentration < 150 pmol/L (Refsum et al., 2001a). Elevated MMA was defined as MMA levels > 0.26 µmol/L (Refsum et al., 2001a). Elevated Hcy was defined as Hcy levels > 10.0 µmol/L. Since functional vitamin B_{12} deficiency may exist with normal plasma vitamin B_{12} concentrations and plasma vitamin B_{12} may not reliably indicate vitamin B_{12} status (Hermann et al., 2003), and the use of MMA being a relatively specific indicator of vitamin B_{12} deficiency (Carmel et al., 1996), we created a composite variable termed as impaired vitamin B_{12} status (low B_{12} concentration and elevated MMA level) to identify women with confirmed B_{12} deficiency.

Erythrocyte folate

Erythrocyte folate analysis was done by a trained biochemist at Sagar Apollo Hospital, Bangalore. Erythrocyte folate was measured by a competitive immunoassay with direct chemiluminescence detection on an automatised immunoanalyser (ADVIA Centaurs, Bayer Health Care Diagnostics, Tarrytown, New York) (Polito et al., 2005). The folate concentration in the hemolysate was converted to values for whole blood by adjusting for the hematocrit. Low erythrocyte folate concentration was defined as < 283 nmol/L (Refsum et al., 2004). The intra- and inter-day assay coefficients of variation for erythrocyte folate were 2.8 and 5.0, respectively.

Microbiological examination of stool samples for ova, cysts and parasite

A single stool sample was collected from the pregnant women and analyzed immediately at the division of Microbiology, St. Johns Research Institute, Bangalore by trained microbiologists for the presence of helminthic ova, cysts and trophozoites by the wet mount method (Parija 2008). We primarily tested the stool samples for the presence or absence of Giardia lamblia.

Breast milk zinc

Women were asked to provide three mid-feeding breast milk samples (5 ml), one each in the morning, afternoon and evening. Breast milk samples were collected in acid washed plastic bottles to avoid zinc contamination, by hand expression after the nipple and areola were cleaned with deionised water and dried. The samples were centrifuged and stored immediately at -20^{o}C in tightly sealed containers until analysis. Breast milk zinc was determined using Flame atomic absorption Spectrophotometry (Ice 3500, Thermo, Cambridge, UK) (Rajalaksmi and Srikantia 1980) at St. Johns Research Institute, Bangalore by trained biochemists. Briefly, breast milk samples were centrifuged to remove solid particles and supernatant was diluted with 0.2% nitric acid solution. The zinc in the sample was emulsified with a drop of 1% triton X-100 (Sigma, Aldrich, St. Louis, MO), aspirated into an acetylene flame and measured at 213.9 nm. Zinc standards (Merck, Darmstadt, Germany) ranging from 0.2 to 1.0 ppm of zinc, prepared in 0.2% nitric acid solution , were used to create calibration curves. The intra- and inter-day assay coefficient of variations were 1.6% and 2.0%, respectively

Serum zinc

A 2 ml fasting blood sample from the mother and 1 ml non-fasting blood sample from the infant was drawn by venipuncture and collected into trace metal free vacuette tube (Becton, Dickinson and company, Japan) at 3 and 6 months postpartum. Blood samples for zinc analysis were centrifuged at 3000 rpm for 10 minutes, and the serum was kept frozen at -80 ° C until analysis. The serum zinc concentration was measured using flame absorption spectrophotometer by trained biochemists at St. Johns Research Institute, Bangalore (Ice 3500, Thermo, Cambridge, UK) (Smith et al., 1979). We were able to obtain infant blood samples only for 31 and 32 infants at 3 and 6 months, respectively. The intra- and inter-day assay CVs were 1.4% and 1.4%, respectively. An infant was considered to be zinc deficient if the serum zinc level was below 65 µg/dL, while the mother was considered to be zinc deficient if the serum zinc level was below 70 µg/dL (IZiNCG 2007)

4.12. Statistical analyses

Papers 1 and 2

Continuous data were summarised as means (SD) and categorical data as numbers (%). Subjects who had missing data on any variable were not considered for the analysis involving that variable.

The association of anaemia with maternal socio-demographic characteristics and energy-adjusted nutrient intakes, categorized by terters, were examined using the Chi-square test. The parameters which were found significant in this analysis were considered in multivariate log-binomial regression analysis to examine their independent effects, while adjusting for possible confounding effects of maternal socio-demographic characteristics. All maternal socio-demographic characteristics with $P < 0.20$ in the univariate analyses were considered in the multivariate model. Characteristics contributing to >5% change in the adjusted estimate after exclusion of the covariate from the full model were retained as confounders in the final model. Separate models were constructed for calcium and phosphorus as they were collinear and could not be considered in the same model. Similar analyses were performed with tertiles of intakes of certain food groups such as meat, poultry and fish green leafy vegetables which were likely to be associated with anaemia. In addition iron intake was also considered as a confounder in the analysis of nutrient intake. The presence of effect modification between the maternal socio-demographic characteristics, intake of nutrients and food groups was examined using stratified analysis. There was no

such effect modification observed and therefore not considered in the multivariable model. Relative risk (RR) with 95% confidence intervals and corresponding P values for both unadjusted and adjusted models are presented.

Predictors of women with low plasma vitamin B_{12} concentration alone, as well as predictors of women with impaired vitamin B_{12} status were analysed. The association of impaired vitamin B_{12} status with maternal sociodemographic and anthropometric characteristics and intake of specific foods were examined using multivariable log-binomial regression analyses. A Poisson link function was used to identify predictors of low vitamin B_{12}concentration. The food groups consumed by less than 50% of the respondents were considered as binomial (consumed/not consumed) and the others were considered as three categories as not consumed, below median consumption and above median consumption. The characteristics found significant in the univariate analysis with $P < 0.20$ were considered for the multivariable analysis. Prevalence Ratio (PR) with 95% confidence intervals and corresponding P values for both unadjusted and adjusted models are presented. Variables were retained in the final adjusted regression model if they had a $P<0.05$.

Statistical analyses were carried out with SPSS (version16, SPSS, Chicago, IL, USA). Log-binomial regression analysis was carried out using the PROC GENMOD program in SAS software (version 9.2 SAS, Cary, NC).

Papers 3 and 4

Data are expressed as means and standard deviations. The maternal body weight and infant WAZ, HAZ, WHZ, HCZ were compared between delivery/birth and postpartum months 1, 3 and 6 using Repeated Measures ANOVA (RMANOVA) and posthoc pair wise comparisons were performed by paired t-test using Bonferroni adjustment. Only valid infant standardized values were considered for the analysis. The weight of the infant was compared with the 50^{th} percentile value for WHO age and gender specific standard population by t-test for single mean. The comparison of breast milk and non breast milk water intake at months 1, 3 and 6 were done using Repeated measures ANOVA (RMANOVA). The proportion of infants who were exclusively breastfed as well as those who were given oral water other than breast milk based on the deuterium dilution method and those who were given liquids and solids other than breast milk based on the 24 hour dietary recall method were calculated. The 95% CI were also computed. Pearson's correlations were performed to examine associations between

breast milk and non breast milk water intake of the infant and WAZ, WHZ, HAZ and HCZ at different time points. The level of significance was set at 0.05.

As the breast milk zinc concentrations of the morning, afternoon and evening breast milk samples did not differ significantly from each other at any of the time points, the mean breast milk zinc for each month was used in further analysis. The breast milk zinc concentrations and breast milk zinc intakes were compared between months 1, 3 and 6 using Friedman test and pairwise comparisons were performed by Wilcoxon rank test using Bonferroni adjustment. The weight and the length gain from months 1 to 3 and 3 to 6 was calculated as the difference in the weights and lengths between these time points. Separate linear regressions of weight and length gain on breast milk zinc intakes were performed. Potential confounders such as non breast milk water intake, infant age, gender and infant weight and length at birth and month 3 were adjusted for in the model. Two sided P value <0.05 was considered statistically significant.

Statistical analyses were carried out with SPSS (version 16.0, SPSS, Chicago, IL).

5. STUDY RESULTS

DATA FROM CROSS SECTIONAL STUDY ON PREGNANT WOMEN (Papers 1 & 2)

5.1 Haematological and biochemical characteristics of pregnant women in early pregnancy.

Table 9: Haematological and biochemical characteristics of pregnant women in early pregnancy.

Parameter(n = 366)	*Mean (SD)/Median (25^{th}, 75^{th} percentile)*
Haematology	
Hemoglobin (g/dL)[1]	11.5 ± 1.5
Hematocrit (%)[1]	34.6 ± 5.1
Mean corpuscular volume (fL)[1]	81.1 ± 8.9
Biochemical profile	
Plasma B_{12} level (pmol/L) (n = 352)[2]	149.3 (109.4, 204.5)
Methylmalonic acid level (µmol/L) (n = 360) [2]	0.47 (0.28, 0.67)
Homocysteine level (µmol/L) (n = 360) [2]	9.22 (5.74, 15.08)
Erythrocyte folate (nmol/L) (n = 359) [2]	386.9 (290.6, 496.4)

[1] Mean ± SD
[2] Median (25th, 75th percentile)

The mean Hb and the hematocrit levels on study entry were 11.5 g/dL and 34.6 %, respectively. The mean plasma vitamin B_{12} concentration was just below the vitamin B_{12} deficiency cut off of 150 pmol/l. Pregnant women in this cohort also had high MMA and Hcy levels. (**Table 9**)

5.2 Prevalence of anaemia and associated parameters in early pregnancy

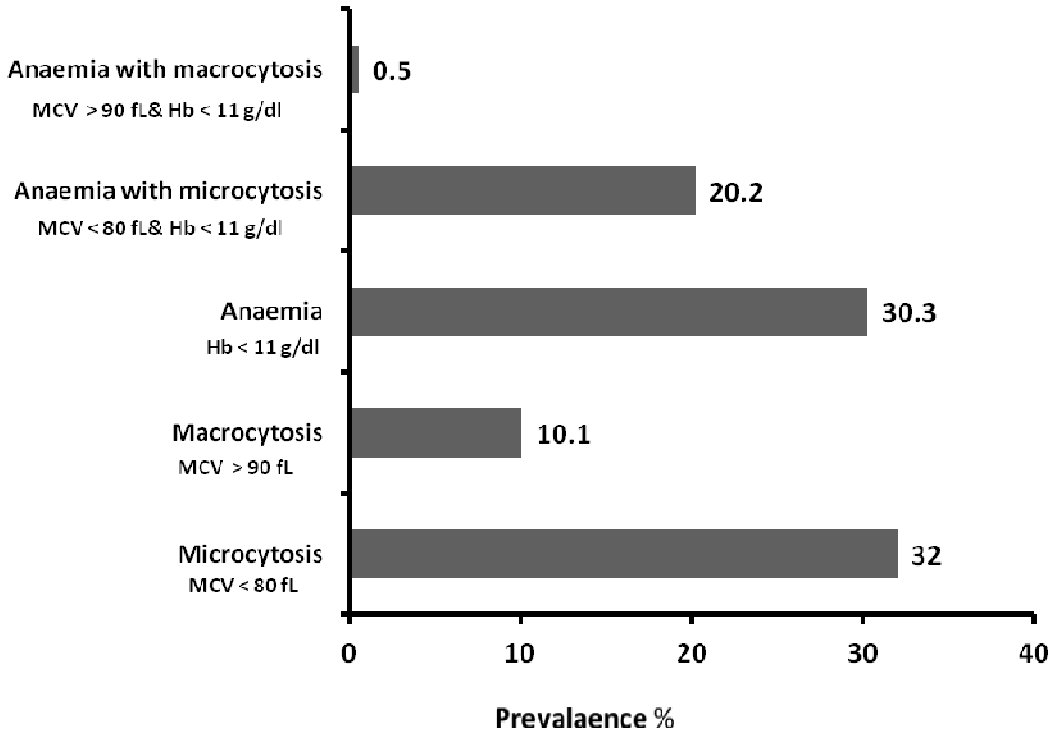

Figure 14: Prevalence of anaemia and associated parameters in early pregnancy

MCV: Mean Corpuscular Volume
Hb: Haemoglobin
Fl: femtolitres

Figure 14 illustrates the prevalence of anaemia and related disorders among pregnant women in early pregnancy. The prevalence of anaemia (Hb < 11.0 g/dL) at baseline was 30.3%. Microcytosis (MCV < 80 fl) alone was present in approximately one-third of the women, while anaemia with microcytosis was present in approximately one-fourth of the pregnant women. Macrocytosis (MCV > 90 fl) was present in 37 of 366 women, while anaemia with macrocytosis, was not very common and had a low prevalence of only 0.5%.

5.3 Prevalence of vitamin B_{12} deficiency and associated biochemical indicators in early pregnancy

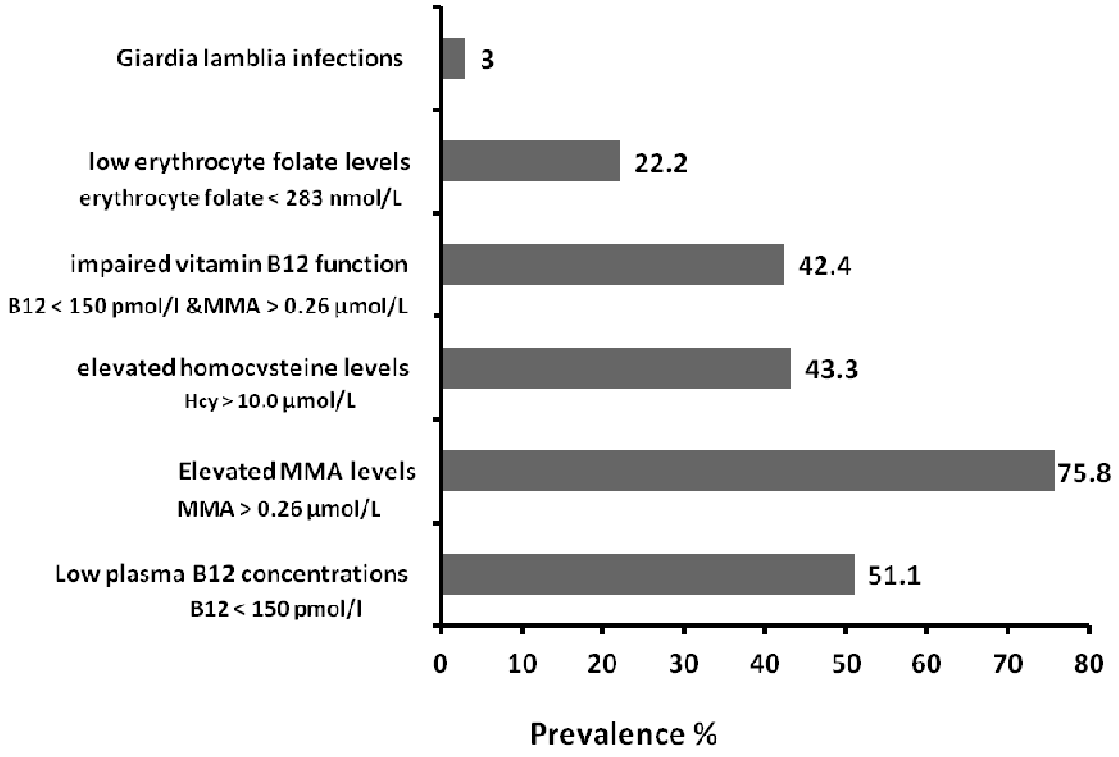

Figure 15: Prevalence of vitamin B_{12} deficiency and associated biochemical indicators in early pregnancy (n=366).

MMA: Methylmalonic acid
Hcy: Homocysteine
Pmol: picomol, µmol: micromole, nmol: nanomol

Figure 15 illustrates the prevalence of vitamin B_{12} deficiency as well as biochemical indicators of the deficiency. Nearly one-half of the pregnant women were deficient in vitamin B_{12}. Elevated MMA concentration was observed in three-fourth of the pregnant women. About half of the women had elevated Hcy concentration.Nearly a quarter of the pregnant women in our cohort had low erythrocyte folate concentration. Impaired vitamin B_{12}status as evidenced by plasma was observed among (148/366, 42.4%) of the women. Infection due to Giardia lamblia was rare in this group of pregnant women.

5.4 Nutrient intake in pregnant women during early pregnancy

Table 10: Mean (SD) nutrient intake in pregnant women during early pregnancy.

Macro and micronutrients	Mean intake (SD)	RDA
Energy (Kcal/d)	1736 (444)	2250
Protein (g/d)	51.8 (14.2)	82.2
Fat (g/d)	47.4 (15.3)	30
Thiamine (mg/d)	1.2 (0.3)	1.2
Riboflavin (mg/d)	1.2 (0.3)	1.4
Niacin (mg/d)	11.8 (3.5)	14
Vitamin B6 (mg/d)	1.7 (0.5)	2.5
Vitamin B_{12} (µg/d)	1.9 (2.6)	1.2
Folate (µg/d)	280.5 (90.1)	500
Vitamin C (mg/d)	105.7 (46.8)	60
Iron (mg/d)	15.2 (4.9)	35
Calcium (mg/d)	827 (286)	1200

SD: Standard deviation
RDA: Recommended Daily Allowances.
Source of RDA: Nutrient requirements and recommended dietary allowances for Indians. A report of the expert group of Indian Council of Medical Research. 2009, page 280

Table 10 shows the mean (SD) intake of macro and micronutrients among pregnant women in early pregnancy. Energy and protein intake was below the RDA, while intake of fat was higher. Intake of vitamin B6, folate, iron and calcium met approximately only half of the requirements during pregnancy. Although vitamin C intake was high among these women, prolonged cooking practices were also observed, which would destroy the vitamin C content of foods.

5.5 Food intake among pregnant women in early pregnancy

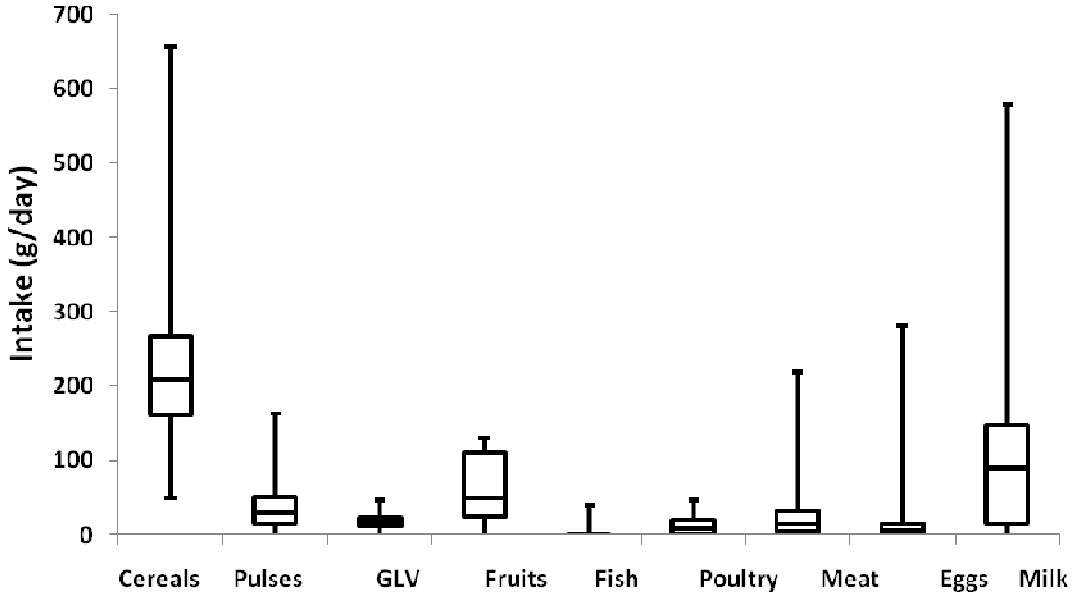

Figure 16: Median intakes of food groups among pregnant women in early pregnancy

GLV: Green leafy vegetables
The middle line in the box represents the median value, while the upper line in the box is the 75th percentile and the lower line in the box is the 25th percentile. The upper intake represents the maximum value, while the lower intake represents the minimum value.

Figure 16 represents the median (25, 75th percentile) of intake of food groups, along with minimum and maximum values. The median (25th, 75th percentile) of food groups in g/day were cereals 208 (162,267), pulses 30 (15, 52), GLV 18 (12, 24), fruits 50 (25, 111), fish 0 (0, 2.1), poultry 10 (2, 20), meat 16 (5, 32), eggs 7 (0, 15), milk and milk products 90 (16, 147). Among pregnant women in this cohort consumption of animal products was very low, which supports the presence of micronutrient deficiencies among them. Fish was hardly consumed, while consumption of poultry and meat was only slightly better.

5.6 Nutritional and sociodemographic risk factors for anaemia among pregnant women in early pregnancy

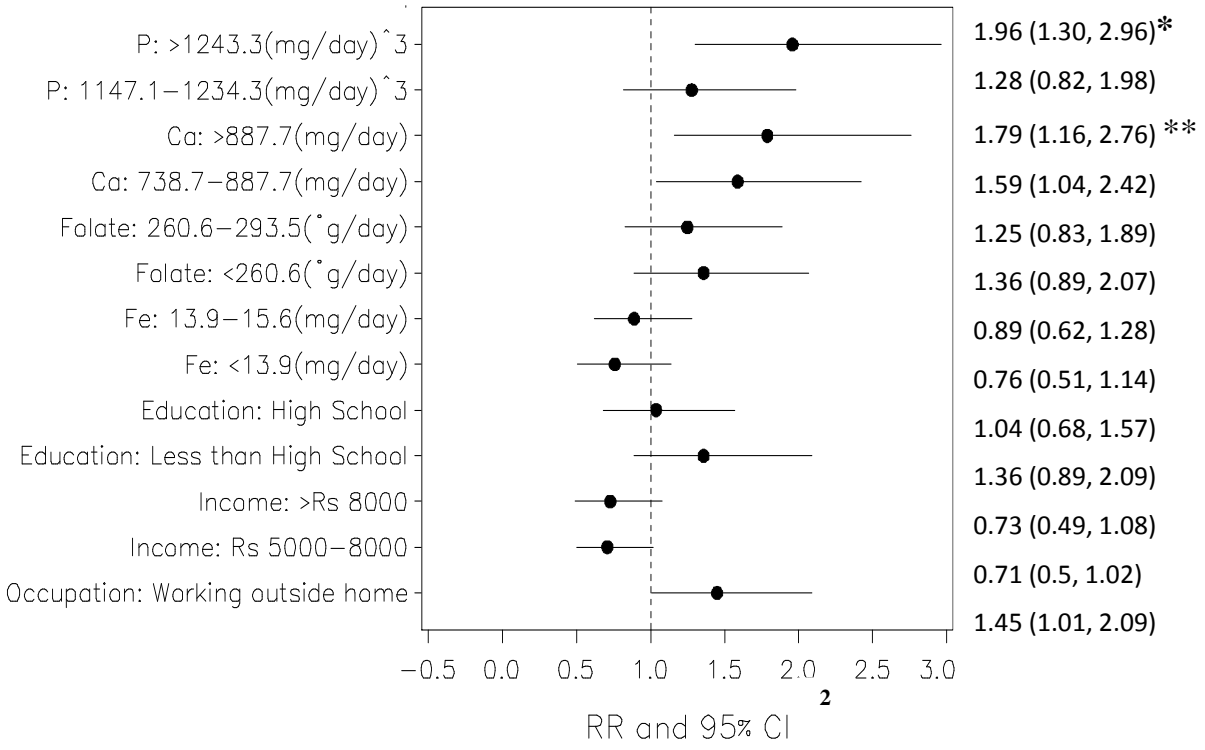

Figure 17: Nutritional and sociodemographic risk factors for anaemia among pregnant women in early pregnancy in the multivariable log binomial regression analysis[1]

P: Phosphorus, Ca: Calcium, Fe: Iron
RR: Relative Risk, CI: Confidence interval
Income measured in INR
* p < 0.001, ** p = 0.006 (p- Values (two-sided) have been reported for the adjusted RR)
[1] Multivariate log binomial regression of anaemia with occupation, monthly family income, education, iron, calcium, folate and phosphorus intake.
[2] RR from a log binomial regression model has been adjusted for the effects of the other variables in the model.

Results of multivariable log binomial regression analyses of anaemia are presented in **Figure 17.** In the multivariable model the significant risk factors for anaemia were high intakes of calcium and phosphorus. The multivariable model presented here is based on a univariate analyses that was done. In the univariate analyses the sociodemographic variable that was significantly associated with a higher prevalence of anaemia was working outside home [RR (95% CI)]: 1.45 (1.09, 2.09). Maternal age, parity, income and education were not

associated with anaemia. Energy-adjusted nutrient intakes associated with higher prevalence of anaemia in the univariate analyses were higher intake of calcium [RR (95% CI)]: 1.89 (1.23, 2.90) and phosphorus 2.06 (1.37, 3.09). The relation of the milk intake with anemia did not show a monotonic trend and was not significant in either the univariate or multivariable analyses.

5.7 Nutritional, sociodemographic and anthropometric risk factors for low plasma vitamin B_{12} concentrations among pregnant women in early pregnancy

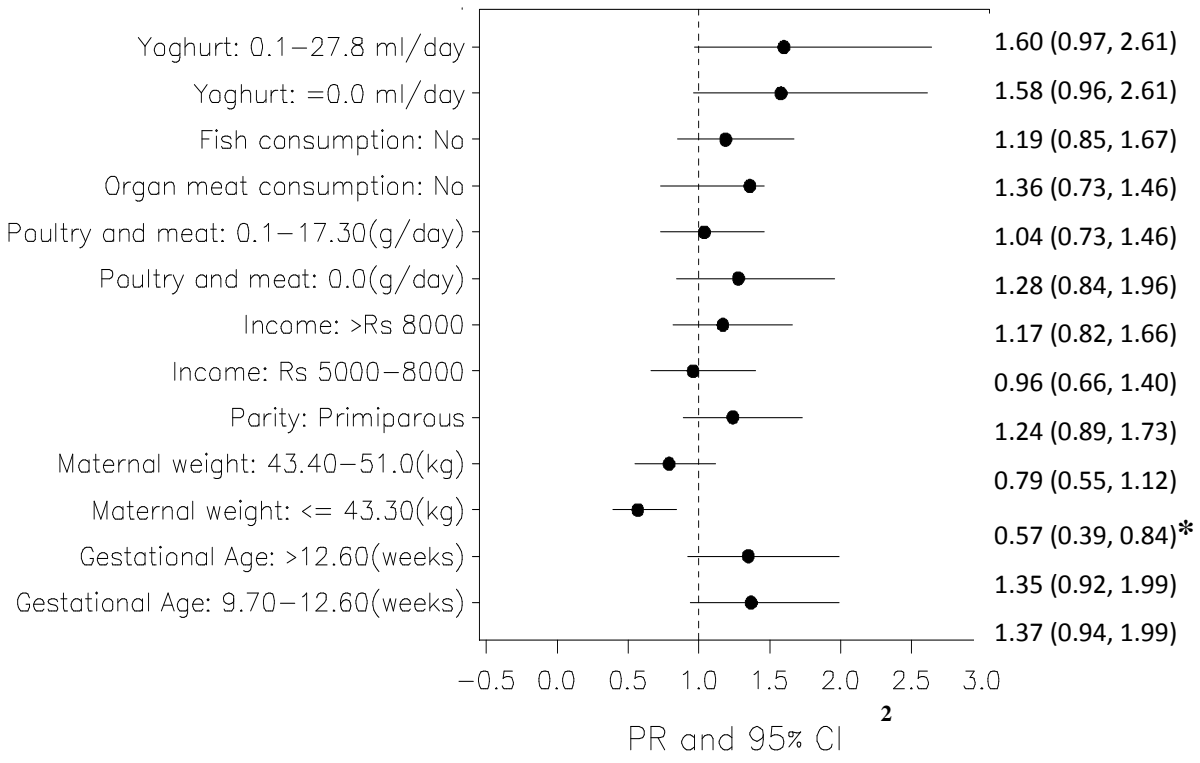

Figure 18: Nutritional, sociodemographic and anthropometric risk factors for low plasma vitamin B_{12} concentrations among pregnant women in early pregnancy in the multivariable Poisson regression analysis [1].

PR: Prevalence Ratio, CI: Confidence interval
Income measured in INR
* p = 0.004 (p- Values (two-sided) have been reported for the adjusted PR)
[1] Multivariable Poisson regression of low vitamin B_{12} concentration, maternal age, maternal weight, Gestational age at recruitment, education, income, parity, intake of poultry and meat, eggs, organ meat, fish, milk, yoghurt and other dairy products.
[2] Adjusted PR from a Poisson regression model adjusted for the effects of the other variables in the model.

Results of multivariable Poisson regression analyses of low vitamin B_{12} concentrations are presented in **Figure 18.** In the multivariable analysis lower maternal body weight was protectively associated with low vitamin B_{12} concentration. The multivariable model presented here is based on a univariate analyses that was done. In the univariate analyses, pregnant women in the lowest terter of body weight had the lowest risk for low vitamin B_{12}concentration [PR (95% CI)]: 0.59 (0.46, 0.78). Intake of milk and milk products,

red meat and poultry, eggs, organ meat, and fish were not associated with low vitamin B_{12} concentration.

5.8 Nutritional, sociodemographic and anthropometric risk factors for impaired vitamin B_{12} status among pregnant women in early pregnancy

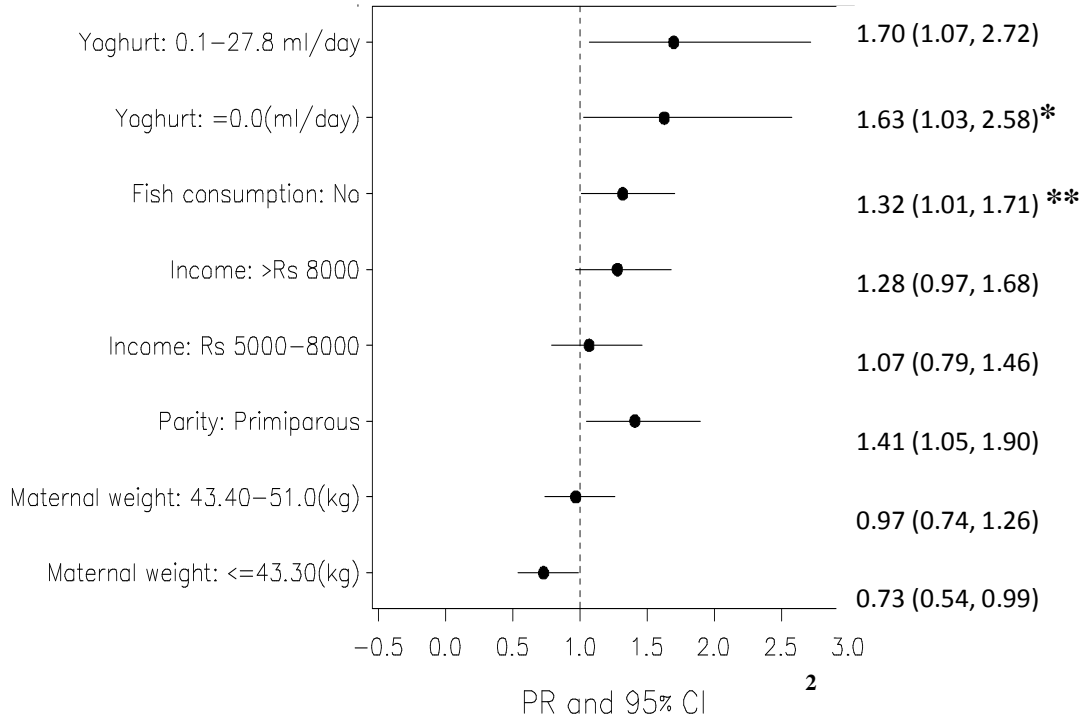

Figure 19: Nutritional, sociodemographic and anthropometric risk factors for impaired vitamin B_{12} status among pregnant women in early pregnancy in the multivariable log binomial regression analysis [1]

PR: Prevalence Ratio, CI: Confidence interval
Income measured in INR
* p = 0.038, ** p = 0.041 (p Values (two-sided) have been reported for the adjusted PR)
[1] Multivariable Poisson regression of low vitamin B_{12} concentration, maternal age, maternal weight, Gestational age at recruitment, education, income, parity, intake of poultry and meat, eggs, organ meat, fish, milk, yoghurt and other dairy products.
[2] Adjusted PR from a Poisson regression model adjusted for the effects of the other variables in the model.

Results of multivariable log binomial regression analyses of impaired vitamin B_{12} status are presented in **Figure 19.** In the multivariable model the risk for impaired vitamin B_{12} status was higher among primiparous women and pregnant women with no intake of yoghurt. Non-use of fish was also associated with a greater risk for impaired vitamin B_{12} status in the multivariable analyses. The multivariable model presented here is based on a univariate analyses that was done. In the univariate analyses we observed that primiparous

women had a significantly higher risk of impaired vitamin B_{12} status in comparison to multiparous women [PR (95% CI)]: 1.41 (1.05, 1.90). Intake of milk and milk products was also associated with the higher prevalence of impaired vitamin B_{12} status [PR (95% CI)]: 3.04 (1.17, 7.90). Since we were interested to know the specific milk or milk product that was associated with vitamin B_{12} deficiency, we categorized them as milk, yoghurt and other dairy products (cottage cheese, buttermilk and payassam). Pregnant women who did not consume yoghurt also had a higher risk for impaired B_{12} status in comparison to those who consumed it above median intake in the univariate analyses [PR (95% CI)]:.1.64 (1.03, 2.61).

5.9 Association between plasma vitamin B_{12} concentration and MMA level and vitamin B_{12} concentration and Hcy level among South Indian pregnant women

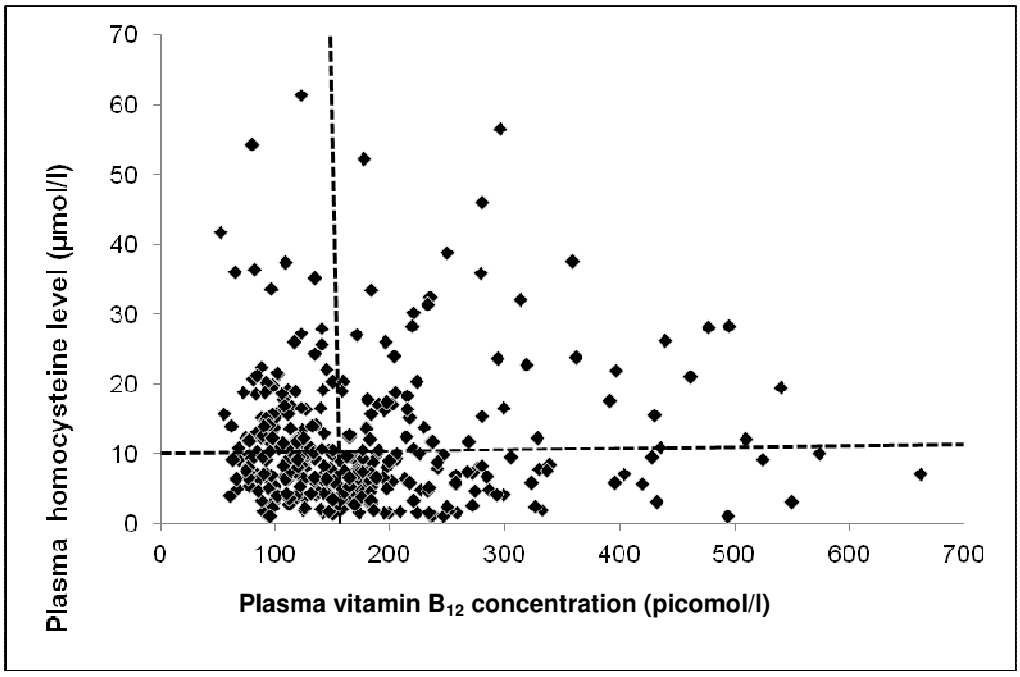

Figure 20a: Association between plasma vitamin B_{12} concentration and MMA level and vitamin B_{12} concentration and Hcy level among South Indian pregnant women.

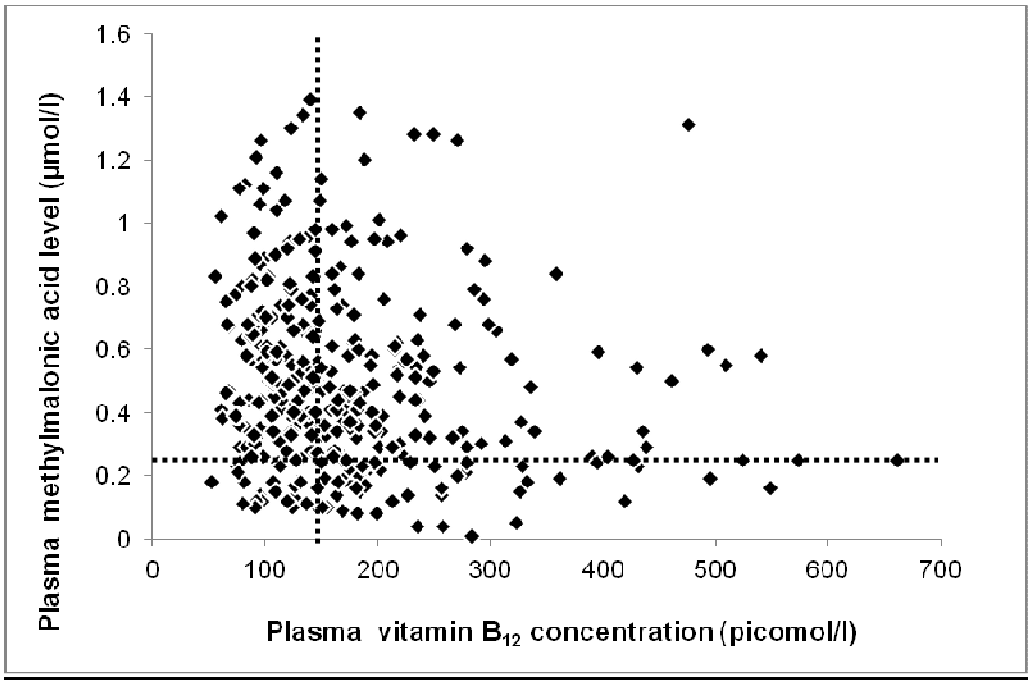

Figure 20b. Association between plasma vitamin B_{12} concentration and Hcy level among South Indian pregnant women.

Figure legend

Figure 20a. Plasma vitamin B_{12} concentration versus concentration of methylmalonic acid (MMA) in pregnant South Indian women(r = -0.184, P = 0.001, n = 349). The dotted line on the x axis denotes the cut off level for vitamin B_{12} deficiency (< 150 picomol/L), while the dotted line on the y axis denotes the cut off level for elevated MMA (> 0.26 µmol/L).

Figure 20b. Plasma vitamin B_{12} concentration versus concentration of homcysteine (Hcy) in pregnant South Indian women(r = -0.097, P = 0.069, n = 349). The dotted line on the x axis denotes the cut off level for vitamin B_{12} deficiency (< 150 picomol/L), while the dotted line on the y axis denotes the cut off level for elevated Hcy (> 10 µmol/L).

Figure 20a shows the association between plasma vitamin B_{12} concentration and MMA level. There was a significant inverse correlation between plasma vitamin B_{12} concentration and levels of MMA. **Figure 20b** shows the association between plasma vitamin B_{12} concentration and level of Hcy. There was an inverse correlation between plasma vitamin B_{12} concentration and level of Hcy, which, however, was of borderline statistical significance

DATA FROM PROSPECTIVE OBSERVATIONAL STUDY IN INFANTS (Papers 3 & 4)

5.10 Infant weight and length at birth and up to 6 months of age

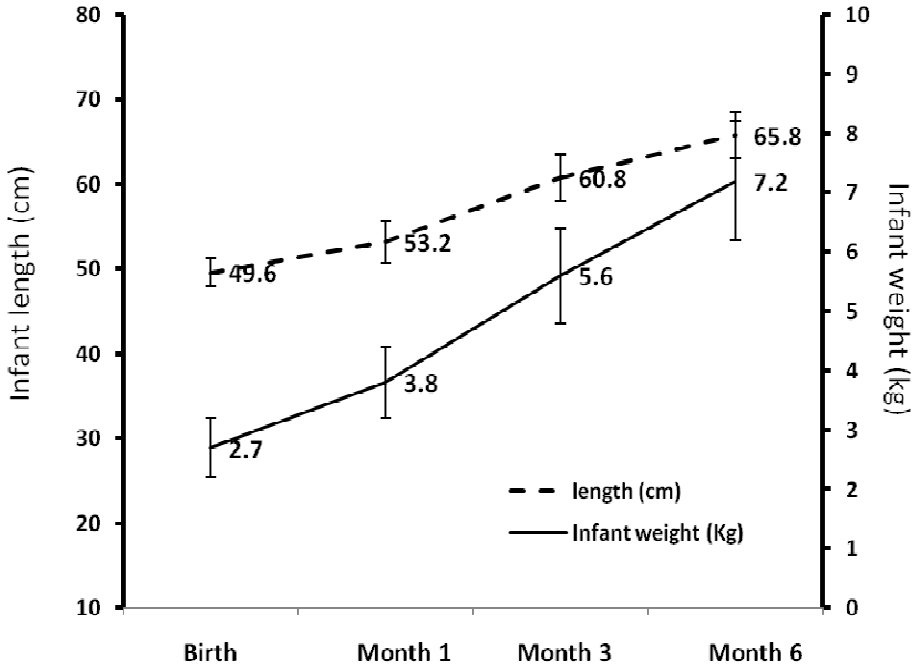

Figure 21: Infant weight and length at birth and up to 6 months of age
All values are mean with SD error bars
Infant weight measured in kg
Infant length measured in cm

Figure 21 represents the infant weight and length with different y axes at birth and months 1, 3 and 6.maternal and infant anthropometric measurements. The body weights of the infants were significantly lower in comparison to WHO standards throughout the early postnatal period of 6 months for boys and from birth to month 3 for girls($P<0.05$ for both). The mean weight gain of infants was 1.88 ± 0.59 kg and 1.47 ± 0.59 kg translating to a daily weight gain of 31.3 g/day and 15.2 g/day during the 1 to 3 months and 3 to 6 months periods, respectively. The mean length gain among infants was 7.1 ± 2.4 cm and 5.7 ± 1.8 cm in the 1 to 3 months and 3 to 6 months periods, respectively. The weight and length gain were not significantly different between boys and girls except for the weight gain from 1 to 3 months ($P = 0.01$) which was higher in boys.

5.11 WHO standardised Z scores of infants at birth and until 6 months of age

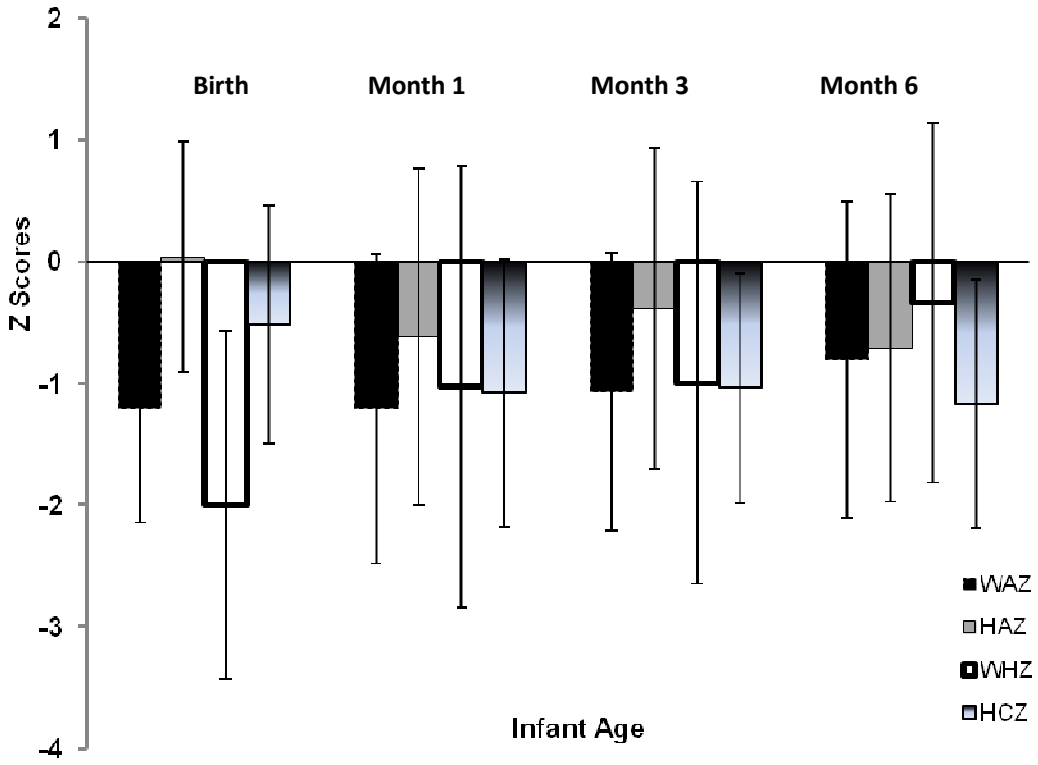

Figure 22: WHO standardised Z scores of infants at birth and until 6 months of age

All values are mean with SD error bars
WAZ: weight for age Z score
HAZ: height for age Z score
WHZ: weight for height Z score
HCZ: head circumference for age Z score.

Figure 22 represents the WAZ, WHZ, HAZ and HCZ of infants at birth and months 1, 3 and 6. There was a significant decline in the HAZ (P=0.001) and HCZ (P<0.001) among the infants from birth to 6 months of age. However, the WHZ significantly increased (P<0.001) from birth to month 6. At 6 months of age, 20% of the infants were underweight (WAZ<-2SD). Stunting (HAZ<-2SD) increased from 2% at month 1 to 16% at month 6.

5.12 Breast milk and non breast milk water intake of infants at months 1, 3 and 6

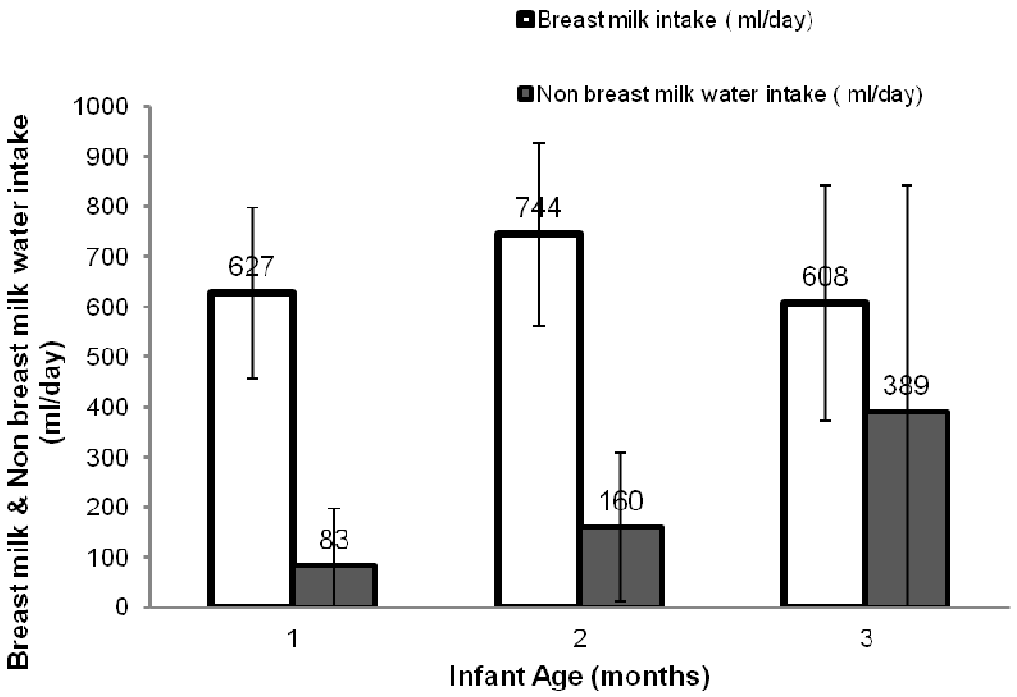

Figure 23: Breast milk and non breast milk water intake of infants at months 1, 3 and 6
All values are mean with SD error bars
BM intake and NBM intake measured using "dose to mother" deuterium dilution method

Figure 23 represents the breast milk and non breast milk water intake of infants at months 1, 3 and 6. The intake of breast milk significantly declined from months 1 to 6 while the intake of non breast milk water significantly increased from months 1 to 6 ($P < 0.01$ for both). The breast milk intake at month 3 was significantly lower than intake at month 1 while BM intake at month 6 was significantly lower than intake at month 3 ($P<0.05$) by Bonferroni adjusted pair wise comparisons in RMANOVA. The non breast milk water intake at month 6 was significantly higher than intake at month 1 and 3 ($P<0.05$) by Bonferroni adjusted pair wise comparisons in RMANOVA .There was also a significant negative correlation between breast milk and non breast milk water at months 3 ($r=-0.59$, $P<0.001$) and 6 ($r =-0.61$, $P<0.001$) indicating that liquid and solid foods introduced by the mothers displaced breast milk.

5.13 Compliance to exclusive breastfeeding based on "dose-to-mother" deuterium dilution method

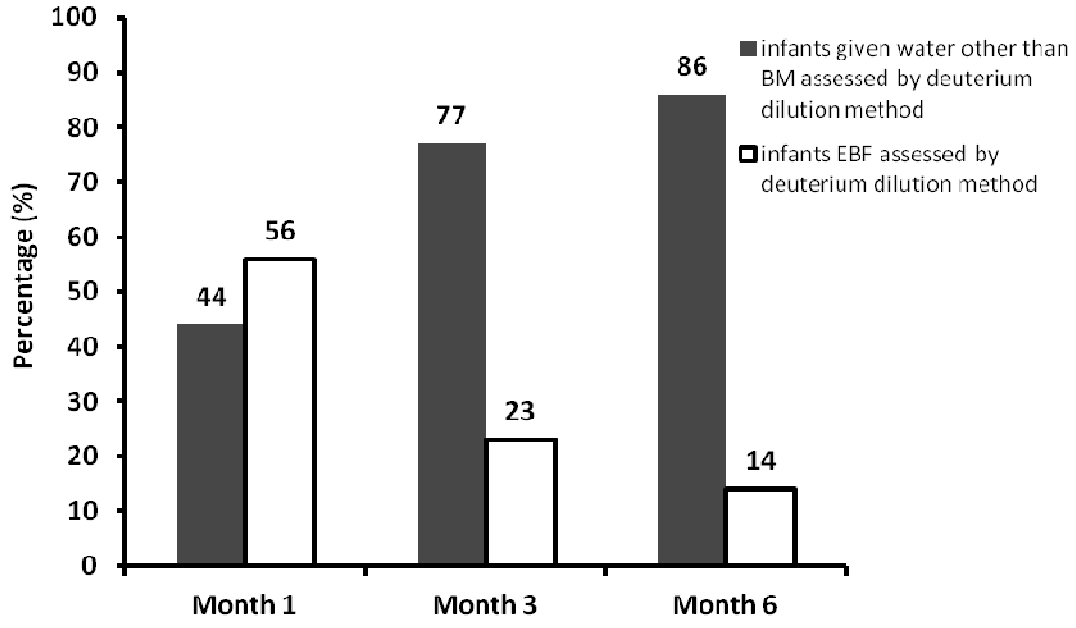

Figure 24: Compliance to exclusive breastfeeding based on "dose-to-mother" deuterium dilution method

BM: Breast milk
EBF: Exclusively breastfed infants
Compliance to EBF assessed using "dose to mother" deuterium dilution method

Figure 24 shows the compliance to exclusive breastfeeding based on "dose-to-mother" deuterium dilution method. The isotopic method showed that 44% of the babies had received oral water (and presumably accompanying foods) and only 56% were exclusively breastfeeding even as early as month 1. At month 3, data from the isotopic method showed that 77% of the babies had received oral water. By month 6, only 14 % of infants were being exclusively breastfed based on isotopic data. This data is in stark contrast to the reported exclusive breastfeeding practices by mothers, which were captured using a questionnaire. Based on the questionnaire method none of the mothers reported having started introducing complementary foods at month 1, while at month 3 only 10% of the mothers reported having introduced complementary foods and at 6 months of age, 36% of the infants were reported to be exclusively breastfed.

5.14 Transfer of breast milk zinc from mother to the infant and maternal and infant serum zinc measures at months 1, 3 and 6

Table 11: Transfer of breast milk zinc from mother to the infant and maternal and infant serum zinc measures at months 1, 3 and 6

Parameters [1]	Month 1	Month 3	Month 6
Breast milk zinc content (mg/l) [2]	2.50 (2.03, 3.26) (n = 50)	1.37 (0.89, 1.79) (n = 47)	1.17 (0.80, 1.60) (n=50)
Zinc intake from breast milk (mg/day) [2]	1.60 (1.13, 2.19) (n=50)	0.88 (0.67, 1.36) (n = 47)	0.68 (0.51, 1.13) (n=49)
Zinc intake from breast milk (mg/kg/day) [2]	0.43 (0.32, 0.53) (n=50)	0.15 (0.11, 0.22) (n = 47)	0.10 (0.06, 0.16) (n=49)
Infant zinc intake from reported complementary foods (mg/d) [3]	-	-	0.90 (0.45, 1.12) (n = 16)
Maternal zinc intake (mg/day)	7.6 ± 2.9 (n =50)	7.7 ± 2.3 (n =48)	7.8 ± 2.8 (n =50)
Maternal serum zinc (µg/dL)	-	83.5 ± 26.5 (n=40)	92.2 ± 25.1 (n=43)
Infant serum zinc (µg/dL)	-	93.0 ± 27.1 (n=31)	99.6 ± 30.1 (n=32)

[1] Parameters are Median (25^{th}, 75^{th} percentile)

[2] Breast milk zinc content and breast milk zinc intake significantly different between months 1, 3 and 6 by Friedman test, P<0.001. All pair wise comparisons were significant by Wilcoxon rank test, Bonferroni adjusted P value<0.001.

[3] The median Zn intake from complementary foods has been reported only for those infants whose mothers reported having given complementary foods.

Table 11 represents the breast milk zinc content of mothers at months 1, 3 and 6 postpartum, breast milk zinc intakes of infants at these times, maternal zinc intake through diet and maternal and infant serum zinc concentration. There was a significant decline in the breast milk zinc concentration from month 1 to 6 ($P < 0.001$), and also a significant decline in zinc

intake from breast milk from months 1 to 6 ($P<0.001$). The maternal zinc intake was much lower than the RDA for lactating women throughout the postpartum period of 6 months; however the mean maternal serum zinc concentration was above the cut off levels for zinc deficiency.

6. DISCUSSION

Pregnancy and infancy are critical time periods in the life cycle, when there is an increased physiological demand and increased requirement of nutrients for the growth and development of the foetus, well being of the mother, as well as to meet the needs for growth in an infant. Micronutrients have a critical role to play in enzymes and transcription factors, and signal transduction pathways that regulate development, therefore, any imbalance in micronutrients can affect pregnancy outcome, cause alterations in maternal and foetal metabolism and disturb the balance between the generation of free oxygen radicals and the production of antioxidants that scavenge them (Shah and Sachdev 2004). Epidemiological studies in India have indicated that maternal macro- and micronutrient deficiencies in pregnancy can have short and long term impact on the nutrition and health status of the offspring leading to poor intrauterine growth, low stature of the offspring in adult life, and in addition LBW in the third generation (Kalaivani 2009). The effect of maternal micronutrient deficiencies does not end with the infant birth outcome, but has also repercussions throughout the period of infant postnatal growth, in her ability to feed and nurture the infant. Considering that mothers and infants form a biological and social unit, and also share problems of malnutrition and ill-health, any efforts to address these problems should concern both mothers and children together in a coherent manner (WHO 2003, Global strategy for infant and young child feeding). Understanding the aetiology of micronutrient deficiencies during pregnancy, lactation and infancy, and additionally, the infant feeding practices that determine the long term health of a child, offers tremendous potential and opportunity for intervening and thereby preventing under nutrition and offering them the best chance to survive and reach optimal growth and development.

This thesis has attempted to assess the prevalence and modifiable risk factors of some key micronutrient deficiencies among pregnant women from urban South Indian, which has the potential for possible nutrient interventions to improve pregnancy and birth outcomes. In addition, we also assessed infant feeding practices such as exclusive breastfeeding that offers tremendous potential to reduce under five mortality, and infant dietary intakes of zinc through breast milk that would be instrumental to understand the aetiology of zinc deficiency among breastfed Indian infants. The results of our study are discussed in light of the relevant existing literature from India, as well as other countries under the following sub-sections.

6.1 High prevalence of anaemia and high intake of iron absorption inhibitors among pregnant Indian women

Among pregnant women ≤14 weeks of gestation from urban South India, the prevalence of anaemia and microcytic anaemia was 30% and 20%, respectively. Studies from African and Asian countries such as Malawi, Nigeria, Ghana, Sudan, Senegal Bangladesh and China have all reported a higher prevalence of anaemia than reports from India (Verhoeff et al., 1999, Oboro et al., 2002, Ahmed et al., 2003, Engmann et al., 2008, Abdelrahim et al., 2009, Zhang et al., 2009, Seck and Jackson 2010). Nevertheless, these studies were carried out among pregnant women in their second and third trimester of pregnancy, while our study has identified the prevalence and dietary correlates of anaemia in early gestation, when the effects of hemodilution on biochemical markers and nutrient concentrations are minimal. The National Family Health Survey 3 reports an anaemia prevalence of 59% among pregnant Indian women (National Family Health Survey 2005-2006), and 32% for moderate to severe anaemia among pregnant women in Karnataka (District Level Household Survey 2002-2004). A study from the urban areas of Udipi in Karnataka (state in South India) found the prevalence of anaemia to be 50% (Hb determined by cyanmethemoglobin method) among pregnant women ≤14 weeks of gestation (Noronha et al., 2010). This difference in prevalence may be explained by the fact that ours was a hospital-based study recruiting urban women in early pregnancy based on strict inclusion and exclusion criteria, and possible differences in the method of Hb analysis.

Pregnant women in our cohort received several macro- and micronutrients in amounts well below the RDA. Similar data has been reported from pregnant women belonging to rural areas of Delhi, where intake of energy, protein, iron, folic acid and vitamin C was found to be less than the RDA in 65 to 100 % of the pregnant women, indicating the presence of an overall food gap rather than isolated deficiency of any particular nutrient (Gautam et al., 2008). Anaemia is often a manifestation of overall maternal dietary inadequacy and consequent under nutrition (Kalaivani 2009). Specifically, the mean dietary intake of iron was less than 50% of the RDA among pregnant women in our cohort. In addition to low intake, low iron bioavailability with high phytate and low ascorbic acid/iron ratio is a major aetiological factor for anaemia among pregnant women in India due to the habitual cereal pulse based diets consumed by them (Nair and Iyengar 2009).

Intake of iron absorption inhibitors such as manganese, phosphorus and zinc were higher in anaemic pregnant women in the present study. It is important to consider the

increased risk of anaemia through possible interactions of other nutrients with iron, in the setting of low dietary iron intake. For example, higher intake of manganese may increase the risk for reduced iron utilization by affecting iron absorption, while higher doses of zinc in may impair iron absorption (Rossander Hulten et al., 1991). In a cross-sectional study of 480 pregnant women from Senegal in their second trimester, mean hemoglobin and serum ferritin levels were higher and erythrocyte protoporphyrin levels were lower in women who consumed iron inhibitors less frequently (Seck and Jackson 2010). Similarly, consumption of more than three cups of tea per day prior to pregnancy was associated with anaemia among pregnant women from Pakistan (POR, 3.2; 95% CI, 1.3 to 8.0) (Baig Ansari et al., 2008).

Equally interesting was the observation that among these pregnant women, who had a low intake of iron and several other nutrients, higher intake of calcium and phosphorus (dietary components known to inhibit iron absorption), were independently associated with a higher risk for anaemia. Single meal studies on adult men and women have demonstrated a dose dependent effect of on the absorption of 5 mg non-heme iron (Hallberg et al., 1991, 1993). Additionally, calcium chloride at doses ≥ 1000 mg and at 800 mg has been found to have an isolated effect on both 5 mg non heme iron and heme iron respectively, among non pregnant women (Gaitan et al., 2011). Calcium is known to inhibit iron absorption by the formation of poorly soluble calcium phytate complexes (Zhou and Erdman 1995) or at the cellular level by its effect on divalent metal transporter 1 receptors (Shawki and Mackenzie 2010). Higher intake of phosphorus was also observed to be an independent risk factor for anemia among pregnant women in the present study. Phosphorus may have a direct inhibitory effect on iron absorption (Monsen and Cook 1976). Even relatively small quantities of residual phytates which are the storage form of phosphorus (<10 mg phytic acid/meal) are known to strongly inhibit iron absorption (Hurrell et al., 2003). The significant association of dietary calcium and phosphorus intake with anaemia may be more relevant among pregnant women from India because of their inadequate iron stores, overall low intakes of iron and other nutrients, a predominantly cereal pulse-based diet, and the relative low iron bioavailability of Indian meals.

The results on the prevalence and determinants of anaemia among pregnant Indian women must be also interpreted in the economic and socio-cultural context. There have been impressive improvements in most health indicators in the last two decades such as a reduction in maternal mortality rate in Indian pregnant women (Lozano 2011). However, improvements in nutritional status have not been that impressive. For instance, although India became the

first developing country to take up the National Nutritional Anaemia Prophylaxis programme in 1970 and the government recommends a minimum of 100 iron and folic acid tablets to be prescribed during pregnancy, and in addition, there are public health programmes from Maternal and Child Health Services for distribution of iron tablets in the last trimester of pregnancy (National Family Health Survey 2005-2006), there is still a high prevalence of anaemia. In this context, there may be other important factors to consider such as socioeconomic status, income and education. Studies from India have found that low socioeconomic status affecting knowledge and health seeking behaviour, and poor education were important determinants of anaemia among pregnant women (Lokare et al., 2012, Bharati et al., 2008a, Noronha et al., 2010). A study to determine the effectiveness of a health information package in terms of empowering the pregnant women to modify their health-care behaviour, has shown that health education contributed significantly in modifying their health seeking behaviour and their perception about significance of anaemia (Noronha et al., 2012). Apart from socio-economic factors access to and use of health care facilities may be important. For example, booking in late pregnancy has been found to be important determinant of anaemia among pregnant women from rural and semi urban states of South Nigeria (Oboro et al., 2002). Frequently visiting the antenatal clinic was also shown to lower the prevalence of anaemia among pregnant women from Jamaica due to routine education, iron and folic acid supplementation and anaemia screening at the initial visit (Charles et al., 2010). Similarly, among pregnant women from China peri-conception folic acid use was associated with a reduced risk for anaemia in the 1st trimester, while initiating prenatal care after the 1st trimester was associated with increased risk of anaemia in the 2nd and 3rd trimesters, suggesting that initiating prenatal care in the 1st trimester may be able to prevent or diagnose and treat anaemia early during pregnancy (Zhang et al., 2009). None of the pregnant women in our cohort were receiving iron or folic acid supplements, at the time of enrolment. There was also a lack of general awareness about the need for antenatal visits and consuming supplements, as understood through interactions with them.

The results of this study has to be interpreted in the light of limitations such as recording the dietary data with a FFQ and not via alternative methods including prospective food weighing and other techniques (although we employed a validated FFQ with a trained interviewer with portion sizes to minimize recall bias), limiting the assessment of iron status to red blood cell morphologic indices, having a moderate sample size and recruiting pregnant women from a single hospital and thus not being able to generalize the study findings to the

general population. In conclusion, the results of this study highlight the need for improving the overall nutritional status of pregnant women from India. There should be increased accessibility, availability and affordability to diverse foods to enhance absorbability of iron. Education and knowledge regarding iron absorption enhancers and inhibitors should be given to all pregnant women.

6.2 High prevalence of biochemical vitamin B_{12} deficiency

In the same cohort of pregnant women ≤ 14 weeks of gestation, we also observed a high prevalence of biochemical vitamin B_{12} deficiency, in addition to anaemia. Low plasma vitamin B_{12} concentration was observed in 51%, while impaired vitamin B_{12} status was observed 42% of the pregnant women. Studies done in Danish, German and Spanish pregnant women in the second and third trimester of pregnancy have reported a lower prevalence of low plasma vitamin B_{12} concentration and elevated MMA in comparison to pregnant women from our cohort (Milman et al., 2006, Koebnick et al., 2002, and Murphy et al., 2007). Other developing countries have reported a higher prevalence of vitamin B_{12} deficiency. For example, among pregnant women from Bangladesh a 46% prevalence of B_{12} deficiency at 14 weeks of gestation was observed (Lindstorm et al., 2011). Among pregnant women from Nepal coming for their first antenatal visit (mean gestational age 18.5 weeks), prevalence of functional B_{12} deficiency was high (elevated MMA in 61% of women (>0.26 mmol/l) and low cobalamin values in 49% of the women (<150pmol/l) (Bondevick et al., 2001). However, these values for prevalence are lower than those for pregnant women in our cohort.

Studies from various parts of India in general point towards a high prevalence of vitamin B_{12} deficiency. The findings from a prospective cohort study among pregnant women from urban Bangalore in South India are in lieu with our findings, and report only a marginally higher value for serum B_{12} concentration (171 pmol/l) in comparison with ours when assessed in early pregnancy (at 13 weeks). In another study from rural areas of Pune in South India, 60% of the pregnant women had plasma B_{12} <150 pmol/l, while 94% and 28% had MMA > 0.26 µmol/l and Hcy > 10 µmol/l at 18 weeks of gestation (Yajnik et al., 2006). Studies done in other parts of both rural and urban India also report low plasma vitamin B_{12} concentrations among pregnant women, however, they have been done later in pregnancy and have limited their analyses to plasma B_{12} concentrations without studying the biochemical indicators of B_{12} deficiency such as MMA (Pathak et al., 2004, Katre et al., 2010, Veena et al., 2010). Our study assessed the vitamin B_{12} concentration and the concentration of vitamin

B_{12} dependent metabolites such MMA and Hcy in early pregnancy. This is important considering that the concentration of vitamin B_{12} and vitamin B_{12} dependent metabolites such as Hcy and MMA decline during the course of pregnancy (Koebnick et al., 2002, Milman et al., 2006, Cikot et al., 2001).

The FAO/WHO recommends an intake of 2.6 µg/day of vitamin B_{12} during pregnancy (WHO/FAO 2004), while the Indian Council of Medical Research has defined an intake of 1.2 µg/day of vitamin B_{12} to be adequate to meet the requirements of all pregnant women in India since Indians consume a predominantly vegetarian diet (Nutrient requirements and recommended dietary allowances for Indians 2012, Antony 2003). Among pregnant women in our cohort not only was the diet low in vitamin B_{12} (median dietary intake of vitamin B_{12} was only1.25 µg/day), but also they were not consuming any vitamin B_{12} supplements at enrolment. Even at low intakes of vitamin B_{12}, there was a weak but significant correlation with plasma B_{12} concentrations.

Studies from other countries have reported a much higher intake of vitamin B_{12}. For instance, in the Framingham offspring study the mean daily vitamin B_{12} intake among young and old women aged 23 to 85 years was 8.7 µg, and intake correlated strongly with plasma B_{12} concentration (Tucker et al., 2000). Among healthy men and women from Florida aged 18 to 50 years the median intake of vitamin B_{12} was 4.2 µg/day (Bor et al., 2010). Similarly, in the Hordaland homocysteine study conducted in men and women aged 47 to 74 years, the median intake of vitamin B_{12} ranged from 5 to 7.3 µg/day (Vogiatzoglou et al., 2009). Studies from pregnant women in both rural and urban Pune in India have reported the intake of vitamin B_{12} not to exceed 0.4 µg/day even with consumption of milk and non-vegetarian foods (Katre et al., 2010). However, this was based on estimation since in that study a structured FFQ was not used to assess intake of B_{12} and only intake of specific foods were assessed.

Among food groups, intake of yoghurt was a significant predictor of B_{12} status. Certain lactobacilli such as Leuconostoc and Propionibacterium have shown to synthesize B-vitamins during fermentation of milk. (Cerna and Hrabova 1977). Among Egyptian children with elevated Hcy levels, yoghurt containing Lactobacillus acidophilus administered over a period of 42 days was effective in increasing B_{12} producing bacteria in the gut, increasing plasma levels of vitamin B_{12}, folate and reducing Hcy and urinary MMA (Mohammad et al., 2006). Consumption of foods from animal sources or milk was not associated with low

vitamin B_{12} concentration among pregnant women in our cohort unlike in the Pune Maternal Nutrition study where higher frequency of intake of dairy products and non-vegetarian foods was associated with higher plasma vitamin B_{12} concentration, lower Hcy and MMA concentration (Yajnik et al., 2008). Similarly, among adult and elderly men and women from Norway the dietary intake of milk was a significant contributor to vitamin B_{12} status (Vogiatzoglou et al 2009).

Higher body weight was also significantly associated with impaired B_{12} status among pregnant women in our cohort. It is possible that overweight or obesity may lead to changes in the absorption, excretion or metabolism of vitamin B_{12} (Ruxton 2011). Equally, the accumulation of MMA in a vitamin B_{12} deficient person can affect mitochondrial respiration and therefore impair substrate oxidation (Melo et al., 2012).

This study has to be interpreted in the light of limitations such as difficulty in generalizing the findings to the general population since there was a high exclusion rate among pregnant women in our cohort. This was due to the reason that many of the women were planning to deliver outside Bangalore in their maternal homes and therefore had to relocate. In addition, our cohort was an urban, health facility-based one and therefore extending the results to rural areas or at the community level needs to be done with caution. Nevertheless, this study is the first of its kind from India that investigates the biochemical indicators of vitamin B_{12} status including MMA and Hcy, in addition to plasma B_{12} concentration among pregnant women early in pregnancy. In addition, we used a validated FFQ with a trained interviewer with portion sizes to minimize recall bias.

6.3 Poor compliance to exclusive breastfeeding in a baby friendly hospital in urban South India

In a cohort of term infants less than 6 months of age and their mothers from a BFH in urban South India, we observed poor compliance to exclusive breastfeeding with low intakes of breast milk and early introduction of complementary foods. Community studies from rural parts of North India, have shown that only 30% and 10% of the women exclusively breastfed their infants by 4 and 6 months of age respectively, and these low rates of exclusive breastfeeding was attributed to the lack of breastfeeding counselling (Kishore *et al.*, 2008). In a tertiary care hospital in South India only 52% of the infants were exclusively breastfed for 6 months (Renitha et al., 2012), while a similar tertiary care hospital in North India reported even a lower rate of 1% exclusive breastfeeding at 6 months (Oomen et al., 2009). In

general, rates of exclusive breastfeeding are low in India, and this is pointed out by the National Family Health Survey in India which states that only 46% of the infants under 6 months and 59% of infants under 4 months of age are exclusively breastfed (Patel *et al.*, 2010). Not only is the problem rampant in India, but similar demographic and health surveys carried out in neighbouring countries such as Bangladesh and Nepal also report the prevalence of exclusive breastfeeding among infants under 6 months of age to be as low as 43 % and 53 % respectively (Mihrshahi *et al.*, 2010; Pandey *et al.*, 2010). It is noteworthy that all these aforementioned studies have used a structured or a semi structured questionnaire to capture subjective information on exclusive breastfeeding. Our study is unique in that we have used an accurate and objective isotopic method (deuterium dilution method) to capture home based compliance to exclusive breastfeeding for the first time in India. Nevertheless, our study with rates of only 14% exclusive breastfeeding at 6 months of infant age reconfirms the findings of others.

Towards an attempt to improve the rates of exclusive breastfeeding, The BFHI has shown to be successful with a significant annual increase in rates of exclusive breastfeeding among infants under 2 and 6 months in 14 developing countries (Abrahams *et al.*, 2009). Studies conducted by the breastfeeding Promotion Network of India also show that in BFH in India, breastfeeding was initiated early, prelacteal feeds were less common and the intake of supplementary feeds like milk and fluids during the hospital stay was significantly lower, however there were no significant differences in maternal plans to breastfeed exclusively in comparison to non BFH hospitals (Breastfeeding Promotion Network of India, 2000). Similarly, in an interview based cross sectional study conducted in two BFH Hospitals of Indore, it was observed that none of the hospitals had a written breastfeeding policy that could be communicated to all the health workers, and in addition, no regular training regarding the programme was being imparted (Nigam et al., 2010). In a study that was conducted using a semi structured questionnaire in the paediatric outpatient department of the same hospital as where our study was conducted (BFH), it was observed that there were no significant differences in the duration and success of lactation between mothers of babies born inside the BFH as against those babies who were not born there, but came for subsequent paediatric visits (Shilpa et al., 2009). All these data point towards the need to develop a strong BFHI Monitoring System so as to ensure that all the components of a BFH are met in practice, and are not merely policies that are written.

Among mothers in our cohort, the primary reason for the early introduction of complementary foods was a crying infant, perceived by her to be due to insufficient breast milk, while other reasons included demands of workplace, advice by elders in the family and the perceptions of the mother herself. Other studies from India have reported lack of breastfeeding counselling (Kishore et al., 2008), higher household wealth quintile index, delivery in a health care facility and being born in northern parts of the country (Patel et al., 2010) and poor awareness regarding the benefits of breastfeeding (Garg et al., 2010) as key risk factors for poor compliance to exclusive breastfeeding. In this regards antenatal counselling of mothers regarding feeding becomes critical. In a study conducted in a tertiary care hospital in South India, 79 % of the mothers had not received antenatal counselling, and even among those who were counselled, there was poor knowledge regarding the correct breastfeeding technique and also a lack of awareness regarding the need to continue breastfeeding (Dhandapany et al., 2008). In India, counselling mothers regarding breastfeeding during the antenatal period is often not practiced or is very poor (Singh et al., 2006, Chaturvedi and Banait 2000). In addition to antenatal counselling, social support for the breastfeeding mothers is very crucial. Educating the immediate family of the mother who is likely to influence her decision to breastfeed, through community based approaches is also important. A meta-analysis of qualitative studies in developed countries on breastfeeding rates has revealed that mothers tended to rate social support as more important than health service support (Mclnnes *et al.*, 2008). Additionally, in India the decision to breastfeed may also be influenced by gender differences, with a greater likelihood to stop breastfeeding earlier among females in comparison to males, with this difference being attenuated by increasing maternal education, reiterating that educating the mother is also critical (Malhotra et al, 2008)

In addition to poor compliance to exclusive breastfeeding, the overall intake of breast milk among infants in our cohort was low. Our isotopic method allowed us to also capture the amount of water contributed from sources other than breast milk such as other beverages or solid foods. We observed that over the period of 6 months while there was a significant decline in the weight specific breast milk intake of the infant, there was also a proportional increase in non breast milk water intake. Breast milk intake from infants in other parts of the world has been reported using the test weighing method (Islam *et al.*, 2008; Coulibaly *et al.*, 2004). However, this is a time consuming method, not highly accurate and interferes with the normal feeding patterns of the infant (IAEA 2010). Other studies have

used a "dose to mother" deuterium dilution method to overcome the practical problems associated with the test weighing method and have reported breast milk intake volumes among Brazilian and Bangladeshi infants (Haisma et al 2003, Albernaz et al 2003, Galpin *et al.*, 2007; Moore *et al.*, 2007). Nevertheless, the breast milk intake volume that we report from India is lower than all reports from other countries, using either the test weighing or dose to mother deuterium dilution method. Our findings are also in stark contrast to the most recent findings of the pooled analysis of data across five continents, which reported the human milk intake to remain above 800 ml/day until 6-7 months of age among infants from rural areas (Da Costa *et al.*, 2010).

We also assessed the growth of our children in relation to intake of breast milk and non breast milk water using the WHO growth standards (WHO 2006). The WHO standards have been developed based on the finding that healthy children from around the world who are raised in healthy environments and follow recommended feeding practices have strikingly similar patterns of growth (De Onis et al., 2009). Among infants in our cohort breast milk intake was related to WAZ and WHZ until 3 months of age, however, beyond 3 months, growth was primarily driven by complementary foods and infants with higher intakes of non breast milk water showed higher WAZ and HAZ. The introduction of additional water in very early postnatal life has not been shown to confer any additional benefits on infant growth (Cohen *et al.*, 1994, Becker et al., 2011), and for infants ≥2.5 kg of middle socioeconomic group, exclusive breast feed for 6 months has been shown to be sufficient for optimal growth (Agarwal et al., 2012b). Since morbidity such as diarrhoea was not a primary concern among our infants, the growth of the children was not affected by intake of non breast milk water so early. However, the effect of early introduction of non breast milk water in increasing morbidity and therefore causing weight faltering has been well documented (Adair *et al.*, 1993)

The limitation of our study is its small sample size and additionally the lack of qualitative research to provide an in-depth understanding of the psychosocial and biological factors behind early introduction of complementary foods. Nevertheless, this is the first study from India using the accurate and objective deuterium dilution technique to assess breast milk and non breast milk water intakes among infants and thereby capture home-based compliance to exclusive breastfeeding.

6.4 Low intakes of breast milk zinc owing to low volumes of milk intake

In the cohort of term infants from urban South India, we also observed low intakes of zinc through breast milk, and this was primarily due to low overall volumes of breast milk intake. We had measured volumes of breast milk using the "dose to mother" deuterium dilution method as well as concentrations of zinc in the breast milk samples. It is generally considered beneficial to obtain more than one sample of breast milk over different times of the day to get a more representative information on the total amount of zinc transferred (Brown et al., 2009b), since some studies have found > 15% increase in zinc concentrations in the morning samples of breast milk (Hussain et al., 1996), while other older studies have reported no such diurnal variations (Krebs et al., 1985). We had collected morning, afternoon and evening samples to overcome this problem, however, in our cohort of mothers we failed to observe any such diurnal variations. Several older studies have also investigated whether breast milk zinc concentration varies by the portion of feeding (foremilk or hindmilk) (Krebs et al., 1985) or the left or right breast (Neville et al., 1984), however have failed to observe any such associations. We had not collected the foremilk and hindmilk separately and therefore could not assess any such associations. Among other factors known to affect the breast milk zinc concentrations are parity, gestational age at birth, age and zinc status of the mother, however none of these have yielded consistent results and the only major determining factor remains the time at postpartum when the sample is collected, with breast milk zinc concentrations declining over time (Brown et al., 2009b). The physiological pattern of decline in breast milk zinc concentrations as lactation progresses has been previously reported from other studies as well (Krebs *et al*. 1995, 1999).

The concentrations of zinc in the breast milk that we report are higher in comparison to the pooled analysis of data from 33 studies of breast milk zinc content of non supplemented mothers of healthy term infants at comparable infant ages of 1, 3 and 6 months. However, in these studies there could have been an overestimation in the distribution of usual intakes of children, since no adjustment was done for day to day variability in breast milk zinc transfer (Brown et al., 2009b). Studies in Guatamalan (Dhonukshe Rutten et al., 2005) and Iranian (Khaghani et al., 2010) women have shown higher concentrations of breast milk zinc at 1, 2 and 6 months postpartum. The methodology used in these studies, age and occupation of the mothers were similar to that of mothers in our cohort, however education among Iranian mothers were higher. There could have been other factors such as the overall nutritional status of the mother and cultural practices which remain unexplored. Among 142

mother-infant pairs from India, of which 17 were preterm and nine were LBW, the median breast milk zinc concentration was lower than reports from ours, when assessed at 2 months postpartum (0.625 mg/l in comparison to 1.37 mg/l (3 months postpartum), respectively) (Orun et al., 2012). Among these mothers breast milk zinc concentrations were not related to maternal age, parity, smoking habits, iron and vitamin/mineral supplementation, birth weight, gestational age, or feeding type, and also had no effect on anthropometric measurements of infants at 2 months of age.

Studies have shown that as infant age increases, volumes of breast milk consumed also increase (Islam and Brown 2010). Additionally, the pooled analyses of data from across five continents show that breast milk intake increases and remains > 800 ml/day until 6 to 7 months of age (Da Costa et al., 2010). However, in our cohort over the period of 6 months there was a decline in the weight specific breast milk intake of the infant and a proportional increase in non breast milk water intake. Volumes of milk consumed also remained lower than those reported by others (Islam et al., 2008, Coulibaly et al., 2004, Galpin et al., 2007, Moore et al., 2007). It is but natural then to see a corresponding decrease in zinc intakes from breast milk. Although complementary foods were introduced early, they did not contribute to the total zinc intake considering that they were primarily cereal based. The zinc intakes of infants from breast milk reported in our study was lower in comparison reports from other studies (Simmer et al., 1990, Islam and Brown 2010). Even, intakes are lower in comparison to the calculated zinc intakes based on measured breast milk zinc concentrations and an assumed breast milk intake volume of 780 ml/day (Food and Nutrition Board & Institute of Medicine, 2001). The intakes we report are also low in comparison to the estimated average physiological requirement of zinc (Brown et al., 2009b).

Among the mothers in our cohort, maternal dietary zinc intake was not related to breast milk zinc concentrations. Despite the lower maternal dietary zinc intake, the lactating women in this study were able to maintain higher levels of breast milk zinc concentration at months 1, 3 and 6 in comparison to the values reported in the combined analyses by Brown and colleagues (Brown et al., 2009b). Similar observations have been reported among Chinese lactating women, who were able to maintain zinc concentration of 2 mg/day, despite having low dietary zinc intakes of 7.6 mg/day (Sian et al., 2002). As stated before, breast milk zinc concentrations have a wide variability; and does not depend on maternal zinc intake, but is affected by factors stage of lactation, type (fore or hind milk) of feeding (Dorea 1993), and maternal zinc intake is not a major determinant of breast milk zinc concentrations

(Brown et al., 2009b). Equally, there may be physiological adaptive response during lactation such as increased absorption and reduced endogenous faecal zinc excretion (Donangelo and King 2012).

Low maternal zinc intake or low intake of breast milk zinc did not reflect in low zinc status among either of them. The prevalence of zinc deficiency (serum Zn level < 65 µg/dL for the infant and < 70 µg/dL for the mother) was only 16% among mothers and 9% among infants at 6 months of age in our cohort. Other cross-sectional studies from India to assess the prevalence of zinc deficiency in children aged 6-60 months and between 2 and 10 months of age, observed a much higher prevalence of zinc deficiency ranging from 42.4% to 79%. (Kapil and Jain 2011, Agarwal et al., 2012a). Among infants in our cohort there could have been utilization of hepatic zinc bound to metallothionein in the first months of life (Zlotkin and Cherian 1988) or less prevalence of diarrhoeal diseases thereby reducing faecal losses of zinc.

Our study is limited in that we have used serum zinc as a measure of infant zinc status which may not be a reliable indicator of individual zinc status (Hess et al., 2007), and not alternative measures such as exchangeable zinc pool which is a more robust marker of zinc status. Additionally, we have a small sample size from which we draw these conclusions. Therefore, the results need to be generalized with caution. Nevertheless, to our knowledge, this study is the first of its kind from India using the accurate and objective deuterium dilution technique to assess breast milk zinc intake among infants less than 6 months of age.

Considering the current paucity of information on prevalence of micronutrient deficiencies as well as an understanding of the role of nutrition in combating them, especially in early pregnancy, the results from this thesis would help guide policy makers, practitioners, nutritionists and counsellors for developing recommendations and planning action oriented approaches to combat maternal nutritional deficiencies. In addition, we report for the first time in India, the biochemical indicators of vitamin B_{12} status including MMA and Hcy in addition to plasma B_{12} concentrations among pregnant women in early pregnancy. We have used a unique cohort wherein we have recruited pregnant women ≤ 14 weeks of gestation to understand the contribution of diet to the aetiology of micronutrient deficiencies when the effects of hemodilution on nutrient concentrations are minimal.

This thesis also reports the intake of breast milk and non breast milk water using an accurate and objective deuterium dilution method for the first time in India among infants less than 6 months of age. The objective assessment of breast milk intake as well as water from other sources is far more accurate than the subjective method of assessment using questionnaires and is a non-invasive and simple method to capture home based compliance to exclusive breastfeeding. This information is very critical to policy makers as well as practitioners, since exclusive breastfeeding up to 6 months has been ranked as the top most intervention with the potential to reduce under 5 mortality. This would also shed light on the country's progress towards the Millennium Development Goal and direct future efforts towards improving infant nutritional status. In addition, for the first time in India we have used the objective deuterium dilution technique to assess breast milk zinc intakes among infants less than 6 months of age. Zinc deficiency is a public health concern and results from this thesis would shed light on the zinc intake and zinc status of Indian infants. Collectively, this thesis focuses on two most important maternal micronutrient deficiencies as well as two most critical issues related to infant nutrition, and directs the attention of the policy makers towards strategies that could improve the maternal and child nutritional status in India.

7. CONCLUSION

Despite the rapid economic growth in India, the progress achieved in reproductive and child health and nutrition does not compare favourably with some other countries in Asia and there remains a long path to traverse for the achievement of its declared goals (Paul et al., 2011). This thesis has made an attempt to evaluate the maternal and infant nutritional status in urban South India. Primarily, we studied the prevalence and modifiable risk factors for anaemia and vitamin B_{12} deficiency in early pregnancy ($\leq$ 14 weeks of gestation) in an urban cohort from South India. Our salient finding was a high prevalence of anaemia attributable to high dietary intake of iron absorption inhibitors such as calcium and phosphorus, with low intake of iron, and also a high prevalence of vitamin B_{12} deficiency, that was associated with low intake of yoghurt and fish, in addition to being primiparous. This has important implications when translated to day to day practices at the level of individual women and households. Increasing the overall intake of iron rich foods during pregnancy, such as meat, poultry and green leafy vegetables, increasing the bioavailability of iron in the diet by consuming foods that enhance iron absorption such as vitamin C rich foods (citrus fruits, sprouts) and meat, and at the same time reducing consumption of foods that inhibit iron absorption such as phytates and tea can improve iron absorption among these women. Equally improved cooking practices such as germination, fermentation and boiling of vegetables rather than deep frying would help to conserve the nutritive value of foods. Similarly, increasing the intake of vitamin B_{12} rich foods such as foods from animal sources, milk and yoghurt would go a long way in improving their B_{12} status. Such knowledge should be imparted to all pregnant women through community based awareness programmes or focus group discussions. Often, even if women are aware of these, the translation in to practice does not happen. For instance, in a community based cross sectional study in women of reproductive age from India, wide gaps were observed between awareness and practices related with maternal and child health (Kumar et al., 2008). There is a need to impart education, motivate the women to translate knowledge in to practice, address the elders in the family or the community who are often the decision makers and also address social and cultural barriers and misconceptions prevailing among them. Equally, planning and implementing well designed targeted micronutrient supplementation programme for pregnant women with sub-optimal nutritional status and multiple micronutrient deficiencies may go a long way in improving their pregnancy and birth outcomes.

In addition, we have demonstrated for the first time in India using an objective and accurate "dose to mother" deuterium dilution method, that there is poor compliance to exclusive breastfeeding among mothers of infants under 6 months of age, as well as low intakes of zinc from breast milk due to overall low volumes of breast milk consumption among these infants. Early introduction of complementary foods due to in-adequate awareness on the part of the mother as well as the elders in her family, lack of effective antenatal and postnatal counselling and poor motivation are some of the key concerns that need to be addressed. Exclusive breastfeeding needs to be scaled up in India based on evidence based policy and a sound implementation strategy in all strata of the society. This would primarily require adequate data on rates of exclusive breastfeeding using more accurate methods in addition to strong political will, advocacy, enabling policies, sustained financial support, and more frequent contacts with the mother through community mobilization efforts and continuous monitoring and evaluation (Bhandari et al., 2008). Exclusive breastfeeding practices may increase volumes of breast milk consumed, and thereby increase intake of necessary micronutrients such as zinc. Equally important is to introduce nutrient dense complementary foods by 6 months of age, in addition to breast milk to prevent growth faltering in the child.

Results of a global literature review from case studies in Ethiopia, India and Nigeria has indicated that improving the antenatal care, community based nutrition programmes for the mother and child, conditional cash transfer programmes, improving iron-folate and multiple micronutrient supplementation and food fortification offers opportunities for improving maternal nutrition and birth outcomes in these countries (Mason et al., 2012). Although, India has a wide portfolio of programmes that address maternal health and nutrition, there are systematic weaknesses, logistical gaps, scarcity and poor utilization of resources. The opportunities for improving maternal nutrition in India include increasing the priority of maternal nutrition in government health programmes, integrating services and ensuring effective procurement measures for micronutrient and food supplements, establishing training facilities to improve program implementation, and strengthening the monitoring and evaluation of these programmes (Ramakrishnan et al., 2012).

8. RECOMMENDATIONS FOR FUTURE RESEARCH

Our studies have given way to understand some of the key issues in maternal and infant nutrition in urban South India. Assessing and understanding the nutritional status of vulnerable groups such as pregnant women and infants is important, however it is equally important that action oriented strategies are in place to address these issues as well. The results of these exploratory studies should give way to intervention studies among the vulnerable groups in this population, to confirm and reassess the results we document. More importantly, it should sensitise the government to prioritise the nutritional and health needs of the mother and child and lead to new and innovative suggestions to improve the present situation.

Further research is also warranted to study exclusive breastfeeding practices across India using accurate isotopic methods such as the deuterium dilution method that would give an objective estimate of the volumes of breast milk consumed by infants as well as a more accurate estimate of compliance to exclusive breastfeeding in comparison to currently available data that reports intake of breast milk using questionnaires. An in depth qualitative assessment of the barriers to successful breastfeeding practices is also warranted. This would help to identify the gaps in exclusive breastfeeding practices and thereby sensitise the government to design strategies towards improving compliance. Additionally, it is important to understand the short and long term outcomes of exclusive breastfeeding and early introduction of complementary foods on the body composition of the infant.

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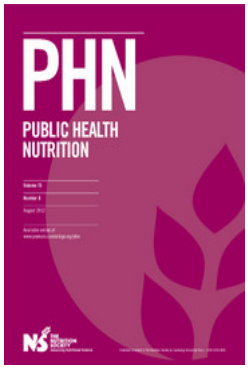

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Correlates of anaemia in pregnant urban South Indian women: a possible role of dietary intake of nutrients that inhibit iron absorption

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Abstract

Objective: To identify correlates of anaemia during the first trimester of pregnancy among 366 urban South Indian pregnant women.
Design: Cross-sectional study evaluating demographic, socio-economic, anthropometric and dietary intake data on haematological outcomes.
Setting: A government maternity health-care centre catering predominantly to the needs of pregnant women from the lower socio-economic strata of urban Bangalore.
Subjects: Pregnant women (*n* 366) aged ≥18 and ≤40 years, who registered for antenatal screening at ≤14 weeks of gestation.
Results: Mean age was 22·6 (SD 3·4) years, mean BMI was 20·4 (SD 3·3) kg/m^2 and 236 (64·5 %) of the pregnant women were primiparous. The prevalence of anaemia (Hb <11·0 g/dl) was 30·3 % and of microcytic anaemia (anaemia with mean corpuscular volume <80 fl) 20·2 %. Mean dietary intakes of energy, Ca, Fe and folate were well below the Indian RDA. In multivariable log-binomial regression analysis, anaemia was independently associated with high dietary intakes of Ca (relative risk; 95 % CI: 1·79; 1·16, 2·76) and P (1·96; 1·31, 2·96) and high intake of meat, fish and poultry (1·94; 1·29, 2·91).
Conclusions: Low dietary intake of multiple micronutrients, but higher intakes of nutrients that inhibit Fe absorption such as Ca and P, may help explain high rates of maternal anaemia in India.

Keywords
Anaemia
Pregnancy
Correlates
South India

Anaemia is a global public health problem that affects nearly 2 billion people in both developed and developing countries[1]. Anaemia is highly prevalent among pregnant women in developing countries and is associated with poor pregnancy outcomes[2]. Anaemia in early pregnancy is associated with a 50 % greater risk of inadequate weight gain for gestation[3]. The risk of preterm delivery is increased by 10–40 % with mild anaemia[4]. The National Family Health Survey reported the prevalence of anaemia among pregnant women in India to be 59 %[5]. A survey among pregnant women from sixteen districts of eleven states in India showed that 61·0 % to 96·8 % of pregnant women were anaemic (Hb < 11 g/dl)[6]. Despite the ongoing national anaemia prophylaxis programme of Fe and folic acid supplementation to pregnant women, two large national surveys[5,7] have indicated that there has been little change in the prevalence of anaemia and the adverse consequences associated with it.

Studies conducted in India suggested that micronutrient deficiencies (Fe, folate and vitamin B_{12}) are the primary cause of anaemia in pregnancy[8]. Dietary intake surveys among pregnant women show low intake of several key micronutrients[9] and subclinical deficiencies of riboflavin, pyridoxine and folic acid have been reported[10]. The relative contribution of each of these factors to anaemia in pregnancy often varies by geographical location, season and dietary intake[11]. Even within India there are wide state-level variations in the

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prevalence of moderate and severe anaemia[7,12], often attributed to differences in the simultaneous presence of several micronutrient deficiencies in addition to Fe deficiency, acute and chronic infections and differences in bioavailability of various micronutrients in the diet[13]. Despite a literacy rate of 68·1 % among women in Karnataka[14] and 85·3 % of the women having received antenatal care[15], the prevalence of anaemia among pregnant women in Karnataka remains 47·6 %[16]. We therefore were interested to determine the modifiable risk factors associated with anaemia among pregnant women in urban Karnataka early in their pregnancy.

Materials and methods

Study design

We performed a cross-sectional study among a cohort of pregnant women enrolled in a randomized controlled trial of vitamin B_{12} supplementation (NCT00641862). The study was conducted at Hosahalli Referral Hospital, Bangalore, Karnataka, South India. This government maternity health-care centre caters predominantly to the needs of pregnant women from the lower socio-economic strata of urban Bangalore and has approximately 200 deliveries per month. Pregnant women were enrolled in early pregnancy (≤14 weeks of gestation) from December 2008 to November 2010. The pregnant women are currently being followed through 2 years postpartum as part of the ongoing trial. The institutional review boards at St John's Medical College Hospital and Harvard School of Public Health approved all study procedures, and written informed consent was obtained from each participant at enrolment.

Study population

All pregnant women aged ≥18 and ≤40 years, who were ≤14 weeks of gestation and registered for antenatal screening at the Hosahalli Referral Hospital, were invited to participate in the study. Pregnant women with multiple gestation, those with a clinical diagnosis of chronic illness (diabetes mellitus, hypertension, heart disease or thyroid disease), those who tested positive for HbSAg (surface antigen of the hepatitis B virus), HIV or syphilis, those who anticipated moving out of the city before delivery, those who were already consuming vitamin B_{12} supplements and those who had been treated for infertility were excluded.

Of the 1376 pregnant women who were contacted at the antenatal clinic during the study period, 958 were excluded for the following reasons: planning to deliver outside Bangalore at maternal home town (*n* 836); wishing to terminate the pregnancy (*n* 67); age <18 years (*n* 4); history of hypertension (*n* 7); previous Caesarean section (*n* 4); and pregnancy was not confirmed (*n* 40). Of the remaining 418 pregnant women who were considered eligible fifty-two declined to participate, leaving 366 who consented.

Sociodemographic and anthropometric information

The pregnant women were interviewed by trained research assistants to obtain sociodemographic information. Gestational age (in weeks) was calculated from the reported first day of the last menstrual period. A digital balance (Salter's 9016, Tonbridge, Kent, UK) was used to record the weights of all mothers to the nearest 100 g. Measurements of height were made using a stadiometer to the nearest 0·1 cm. Mid-upper arm circumference was measured to the nearest 0·1 cm using a plastic measuring tape and triceps skinfold was measured using a Holtain calliper (Crosswell, Crymych, UK). BMI was calculated as weight in kilograms divided by the square of height in metres (kg/m^2).

Haematological data

Blood was drawn from pregnant women after an overnight fast by venepuncture and collected in both EDTA-containing and plain Vacutainer™ tubes (BD Diagnostics, Franklin Lakes, NJ, USA). Hb and complete blood count were measured on an automated Coulter counter (ABX Pentra 60C+; Horriba Ltd, Kyoto, Japan). Anaemia was defined as Hb < 11·0 g/dl and severe anaemia as Hb < 7·0 g/dl[1]. Microcytosis was defined as mean corpuscular volume (MCV) < 80 fl[17], while microcytic anaemia was defined as anaemia with MCV < 80 fl. Haematocrit was considered to be low in pregnancy at values <33 %[18] while the reference range for red-cell distribution width was considered to be 11·5 % to 14·5 %[17].

Dietary data

A pre-tested interviewer-administered FFQ was used to assess the habitual dietary intake for the three months preceding the date of the pregnant women's enrolment into the study. Standard measures were placed before the respondent to quantify the portion size of each food item when administering the questionnaire. The questionnaire was adapted from a questionnaire developed for the urban population residing in South India[19] and has a food list of 127 items, derived from a food database developed from studies at St John's Medical College. Nutrient scores were computed by multiplying the relative frequency of consumption of each food item by the nutrient content of the standard portion size. Nutrient information was obtained on twenty-seven macro- and micronutrients. Energy-adjusted nutrient intakes were calculated by the residual method[20]. Data on the amount of food groups consumed were calculated as the total grams of the food groups consumed daily.

Statistical analysis

Continuous data were summarised as means and standard deviations, and categorical data as numbers and percentages.

Women who had missing data on any variable were not considered for the analysis involving that variable. The association of anaemia with maternal sociodemographic characteristics and energy-adjusted nutrient intakes, categorized by tertiles, was examined using the χ^2 test. The parameters that were found significant in this analysis were considered in multivariable log-binomial regression analysis to examine the independent effect of certain dietary nutrient intakes and food group intakes while adjusting for possible confounding effects of maternal sociodemographic characteristics. All maternal sociodemographic characteristics with $P < 0{\cdot}20$ in the univariate analyses were considered in the multivariable model. Characteristics contributing a >5 % change in the adjusted estimate after exclusion of the covariate from the full model were retained as confounders in the final model. Separate models were constructed for Ca and P as they were collinear and could not be considered in the same model. Similar analyses were performed with tertiles of intakes of certain food groups which were likely to be associated with anaemia. In addition, Fe intake was retained in the model as an exposure of interest. The presence of effect modification by maternal sociodemographic characteristics, dietary intake of nutrients and food groups was examined using stratified analyses. No effect modification was observed (data not shown). Relative risks (RR) with 95 % confidence intervals and corresponding *P* values for both unadjusted and adjusted models are presented. Statistical analyses were carried out with the SPSS statistical software package version 16 (SPSS Inc., Chicago, IL, USA). Log-binomial regression analysis was carried out using the PROC GENMOD program in the SAS statistical software package version 9·2 (SAS Institute, Cary, NC, USA).

Results

The mean age of the pregnant women was 22·6 (SD 3·4) years and the mean gestational age was 11·2 (SD 2·4) weeks (Table 1). High-school graduation was attained by at least 95·8 % of the women. A majority of them (83·8 %) were unemployed. Primiparous pregnant women made up 64·5 % of the cohort. The mean weight and height of the women were 47·8 (SD 8·1) kg and 153·0 (SD 5·6) cm, respectively. BMI was less than 18·5 kg/m^2 in 31·3 % of the women[21]. The mean mid-upper arm circumference (an estimate of peripheral muscle and fat mass) and triceps skinfold (an estimate of peripheral fat mass) of the women were 23·6 (SD 3·1) cm and 15·6 (SD 5·7) mm, respectively. None of the women were consuming Fe or folate supplements on study entry.

The mean Hb and haematocrit levels on study entry were 11·5 (SD 1·5) g/dl and 34·6 (SD 5·1) %, respectively (Table 2). The prevalence of anaemia (Hb < 11·0 g/dl) at baseline was 30·3 % (111/366). Very few women (0·8 %) had severe anaemia (Hb < 7·0 g/dl). Nearly one-third of the women had microcytosis (MCV < 80 fl) and 20·2 %

Table 1 Baseline sociodemographic and anthropometric characteristics of the study population: pregnant Indian women aged ≥18 and ≤40 years (*n* 366), enrolled in early pregnancy (≤14 weeks of gestation), urban Bangalore, December 2008 to November 2010

Parameter	*n*	%
Age (years) (*n* 366)		
<20	102	27·9
20–24	170	46·4
25–29	77	21·0
≥30	17	4·6
Level of education (*n* 364)		
No formal education	15	4·1
Finished high school (10th grade)	257	70·5
Post high school	71	19·5
University degree and above	21	5·8
Occupation (*n* 364)		
Unemployed	305	83·8
Unskilled worker	21	5·8
Skilled worker	27	7·4
Others (secretarial jobs, teachers, business, shop owners)	11	3·1
Parity (*n* 366)		
0	236	64·5
≥1	130	35·6
BMI < 18·5 kg/m^2 (*n* 364)	114	31·3
	Mean	SD
Age (years) (*n* 366)	22·6	3·4
Gestational age at recruitment by LMP (weeks) (*n* 366)	11·2	2·4
Anthropometry (*n* 364)		
Weight (kg)	47·8	8·1
Height (cm)	153·0	5·6
BMI (kg/m^2)	20·4	3·3
Mid-upper arm circumference (cm)	23·6	3·1
Triceps skinfold (mm)	15·6	5·7
	Median	25th, 75th percentile
Total monthly household income (Rs)* (*n* 365)	6000	4500, 9000

LMP, last menstrual period; Rs, rupees.
*$US 1 = Rs 50·2 as of 21 January 2012.

Table 2 Haematological characteristics of the study population: pregnant Indian women aged ≥18 and ≤40 years (*n* 366), enrolled in early pregnancy (≤14 weeks of gestation), urban Bangalore, December 2008 to November 2010

Parameter	Mean	SD
Hb (g/dl)	11·5	1·5
Haematocrit (%)	34·6	5·1
MCV (fl)	81·1	8·9
RDW (%)	13·3	2·2
	n	%
Anaemia (Hb < 11·0 g/dl)	111	30·3
Hb < 8·5 g/dl	18	4·9
MCV < 80 fl	117	32·0
MCV > 90 fl	37	10·1
RDW > 14·5 %	81	22·1
Anaemia and MCV < 80 fl	74	20·2
Anaemia and MCV > 90 fl	2	0·5

MCV, mean corpuscular volume; RDW, red-cell distribution width.

Mean (SD)	
Anaemic	Non-anaemic
7513 (1565)	7162 (1963)
55·0 (14·1)	50·4 (14·0)
49·6 (14·1)	46·4 (14·0)
102 (32)	107 (51)
283 (272)	279 (96)
1·96 (2·33)	1·92 (2·80)
895 (262)	797 (291)
15·6 (3·9)	14·9 (5·2)
2·19 (0·66)	2·06 (0·77)
8·05 (1·94)	7·39 (2·18)
6·9 (3·1)	6·0 (3·2)
1·26 (3·4)	1·16 (2·8)

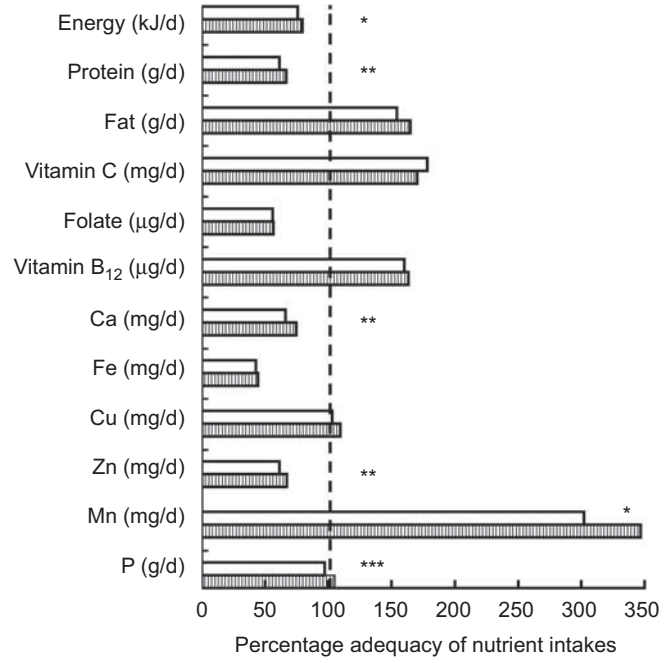

Fig. 1 Dietary intakes, means with standard deviations (left) and as percentage adequacy of the corresponding Indian RDA (right), among anaemic (▥, *n* 110) and non-anaemic (□, *n* 252) pregnant Indian women aged ≥18 and ≤40 years, enrolled in early pregnancy (≤14 weeks of gestation), urban Bangalore, December 2008 to November 2010. Mean values were significantly different between anaemic and non-anaemic pregnant women using the independent *t* test (two-sided): $^{*}P<0{\cdot}05$, $^{**}P<0{\cdot}01$, $^{***}P<0{\cdot}001$

had anaemia with microcytosis. The percentage of women with a red-cell distribution width >14·5 %, suggestive of Fe-deficiency anaemia, was 22·1 %.

The dietary intakes of the anaemic and non-anaemic pregnant women, as means with standard deviations and as percentage adequacy compared with the Indian RDA, are presented in Fig. 1. The mean Fe intakes of anaemic (15·6 mg/d) and non-anaemic women (14·9 mg/d) were not significantly different from each other; however, intakes were very low and met only 44·5 % and 42·6 % of the RDA, respectively, in the two groups. Although the overall mean energy intake was low among both anaemic (7513 kJ/d) and non-anaemic women (7162 kJ/d), it was significantly higher among pregnant women with anaemia compared with those without anaemia ($P<0{\cdot}05$). Protein intake met between only 60 % and 70 % of the RDA, although it was significantly higher among anaemic pregnant women ($P<0{\cdot}01$). The percentage adequacy of fat intake was greater than 100 % in comparison to the RDA among both anaemic and non-anaemic pregnant women. Mean dietary intake of Ca was 895 mg/d among anaemic pregnant women compared with 797 mg/d among pregnant women without anaemia ($P<0{\cdot}01$). The dietary intakes of other nutrients known to have an inhibitory effect on Fe absorption, such as P, Mn and Zn, were significantly higher among the anaemic pregnant women than those without anaemia ($P<0{\cdot}001$, $P=0{\cdot}03$ and $P=0{\cdot}002$, respectively). The primary food groups contributing towards higher intake of P in anaemic pregnant women were cereals, pulses and milk and milk products; mean P intakes from these were 0·41 (SD 0·13) g/d, 0·11 (SD 0·07) g/d and 0·25 (SD 0·23) g/d, respectively. The main contributors towards high Mn intake in anaemic pregnant women were cereals and tea, mean Mn intakes from these being 2·6 (SD 0·8) mg/d and 4·2 (SD 2·3) mg/d, respectively. The significant difference in intakes of protein, Ca, P, Mn and Zn between anaemic and non-anaemic pregnant women persisted even after energy adjustment of nutrients. The intakes of vitamin C, vitamin B_{12} and Cu were all greater than the RDA and were not significantly different between the two groups. The primary food groups contributing towards higher intake of vitamin C were vegetables and fruits, with mean vitamin C intakes of 40·4 (SD 22·5) and 23·0 (SD 26·7) mg/d, respectively. The main contributor to Cu intake was cereals (mean 0·84 (SD 0·33) mg/d); while milk and meat contributed towards vitamin B_{12} intake, mean intakes from the latter being 0·40 (SD 0·40) and 0·36 (SD 0·38) µg/d, respectively.

Results of log-binomial regression analyses of anaemia are presented in Table 3. Two separate multivariable models are presented: Model 1 (sociodemographic factors and energy-adjusted micronutrient intakes) and Model 2 (sociodemographic factors and food dietary intakes from

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Table 3 Sociodemographic and nutritional correlates of anaemia among the study population: pregnant Indian women aged ≥18 and ≤40 years (*n* 366), enrolled in early pregnancy (≤14 weeks of gestation), urban Bangalore, December 2008 to November 2010

		Univariate analyses			Multivariable Model 1* (*n* 358)			Multivariable Model 2† (*n* 358)		
Parameter	*n*/*N*	RR	95 % CI	*P* value	Adjusted RR	95 %CI‡	*P* value	Adjusted RR	95 %CI‡	*P* value
Occupation										
Working at home	86/305	1·00	Ref.	0·016	1·00	Ref.	0·06	1·00	Ref.	0·05
Working outside the home	25/58	1·53	1·09, 2·16		1·45	1·01, 2·09		1·40	1·01, 1·94	
Monthly family income										
Tertile 1 (<Rs 5000)	43/155	1·00	Ref.	0·15	1·00	Ref.	0·14	1·00	Ref.	0·220
Tertile 2 (Rs 5000–8000)	26/98	0·74	0·52, 1·05		0·71	0·50, 1·02		0·76	0·55, 1·05	
Tertile 3 (>Rs 8000)	42/112	0·71	0·47, 1·06		0·73	0·49, 1·08		0·79	0·53, 1·17	
Education										
Less than high school	37/95	1·43	0·94, 2·18	0·12	1·36	0·89, 2·09	0·24	1·49	0·98, 2·26	0·11
High school	49/177	1·02	0·67, 1·54		1·04	0·68, 1·57		1·10	0·73, 1·64	
More than high school	25/92	1·00	Ref.		1·00	Ref.		1·00	Ref.	
Fe intake (mg/d)										
Tertile 1 (<13·9)	32/120	0·83	0·56, 1·23	0·55	0·76	0·51, 1·14	0·41	–		–
Tertile 2 (13·9–15·6)	39/121	1·00	0·69, 1·44		0·89	0·62, 1·28		–		
Tertile 3 (>15·6)	39/121	1·00	Ref.		1·00	Ref.		–		
Folate intake (μg/d)										
Tertile 1 (<260·6)	42/120	1·46	0·98, 2·18	0·15	1·36	0·89, 2·07	0·34	–		–
Tertile 2 (260·6–293·5)	39/121	1·34	0·89, 2·02		1·25	0·83, 1·89		–		
Tertile 3 (>293·5)	29/121	1·00	Ref.		1·00	Ref.		–		
Ca intake (mg/d)										
Tertile 1 (<738·7)	24/121	1·00	Ref.	0·006	1·00	Ref.	0·02	–		–
Tertile 2 (738·7–887·7)	41/121	1·71	1·10, 2·64		1·59	1·04, 2·42		–		
Tertile 3 (>887·7)	45/120	1·89	1·23, 2·90		1·79	1·16, 2·76		–		
P intake (mg/d)§										
Tertile 1 (<1147·1)	25/121	1·00	Ref.	<0·001	1·00	Ref.	0·002	–		–
Tertile 2 (1147·1–1234·3)	34/121	1·36	0·87, 2·13		1·28	0·82, 1·98		–		
Tertile 3 (>1243·3)	51/120	2·06	1·37, 3·09		1·96	1·30, 2·96		–		
Fibre intake (g/d)										
Tertile 1 (<4·1)	30/121	1·00	Ref.	0·15	–		–	1·00	Ref.	0·11
Tertile 2 (4·1–6·2)	36/120	1·21	0·80, 1·83		–		–	1·18	0·80, 1·74	
Tertile 3 (>6·2)	44/121	1·47	0·99, 2·17		–		–	1·46	1·01, 2·11	
Meat, poultry & fish intake (g/d)										
Tertile 1 (<9·2)	24/121	1·00	Ref.	0·002	–		–	1·00	Ref.	0·002
Tertile 2 (9·2–25·6)	37/123	1·52	0·97, 2·37		–		–	1·35	0·87, 2·10	
Tertile 3 (>25·6)	49/118	2·09	1·38, 3·18		–		–	1·94	1·29, 2·91	

RR, relative risk; Ref. referent category.
P values (two-sided) are reported for the adjusted RR.
*Multivariable log-binomial regression of anaemia *v.* occupation, monthly family income, education, Fe, Ca, folate and P intakes.
†Multivariable log-binomial regression of anaemia *v.* occupation, monthly family income, education, fibre and meat, poultry & fish intakes.
‡Adjusted RR from a log-binomial regression model adjusted for the effects of the other variables in the model.
§Due to collinearity of Ca and P intakes ($r = 0·8$), the adjusted RR for dietary P intakes were obtained from a separate model adjusted for the effects of the other variables in the model.

food groups). In the univariate analyses, the socio-demographic variable that was significantly associated with a higher prevalence of anaemia was working outside the home ($P = 0{\cdot}016$). Maternal age, parity, income and education were not associated with anaemia. Energy-adjusted nutrient intakes associated with higher prevalence of anaemia included higher intake of Ca and P ($P = 0{\cdot}006$ and $P < 0{\cdot}001$, respectively). In multivariable Model 1, working outside the home was no longer associated with anaemia. The significant association of Ca and P intakes with anaemia observed in the univariate analyses persisted in the multivariable Model 1 (Table 3).

In multivariable Model 2 (Table 3), the RR of anaemia was higher for pregnant women with the greatest intakes of meat, fish and poultry (RR = 1·94; 95 % CI 1·29, 2·91), consistent with the univariate analyses. The relationship of milk intake with anaemia did not show a monotonic trend and was not significant in either the univariate or multivariable analyses.

Discussion

In a cross-sectional analysis of 366 urban pregnant Indian women, we found a high prevalence of both anaemia (30·3 %) and microcytic anaemia (20·2 %) during the first trimester of pregnancy. Among these pregnant women, who had low intakes of Fe and several other nutrients, higher intakes of Ca and P (dietary components known to inhibit Fe absorption) were independently associated with a higher prevalence of anaemia.

Our study is unique in that we have identified the prevalence and dietary correlates of anaemia as early as ≤14 weeks of gestation. A study from the urban areas of Udipi, Karnataka found the prevalence of anaemia to be 50·1 % (Hb determined by the cyanmethaemoglobin method) among pregnant women at ≤14 weeks of gestation, and identified age, parity, education, socio-economic status, compliance with Fe supplements, history of bleeding and food selection ability as significant determinants of anaemia; however, dietary factors were not studied[22]. Other studies have reported a higher prevalence of anaemia than our reported rate of 30·3 % but these have been carried out among pregnant women later in pregnancy and have used different methods of measuring Hb[23–25]. The present anaemia prevalence is also lower than the published figures of 59 % for any anaemia among pregnant Indian women[5] and 32 % for moderate to severe anaemia among pregnant women in Karnataka[7]. This may be attributed to the fact that our study was a hospital-based study recruiting urban women in early pregnancy based on strict inclusion and exclusion criteria, and possible differences in the method of Hb analysis. It is also noteworthy that the years of schooling attained by pregnant women in our cohort were generally higher in comparison to the overall level of education attainment by pregnant women from urban Karnataka[26]. Nevertheless, a prevalence of 30·3 % anaemia is in itself an indication that anaemia continues to be a problem of public health significance among pregnant Indian women.

Our results indicate that nearly one-third of the pregnant women had BMI < 18·5 kg/m^2. This is in accordance with nationally representative data from the National Family Health Survey that has shown more than one-third of women to have a BMI < 18·5 kg/m^2, reflecting chronic energy and micronutrient deficiencies[27]. The mean intakes of several macro- and micronutrients were well below the RDA for both pregnant anaemic and non-anaemic women. For example, the energy intake was low in comparison to the RDA among all pregnant women in our cohort, although it was higher among the anaemic pregnant women compared with the non-anaemic pregnant women possibly reflecting a diet high in energy but poor in quality. Similar low intake of energy has been reported among pregnant women from urban South India during early pregnancy[28]. The mean dietary intake of Fe was less than 50 % of the RDA. It is noteworthy that all of the pregnant women were ≤14 weeks of gestational age and at enrolment none was receiving Fe or folic acid supplements. Perhaps because of the low and narrow range of dietary Fe intake, we did not observe any association of Fe intake with anaemia. These results are consistent with data from the National Nutrition Monitoring Bureau surveys in India that also showed no correlation of Fe intake with anaemia[13]. Vitamin C is known to have an enhancing and dose-dependent effect on Fe absorption in many single-meal radioisotope studies in human volunteers[29], and can overcome the negative effect of inhibitors on Fe absorption[30]. However prolonged cooking and storage of food can reduce its enhancing effect[31]. Although the mean intake of vitamin C among pregnant women in our cohort exceeded the RDA, it was apparently not sufficient to overcome the inhibitory effect of Fe absorption inhibitors such as P and Ca. This may be due to practices commonly observed among these women, such as prolonged cooking and allowing the meals to stand for longer durations. On the contrary, intakes of Fe absorption inhibitors such as Mn, P and Zn were higher in anaemic pregnant women.

In the setting of low dietary Fe intake, the intakes of several other nutrients and their role in increasing the risk of anaemia through possible interactions with Fe are important to consider. A higher intake of Mn may impose a risk for reduced Fe utilization by affecting Fe absorption, while higher doses of Zn in aqueous solutions are known to impair Fe absorption, although this effect has not been observed when Zn was added to meals[32]. Among pregnant women we observed that higher intake of Ca was associated with a greater risk of anaemia. In single-meal studies conducted in adult men and women, $CaCl_2$ at doses ranging from 40 to 300 mg had a dose-dependent inhibitory effect on the absorption of 5 mg

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non-haem Fe, and 165 mg of Ca given as milk, cheese or $CaCl_2$ diminished absorption of 5 mg haem Fe[33,34]. More recently, an isolated effect of $CaCl_2$ on absorption of both 5 mg non-haem Fe and haem Fe was observed at Ca doses ≥1000 mg and 800 mg, respectively, among non-pregnant women[35]. The effect of Ca supplementation on the uptake of haem Fe is independent of Fe bioavailability in the meal[36] and is a direct effect on haem Fe absorption rather than an indirect counteracting effect on the enhancing effect of meat[37]. This effect is not only seen when Ca and Fe are taken together in the same meal[38,39]. Ca is known to inhibit Fe absorption by the formation of poorly soluble calcium phytate complexes[40] or at the cellular level by its effect on DMT 1 (divalent metal-ion transporter-1) receptors[41]. The competitive inhibition between Ca and Fe in the final transport steps from the mucosal cells to the plasma, common for haem and non-haem Fe, has also been suggested[33]. This significant association of dietary Ca intake with anaemia may have a greater relevance among pregnant women from India because of their inadequate Fe stores, overall low intakes of both Fe and Ca, a predominantly cereal/pulse-based diet and the relative low Fe bioavailability of Indian meals.

Higher intake of P was also observed to be an independent risk factor for anaemia among pregnant women in the present study. P may have a direct inhibitory effect on Fe absorption[42]. Even relatively small quantities of residual phytate, which is the storage form of P (<10 mg phytic acid/meal), are known to strongly inhibit Fe absorption[43]. Although we did not analyse the phytate content in the meals of women in this current cohort, results from another study in a similar group of young women of low socio-economic status from Bangalore showed that the phytate content in their diets was as high as 1287 mg/d[44]. We also observed that a higher intake of meat was associated with a greater risk of anaemia. It is, however, noteworthy that the median (25th, 75th percentile) intake of meat even in the highest group of meat intake was only 39 (32, 50) g/d, making it difficult to draw any conclusions about the relationship between meat intake and anaemia.

In addition to the nutritional factors that cause anaemia, chronic Fe losses due to parasitic infections such as hookworm and schistosomiasis may cause anaemia[44]. We did not evaluate for the presence of parasitic infections among pregnant women in our cohort; however, a low prevalence of parasitic infections has been previously reported among women from urban slums in South India[44]. Our study is limited in that the dietary data were recorded with an FFQ and not via alternative methods including prospective food weighing and other techniques. However, we did employ a validated FFQ with a trained interviewer and portion sizes to minimize recall bias. Fluctuations in appetite and nausea commonly experienced by pregnant women in the first trimester may have influenced reported dietary intakes[45]. Further, our assessment of Fe status was limited to red-blood-cell morphologic indices. The results of our study are based on cross-sectional data with a moderate sample size, so caution should be exercised when drawing conclusions, especially for different populations. In addition, our study results are based on a sample of pregnant women enrolled in a clinical trial at a single hospital and therefore the findings may not be representative of all urban Indian pregnant women.

Conclusions

The results of the present study indicate that higher intakes of Ca and P may be important correlates of anaemia among women with low Fe intake. Further research is warranted to elucidate the modulatory effect of Ca and P on Fe absorption and the levels at which these effects are pronounced among pregnant women in the Indian subcontinent, where such effects have not been previously documented.

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Vitamin B_{12} Intake and Status in Early Pregnancy among Urban South Indian Women

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Key Words
Pregnancy · Vitamin B_{12} deficiency · Methylmalonic acid · Homocysteine

Abstract
Aim: To evaluate the vitamin B_{12} status of South Indian women in early pregnancy and its relationship with sociodemographic, anthropometry and dietary intake. ***Methods:*** Cross-sectional study among 366 pregnant urban South Indian women ≤14 weeks of gestation with outcome variables defined as low vitamin B_{12} blood concentration (<150 pmol/l) and impaired vitamin B_{12} status [low vitamin B_{12} plus elevated methylmalonic acid (MMA) >0.26 μmol/l)]. ***Results:*** Low plasma vitamin B_{12} concentration was observed in 51.1% of the women, while 42.4% had impaired B_{12} status. Elevated MMA, elevated homocysteine (>10 μmol/l) and low erythrocyte folate (<283 nmol/l) were observed among 75.8, 43.3 and 22.2% of the women, respectively. The median (25th, 75th percentile) dietary intake of vitamin B_{12} was 1.25 (0.86, 1.96) μg/day. Lower maternal body weight was associated with higher vitamin B_{12} concentration [prevalence ratios (PR) (95% CI) 0.57 (0.39, 0.84)]. The predictors of impaired vitamin B_{12} status were no consumption of yoghurt [PR (95% CI) 1.63 (1.03, 2.58)] or fish [PR (95% CI) 1.32 (1.01, 1.71)] and primiparity [PR (95% CI) 1.41 (1.05, 1.90)]. ***Conclusion:*** A high prevalence of vitamin B_{12} deficiency in early pregnancy among urban South Indian women was related to primiparity and to a low consumption of yoghurt and fish.

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Introduction

Deficiency of vitamin B_{12} is considered to be highly prevalent in India and its metabolic signs have been reported in 75% of adult men and women from urban areas of West India [1]. Pregnancy is a critical stage when the requirement for vitamin B_{12} increases due to the rapid cell multiplication resulting from the enlargement of the uterus, placental development and fetal growth [2]. Vitamin B_{12} deficiency during pregnancy may elevate plasma homocysteine (Hcy) levels [3], and is associated with an increased risk for adverse outcomes including neural tube defects [4, 5], small-for-gestational-age [6], intrauterine growth retardation [7], early miscarriage [8, 9] and preeclampsia [1].

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In addition to increased requirements during pregnancy, chronic low intakes of dietary vitamin B_{12} [10] and/or malabsorption due to parasitic infections and other causes [11] may lead to a negative vitamin B_{12} balance and depletion of tissue stores leading to a deficiency state. Pregnant women who are vegetarian or those who consume low amounts of animal products are more likely to become vitamin B_{12}-deficient, to give birth to infants who develop clinical or biochemical signs of B_{12} deficiency and to have low levels of this vitamin in their breast milk [12]. The vitamin B_{12} status of an infant at birth, as well as stores during infancy, is strongly determined by the amount of vitamin B_{12} that is accumulated by the fetus during pregnancy [13]. Assessing the vitamin B_{12} intake and status of pregnant women consuming diets low in foods from animal sources may allow the identification of those with sub-optimal status and at risk for adverse pregnancy and birth outcomes. In addition, early pregnancy may be a better time to assess the vitamin B_{12} status as later in pregnancy there may be reductions in the plasma concentrations of vitamin B_{12} and its metabolites due to the expansion of plasma volume [14].

We therefore conducted a cross-sectional study among 366 pregnant urban South Indian women ≤14 weeks of gestation to assess vitamin B_{12} status based on blood concentrations of plasma B_{12}, methylmalonic acid (MMA) and Hcy. In addition, we evaluated the relationship between demographic, anthropometric and dietary intake patterns with biochemical assessment of B_{12} status.

Subjects and Methods

Study Design and Study Population

A cross-sectional study was performed among a cohort of pregnant women enrolled in a randomized controlled trial of vitamin B_{12} supplementation (NCT00641862). The study was conducted at Hosahalli Referral Hospital, Bangalore, which is a government maternity health care center predominantly catering to the needs of the women from the lower socioeconomic strata of urban Bangalore. Pregnant women were enrolled in early pregnancy (≤14 weeks of gestation) from December 2008 to November 2010. The institutional review boards at St. John's Medical College Hospital and the Harvard School of Public Health approved all study procedures, and written informed consent was obtained from each subject at enrolment.

Inclusion and Exclusion Criteria

Pregnant women aged ≥18 and ≤40 years (≤14 weeks of gestation) and registered for antenatal screening at the hospital were included in the study. Women who were diagnosed with chronic illness such as diabetes mellitus, hypertension, heart disease or thyroid disease, who tested positive for HbSAg, HIV or syphilis, who were likely to move out of the city prior to delivery, with multiple gestation, treated for infertility, with previous Cesarean section or who were already consuming vitamin B_{12} supplements were excluded from the study.

Recruitment and Lost to Follow-Up

We contacted 1,376 women at the antenatal clinic during the study period. Of these, 958 were excluded for the following reasons: 836 planned to deliver outside Bangalore in their maternal home town, 67 wanted to terminate the pregnancy, 7 had a history of hypertension, 4 were <18 years, 4 had previously had a Caesarean section and pregnancy was not confirmed among 40. There were 418 women who were eligible to participate in the study. Of these, 52 declined to participate, i.e. 366 consented.

Sociodemographic and Anthropometric Information

Sociodemographic information was obtained by trained research assistants via interviews. Gestational age (in weeks) was calculated from the reported first day of the last menstrual period. Body mass was recorded using a digital balance (Salter's 9016, Tonbridge, Kent, UK) to the nearest 100 g, while height was measured using a stadiometer to the nearest 0.1 cm and the body mass index (BMI) was calculated.

Dietary Data

A pretested interviewer-administered food frequency questionnaire (FFQ) was used to assess the habitual dietary intake for the 3 months preceding the date of the subject's enrolment into the study. Standard measures were placed before the respondent to quantify the portion size of each food item when administering the questionnaire. The questionnaire was adapted from one developed for the urban population residing in South India [15], and has a food list of 127 items derived from a food database developed from studies at St. John's Medical College. To develop this database, the raw food items required for each recipe were entered and the nutrient and food group values were obtained for the cooked weight of that recipe which was obtained by a recipe collection and standardization process. Databases of recipes were obtained from urban and rural groups. The food items were cooked in the metabolic kitchen according to the recipes provided and weights were obtained for the edible portion of the food used. The Indian Food Composition tables were used to estimate the nutrient content of the raw ingredients reported in the recipes [16]. The nutrient content of the cooked recipe was obtained by using a conversion factor to account for the weight/volume changes in cooking [15]. Nutrient and food group values were computed by multiplying the frequency of intake by the portion size and the nutrient or food group value of each food item. These were then summed up to obtain the nutrient and food group values for all foods consumed per day. The total amount of the food groups consumed per day was also calculated (in grams). The food groups were cereals (whole and processed grains), lentils, green leafy vegetables, roots and tubers, fruit, milk and milk products, red meat, organ meat, poultry, fish, nuts, coffee and tea. For further analyses, the milk and milk products group was subdivided into milk, yoghurt and other dairy products.

Biochemical Data

Approximately 10 ml of blood was drawn from subjects after an overnight fast by venipuncture and collected in both ethylene diaminetetraacetate and plain vacutainers (BD Franklin Lakes,

N.J., USA). Hemoglobin (Hb) and complete blood count were analyzed on whole blood samples in an automated Coulter counter (ABX Pentra C+; Horiba Medicals, Calif., USA). The plasma and red blood cells were separated and stored at –80°C until they could be analyzed for vitamin B_{12}, Hcy, MMA and erythrocyte folate.

Vitamin B_{12} was measured by the electrochemiluminescence method (Elecsys 2010; Roche Diagnostics, Mannheim, Germany), while the combined measurement of Hcy and MMA was performed by the gas chromatography-mass spectrometry (GC-MS) method (Varian 3800; Palo Alto, Calif., USA) [17]. The intra- and interday assay coefficients of variation (CV) for vitamin B_{12} were 0.54 and 2.44, respectively. The interday assay CV for MMA and Hcy were 5.57 and 5.04, respectively, while the intraday assay CV were 6.92 and 5.60, respectively. Erythrocyte folate was measured by a competitive immunoassay with direct chemiluminescence detection on an automatised immunoanalyser (ADVIA Centaurs; Bayer Health Care Diagnostics, Tarrytown, N.Y., USA) [18]. The folate concentration in the hemolysate was converted to values for whole blood by adjusting for the hematocrit.

A single stool sample was collected from the pregnant women and analyzed immediately for the presence of helminthic ova, cysts and trophozoites by the wet mount method [19]. We primarily tested the stool samples for the presence or absence of *Giardia lamblia* and *Enterobius vermicularis*.

Statistical Analysis

Continuous data were summarized as means (SD) and categorical data as number (%). Data that are normally distributed were expressed as means (SD). Skewed variables such as dietary intake variables, biochemical variables, household income and gestational age at recruitment were reported as medians (25th, 75th percentile). Anemia was defined as Hb <11.0 g/dl [20]. In the absence of validated cut-offs for plasma vitamin B_{12}, MMA and Hcy concentrations in pregnant Indian women, we used the cut-offs available from the literature. Low vitamin B_{12} concentration was defined as plasma vitamin B_{12} concentration <150 pmol/l [1]. Elevated MMA was defined as >0.26 μmol/l [1]. Elevated Hcy was defined as >10.0 μmol/l. Low erythrocyte folate concentration was defined as <283 nmol/l [21]. As vitamin B_{12} deficiency may exist with a normal plasma vitamin B_{12} concentration and as plasma vitamin B_{12} may not reliably indicate vitamin B_{12} status [22], and because the use of MMA is a relatively specific indicator of vitamin B_{12} deficiency [23], we created a composite variable termed 'impaired vitamin B_{12} status' (low B_{12} concentration and elevated MMA level) to identify women with confirmed B_{12} deficiency. Secondary analyses included evaluating predictors of women with low plasma vitamin B_{12} concentration alone as well as predictors of women with impaired vitamin B_{12} status.

The association of impaired vitamin B_{12} status with maternal sociodemographic and anthropometric characteristics and the intake of specific foods were examined using multivariable log-binomial regression analyses. For the analysis to identify predictors of low vitamin B_{12} concentration, the log-binomial model did not converge and the Poisson regression was applied. The food groups that were consumed by less than 50% of the respondents were considered as binary (consumed/not consumed) and the others were considered as three categories (not consumed, below median consumption and above median consumption). All sociodemographic, anthropometric and dietary variables were examined with low B_{12} concentrations and impaired B_{12} status. The variables that were significant in this analysis with $p < 0.20$ were considered for log-binomial regression analyses or the Poisson regression. The characteristics found significant in the univariate analysis with $p < 0.20$ were considered for the multivariable analysis. Prevalence ratios (PR) with 95% confidence intervals and corresponding p values for both unadjusted and adjusted models are presented. Variables were retained in the final adjusted regression model if they had a $p < 0.05$. Statistical analyses were carried out with SPSS (version 16; SPSS, Chicago, Ill., USA). Log-binomial regression analysis was carried out using the PROC GENMOD program in SAS software (version 9.2; SAS, Cary, N.C., USA).

Results

The sociodemographic, anthropometric, dietary intake and biochemical data are presented in table 1. The mean (SD) age of the pregnant women was 22.6 (3.7) years and the mean (SD) gestational age at the time of the study was 11.2 (2.4) weeks. Primiparous women made up 64.5% of the cohort. The median dietary intake of energy, protein and folate among pregnant women was lower in comparison to the recommended dietary allowances (RDA) for this group [24]. The median (25th, 75th percentile) dietary intake of vitamin B_{12} was 1.25 (0.86, 1.96) μg/day. On the study entry, none of the women used iron or folate or vitamin B_{12} supplements.

The mean (SD) Hb was 11.5 (1.5) g/dl and the prevalence of anemia (Hb <11.0 g/dl) was 30.3% (111/366). Mean corpuscular volume was >90 fl in 10.1% of the women. A very small percentage of the women (0.5%) had macrocytic anemia (anemia with mean corpuscular volume >90 fl). About half (51.1%) of them had low plasma vitamin B_{12} concentrations (<150 pmol/l), and MMA levels >0.26 μmol/l were observed in three quarters (75.8%). About half (43.3%) of them had Hcy levels >10.0 μmol/l, and nearly a quarter (22.2%) had erythrocyte folate concentrations <283 nmol/l. Impaired vitamin B_{12} status as evidenced by plasma B_{12} <150 pmol/l and MMA >0.26 μmol/l was observed among 42.4% of the women. *G. lamblia* cysts were present in 3.0%.

In the women in the cohort, energy-adjusted dietary vitamin B_{12} intake correlated significantly with plasma B_{12} concentration ($r = 0.164$, $p = 0.002$), but not with the plasma MMA ($p = 0.260$) or Hcy ($p = 0.902$) levels. The dietary intake of folate did not correlate with either the erythrocyte folate ($p = 0.276$) or Hcy ($p = 0.532$) level. Figure 1a shows the association between plasma vitamin B_{12} concentration and MMA level. There was a significant inverse correlation between plasma vitamin B_{12} concentration and plasma MMA ($r = –0.184$, $P = 0.001$). Fig-

Table 1. Sociodemographic, anthropometric, dietary and biochemical characteristics of 366 pregnant South Indian women

Parameter	
Sociodemographic characteristics	
Age, years (n = 366)[1]	22.6 ± 3.7
Level of education (n = 364)[2]	
No formal education	15 (4.1%)
Finished high school (10th grade)	257 (70.5%)
Tertiary education	71 (19.5%)
University degree or more	21 (5.8%)
Occupation (n = 364)[2]	
Unemployed	305 (83.8%)
Unskilled worker	21 (5.8%)
Skilled worker	27 (7.4%)
Other business (secretarial jobs, teachers or shop owners)	11 (3.1%)
Total monthly household income, INR (n = 365)[3]	6,000 (4,500, 9,000)
Parity (n = 366)[2]	
0	236 (64.5%)
≥1	130 (35.6%)
Gestational age at recruitment by LMP, weeks (n = 366)[1]	11.2 ± 2.4
Anthropometric characteristics (n = 364)[1]	
Weight, kg	47.8 ± 8.1
Height, cm	153.0 ± 5.6
BMI	20.4 ± 3.3
Dietary intake characteristics (n = 362)[3]	
Energy, Kcal/day	1,695 (1447, 1977)
Protein, g/day	51.1 (42.4, 58.9)
Fat, g/day	46.2 (38.3, 55.1)
Carbohydrates, g/day	269 (225, 315)
Vitamin B_{12}, μg/day	1.25 (0.86, 1.96)
Folate, μg/day	272 (226, 318)
Intake of B_{12} or folate supplements	Nil
Biochemical characteristics	
Anemia (Hb <11.0 g/dl) (n = 366)[2]	111 (30.3%)
Macrocytosis, MCV >90	37 (10.1%)
Anemia and MCV >90[2]	2 (0.5%)
Plasma B_{12} level, pmol/l (n = 352)[3]	149.3 (109.4, 204.5)
MMA level, μmol/l (n = 360)[3]	0.47 (0.28, 0.67)
Hcy level, μmol/l (n = 360)[3]	9.22 (5.74, 15.08)
Erythrocyte folate, nmol/l (n = 359)[3]	386.9 (290.6, 496.4)
Plasma B_{12} <150 pmol/l, (n = 352)[2]	179 (51.1%)
Prevalence of elevated MMA levels (>0.26 μmol/l) (n = 360)[2]	273 (75.8%)
Prevalence of elevated Hcy levels (>10.0 μmol/l) (n = 360)[2]	157 (43.3%)
Prevalence of impaired vitamin B_{12} status (plasma B_{12} <150 pmol/l; MMA >0.26 μmol/l) (n = 349) [2]	148 (42.4%)
Prevalence of low erythrocyte folate levels (<283 nmol/l) (n = 351) [2]	80 (22.2%)
Prevalence of *G. lamblia* infections (n = 328)[2]	10 (3.0%)

LMP = Last menstrual period; MCV = mean corpuscular volume.
[1] Mean ± SD; [2] number (%); [3] median (25th, 75th percentile).

ure 1b shows the association between plasma vitamin B_{12} concentration and level of Hcy. There was an inverse correlation between plasma vitamin B_{12} concentration and plasma Hcy, which, however, was of borderline statistical significance (r = –0.097, p = 0.069).

Table 2 shows the results of the Poisson regression analyses of low vitamin B_{12} concentration as well as log-binomial regression analyses of impaired vitamin B_{12} status (defined by plasma B_{12} concentration <150 pmol/l plus MMA >0.26 μmol/l) with sociodemographic and anthropometric factors. In the univariate analyses, the pregnant women in the lowest third of body weight had the lowest risk for low vitamin B_{12} concentration [PR (95% CI) 0.59 (0.46, 0.78)]. Maternal age, gestational age in weeks, parity, income and education were not significantly associated with low vitamin B_{12} concentrations in the univariate analyses. When we considered impaired vitamin B_{12} status as the outcome variable, we observed that primiparous women had a significantly higher risk of impaired vitamin B_{12} status in comparison to multiparous women [PR (95% CI) 1.55 (1.16, 2.08)].

In the multivariable model after adjusting for the other variables in the model, a lower maternal body weight was associated with a lower risk for low vitamin B_{12} concentration [PR (95% CI) 0.57 (0.39, 0.84)]. The risk for impaired vitamin B_{12} status was higher among primiparous women [PR (95% CI) 1.41 (1.05, 1.90)].

Table 3 shows the results of the Poisson regression analyses of low vitamin B_{12} concentration as well as the log-binomial regression analyses of impaired vitamin B_{12} status with nutritional factors. In the univariate analyses, intake of milk and milk products, red meat and poultry, eggs, organ meat and fish were not associated with low vitamin B_{12} concentration. Intake of milk and milk products was associated with the higher prevalence of impaired vitamin B_{12} status [PR (95% CI) 3.04 (1.17, 7.90)]. Since we were interested to know the specific milk or milk product that was associated with vitamin B_{12} deficiency, we categorized them as milk, yoghurt and other dairy products (cottage cheese, buttermilk and payassam). Pregnant women who did not consume yoghurt had a higher risk for impaired B_{12} status in comparison to those who had an intake above the median [PR (95% CI) 1.64 (1.03, 2.61)].

In the multivariable model, women reporting no intake of yoghurt had a higher risk for impaired vitamin B_{12} status [PR (95% CI) 1.63 (1.03, 2.58)]. A report of no dietary intake of fish was also associated with a greater risk for impaired vitamin B_{12} status in the multivariable analyses [PR (95% CI) 1.32 (1.01, 1.71)].

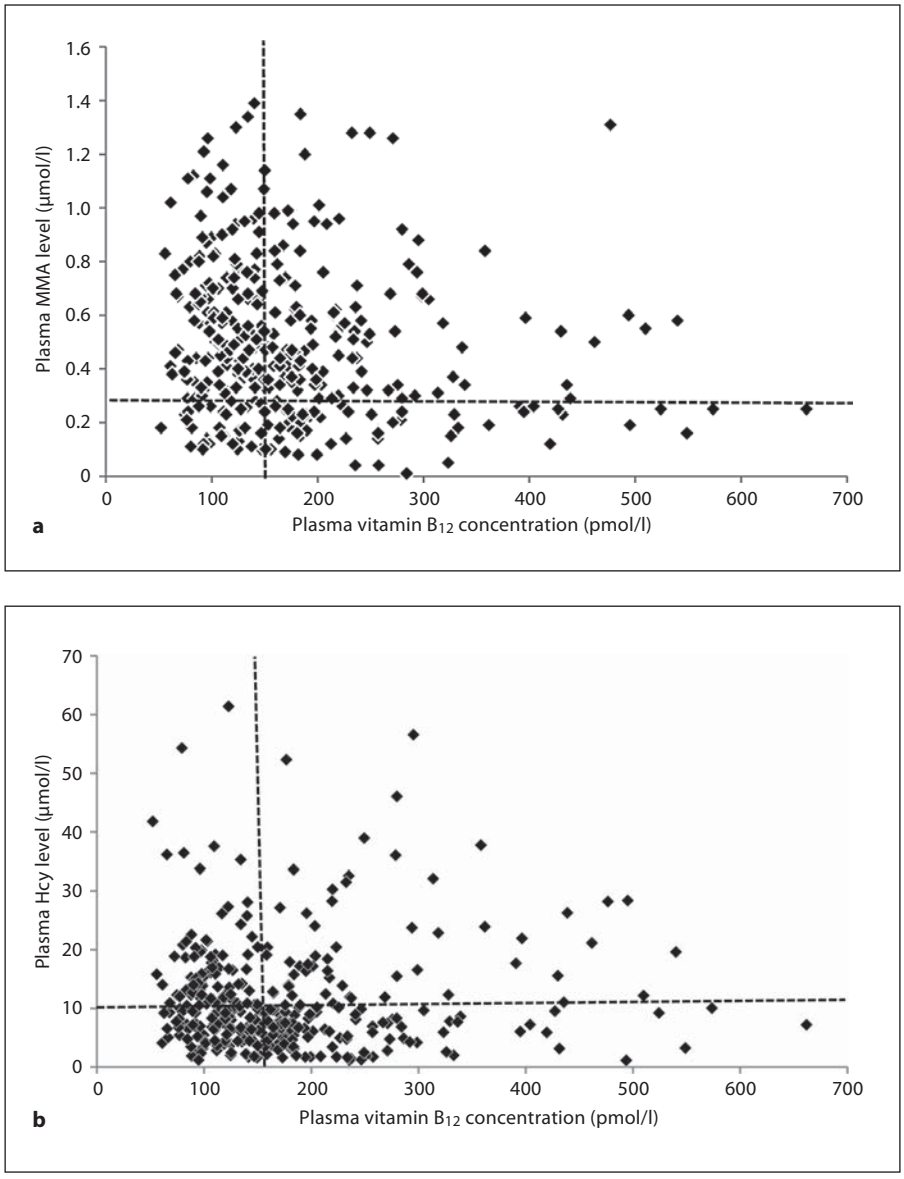

Fig. 1. a Plasma vitamin B_{12} concentration versus concentration of MMA in pregnant South Indian women (r = –0.184, p = 0.001, n = 349). The dashed line on the x-axis denotes the cut-off level for vitamin B_{12} deficiency (<150 pmol/l), while the dashed line on the y-axis denotes the cut-off level for elevated MMA (>0.26 μmol/l). **b** Plasma vitamin B_{12} concentration versus concentration of Hcy in pregnant South Indian women (r = –0.097, p = 0.069, n = 349). The dashed line on the x-axis denotes the cut-off level for vitamin B_{12} deficiency (<150 pmol/l), while the dashed line on the y-axis denotes the cut-off level for elevated Hcy (>10 μmol/l).

Discussion

In a cohort of pregnant women from urban South India recruited early in pregnancy, we found a high prevalence of biochemical vitamin B_{12} deficiency. Low plasma vitamin B_{12} concentration and impaired vitamin B_{12} status were observed in 51.1 and 42.4% of the pregnant women, respectively. The pregnant women in our cohort had a higher prevalence of low plasma vitamin B_{12} concentration and elevated MMA levels in comparison to pregnant women from Nepal [25] and other developed countries when assessed in early pregnancy [26–28].

Studies from both developed and developing countries have documented a high prevalence of low vitamin B_{12} concentration as well as high level of MMA among pregnant women in the 2nd and 3rd trimester of pregnancy [29, 30]. A low plasma vitamin B_{12} concentration has also been reported among pregnant women from both rural and urban India in the later half of pregnancy [31–33]. However, the concentration of vitamin B_{12} and vitamin B_{12}-dependent metabolites such as Hcy and MMA are known to decline during the course of pregnancy [34–37], mainly due to factors such as hemodilution and hormonal influences in addition to nutritional

Table 2. Sociodemographic and anthropometric factors associated with low vitamin B_{12} concentration and impaired vitamin B_{12} status among pregnant Indian women

Parameter	Low vitamin B_{12} concentration[1] (plasma vitamin B_{12} <150 pmol/l)					Impaired vitamin B_{12} status [2] (plasma vitamin B_{12} <150 pmol/l and MMA >0.26 μmol/l)				
	% low plasma B_{12}	univariate PR 95% CI	p value	adjusted PR[3] 95% CI	p value	% impaired B_{12} status	univariate PR 95% CI	p value	adjusted PR[4] 95% CI	p value
Sociodemographic and anthropometric characteristics										
Maternal age, years			0.732					0.791		
Lowest third (≤20.90) (n = 122)	48.3	1.02 (0.77, 1.36)	0.889			43.0	1.16 (0.82, 1.64)	0.400		
Middle third (21.0–23.40) (n = 125)	53.7	1.03 (0.80, 1.33)	0.819			44.7	1.13 (0.82, 1.55)	0.449		
Highest third (<23.40) (n = 119)	50.9	1.00				40.2	1.00	0.791		
Gestational age, weeks			0.053		0.144			0.415		
Lowest third (≤9.60) (n = 123)	41.7	1.00		1.00		38.9	1.21 (0.90, 1.63)	0.203		
Middle third (9.70–12.60) (n = 130)	55.6	1.33 (1.02, 1.74)	0.036	1.37 (0.94, 1.99)	0.101	47.2	1.13 (0.60, 2.15)	0.703		
Highest third (>12.60) (n = 113)	55.5	1.77 (1.02, 3.05)	0.042	1.35 (0.92, 1.99)	0.126	41.4	1.00			
Maternal weight, kg			0.003		0.024			0.064		0.082
Lowest third (≤43.30) (n = 123)	38.5	0.59 (0.46, 0.78)	0.001	0.57 (0.39, 0.84)	0.004	34.5	0.70 (0.51, 0.95)	0.022	0.73 (0.54, 0.99)	0.043
Middle third (43.40–51.0) (n = 121)	49.6	0.59 (0.37, 0.93)	0.022	0.79 (0.55, 1.12)	0.186	43.6	0.77 (0.99, 1.34)	0.362	0.97 (0.74, 1.26)	0.814
Highest third (>51.0) (n = 121)	64.7	1.00		1.00		49.6	1.00		1.00	
Parity										
Primiparous (n = 227)	54.6	1.23 (0.98, 1.55)	0.076	1.24 (0.89, 1.73)	0.335	48.9	1.55 (1.16, 2.08)	0.003	1.41 (1.05, 1.90)	0.023
Multiparous (n = 124)	44.4	1.00		1.00		31.5	1.00		1.00	
Income, INR			0.330		0.655			0.165		0.208
Lowest third (n = 152)	48.0	1.00		1.00		38.0	1.00		1.00	
Middle third (n = 93)	49.5	1.03 (0.94, 1.50)	0.827	0.96 (0.66, 1.40)	0.830	42.6	1.12 (0.82, 1.53)	0.476	1.07 (0.79, 1.46)	0.639
Highest third (n = 105)	57.1	1.19 (0.95, 1.69)	0.146	1.17 (0.82, 1.66)	0.390	50.0	1.32 (0.99, 1.74)	0.055	1.28 (0.97, 1.68)	0.082
Education			0.411					0.267		
Illiterate/primary school (n = 90)	50.0	0.86 (0.64, 1.17)	0.335			40.0	0.81 (0.56, 1.17)	0.271		
High school (n = 170)	50.0	1.02 (0.80, 1.31)	0.849			50.0	1.05 (0.79, 1.40)	0.739		
>High school (n = 87)	50.0	1.00				40.0	1.00			

All values are PRs and 95% CIs. p values (2-sided) have been reported for the adjusted PR.

[1] Multivariable Poisson regression of low vitamin B_{12} concentration, maternal age, maternal weight, gestational age at recruitment, education, income and parity. [2] Multivariable log-binomial regression of impaired vitamin B_{12} status, maternal age, maternal weight, gestational age at recruitment, education, income and parity. [3] Adjusted PR from a Poisson regression model adjusted for the effects of the other variables in the model. [4] Adjusted PR from a log-binomial regression model adjusted for the effects of the other variables in the model.

deficiencies [2]. Therefore, low values of vitamin B_{12} and its metabolites later in pregnancy must be interpreted with caution. Our study partially overcomes this issue of declining B_{12} concentration by recruiting women as early as ≤14 weeks of gestation. Although maternal plasma volume begins to increase by 6 weeks of gestation, a peak increase is observed only between 30 and 34 weeks of gestation [14]. The pregnant women in our cohort also had a higher prevalence of folate deficiency (22.2%) in early pregnancy in contrast to pregnant women from rural Pune for whom a very low prevalence of folate deficiency (0.2%) was reported even in the 2nd and 3rd trimester of pregnancy [38]. It may be that the women in our study had an overall poor dietary intake, reflected not just in their low consumption of foods from animal sources but also a low intake of green leafy vegetables. Access to and consumption of fresh vegetables may also be limited in an urban compared to a rural setting.

Inadequate dietary intake is one of the main causes of vitamin B_{12} deficiency. In our cohort, vitamin B_{12} dietary intake was significantly related to plasma B_{12} concentration, although the correlation was weak. Other studies in postmenopausal women as well as young and elderly men and women have reported a much stronger correlation between vitamin B_{12} dietary intake and plasma or serum B_{12} levels [39, 40]. However, the vitamin B_{12} intake among these men and women was much higher than that reported by the pregnant women in our cohort, and was contributed by vitamin B_{12} supplements in addition to diet. The women in our cohort not only had a low dietary intake of vitamin B_{12}, but were also not

Table 3. Nutritional factors associated with a low vitamin B_{12} concentration and an impaired vitamin B_{12} status among pregnant Indian women

Parameter	Low vitamin B_{12} concentration[1] (plasma vitamin B_{12} <150 pmol/l)					Impaired vitamin B_{12} status [2] (plasma vitamin B_{12} <150 pmol/l and MMA >0.26 μmol/l)				
	% low plasma B_{12}	univariate PR 95% CI	p value	adjusted PR [3] 95% CI	p value	% impaired B_{12} status	univariate PR 95% CI	p value	adjusted PR[4] 95% CI	p value
Intake of foods										
Poultry and meat, g/day			0.182		0.419			0.422		
0.0 (n = 74)	60.6	1.28 (0.99, 1.65)	0.063	1.28 (0.84, 1.96)	0.258	49.3	1.16 (0.85, 1.58)	0.34		
0.1–17.30 (n = 146)	49.6	1.05 (0.82, 1.33)	0.717	1.04 (0.73, 1.46)	0.865	39.7	0.94 (0.71, 1.24)	0.64		
>17.30 (n = 146)	47.5	1.00		1.00		42.4	1.00			
Eggs, g/day			0.616					0.899		
0.0 (n = 112)	53.8	1.13 (0.88, 1.46)	0.347			43.8	1.07 (0.79, 1.45)	0.667		
0.1–10.17 (n = 125)	52.1	1.09 (0.85, 1.41)	0.479			43.2	1.05 (0.78, 1.42)	0.726		
>10.17 (n = 125)	47.5	1.00				41.0	1.00			
Organ meat consumption										
No (n = 330)	52.2	1.35 (0.86, 2.13)	0.150	1.36 (0.73, 1.46)	0.330	43.6	1.35 (0.80, 2.29)	0.259		
Yes (n = 32)	38.7	1.00		1.00		32.3	1.00			
Fish consumption										
No (n = 234)	54.2	1.20 (0.96, 1.51)	0.104	1.19 (0.85, 1.67)	0.314	45.7	1.24 (0.94, 1.63)	0.111	1.32 (1.01, 1.71)	0.041
Yes (n = 128)	45.1	1.00		1.00		36.9	1.00		1.00	
Milk, ml/day			0.284					0.313		
0.0 (n = 175)	55.4	1.21 (0.86, 1.72)	0.269			45.8	1.37 (0.88, 2.14)	0.162		
0.1–120.0 (n = 144)	47.5	1.04 (0.73, 1.49)	0.831			42.0	1.26 (0.79, 1.99)	0.321		
>120.0 (n = 47)	45.7	1.00				33.3	1.00			
Yoghurt, ml/day			0.031		0.263			0.021		0.035
0.0 (n = 171)	51.8	1.45 (0.99, 2.12)	0.056	1.58 (0.96, 2.61)	0.071	43.9	1.64 (1.03, 2.61)	0.038	1.63 (1.03, 2.58)	0.038
0.1–27.8 (n = 135)	56.5	1.58 (1.08, 2.32)	0.019	1.60 (0.97, 2.64)	0.066	48.1	1.79 (1.23, 2.87)	0.015	1.70 (1.07, 2.72)	0.025
>27.8 (n = 60)	35.7	1.00		1.00		26.8	1.00		1.00	
Other dairy products										
No (n = 69)	51.5	1.01 (0.78, 1.31)	0.926			47.0	1.13 (0.84, 1.51)	0.423		
Yes (n = 297)	50.9	1.00				41.7	1.00			

All values are PR and 95% CI. p values (2-sided) have been reported for the adjusted PR.

[1] Multivariable Poisson regression of low vitamin B_{12} concentration, intake of poultry and meat, eggs, organ meat, fish, milk, yoghurt and other dairy products. [2] Multivariable log-binomial regression of impaired vitamin B_{12} status, intake of poultry and meat, eggs, organ meat, fish, milk, yoghurt and other dairy products. [3] Adjusted PR from a Poisson regression model adjusted for the effects of the other variables in the model. [4] Adjusted PR from a log-binomial regression model adjusted for the effects of the other variables in the model.

consuming either B_{12} supplements or foods fortified with vitamin B_{12} at the time of recruitment. The weak correlation observed between diet and plasma vitamin B_{12} concentration may also be due to nondietary sources contributing to B_{12} intake among the women in the cohort. For example, it has been speculated that small intestinal microbes such as *Pseudomonas* and *Klebsiella* may synthesize nutritionally significant amounts of vitamin B_{12} for terminal ileal absorption [41]. In addition, since the food tables we used were based on raw foods, micronutrient losses during cooking and food preparation may be one of the causes of poor correlation between calculated dietary intake and plasma B_{12}. As data on the cooking duration was not available in this sample, applying a uniform correction factor for cooking losses could only adjust for the systematic error and did not alter the observed association between diet and plasma B_{12}. With a dietary intake of 1.25 μg/day of vitamin B_{12}, and based on the assumption that 50% of the dietary B_{12} is absorbed by healthy adults with normal gastric function [42], the amount of absorbed vitamin B_{12} would only be 0.63 μg/day. More recently, the Indian Council of Medical Research has defined an intake of 1.2 μg/day of vitamin B_{12} to be adequate to meet the requirements of all pregnant women in India since Indians consume a predominantly vegetarian diet [24, 43]. This is in contrast to the FAO/WHO recommendation of 2.6 μg/day for pregnant women [44], where requirements are based on intakes of a population subsisting mainly on animal foods.

From a food-group perspective, the intake of foods from animal sources was not associated with low vitamin B_{12} concentrations. However, the intake of fish was associated with a lower risk for impaired vitamin B_{12} status. Dairy products are important sources of vitamin B_{12}, and the absorption of vitamin B_{12} from them is considered to be more efficient than that from poultry, fish or meat sources [42]. However, we did not observe any significant relation between the intake of milk and low vitamin B_{12} concentration or impaired B_{12} status. This is in contrast to the observations of a study among adult and elderly men and women from Norway where the dietary intake of milk was a significant contributor to vitamin B_{12} status [45]. It may be that the intake of milk among our pregnant women was not as high as the milk intake reported in the Norwegian study, which ranged from 173 to 320 ml/day. In addition, milk in Norway is primarily consumed in the raw form (mild pasteurization) and along with meals, unlike in India where it is boiled prior to consumption. Boiling milk for 5–30 min leads to a 30–50% loss in its vitamin B_{12} content [46].

More interestingly, we found that yoghurt intake was a significant predictor of B_{12} status. An intake of 100 ml of yoghurt would provide approximately the same amount of B_{12} as a similar portion of milk (0.357 μg); however, there is a possibility that certain *Lactobacilli* may synthesize B vitamins during the fermentation of milk. For example, particular strains of *Leuconostoc* and *Propionibacterium* have been shown to increase the vitamin B_{12} content substantially during milk fermentation [47]. In Egyptian children with elevated Hcy levels, yoghurt containing *Lactobacillus acidophilus* administered over a period of 42 days was effective in increasing B_{12}-producing bacteria in the gut, increasing plasma levels of vitamin B_{12} and folate, and reducing Hcy and urinary MMA [48]. In addition to a poor diet, parasitic infestations may result in suboptimal vitamin B_{12} status. For instance, infection with *G. lamblia* is known to result in vitamin B_{12} malabsorption in children [49]. *G. lamblia* infections were also significant predictors of low holotranscobalamin II concentrations among lactating women from Guatemala at 3 months postpartum [50]. However, among the pregnant women in our cohort, the prevalence of *G. lamblia* infections was very low (3%) and was not significantly associated with a low B_{12} concentration. Equally interesting in our cohort was the observation that a higher body weight was significantly associated with an impaired B_{12} status. The link between maternal body weight and impaired B_{12} status may be bidirectional. Vitamin B_{12} acts as a coenzyme for methylmalonylCoA mutase that converts methylmalonyl CoA to succinyl CoA. The accumulation of MMA in a vitamin B_{12}-deficient person can affect mitochondrial respiration [51, 52], and therefore impair substrate oxidation. It may also be possible that being overweight or obese may lead to changes in the absorption, excretion or metabolism of vitamin B_{12}, and this may be exacerbated by a diet low in this vitamin [53].

The strength of our study is that it is the first of its kind from India that investigates the biochemical indicators of vitamin B_{12} status including MMA and Hcy, in addition to the plasma B_{12} concentration among women in early pregnancy. In addition, we used a validated FFQ with a trained interviewer with portion sizes to minimize recall bias. However, it may be difficult to generalize our study findings to all Indian pregnant women as our cohort was an urban, health-facility-based one. In addition, the generalizability of our findings may be affected by the high exclusion rate in the cohort due to mothers planning to deliver outside Bangalore.

In conclusion, we found a high prevalence of vitamin B_{12} deficiency among urban South Indian pregnant women ≤14 weeks of gestation. The association of higher maternal body weight with low vitamin B_{12} concentration merits further evaluation. Efforts towards improving the vitamin B_{12} status of pregnant women should focus on improving the overall quality of the diet and on increasing the intake of specific foods that are high in vitamin B_{12} and commonly consumed in a vegetarian population. In addition, it remains to be explored with well-designed intervention studies, whether supplementing vulnerable groups such as pregnant women with vitamin B_{12} in addition to iron and folate would improve hematological and clinical outcomes during pregnancy.

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Disclosure Statement

A.V.K. is a member of the Kraft Health and Wellness Board. His honoraria go entirely to charity. None of the other authors has any personal or financial conflict of interest.

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ORIGINAL ARTICLE

Are infants born in baby-friendly hospitals being exclusively breastfed until 6 months of age?

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Background/Objectives: To objectively measure rates of breast-feeding to infants born in a baby-friendly hospital in Bangalore, India, and to capture home-based compliance to exclusive breastfeeding (EBF).
Subjects/Methods: Breast-milk (BM) and non-breast-milk (NBM) water intake were assessed in 50 mother–infant pairs using a deuterium dilution technique at months 1, 3 and 6.
Results: Complementary feeding (CF) was introduced as early as 1 month among 44% of the infants, and only 14.2% remained as exclusively breastfed by month 6. Intake of BM significantly declined from 166 to 87 ml/kg/day and NBM significantly increased from 23 to 51 ml/kg/day from month 1–6 ($P<0.01$). There was a significant negative correlation between BM and NBM at months 3 ($r=-0.59$, $P<0.001$) and 6 ($r=-0.61$, $P<0.001$). The most common barrier to EBF was 'a persistently crying infant'. BM intake significantly correlated with weight for age (WAZ; month 1: $r=0.56$, $P<0.001$; month 3: $r=0.60$, $P<0.001$) and weight for height (WHZ; month 1: $r=0.59$, $P<0.001$; month 3: $r=0.57$, $P<0.001$). NBM intake showed a significant negative correlation with WHZ ($r=-0.33$, $P=0.02$) at month 3 and correlated positively with WAZ ($r=0.37$, $P=0.01$) and height for age ($r=0.30$, $P=0.03$) at month 6.
Conclusions: Despite intensive counseling at birth and during the immediate postnatal period in a baby-friendly hospital, early CF was observed at home. Reason for the early introduction of CF was primarily a crying infant. Home- and community-oriented approaches should be designed to address barriers and improve EBF rates.
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Keywords: exclusive breastfeeding; breast-milk intake; non-breast-milk water intake; breastfeeding barriers

Introduction

The WHO recommends exclusive breastfeeding (EBF) until 6 months of age with the introduction of complementary foods thereafter, and continued breastfeeding until 2 years of age (Kramer and Kakuma, 2004). Despite this recommendation, the rates and duration of EBF are still low and unsatisfactory, and <35% of all infants worldwide are exclusively breastfed for at least 4 months (Heinig, 2004). In many societies, infants <6 months receive water, tea and juices (Labbok and Krasovec, 1990) in addition to breast milk (BM). Demographic and health surveys carried out in the Bangladesh and the Nepal report the prevalence of EBF among infants under 6 months of age to be as low as 42.5% and 53.1%, respectively (Mihrshahi *et al.*, 2010; Pandey *et al.*, 2010). Reports from the National Family Health Survey of India (Patel *et al.*, 2010) indicate that only 46.4% of the infants <6 months and 58.3% of infants <4 months of age are exclusively breastfed. EBF up to 6 months of age and continued breastfeeding up to 12 months has been ranked the most effective child survival intervention for preventing under-five mortality (Jones *et al.*, 2003).

The Baby-Friendly Hospital initiative has been associated with a statistically significant annual increase in rates of EBF among infants <2 and 6 months in 14 developing countries (Abrahams and Labbok, 2009). An Indian study has shown that when breastfeeding was initiated early, prelacteal feeds were less common and the intake of supplementary feeds like milk and fluids during the hospital stay was significantly lower in baby-friendly hospitals (Breastfeeding Promotion Network of India, 2000). However, the actual volumes of BM

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and non-breast-milk (NBM) water intakes (indicating liquids or solids introduced other than BM) of infants born in these settings have not been studied at home, to confirm whether these practices were continued by mothers.

This study was therefore designed to capture the compliance to EBF at home in the first 6 months of life, and the barriers to successful breastfeeding, in an urban setting where breastfeeding counseling is a routine practice in the hospitals. A prospective observational study among pregnant women in the last trimester of pregnancy and their infants until 6 months of postnatal life was conducted to assess intakes of BM and NBM water at 1, 3 and 6 months of age using deuterium dilution and a compartmental analysis method (IAEA, 2010), and to assess their role in supporting infant growth.

Materials and methods

Study setting and population

The present study was a prospective observational study of infants from birth to 6 months of age, in St John's Medical College Hospital, Bangalore, India, which is a baby-friendly tertiary care urban hospital drawing patients of diverse socioeconomic status, from urban slums to high income residential areas. The Institutional Ethical Review Board at St John's Medical College Hospital approved all study procedures, and a signed informed consent was obtained from each study subject at enrollment.

Study population

Pregnant women aged 18–40 years, who planned to exclusively breastfeed their infants until 6 months of age, and who were in the last trimester of pregnancy, were invited to participate in the study. Women with multiple pregnancies and any illness were excluded. Of 65 women who consented to be part of the study, 58 were recruited into the study. During the study, eight mother–infant pairs were lost to follow-up such that 50 mothers and their infants completed the study successfully. Information on sociodemograpy at baseline, and anthropometric data in the last trimester of pregnancy and postpartum were collected. A single 24-h dietary recall from the mother, of the infant's solid or liquid intake on a typical day of the week during the deuterium study, was captured at 1, 3 and 6 months. In addition, maternal and infant morbidity details were collected, while BM and NBM water intakes were assessed for the mother–infant pairs at 1, 3 and 6 months.

Sociodemographic and anthropometric information

Information on age, education, parity, occupation, income and obstetric history was obtained. A digital balance (Soehnle, Murrhardt, Germany) was used to record the weights of all mothers to the nearest 100 g, and height was measured to the nearest 0.1 cm. The nude weight of the infant was measured using a portable pediatric weighing scale (accurate to 100 g, Salters, Kent, UK), within 48 h of birth. Weights at the following months were measured on the days fixed for appointment when each child would attain 1, 3 and 6 months of age. Infant length was measured using an infantometer (locally constructed) to the nearest 0.1 cm. Mid upper-arm circumference was measured to the nearest 0.1 cm using a plastic measuring tape. Head circumference was measured at a level passing from supraorbital protuberance anteriorly and occipital protuberance posteriorly using a non-elastic accurately scaled standard tape to the nearest 0.1 cm. All anthropometric measurements within 48 h of birth and at months 1, 3 and 6 of infant age were done by a trained research assistant in duplicates and the mean was recorded. Infant weight for height (WHZ), height for age (HAZ), weight for age (WAZ) and head circumference for age (HCZ) were calculated and presented as deviations in *Z*-scores (standard deviation) from the WHO reference population mean value for age and sex (World Health Organization, 2006).

BM and NBM oral intakes

BM and NBM water intake was measured using the 'dose-to-the mother' deuterium-oxide turnover technique at infant age of 1, 3 and 6 months (IAEA, 2010). A baseline sample of 2 ml of saliva from the mother and infant were collected on day 0, after which the mother received an oral dose of 30 g ^2H_2O. The dose was measured to the nearest 0.01 g. Saliva samples from the mother (days 1, 2, 13 and 14) and from the infant (days 1, 2, 3, 4, 13 and 14) were then collected over a period of 14 days. Adsorbant sorbettes (Salimetrics, Suffolk, UK) were used to collect saliva samples (2 ml), which were centrifuged at 3500 r.p.m., and stored at $-20\,^{\circ}C$ for subsequent analysis of their ^2H enrichment, using Fourier Transformed Infrared Spectrophotometry (IAEA, 2010). The BM and NBM water intake by isotopic method was not available for two mother–infants pairs at month 3 and one mother–infant pair at month 6. The weight of the infant was recorded on day 0 and day 14. Intake of BM and water from NBM sources was calculated by fitting the isotopic enrichment data to a mathematical model for water turnover in the mother–infant pair and the transfer of water from mother to the baby, based on assumptions as described earlier (IAEA, 2010). An infant was considered to be exclusively breastfed by the deuterium dilution method if the NBM water intake was 0 ml/day, and by the 24-h dietary recall method, if the mother reported that she was not feeding her infant any solid/liquid food or water other than BM.

Dietary intake and morbidity details

A single 24-h dietary recall from the mother, of the infant's solid or liquid intake (if any) on a typical day of the week during the deuterium study, was captured at months 1, 3 and

6 of infant age. Infant intake of tonics and vitamin/mineral supplements was also recorded. Maternal and infant morbidity symptom data (for diarrhea, dysentery, upper respiratory illness and fever) were collected by maternal recall at months 1, 3 and 6. The proportion of infants with any particular illness was calculated.

Statistical analysis

Statistical analyses were carried out with SPSS (version 16.0, SPSS, Chicago, IL, USA). Data are expressed as means and standard deviations. The maternal body weight and infant WAZ, HAZ, WHZ and HCZ were compared between delivery/birth and postpartum months 1, 3 and 6 using repeated measures analysis of variance and *post hoc* pair wise comparisons were performed by paired *t*-test using Bonferroni adjustment. Only valid infant standardized values were considered for the analysis. The weight of the infant was compared with the 50th percentile value for WHO age- and gender-specific standard population by *t*-test for single mean. The comparison of BM and NBM water intake at months 1, 3 and 6 were done using repeated measures analysis of variance. The proportion of infants who were exclusively breastfed as well as those who were given oral water other than BM based on the deuterium dilution method and those who were given liquids and solids other than BM based on the 24-h dietary recall method were calculated. The 95% confidence intervals were also computed. Pearson's correlations were performed to examine associations between BM and NBM water intake of the infant and WAZ, WHZ, HAZ and HCZ at different time points. The level of significance was set at 0.05.

Results

Maternal baseline characteristics

The study mothers were on average 23.0 ± 2.9 years old, and ~76% were primiparous. All of the women had attained some level of education at school level or beyond. The women were not from a lower socioeconomic group as indicated by their median monthly household income of 9000 INR. Maternal employment status and gestational age at delivery are detailed in Table 1.

Table 1 Maternal sociodemographic characteristics at baseline[a]

Characteristics	
Age at delivery (years)[b]	23.0 ± 2.9
Gestational age at delivery (weeks by USG)[b]	39.1 ± 0.9
Parity[c]	
Primiparous	38 (76)
Multiparous	12 (24)
Educational level[c]	
Up to high school (10th grade)	24 (48)
12th grade and above	26 (52)
Employment status[c]	
Employed outside the home	9 (18)
Homemaker	41 (82)
Monthly household income (INR)[d]	9000 (5000, 15000)

Abbreviations: INR, Indian Rupee; USG, ultrasonography.
[a]$n = 50$.
[b]Mean (s.d.).
[c]*n* (%).
[d]Median (lower bound, upper bound).

Mother and infant anthropometric measurements

Table 2 represents the maternal and infant anthropometric measurements. The maternal body weight at delivery was significantly different from the body weights at postpartum months 1, 3 and 6 (all $P<0.001$). However, the weights at months 1, 3 and 6 were not different from each other. The body weights of the infants were significantly different ($P<0.05$) in comparison with WHO standards throughout the early postnatal period of 6 months for boys and from birth to month 3 for girls. There was a significant decline in the HAZ ($P=0.001$) and HCZ ($P<0.001$) among the infants from birth to 6 months of age. However, the WHZ significantly increased ($P<0.001$) from birth to month 6. At 6 months of age, 20% of the infants were underweight (WAZ < −2s.d.). Stunting (HAZ < −2 s.d.) increased from 2% at month 1 to 16% at month 6.

Infant BM and NBM water intake

The BM and NBM water intake of the infants have been detailed in Table 3. Intake of BM significantly declined from 166 to 87 ml/kg/day and NBM significantly increased from 23 to 51 ml/kg/day from month 1 to 6 ($P<0.01$). Figure 1 shows that there was a significant negative correlation between BM and NBM water at months 3 ($r=-0.59$, $P<0.001$) and 6 ($r=-0.61$, $P<0.001$) indicating that liquid and solid foods introduced by the mothers displaced BM. There was a considerable discrepancy between reported EBF practices and what was measured by the isotopic measurements. None of the mothers reported having started introducing complementary feeding (CF) at month 1, however, the isotopic method showed that 44% of the babies had received oral water (and presumably accompanying foods) and only 56% were exclusively breastfeeding even as early as month 1. At month 3, only 10% of the mothers reported having introduced CF, whereas data from the isotopic method showed that 77% of the babies had received oral water. By month 6, only 14.2% of infants were being exclusively breastfed based on isotopic data; however, the dietary recall method showed that 36% of the infants were exclusively breastfed.

In the few women who reported having introduced CF at 3 months of age, commercial cereal formula, cow's milk and malted finger millet preparations were commonly used.

Table 2 Anthropometric characteristics of mother and infant[a]

	At delivery/birth	*Month 1*	*Month 3*	*Month 6*
Maternal postpartum anthropometry				
Maternal postpartum body weight (kg)	60.8 ± 13.0 ($n=50$)	57.3 ± 11.6 ($n=50$)[b]	56.3 ± 11.5 ($n=50$)[b]	56.7 ± 12.4 ($n=50$)[b]
Maternal postpartum BMI	25.6 ± 5.2 ($n=50$)	25.1 ± 6.2 ($n=50$)	23.5 ± 4.5 ($n=50$)	23.8 ± 4.8 ($n=50$)
Infant anthropometry				
Infant age (days)	—	30 ± 2 ($n=50$)	94 ± 13 ($n=48$)	182 ± 17 ($n=50$)
Body weight (kg)	2.7 ± 0.5 ($n=50$)	3.8 ± 0.6 ($n=50$)	5.6 ± 0.8 ($n=48$)	7.2 ± 1.0 ($n=50$)
Body length (cm)	49.6 ± 1.7 ($n=48$)	53.2 ± 2.4 ($n=49$)	60.8 ± 2.8 ($n=47$)	65.8 ± 2.7 ($n=50$)
MUAC (cm)	10.2 ± 2.6 ($n=48$)	10.7 ± 0.9 ($n=50$)	12.8 ± 0.9 ($n=48$)	13.6 ± 1.1 ($n=50$)
HC (cm)	33.6 ± 1.2 ($n=48$)	35.8 ± 1.3 ($n=50$)	39.3 ± 1.1 ($n=48$)	41.6 ± 1.3 ($n=50$)
WAZ	−1.21 ± 0.93 ($n=48$)	−1.21 ± 1.27 ($n=50$)	−1.07 ± 1.14 ($n=48$)	−0.80 ± 1.30 ($n=50$)
HAZ	0.04 ± 0.95 ($n=47$)	−0.62 ± 1.38 ($n=49$)[b]	−0.39 ± 1.32 ($n=47$)	−0.71 ± 1.26 ($n=50$)[b]
WHZ	−2.00 ± 1.43 ($n=47$)	−1.03 ± 1.81 ($n=48$)[b]	−1.00 ± 1.65 ($n=47$)[b]	−0.34 ± 1.48 ($n=49$)[b]
HCZ	−0.52 ± 0.98 ($n=48$)	−1.08 ± 1.10 ($n=50$)[b]	−1.04 ± 0.94 ($n=48$)[b]	−1.17 ± 1.02 ($n=50$)[b]
Underweight[c]	11 (22)	8 (16)	13 (26)	10 (20)
Wasting[c]	20 (40)	15 (30)	13 (26)	5 (20)
Stunting[c]	1 (2)	7 (14)	6 (12)	8 (16)

Abbreviations: BMI, body mass index; HAZ, height for age *Z* score; HC, head circumference; HCZ, head circumference for age *Z* score; MUAC, mid upper-arm circumference; WAZ, weight for age *Z* score; WHZ, weight for height *Z* score.
[a]All values are mean ± s.d.
[b]Significantly different from measurements at delivery/birth ($P<0.05$) by Bonferroni-adjusted pair wise comparisons in RMANOVA (repeated measures analysis of variance).
[c]*n* (%).

Table 3 Breast-milk and non-breast-milk water intakes

Infant intakes (1–6 months)	*Month 1*	*Month 3*	*Month 6*
Breast-milk intake (ml/day)[a]	627 ± 170 ($n=50$)	744 ± 183 ($n=48$)	608 ± 235 ($n=49$)
Breast-milk intake (ml/kg/day)[a]	166 ± 38 ($n=50$)	132 ± 29 ($n=48$)[b]	87 ± 34 ($n=49$)[b,c]
Non-breast-milk water intake (ml/day)[a]	83 ± 115 ($n=50$)	160 ± 147 ($n=48$)	389 ± 455 ($n=49$)
Non-breast-milk water intake (ml/kg/day)[a]	23 ± 34 ($n=50$)	28 ± 27 ($n=48$)	51 ± 52 ($n=49$)[b,c]
Proportion of mothers reporting having started complementary foods based on 24-h dietary recall[d]	0 ($n=50$)	0.1 (0.02, 0.18) ($n=50$)	0.64 (0.51, 0.77) ($n=50$)
Proportion of infants given water other than breast milk assessed by deuterium dilution method[d]	0.44 (0.30, 0.58) ($n=50$)	0.77 (0.65, 0.89) ($n=48$)	0.85 (0.75, 0.95) ($n=49$)
Proportion of infants exclusively breastfed assessed by deuterium dilution method[d]	0.56 (0.42, 0.70) ($n=50$)	0.22 (0.10, 0.34) ($n=48$)	0.14 (0.04, 0.24) ($n=49$)

[a]All values are mean ± s.d.
[b]Significantly different from intake at month 1 ($P<0.05$) by Bonferroni-adjusted pair wise comparisons in RMANOVA (repeated measures analysis of variance).
[c]Significantly different from intake at month 3 ($P<0.05$) by Bonferroni-adjusted pair wise comparisons in RMANOVA.
[d]Values are expressed as proportion (95% confidence interval: lower bound, upper bound).

The commonly introduced foods by 6 months of age were commercial milk formula, commercial cereal formula, cow's milk, biscuits, mixed grain porridges and traditional Indian breakfast foods such as steamed rice and lentil cakes. At 6 months, the common barriers to EBF were a crying infant, with the assumption that this was due to inadequate milk (75%), 'working outside the home' (11%) and 'advice by elders in the family' (14%).

BM and NBM water intake in relation to infant growth

Table 4 shows the correlation between BM and NBM water intakes and WAZ, HAZ, WHZ and HCZ. BM intake significantly correlated with WAZ (month 1: $r=0.56$, $P<0.001$; month 3: $r=0.60$, $P<0.001$) and WHZ (month 1: $r=0.59$, $P<0.001$; month 3: $r=0.58$, $P<0.001$) at months 1 and 3. NBM intake showed a significant negative correlation with WHZ ($r=-0.33$, $P=0.02$) at month 3 and correlated positively with WAZ ($r=0.37$, $P=0.01$) and HAZ ($r=0.30$, $P=0.03$) at month 6. There were no differences in the minor symptomatic illnesses reported between EBF and non-EBF infants, and none of the infants received any vitamin/mineral supplements or tonics during the study period.

Discussion

The principal finding of the present study is the low intake of BM and early introduction of CF in infants born to mothers in a baby-friendly hospital, where counseling for

breastfeeding is the norm. The results of our study indicate that over the period of 6 months there was a significant decline in the weight-specific BM intake of the infant and a proportional increase in NBM water intake. BM intake among our infants was lower than those reported by others using test weighing method (Coulibaly *et al*., 2004; Islam *et al*., 2008) and 'dose to mother' deuterium dilution method (Galpin *et al*., 2007; Moore *et al*., 2007). Our urban findings are in contrast to the most recent findings of the pooled analysis of data across five continents, which reported the human milk intake to remain >800 ml/day until 6–7 months of age among infants from rural areas (Da Costa *et al*., 2010). The only Asian country included in this pooled analyses was Bangladesh, which reported a higher mean BM intake of 863 ml/day among all infants ($n=94$) from rural Bangladesh at 2–3 months of age and 883 ml/day among exclusively breastfed infants ($n=73$; Moore *et al*., 2007).

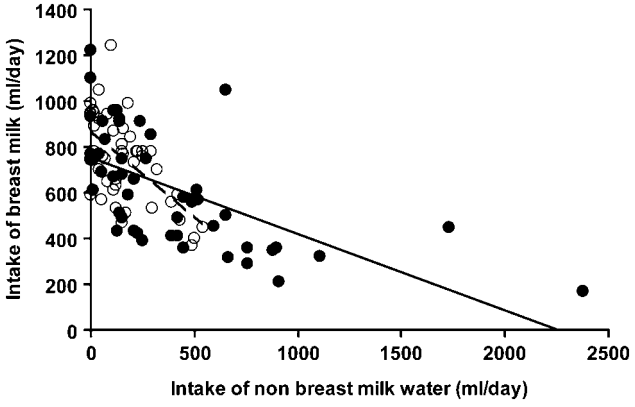

Figure 1 Association between intake of BM and NBM water at 3 and 6 months. Open circles represent the association between BM intake and NBM water intake at month 3 ($n=48$, Pearson's correlation coefficient, $r=-0.587$, $P<0.001$) and the closed circles represent the association between BM intake and NBM intake at month 6 ($n=49$, $r=-0.608$, $P<0.001$).

We studied those mothers who had assured us that they would exclusively breastfeed their infants until 6 months of age. However, data from the isotopic method showed that more than half of the mothers were not exclusively breastfeeding. This is similar to community studies in rural North India, based on questionnaires, which have shown that only 30 and 10% of the women exclusively breastfed their infants by 4 and 6 months of age, respectively. This may have been due to the lack of breast-feeding counseling, as this lack has been shown to be significantly associated with decreased rates of EBF (Kishore *et al*., 2008). However, in this urban sample, intensive breast-feeding counseling was performed for each mother while at the hospital, and education, parity and income were not related to the early introduction of CF.

Based on interviews with the mother, the primary reason for the early introduction of CF was a crying infant, perceived by her to be due to insufficient BM. In addition, having to report back to her job, advice by elders in the family and the perceptions of the mother herself were key reasons for early introduction of CF. All the mothers reported having received counseling during pregnancy and immediately after giving birth. However, back at home, they found it difficult to follow the counseling advice they had been given. It may be that the effectiveness of counseling sessions in bringing about desirable improvement in lactation needs consideration. To emphasize this, for example, no significant differences in maternal plans to breastfeed exclusively were observed in baby-friendly versus non-baby-friendly hospitals (Breastfeeding Promotion Network of India, 2000), and this was attributed to the lack of intensive counseling during the antenatal, perinatal and postnatal period. Therefore, it is possible that they are not motivated enough. It is also possible that mothers are more influenced by elders in their family who have different perceptions, or that they lack confidence in their ability to breastfeed. In this complex sociological setting, a meta-analysis of qualitative studies in developed countries on breastfeeding rates has revealed that

Table 4 Association between breast-milk, non-breast-milk water intakes and WAZ, WHZ, HAZ and HCZ

	Month 1		*Month 3*		*Month 6*	
	r[a]	P-*value*	r	P-*value*	r	P-*value*
Breast-milk intake						
WAZ	0.564 ($n=50$)	<0.001	0.597 ($n=48$)	<0.001	0.165 ($n=49$)	0.258
WHZ	0.585 ($n=48$)	<0.001	0.575 ($n=47$)	<0.001	0.270 ($n=48$)	0.060
HAZ	−0.030 ($n=49$)	0.829	0.054 ($n=47$)	0.721	−0.067 ($n=49$)	0.647
HCZ	0.280 ($n=50$)	0.049	0.212 ($n=48$)	0.147	0.125 ($n=49$)	0.392
Non-breast-milk water intake						
WAZ	−0.068 ($n=50$)	0.640	−0.093 ($n=48$)	0.528	0.366 ($n=49$)	0.010
WHZ	−0.111 ($n=48$)	0.452	−0.333 ($n=47$)	0.020	0.182 ($n=48$)	0.215
HAZ	0.079 ($n=49$)	0.589	0.272 ($n=47$)	0.060	0.299 ($n=49$)	0.030
HCZ	−0.278 ($n=50$)	0.050	0.048 ($n=48$)	0.747	0.220 ($n=49$)	0.129

Abbreviations: HAZ, height for age *Z* score; HCZ, head circumference for age *Z* score; WAZ, weight for age *Z* score; WHZ, weight for height *Z* score.
[a]Pearson's correlation coefficient.

mothers tended to rate social support as more important than health service support (Mclnnes and Chambers, 2008).

Overcoming barriers to breastfeeding probably requires designing of community-oriented approaches that extend beyond the hospital settings and encourage not only women to exclusively breastfeed until 6 months of age but also target the elders in the family who are likely to influence the decision-making process. There may also be gaps in the existing practices followed in the pediatric outpatient departments, wherein little importance is given to breastfeeding counseling during the follow-up visits to the hospital. Therefore, subsequent follow-up visits made by the mother to the hospital for infant immunizations and growth monitoring should be considered as critical points of interaction of the mother with the health practitioner where the importance of EBF is reiterated. The use of mobile phone technology may be another innovative means of keeping in touch with newly delivered mothers to improve their compliance to EBF.

Growth is a good indicator against which lactation performance can be judged (Alam *et al*., 2003). Infants born to mothers in this study were shorter and thinner at birth and throughout the postnatal period of 6 months compared with the reference WHO population; however, neither intakes of BM nor NBM at months 1, 3 and 6 were associated with birth weight. It is critical to note that the WHO standards reflect growth patterns in those who were predominantly breastfed for at least 4 months, whereas infants in our study group received a significant amount of oral water other than BM much earlier. A higher intake of BM was related to higher WAZ and WHZ until 3 months of age, however, beyond 3 months, growth was primarily driven by CF, and infants with higher intakes of NBM water showed higher WAZ and HAZ. The relation of NBM with WAZ and HAZ later may be primarily because BM intakes were inadequate, and that infants who received adequate amounts of hygienically prepared CF, without accompanying morbidity, showed a greater improvement in growth parameters. However, lack of EBF during the first 6 months of life and inappropriate CF have been reported to be important risk factors for infant and childhood morbidity and mortality (World Health Organization/UNICEF, 2003). It is also possible that morbidity associated with CF may be higher in lower socioeconomic groups. In addition, the introduction of NBM water in very early postnatal life has not been shown to confer any additional benefits on infant growth (Cohen *et al*., 1994).

To our knowledge, this study is the first of its kind from India using the accurate and objective deuterium dilution technique to assess BM and NBM water intakes among infants and thereby capture home-based compliance to EBF. The limitation of the present study is its small sample size. In addition, we do not have any qualitative research that might provide an in-depth understanding of the psychosocial and biological factors behind the reasons stated by the mothers for the early introduction of CF. In conclusion, this study has demonstrated low BM intakes and early introduction of CF among infants born to mothers in a baby-friendly hospital in South India. Further research is warranted to understand barriers to EBF and to study the short- and long-term outcomes of infant feeding practices, particularly in terms of body composition of the infant.

Conflict of interest

AVK is a member of the Kraft Health and Wellness Advisory Council; none of the other authors have any personal or financial conflict of interest.

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Author contributions

The authors' responsibilities were as follows: AVK: conception and design of the study, statistical analyses, and writing of manuscript; TMS: data collection, sample analysis and calculations, data entry, statistical analyses and writing of manuscript; TT: statistical analysis; and SB: conception and design of the study and execution of the study.

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Original Article

Breast milk zinc transfer and early post-natal growth among urban South Indian term infants using measures of breast milk volume and breast milk zinc concentrations

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Abstract

Zinc (Zn) deficiency in infancy and early childhood is of public health concern in developing countries. This study aimed to longitudinally assess Zn intake of urban South Indian term infants in the first 6 months of life using measures of breast milk (BM) volume and BM Zn concentrations and, additionally, to study the effect of BM Zn intake on infant length and weight gain. BM intake by the deuterium dilution technique, BM Zn concentration at months 1, 3 and 6, as well as serum Zn level at months 3 and 6 were assessed in 50 mother–infant pairs. BM intake significantly declined from 627 mL day^{-1} at month 1 to 608 mL day^{-1} at month 6 ($P < 0.01$). BM Zn concentration and intake significantly declined from month 1 to month 6 ($P < 0.001$ for both). Mean infant serum Zn level at months 3 and 6 were 93.0 ± 27.1 and 99.6 ± 30.1 μg dL^{-1}, respectively. Infant BM Zn intake at months 1 and 3 was not associated with the weight and length gain between 1–3 and 3–6 months, respectively. Zn intake from BM, maternal BM Zn content and serum Zn levels were not significantly different between small-for-gestational age and appropriate-for-gestational age infants. Therefore, among urban south Indian term infants less than 6 months of age, BM Zn intakes were low, owing to low volumes of BM intake, despite BM Zn concentrations being in the normal range. Promotion of breastfeeding and thereby increasing the volumes of milk produced is a first important step towards improving Zn intake among infants.

Keywords: infant, breast milk zinc content, breast milk zinc intake, serum zinc, length gain and weight gain.

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Introduction

Zinc (Zn) deficiency in infancy and early childhood is an important cause of stunting, increases infectious disease morbidity and mortality due to diarrhoea and pneumonia, and therefore is of public health concern especially in developing countries (Hambidge *et al*. 1997; Hambidge & Krebs 2003). Zn deficiency is responsible for approximately 4% of the worldwide morbidity and mortality burdens of young children (Black *et al*. 2008).

For term infants with normal birthweight, Zn requirements are generally assumed to be met by exclusive breastfeeding due to high bioavailability of Zn in human milk (Krebs *et al*. 1996). However, at 5–6 months of age, infants may become marginally Zn deficient due to the physiological decline in breast milk (BM) Zn concentrations, making the infants vulnerable to sub-optimal Zn intakes and thereby impaired growth (Walravens *et al*. 1992; Krebs 1999). In addition, a decreased intake of Zn from BM due to lower volumes of BM intake, attributed to poor

breastfeeding practices, may predispose infants to an elevated risk of Zn deficiency even before 6 months. Equally, an infant born small-for-gestational age (SGA) may be at increased risk for Zn deficiency in comparison to infants born appropriate-for-gestational age (AGA) as they may either have increased requirements for Zn during the first few months of life which could limit their potential for catch up growth during these months (Castillo-Duran *et al*. 1995), or they may have lower neonatal reserves of Zn (Hambidge & Krebs 2003) and/or have impaired Zn absorption or increased endogenous losses (Krebs *et al*. 2006). As SGA infants form a large proportion of low-birthweight infants in developing countries (Hambidge & Krebs 2003), it would also be of interest to assess intakes and levels of Zn among these infants as well. In general, there is a paucity of data on Zn intakes among breastfed infants less than 6 months of age in India based on measured volumes of BM intake and BM Zn concentrations.

We therefore assessed the longitudinal Zn intake of urban South Indian term infants in the first 6 months of life by measuring actual volumes of BM consumed using a non-invasive and accurate 'dose to mother' deuterium dilution method and measuring the maternal BM Zn concentrations. In addition, we assessed if Zn intake from BM was associated with the infant's length and weight gain.

Methods

Study setting

The present study was a prospective observational study of infants from birth to 6 months of age in St. John's Medical College Hospital, Bangalore, India, which is a tertiary care urban hospital drawing patients of diverse socio-economic status, from urban slums to high-income residential areas. The Institutional Ethical Review Board at St. John's Medical College Hospital approved all study procedures and a signed informed consent was obtained from each study subject at enrolment.

Study population

Pregnant women aged 18–40 years, who planned to exclusively breastfeed their infants until 6 months of age, and who were in the last trimester of pregnancy, were invited to participate in the study. Women with multiple pregnancies and any illness were excluded. Of 65 women who consented to be part of the study, 58 were recruited into the study. During the study, 8 mother–infant pairs were lost to follow-up such that 50 mothers and their infants completed the study successfully and were followed up at months 1, 3 and 6.

Socio-demographic and anthropometric information

Information on age, education, parity, occupation, income and obstetric history was obtained by interview from the study subjects. A digital balance (Soehnle, Murrhardt, Germany) was used to record the weights of all mothers to the nearest 100 g, and height was measured to the nearest 0.1 cm using a locally constructed stadiometer. Maternal body mass index (BMI) was calculated as weight in kilograms by the square of height in meters (kg m^{-2}). The nude weight of the infant was measured to the nearest 10 g using a portable paediatric weighing scale (Salters, Tonbridge, Kent, UK), and length was measured using a locally constructed infantometer to the nearest 0.1 cm within 48 h of birth. Only term infants (gestational age >37 weeks) were considered. The newborn was designated SGA if the birthweight was

Key messages

- Among urban south Indian term infants less than 6 months of age, BM Zn intakes were low; owing to low volumes of BM intake, despite BM Zn concentrations being in the normal range.
- BM Zn intake was not related to infant weight gain and length gain.
- Promotion of breastfeeding and thereby increasing the volumes of milk produced is a first important step towards improving Zn intake among infants.

<10th percentile for the gestational age at birth compared with the reference population and AGA if the birthweight was >10th percentile for the gestational age at birth (World Health Organization 1995). Infant weight and length were also recorded at months 1, 3 and 6. Infant weight-for-length, length-for-age and weight-for-age were calculated and presented as deviations in *z*-scores [standard deviation (SD)] from the World Health Organization (WHO) reference population mean value for age and gender (World Health Organization 2006). All anthropometric measurements were done by a trained research assistant in duplicate and the mean was recorded.

Dietary data

A pre-tested interviewer-administered 24-h recall was used to assess the intake of nutrients in mothers at 1, 3 and 6 months post-partum. While administering the questionnaire, standard measures were placed before the mother to quantify the portion size of each food item. The nutrient values were obtained from the nutrient database developed (Bharathi *et al*. 2008) and intake of Zn was thereby calculated. A 24-h dietary recall from the mother, of the infant's solid or liquid intake (if any) during the previous day of the interview, was also captured at months 1, 3 and 6. Mothers were asked to quantify the amount of the formula used (usually in powder form) to prepare the feed using standard measures and the nutrient composition (Zn content) of that particular formula was recorded. In addition, questions were asked about leftovers and care was taken to record the amount consumed after excluding the leftovers. In the case of biscuits, the number consumed was noted and the nutrient content was calculated using the nutrient information available on the pack. In the case of homemade porridges and other breakfast foods, the nutrient database previously described was used to compute intakes of Zn.

Information on infant morbidity

Infant morbidity symptom data (for diarrhoea, dysentery, fever, cough and cold) was collected by maternal recall at months 1, 3 and 6. The percentage of infants with any particular illness was calculated.

BM and non-breast milk (NBM) water intake

BM intake and NBM water intake was measured using the 'dose-to-the mother' deuterium-oxide turnover technique at the infant age of 1, 3 and 6 months (IAEA 2010). A baseline saliva sample of 2 mL from the mother and infant was collected on day 0, after which the mother received an oral dose of 30 g ^2H_2O, measured to the nearest 0.01 g. Saliva samples were then collected using adsorbent sorbettes (Salimetrics, Suffolk, UK) from the mother and the infant over a period of 14 days. The samples were then centrifuged at 3500 rpm and stored at –20°C for subsequent analysis of their ^2H enrichment using Fourier transformed infrared spectrophotometry (IAEA 2010). Intake of BM and water from NBM sources [an indicator of complementary foods (CFs) introduced] was calculated by fitting the isotopic enrichment data to a mathematical model for water turnover in the mother–baby pair and the transfer of water from mother to the baby, based on assumptions as described earlier (IAEA 2010).

BM Zn and serum Zn analysis

Women were asked to provide three mid-feeding BM samples (5 mL), one each in the morning, afternoon and evening. Milk samples were collected in acid-washed plastic bottles to avoid Zn contamination, by hand expression after the nipple and areola were cleaned with deionised water and dried. The samples were centrifuged and stored immediately at –20°C in tightly sealed containers until analysis. BM Zn was determined using flame atomic absorption spectrophotometry (Ice 3500, Thermo, Cambridge, UK) (Rajalakshmi & Srikantia 1980). Briefly, BM samples were centrifuged to remove solid particles, and supernatant was diluted with 0.2% nitric acid solution. The Zn in the sample was emulsified with a drop of 1% triton X-100 (Sigma-Aldrich, St. Louis, MO, USA), aspirated into an acetylene flame and measured at 213.9 nm. Zn standards (Merck, Darmstadt, Germany) ranging from 0.2 to 1.0 ppm of zinc, prepared in 0.2% nitric acid solution, were used to create calibration curves. The intra- and inter-day assay coefficients of variability (CVs) were 1.6% and

2.0%, respectively. Zinc intake of the infant day^{-1} was calculated as the product of BM intake day^{-1} in mL day^{-1} and BM Zn concentration mL^{-1} of BM. A 2 mL of fasting blood sample from the mother and 1 mL of non-fasting blood sample from the infant was drawn by venipuncture and collected into trace metal-free vacuette tube (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) at 3 and 6 months post-partum. Once the blood samples were obtained, they were immediately stored in cool boxes and transferred to the laboratory for further processing. The median time between the blood draw and the spinning and the separation of serum from the clot was 3 h. Blood samples for Zn analysis were centrifuged at 3000 rpm for 10 min, and the serum was kept frozen at –80°C until analysis. The serum Zn concentration was measured using flame absorption spectrophotometer (Ice 3500, Thermo) (Smith *et al*. 1979). We were able to obtain infant blood samples only for 31 and 32 infants at 3 and 6 months, respectively. The intra- and inter-day assay CVs were 1.4% and 1.4%, respectively. An infant was considered to be Zn deficient if the serum Zn level was below 65 μg dL^{-1}, while the mother was considered to be Zn deficient if the serum Zn level was below 70 μg dL^{-1} (Executive Summary 2007).

Statistical analysis

Statistical analyses were carried out with SPSS (version 16.0, SPSS, Chicago, IL, USA). Data are expressed as numbers and percentages, mean ± SD for normally distributed data and median (25th, 75th percentile) for non-normal data. Infant BM intake and NBM water intake were compared between months 1, 3 and 6 using repeated measures analysis of variance (RMANOVA), and post hoc pairwise comparisons were performed by paired *t*-test using Bonferroni adjustment. As the BM Zn concentrations of the morning, afternoon and evening BM samples did not differ significantly from each other at any of the time points, the mean BM Zn for each month was used in further analysis. The BM Zn concentrations and BM Zn intakes were compared between months 1, 3 and 6 using Friedman test and pairwise comparisons were performed by Wilcoxon rank test using Bonferroni adjustment. The weight and the length gain from months 1 to 3 and 3 to 6 were calculated as the difference in the weights and lengths between these time points. Separate linear regressions of weight and length gain on BM Zn intakes were performed. Potential confounders such as NBM water intake, infant age, gender, and infant weight and length at birth and month 3 were adjusted for in the model. Independent *t*-test was used to examine whether BM intake was significantly different between SGA and AGA infants, while Mann–Whitney *U*-test was used to study whether maternal BM Zn content, BM Zn intakes and serum Zn (mothers and infants) were significantly different between the SGA and AGA groups. Two-sided *P*-value <0.05 was considered statistically significant.

Results

Socio-demographic and anthropometric characteristics of infants and their mothers at birth/delivery

Socio-demographic and anthropometric characteristics of infants and their mothers at birth/delivery are detailed in Table 1. The study mothers were on average 23.0 ± 2.9 years old, and approximately 76% of them were primiparous. The median family income was 9000 Indian Rupees, indicating that the mothers were not from a low socio-economic status. Nearly half of the infants born in the study had birthweight less than the 10th percentile for the gestational age at birth.

Infant and maternal dietary intake, maternal BM Zn content and serum Zn

Maternal and infant dietary intake, serum Zn and anthropometric measurements are presented in Table 2. BM intake significantly declined from 627 to 608 mL day^{-1} and NBM water intake increased from 83 to 389 mL day^{-1} from month 1 to month 6 ($P < 0.01$ for both). The BM Zn concentrations declined significantly from month 1 to month 6 ($P < 0.001$), and so did the Zn intakes from BM ($P < 0.001$). The mothers reported having introduced

Table 1. Socio-demographic and anthropometric characteristics of study infants and their mothers at birth/delivery

Maternal antenatal characteristics (last trimester of pregnancy) ($n = 50$)	Mean ± SD or n (%)
Age (years)	23.0 ± 2.9
Total family income (INR)*	9000 (5000, 15 000)
Parity	
Primiparous	38 (76)
Multiparous	12 (24)
Weight(kg)	60.4 ± 12.1
Height (cm)	154 ± 5.9
Body mass index	25.4 ± 5.1
Type of delivery	
Spontaneous vaginal delivery	34 (68)
Caesarean section	16 (32)
Infant characteristics at birth ($n = 50$)	
Gestational age at birth by ultrasonography (weeks)	39.1 ± 0.9
Birth outcome	
Small-for-gestational age	24 (48)
Appropriate-for-gestational age	26 (52)
Gender	
Male	29 (58)
Female	21 (42)
Birthweight (kg)	2.7 ± 0.5
Birth length (cm)†	49.6 ± 1.7
Weight-for-age z-score	–1.21 ± 0.93 ($n = 48$)
Weight-for-length z-score	–2.00 ± 1.43 ($n = 47$)
Length-for-age z-score	0.04 ± 0.95 ($n = 47$)

INR, Indian Rupees; SD, standard deviation. *Median (25th, 75th percentile). †$n = 48$.

CFs only by 6 months; however, data from the deuterium method indicated that NBM water was introduced among 44% of the infants at month 1, 77% at month 3 and 85% at month 6, leaving only 14.2% of the infants being exclusively breastfed by 6 months of age. The commonly introduced foods by 6 months of age were fortified commercial cereal formula, cow's milk, biscuits, mixed grain porridges and traditional Indian breakfast foods such as steamed rice and lentil cakes. The contribution of Zn from CF among the infants whose mothers reported having started CF ($n = 16$) at 6 months was 0.90 (0.45, 1.12) mg day^{-1}.

The maternal energy and Zn intakes were much lower than the recommended dietary allowance for lactating women throughout the post-partum period of 6 months; however, the mean maternal serum Zn levels were above the cut-off levels for Zn deficiency. The proportion of Zn deficient mothers was 0.32 and 0.16 at months 3 and 6, respectively. In addition, the maternal BM Zn concentrations were not low, nor were they correlated with maternal dietary Zn intake and Zn status. However, the Zn intakes of the infants calculated as the product of the BM volume consumed in mL day^{-1} and the BM Zn concentration mL^{-1} of BM appeared to be low. Nevertheless, the mean infant serum Zn levels appeared to be in the normal range, with a very small proportion of the infants being deficient in Zn. We observed no significant correlations between infant Zn intake and serum Zn levels. The BM Zn intakes as well as the serum Zn concentrations were not significantly different between male and female infants (data not shown). The serum Zn concentrations were also not significantly different among infants who received Zn from CF in addition to BM Zn (data not shown).

Infant BM Zn intake and serum Zn in relation to growth

The bodyweights of the infants were significantly lower ($P < 0.05$) in comparison to WHO standards throughout the early post-natal period of 6 months. Table 3 represents the infant BM Zn intakes in relation to weight and length gain. The mean weight gain of infants was 1.88 ± 0.59 and 1.47 ± 0.59 kg, translating to a daily weight gain of 31.3 and 15.2 g day^{-1} during the 1- to 3-month and 3- to 6-month periods, respectively. The mean length gain among infants was 7.1 ± 2.4 and 5.7 ± 1.8 cm in the 1- to 3-month and 3- to 6-month periods, respectively. The weight and length gain were not significantly different between boys and girls, except for the weight gain from 1 to 3 months ($P = 0.01$) which was higher in boys. The weight and length gain were also not significantly different among infants who received Zn from CF in addition to BM Zn (data not shown).

The weight and length gain from 1 to 3 and 3 to 6 months were not associated with the BM Zn intakes at months 1 and 3, respectively, when examined in separate linear regression analyses. The regression

Table 2. Infant and maternal dietary intake, maternal breast milk zinc content and serum zinc

Parameters*	Month 1	Month 3	Month 6
Infant age (days)	30 ± 2 ($n = 50$)	94 ± 13 ($n = 48$)	182 ± 17 ($n = 50$)
Infant dietary intake			
Infant BM intake (mL day^{-1})	627 ± 170 ($n = 50$)	744 ± 183 ($n = 48$)	608 ± 235† ($n = 49$)
Infant NBM water intake (mL day^{-1})	83 ± 115 ($n = 50$)	160 ± 147 ($n = 48$)	389 ± 455‡ ($n = 49$)
BM zinc content (mg L^{-1})§	2.50 (2.03, 3.26) ($n = 50$)	1.37 (0.89, 1.79) ($n = 47$)	1.17 (0.80, 1.60) ($n = 50$)
Zinc intake from BM (mg day^{-1})§	1.60 (1.13, 2.19) ($n = 50$)	0.88 (0.67, 1.36) ($n = 47$)	0.68 (0.51, 1.13) ($n = 49$)
Zinc intake from BM (mg kg^{-1} day^{-1})§	0.43 (0.32, 0.53) ($n = 50$)	0.15 (0.11, 0.22) ($n = 47$)	0.10 (0.06, 0.16) ($n = 49$)
Zinc intake from reported CFs (mg day^{-1})¶	–	–	0.90 (0.45, 1.12) ($n = 16$)
Maternal dietary intake			
Energy intake/kg bodyweight (kcal kg^{-1} day^{-1})	32.7 ± 12.8 ($n = 50$)	34.6 ± 13.1 ($n = 48$)	32.3 ± 13.8 ($n = 48$)
Zinc intake (mg day^{-1})	7.6 ± 2.9 ($n = 50$)	7.7 ± 2.3 ($n = 48$)	7.8 ± 2.8 ($n = 50$)
Infant and maternal zinc levels			
Maternal serum zinc (μg dL^{-1})	–	83.5 ± 26.5 ($n = 40$)	92.2 ± 25.1 ($n = 43$)
Infant serum zinc (μg dL^{-1})	–	93.0 ± 27.1 ($n = 31$)	99.6 ± 30.1 ($n = 32$)
Proportion of zinc deficient mothers**	–	0.32 (0.18, 0.46) ($n = 40$)	0.16 (0.05, 0.27) ($n = 43$)
Proportion of zinc deficient infants**	–	0.16 (0.03, 0.29) ($n = 31$)	0.09 (–0.01, 0.19) ($n = 32$)
Infant anthropometry			
Bodyweight (kg)	3.8 ± 0.6 ($n = 50$)	5.6 ± 0.8 ($n = 48$)	7.2 ± 1.0 ($n = 50$)
Body length (cm)	53.2 ± 2.4 ($n = 49$)	60.8 ± 2.8 ($n = 47$)	65.8 ± 2.7 ($n = 50$)
Weight-for-age *z*-score	–1.21 ± 1.27 ($n = 50$)	–1.07 ± 1.14 ($n = 48$)	–0.80 ± 1.30 ($n = 50$)
Weight-for-length *z*-score	–0.62 ± 1.38 ($n = 49$)	–0.39 ± 1.32 ($n = 47$)	–0.71 ± 1.26 ($n = 50$)
Length-for-age *z*-score	–1.03 ± 1.81 ($n = 48$)	–1.00 ± 1.65 ($n = 47$)	–0.34 ± 1.48 ($n = 49$)

BM, breast milk; CFs, complementary foods; NBM, non-breast milk. *Parameters are mean ± standard deviation/median (25th, 75th percentile). †BM intake at month 3 is significantly different from that of month 1 ($P < 0.05$) and BM intake at month 6 is significantly different from that of month 3 ($P < 0.05$) by Bonferroni adjusted pair wise comparisons in repeated measures analysis of variance (RMANOVA). ‡NBM water intake at month 6 is significantly different from that of month 1 ($P < 0.05$) and month 3 ($P < 0.05$) by Bonferroni adjusted pairwise comparisons in RMANOVA. §BM Zn content and BM Zn intake significantly different between months 1, 3 and 6 by Friedman test, $P < 0.001$. All pair wise comparisons were significant by Wilcoxon rank test, Bonferroni adjusted *P*-value <0.001. ¶The median Zn intake from CFs has been reported only for those infants whose mothers reported having given CFs. **Values are expressed as proportion (95% confidence interval: lower bound, upper bound).

for 1–3 months was adjusted for infant NBM water intake at month 1, infant age at month 1, gender and birthweight/length. The regression for 3–6 months was adjusted for infant NBM water intake at month 3, infant age at month 3, gender and weight/length at month 3. Serum Zn at months 3 and 6 was not associated with weight or length gain in the months preceding those time points.

Table 3. Infant zinc intakes in relation to post-natal growth*

Months 1–3							
Weight gain†				Length gain†			
	B	95% CI	*P*-value		B	95% CI	*P*-value
BM Zn intake at month 1	–0.03	–0.25, 0.19	0.785	BM Zn intake at month 1	0.34	–0.83, 1.53	0.555
NBM water intake at month 1‡	–0.76	–2.16, 0.63	0.278	NBM water intake at month 1‡	–6.34	–13.5, 0.84	0.082
Infant age at month 1	–0.74	–1.54, 0.06	0.069	Infant age at month 1	4.80	0.70, 8.90	0.023
Infant gender	–0.48	–0.82, –0.15	0.005	Infant gender	–0.08	–1.84, 1.67	0.925
Infant birthweight	0.07	–0.27, 0.42	0.665	Infant birth length	–0.20	–0.72, 0.32	0.437
Months 3–6							
Weight gain†				Length gain†			
	B	95% CI	*P*-value		B	95% CI	*P*-value
BM Zn intake at month 3	–0.13	–0.41, 0.15	0.362	BM Zn intake at month 3	0.62	–0.41, 1.66	0.231
NBM water intake at month 3‡	0.56	–0.67, 1.80	0.363	NBM water intake at month 3‡	–0.74	–5.46, 3.96	0.750
Infant age at month 3	0.06	–0.30, 0.44	0.714	Infant age at month 3	–2.16	–3.65, –0.67	0.006
Infant gender	–0.00	–0.38, 0.38	0.984	Infant gender	–0.88	–2.21, 0.44	0.186
Infant weight at month 3	0.05	–0.17, 0.29	0.609	Infant length at month 3	–0.25	–0.51, 0.01	0.061

BM, breast milk, CI, confidence interval; NBM, non-breast milk. *Multiple linear regression with weight and length gain from month 1 to month 3 and from month 3 to month 6 as dependent variables and BM Zn intakes at months 1 and 3, NBM water intakes at months 1 and 3, infant age at months 1 and 3, infant gender, birthweight and length and infant weight and length at months 1 and 3 as independent variables. †Weight gain and length gain from month 1 to month 3 and from month 3 to month 6 are calculated as the difference in weight and length, respectively, during these time points. ‡NBM water intake at months 1 and 3 is calculated using 'dose-to-mother' deuterium dilution method.

The BM Zn intake and serum Zn levels were not significantly different between infants with and without reported minor symptomatic illnesses such as diarrhoea, fever, cough, cold and dysentery at months 1, 3 and 6. Diarrhoea was reported among 6% (3 out of 50), 12.8% (6 out of 47) and 14% (7 out of 50) of the infants at months 1, 3 and 6, respectively. Fever was reported among 4% (2 out of 50), 8.3% (4 out of 48) and 22% (11 out of 50) of the infants at months 1, 3 and 6, respectively. Cough was reported among 4% (2 out of 50), 29% (14 out of 48) and 20% (10 out of 50) of the infants at months 1, 3 and 6, respectively. Cold was reported among 14% (7 out of 50), 33% (16 out of 48) and 42% (21 out of 50) of the infants at months 1, 3 and 6, respectively. None of the infants had dysentery during the study period (data not shown).

Comparison between SGA and AGA infants

A comparison of the BM intakes and intakes of Zn through BM, maternal BM Zn content, maternal and infant serum Zn levels between SGA and AGA infants is presented in Table 4. None of these parameters were significantly different between the two groups. However, the weight-for-age *z*-scores were significantly lower at birth and months 1, 3 and 6 in SGA infants in comparison to AGA infants ($P < 0.001$ at birth and months 1 and 3, $P = 0.005$ at month 6). Similarly, the SGA infants had lower weight-for-length *z*-scores at birth ($P < 0.001$) and lower length-for-age *z*-scores at months 1 and 3 ($P = 0.034$ and $P = 0.011$, respectively). However, intakes of BM Zn or serum Zn were not related to any of these standardised anthropometric indices in either SGA or AGA infants (data not shown).

Discussion

In a prospective observational study to assess longitudinal concentrations of maternal BM Zn and Zn intakes among their infants at 1, 3 and 6 months of age from urban South India, we observed that BM Zn intakes were low, owing to low volumes of BM intake,

Table 4. Infant Zn intakes and serum Zn levels, maternal BM zinc content and infant anthropometric measures in term SGA and AGA infants

	Birth/Delivery	Month 1	Month 3	Month 6
Infant BM intake (mL day^{-1})				
SGA	–	620 (500, 757) ($n = 24$)	755 (575, 780) ($n = 22$)	500 (390, 750) ($n = 23$)
AGA	–	665 (507, 755) ($n = 26$)	775 (672, 940) ($n = 26$)	660 (440, 841) ($n = 26$)
P-value*	–	0.690	0.153	0.288
BM zinc content (mg L^{-1})				
SGA	–	2.42 (2.08, 3.23) ($n = 24$)	1.37 (0.84, 1.99) ($n = 21$)	1.24 (0.73. 1.57) ($n = 24$)
AGA	–	2.54 (1.83, 3.30) ($n = 26$)	1.35 (0.95, 1.74) ($n = 26$)	1.06 (0.85, 1.63) ($n = 26$)
P–value**	–	0.900	0.856	0.627
Zn intake from BM (mg day^{-1})				
SGA	–	1.48 (1.06, 2.33) ($n = 24$)	0.72 (0.56, 1.57) ($n = 22$)	0.62 (0.54, 1.00) ($n = 24$)
AGA	–	1.67 (1.26, 2.19) ($n = 26$)	0.93 (0.73, 1.33) ($n = 26$)	0.61 (0.43, 1.20) ($n = 26$)
P-value**	–	0.634	0.286	0.946
Maternal serum zinc (μg dL^{-1})				
SGA	–		82.8 (64.5, 106.3) ($n = 18$)	86.2 (72.2, 103.9) ($n = 22$)
AGA	–		76.9 (55.7, 101.5) ($n = 22$)	87.2 (73.0, 117.2) ($n = 21$)
P-value**	–	–	0.348	0.752
Infant serum zinc(μg dL^{-1})				
SGA	–		100.0 (72.6, 114.2) ($n = 11$)	98.3 (82.6, 135.8) ($n = 20$)
AGA	–		90.0 (67.5, 106.5) ($n = 20$)	89.3 (77.3, 109.3) ($n = 12$)
P-value**	–	–	0.433	0.276
Weight-for-age *z*-score				
SGA	–1.94 ± 0.74	–1.84 ± 1.24	–1.68 ± 1.11	–1.33 ± 1.38
AGA	–0.55 ± 0.49	–0.62 ± 1.01	–0.55 ± 0.89	–0.32 ± 1.02
P-value*	0.000	0.000	0.000	0.005
Weight-for-length *z*-score				
SGA	–2.82 ± 1.10	–1.37 ± 1.96	–1.33 ± 1.81	–0.72 ± 1.69
AGA	–1.27 ± 1.29	–0.71 ± 1.65	–0.73 ± 1.50	–0.00 ± 1.19
P-value*	0.000	0.214	0.229	0.162
Length-for-age *z*-score				
SGA	–0.21 ± 0.69	–1.04 ± 1.26	–0.92 ± 1.39	–0.85 ± 1.18
AGA	0.27 ± 1.13	–0.21 ± 1.39	0.03 ± 1.11	–0.42 ± 1.04
P-value*	0.082	0.034	0.011	0.179

AGA, appropriate-for-gestational age; BM, breast milk; SGA, small-for-gestational age. *Two-sided *P*-values using independent *t*-test; **two-sided *P*-values using Mann–Whitney *U*-test.

despite BM Zn concentrations being in the normal range. Despite the lower maternal dietary Zn intake, the lactating women in this study were able to maintain higher levels of BM Zn concentration at months 1, 3 and 6 in comparison to the age-specific BM Zn concentrations generated from the combined analysis of 33 studies that investigated the BM Zn content of non-supplemented mothers of healthy term infants (Brown *et al.* 2009). This may be brought forth by a physiological adaptive response during lactation, such as increased absorption, reduced excretion from the kidney and the intestine, and mobilisation and

re-equilibration from maternal Zn pools (Krebs 1998). A study among Chinese lactating women at 2 months post-partum has demonstrated that they were able to secrete 2 mg Zn day^{-1} into their BM, in the presence of marginal Zn intakes of 7.6 mg Zn day^{-1} (Sian *et al*. 2002). The Zn concentrations in the BM of mothers in our cohort declined from 1 to 6 months post-partum; however, this physiological pattern of decline in BM Zn concentrations as lactation progresses has been previously reported from other studies as well (Krebs *et al*. 1995; Krebs 1999). It is possible that as maternal zinc pools decline, a progressive decline in BM zinc may be seen. The decline in BM Zn concentrations of the mothers in our cohort could not be compensated by an increase in BM intake by the infant, as over the period of 6 months there was a decline in the weight-specific BM intake of the infant and a proportional increase in NBM water intake. The BM intake of our infants was lower than that reported by others using test weighing method (Coulibaly *et al*. 2004; Islam *et al*. 2008) and 'dose–to-mother' deuterium dilution method (Galpin *et al*. 2007; Moore *et al*. 2007).

The Zn intakes of infants from BM reported in our study (calculated as the product of the BM volume consumed in mL day^{-1} and the BM Zn concentration mL^{-1} of BM) were lower at months 1, 3 and 6 in comparison to calculated Zn intakes based on measured BM Zn concentrations and an assumed BM intake volume of 780 mL day^{-1} (FNB & IOM 2001), as well as the measured Zn intake of term, AGA infants born to mothers in the United States (Krebs *et al*. 1994). However, they were in close agreement with the simulated mean daily Zn transfer in BM to exclusively breastfed infants at months 1, 3 and 6 (Brown *et al*. 2009). With the assumptions of urinary and sweat Zn losses to be 20 $\mu g\ kg^{-1}\ day^{-1}$, endogenous fecal Zn losses to be 50 $\mu g\ kg^{-1}\ day^{-1}$ and Zn required for new tissue accretion to be 20 $\mu g\ g^{-1}$ weight gain or 30 $\mu g\ g^{-1}$ lean tissue gain (Butte *et al*. 2002), the total requirement of Zn for infants (boys and girls) would be 0.94, 0.86 and 0.79 mg day^{-1} at 1, 3 and 6 months, respectively. Assuming a mean fractional absorption of 0.55 for BM Zn (Krebs 1999), in the present study, the amount of Zn available for absorption from BM at 1, 3 and 6 months would only amount to 0.88, 0.48 and 0.37 mg day^{-1}, respectively, indicating increasing gap between the measured and recommended dietary intake of Zn.

However, despite the low intakes of BM Zn, the serum Zn levels of the infants appeared to be in the normal range. It may be that the requirements of Zn are partially offset by mobilisation of hepatic Zn bound to metallothionein in the first months of life (Zlotkin & Cherian 1988). It may also be that diarrhoeal diseases were not a major cause of morbidity among our children and, therefore, increased fecal Zn losses may not be an important factor in determining Zn requirements among these infants. In addition to these, we know from the deuterium data that infants were receiving water other than BM (either as water alone or as part of food) as early as month 1, which the mothers were not reporting. The reasons for this need further exploration using qualitative data. It could be possible that these CFs were contributing to the total intake of Zn among infants and thereby resulting in normal serum Zn concentrations. Equally, one may argue that there could have been some leaching of Zn from the red blood cells into the serum between the time of blood draw and the processing of the sample, which may have led to an increase in Zn levels. In addition, serum Zn measurements may not reflect individual Zn status (Golden 1989) and the use of an exchangeable zinc pool may have been a more robust marker of Zn status.

Infants born to mothers in this study were shorter and thinner at birth and throughout the post-natal period of 6 months compared with the reference WHO population; however, the growth velocity appeared adequate. BM Zn intakes at months 1 and 3 were not associated with the change in weight or length during the period of 1–3 or 3–6 months. To support a daily weight gain of 31 g day^{-1} (months 1–3) and 15.5 g day^{-1} (months 3–6) observed among our infants, the estimated Zn retention would be approximately 0.62 and 0.30 mg day^{-1}, respectively. The observed Zn intakes might have been adequate to support this gain in weight in the present study; equally, these intakes may not have been enough to enable catch up growth to the WHO standards, at any point in time during the 6-month study period. In addition, we could speculate effects of low Zn intakes

among these children on body composition, as 10 mg Zn supplements day^{-1} among peri urban Guatemalan children aged 81.5 ± 7.0 months has shown a greater increase in median triceps skin-fold *z*-score and a smaller deficit in median mid-arm circumference *z*-score compared with the placebo group (Cavan *et al*. 1993).

Equally noteworthy is the observation that 48% of the infants born to mothers in our cohort were SGA. Prematurely born SGA infants from United States have shown to have smaller exchangeable Zn pool size at birth in comparison to AGA infants, indicating a compromised Zn status at birth (Krebs *et al*. 2006). Large-scale supplementation studies of Zn among breastfed SGA infants in developing countries have demonstrated significant improvements in growth and reduced morbidity and mortality (Sazawal *et al*. 2001). However, such an effect has not been demonstrated among AGA infants in whom the Zn content of the BM is considered adequate to meet their needs for Zn during the first few months of life (Krebs 1999). Post-natal requirements for Zn may be higher in SGA infants, or they may be consuming lower volumes of BM and thereby lower amounts of Zn, or alternatively their mother may have lower concentrations of Zn in their BM. We explored these possibilities among mother–infant pairs in our cohort; however, results from our study did not demonstrate any significant differences in either intakes of BM Zn, maternal BM Zn concentrations or Zn levels between SGA and AGA infants. Results from a similar study in Bangladesh demonstrate that BM Zn concentrations and total BM Zn transfer were not significantly different between mothers of SGA and AGA infants when assessed at 1, 3 and 6 months post-partum (Islam & Brown 2010). It is very likely that the aetiology of growth retardation is multifactorial and affected by deficits of energy or several other limiting nutrients such as protein, iron and vitamin A, D or C, apart from low Zn intakes.

To our knowledge, this study is the first of its kind from India using the accurate and objective deuterium dilution technique to assess BM Zn intake among infants less than 6 months of age. This study demonstrated that among urban south Indian term infants less than 6 months of age, BM Zn intakes were low, owing to low volumes of BM intake, despite BM Zn concentrations being in the normal range. BM Zn intakes were not related to weight gain and length gain among these infants. However, this study is limited by its small sample size and therefore may have low power to detect potentially clinically significant effects in the multiple linear regression models. The effect of low Zn intakes on weight and length gain in stunted or low weight population merits further evaluation using well-designed intervention studies with greater sample size. Promotion of breastfeeding and thereby increasing the volumes of milk produced is a first important step towards improving Zn intake among infants.

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Conflicts of interest

Dr. Anura V Kurpad is a member of the Kraft Health and Wellness Advisory Council. His honoraria go entirely to charity. None of the other authors have any personal or financial conflict of interest.

Contributions

TMS collected, analysed and interpreted the data, and wrote the first draft of the manuscript; TT provided statistical guidance in data analysis and contributed to the writing of the manuscript; PT guided the analyses of breast milk zinc and serum zinc, and contributed to the writing of the manuscript; SB was involved in the conception, design and execution of the study; SMV contributed to the writing of the manuscript; and AVK conceived and designed the study, interpreted

the results and contributed to the writing of the manuscript. All co-authors participated in manuscript preparation and critically reviewed all sections of the text for important intellectual content.

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