School of Public Health

The Relationship of Maternal Micronutrient Intakes of Vitamin B_{12}, Vitamin $B_{6,}$ Folate and Calcium on Intrauterine Growth Retardation and Birth weight: A Prospective Cohort Study of Urban South Indian Pregnant Women

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STATEMENT OF ORIGINAL AUTHORSHIP

The work contained in this thesis has not been previously submitted for a degree or diploma at any other higher educational institutional. To the best of my knowledge and belief, the thesis contains no material previously written by another except where due reference is made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

Pratibha Dwarkanath

26th September 2011

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ABBREVIATIONS

LBW	Low birth weight
SGA	Small for gestational age
IUGR	Intrauterine growth retardation
WHO	World Health Organisation
ICMR	Indian Council of Medical Research
DOHaD	Developmental origins of adult disease
PIH	Pregnancy induced hypertension
Hcy	Homocysteine
PAL	Physical activity level
AGA	Appropriate for gestational age
IUD	Intrauterine death
24-hours dietary recall	24-HDR

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ABSTRACT

The period of intrauterine growth and development is one of the most vulnerable periods in the human life cycle. The weight of the infant at birth is a powerful predictor of infant growth and survival, and is dependent on maternal health and nutrition during pregnancy. Prevention of low birth weight (LBW; <2500 g), which affects nearly 30% of infants born in India, is a public health priority. Low birth weight includes infants born prematurely (<37 weeks of gestation) or are small for gestational age (SGA; <10^{th} % for gestational age). The majority of LBW infants in India and in most developing countries are a result of SGA. LBW is a strong predictor for size in later life because SGA infants seldom catch-up to normal size during childhood. Maternal nutrition is an important factor from a public health point of view because it is modifiable through appropriate public health interventions. In urban Indian populations, despite the routine antenatal program, there has been a high prevalence of LBW and SGA babies. Micronutrients such as vitamin B_{12}, vitamin B_6 and folate that are involved in the 1-C methyl metabolism are thought to play a key role in fetal programming. Studies have shown that vitamin B_{12} deficiency leads to hyperhomocysteinemia especially in populations that consume predominantly cereal based diet, as seen in India. Dietary calcium is known to be related to pregnancy - induced hypertension (PIH); a morbidity affecting ~11% of first pregnancies. PIH also increases the risk of adverse birth outcomes.

I established a prospective cohort study of 637 pregnant women at St. John's Medical College Hospital, Bangalore, India. These pregnant women were followed antenatally for their dietary intakes, health status and birth outcomes.

The food frequency questionnaire (FFQ) is commonly used in epidemiological studies for assessing dietary intakes. I validated FFQ against multiple 24-hour dietary recalls (24-HDR) and observed a significant correlation between the two methods for vitamin B_{12}, vitamin B_6 and folate intakes. Correlations between the dietary intakes assessed by FFQ and the blood biomarkers (micronutrient status) indicated a good correlation between energy- adjusted vitamin B_{12} and micronutrient status.

Intakes of vitamin B_{12}, vitamin B_6, folate and food groups rich in these vitamins were related to adverse birth outcomes. Intakes of vitamin B_{12} in the 1st and 2nd trimester of pregnancy correlated negatively with homocysteine (Hcy) status and positively with the birth weight. Vitamin B_{12} intakes also correlated negatively with Hcy concentrations in the 1st and 2nd trimester of pregnancy. I found that low intakes of cereals and dairy products in the 2nd trimester of pregnancy were associated with SGA.

Evidence shows that micronutrient deficiencies exist during pregnancy in developing countries. In the 1st trimester approximately 50% of the pregnant women were vitamin B_{12} deficit, 30% had vitamin B_6 deficiency and 12% with folate deficiency. Almost 38% of the subjects had elevated Hcy levels at the 1st trimester. Maternal vitamin B_{12} showed significant negative correlation with plasma Hcy levels within trimester after adjusting for other micronutrients. Women categorised as vitamin B_{12}, vitamin B_6 or folate deficient and those who had hyperhomocysteinemia in the 1st trimester of pregnancy had a high prevalence of adverse birth outcomes such as LBW, SGA and preterm births. High maternal plasma Hcy concentration in the 1st trimester seemed to be a determinant of LBW after adjusting for potential confounders.

Several lines of evidence show that calcium and calcium - rich food groups may play an important role in determining adverse birth outcomes particularly preterm births. I observed that mothers of preterm babies and LBW had low intakes of calcium and calcium - rich food groups. Pregnant women with PIH had significantly higher incidence of preterm births. Calcium - rich food groups include dairy products and cereals and I have seen an association between low dairy intakes and high prevalence of SGA babies. These findings suggest that combination of nutrients as in food groups would be responsible for the adverse birth outcomes.

Collectively, the results presented in this thesis indicate a preventive role for calcium in PIH and a beneficial role of specific food groups (cereals and dairy) in reducing the incidence of SGA and preterm births. Women with low maternal vitamin B_{12} status and hyperhomocysteinemia have higher risk of having a LBW baby. My findings would encourage interventional studies to delineate the cause-

effect relationship between maternal dietary / nutritional influences on maternal morbidity and birth outcomes.

Structure of Thesis

This thesis is presented in eight chapters. One has been re-submitted for publication after review (chapter 4), and is hence presented in the journal format. All others are written as standard chapters in partial readiness for submission as journal papers. I present a general overview of the contents of each chapter and their salient findings.

Chapter 1 Introduction

- I explore the links between diet and birth outcomes with emphasis on India and developing countries where the population consumes predominantly cereal-based vegetarian diets. The importance of micronutrients, vitamin B_{12}, vitamin B_6 and folate is their involvement in the methylation cycle. Dietary calcium is known to contribute to the pathogenesis of PIH and early delivery. The conceptual framework of this PhD thesis is outlined.

  The chapter also clarifies that the term intrauterine growth retardation (IUGR) is now replaced with small for gestational age (SGA). Hence in the subsequent chapters I will refer to SGA as one of the birth outcomes.

Chapter 2 Review of literature

- This chapter covers the details of the methionine cycle, the B vitamins and its importance in the growth and development of the fetus. The interaction of these nutrients causes elevation of the intermediary metabolite called homocysteine (Hcy) and hence the role of Hcy in adverse birth outcomes is also mentioned. The levels of these and other nutrients in Indian diets and the reasons for believing that they may be limiting serves as the basis for initiating the studies described in this thesis. While there are gaps in pointing out the beneficial role of a particular single nutrient, food groups (a combination of nutrients) and their role in determining adverse birth outcomes is discussed. Further this chapter describes in detail the work done so far on the role of calcium in pathogenesis of PIH and adverse birth outcomes.

Chapter 3 Methodology

Details of the study design and the protocols, the questionnaires used, assessment of the biochemical markers etc are described. The general characteristics of the study subjects are described and the statistical tools employed are also mentioned.

Chapter 4

Validity of a semi quantitative food frequency questionnaire in the dietary assessment of south Indian pregnant women.

- This chapter describes the relative validity of the FFQ used in the studies and its usefulness; in comparison to 24 hr dietary recall method (24-HDR), as the reference tool. The dietary intakes of vitamin B_{12}, vitamin B_6 and folate obtained from FFQ were also compared with the biomarkers (micronutrient serum status).
- The pros and cons of the methods are discussed in detail and the advantage of using FFQ in an epidemiological study is mentioned.

Chapter 5

Influence of maternal dietary intakes of B vitamins (vitamin B_{12}, vitamin B_6 and folate) and vitamin B rich food groups on LBW, SGA and preterm births.

This chapter investigates the relationship between dietary intakes of micronutrients of the methionine cycle; vitamin B_{12}, folate and vitamin B_6 and birth outcomes.

- Among all the food groups assessed, it was seen that mothers with SGA babies had significantly lower nutrient intakes of protein, fat, carbohydrate, vitamin B_{12} and folate. They also consumed less cereals and milk products in the 2^{nd} trimester of pregnancy.
- Since cereals and milk products are also rich sources of calcium, and studies indicate that calcium plays a role in pathogenesis of PIH and in addition to birth outcomes particularly the preterm births, further role of calcium in adverse birth outcomes was investigated.

Chapter 6

Relationship of maternal biomarkers of B vitamins (vitamin B_{12}, vitamin B_6, folate) on homocysteine on and its effects on birth weight and birth outcomes; LBW, SGA and preterm births.

This chapter deals with the correlations between each individual micronutrient status (important in the methylation cycle) with an intermediary metabolite; Homocysteine (Hcy) in South Indian pregnant women and further explores its relationship with baby anthropometric parameters and birth outcomes.

- It was seen that antenatal serum levels of vitamin B_{12} and vitamin B_6 correlated negatively with Hcy levels, and also that the maternal status of these micronutrients were reflected in the fetal cord blood implying the transfer of nutrients from mother to the baby.
- Maternal Hcy status in the 1st trimester of pregnancy was a determinant of the neonatal Hcy status and for LBW babies.

Chapter 7

Influence of maternal dietary calcium and calcium rich food group intakes on pregnancy induced hypertension, SGA, LBW and preterm births.

This particular chapter deals with the associations between intakes of calcium rich foods and PIH and birth outcomes. The prevalence of PIH in the study subjects is mentioned. As part of the antenatal care, our hospital has routine supplemented with calcium (1000 mg/d) together with other micronutrients such as iron and folic acid. We have explored a range of ways of expressing calcium intake; from diet alone, from diet plus supplement and from calcium rich food groups in relation to pathogenesis of PIH and adverse birth outcomes.

- Mothers of preterm babies and LBW were found to have lower intakes of calcium and calcium rich food groups.
- Women with PIH had higher incidence of preterm babies.

Chapter 8 Summary

This concluding chapter brings together the overall findings of each chapter on nutrient intake/status and maternal/fetal morbidity. I revisit the postulated scheme of potential pathways and delineate where the outcomes of this thesis have clarified the expected model.

SIGNIFICANCE OF PhD

This PhD thesis has employed three approaches: maternal nutrient intake, maternal food group intakes and maternal & fetal nutrient status, in addressing the adverse birth outcomes of LBW, SGA and preterm births. In developing countries such as India, the prevalence of LBW and SGA has been on the rise. 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. The DOHaD concept suggests that non-communicable diseases have their origins in early life. The risk progressively accumulates throughout the life course and the disease manifest in later life. A number of experiments have highlighted an important role for 1-C metabolism in nutritional programming and suggest that this could be achieved by dietary manipulation of methyl donors like vitamins B_{12} and folate. Therefore this PhD elucidates the role of B vitamin intakes / food group rich in B vitamins and status during pregnancy in unveiling their role individually as well as in combination to adverse birth outcomes. This PhD thesis also describes the role of calcium intake / supplements and calcium - rich food groups in pathogenesis of PIH which in turn increases the severity of adverse birth outcomes. In brief, this proposed PhD program has tried to uncover the interrelationships between certain B vitamins, the metabolic intermediary; homocysteine, and maternal and fetal health by providing a holistic view of the select nutrients. Specifically, efforts have been made in linking the status and intake of these nutrients to birth weight, SGA, preterm births and infant anthropometry at birth in an urban population. This prospective cohort study has tried to contribute valuable knowledge and information in public health nutrition in developing countries. This work has highlighted those food groups that are important in preventing adverse pregnancy (PIH) and birth outcomes (LBW, SGA and preterm births), which will help guide future recommendations for healthy eating practices, and possibly translate into operational procedures for individual counselling and population-based health programs.

CHAPTER 1
INTRODUCTION

1.1 BACKGROUND

India is a country in nutrition transition, in part due to rapid economic and technological growth, and displays the fallout of dual burdens of malnutrition and obesity. The incidence of poor fetal growth (LBW and SGA) is highest in developing countries (Agarwal et al., 2002) and is a public health priority (Engle 2004; WHO 1995). Pregnancy is a state of high nutritional demand due to changing maternal needs and the rising metabolic costs of the growing fetus (King 2000). Maternal nutrition has been a focus of research interest for over 100 years but has gained a tremendous boost since the work of Barker and colleagues came to light (Hales & Barker 1992). It is proposed that maternal characteristics before and during pregnancy influence fetal survival, growth, size and body composition, and function of various systems. Some of these effects are demonstrable at birth. Post-natal growth also makes important contribution to risk of later disease: rapid growth contributes to increased risk. These are manifest in risk factors related to structure (body composition) as well as function (beta cell function and insulin resistance) which contribute to preterm morbidity and mortality. The concept of DOHaD (Developmental origins of health and adult diseases) has revolutionized ideas in the etiology of chronic non-communicable disease. Barker's exposition on fetal origins that has served to highlight the importance of maternal nutrition to pregnancy related morbidities, fetal growth and development, as well as the flow through effects on chronic disease in adulthood. This has led to an explosion of interest in the 'fetal origins' hypothesis or more appropriately, DOHaD.

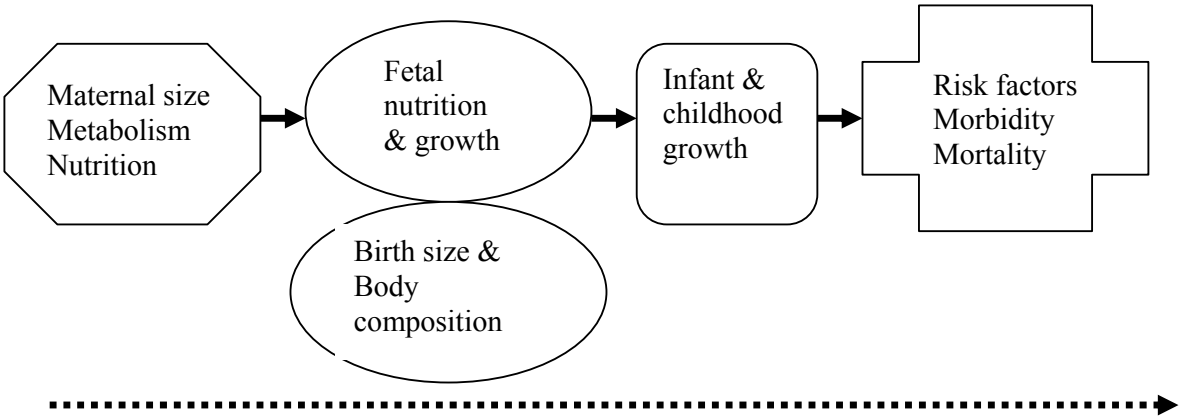


Figure 1. Schematic representation of developmental origins of health and adult diseases (DOHaD) concept.

The model suggests that non-communicable diseases have their origins in early life. The risk progressively accumulates throughout the life course and the disease manifests in later life (WHO 2001).

The majority of LBW infants in India and most developing countries are a result of IUGR (ACC/SCN 2000). Due to both high birth rates and a higher incidence of poor fetal growth, countries in South Asia including India have the highest rates of LBW in the world. Of all LBW infants born each year, 75% are born in Asia, 20% in Africa, and 5% in Latin America. In India, LBW nearly affects 30% of infants born. LBW has been associated with significantly higher perinatal and infant mortality (Ashworth 1998) and with higher risk of hospitalization and diarrhoea (Barros et al., 1992) and pneumonia (Chandra 1975) in the first two years of life. The post natal growth of these babies is also impaired (Barros et al., 1992) and such children remain small at 4 years of age (Gluckman 1993). They may also have developmental disabilities, including sensorineural and learning problems as well as limited cognitive development in childhood (Sorensen et al., 1997). Increasing evidence also has identified LBW as a risk factor for chronic diseases (Stein et al., 1996).

Several maternal factors are known causes of LBW, preterm and SGA namely; maternal pre-pregnancy weight, gestational weight gain, malnutrition (Siega-Riz et al., 1994; Kramer et al., 1995; Muthayya et al., 2006; Spinillo et al., 1998) and morbidity (Kaul et al., 1999) during pregnancy. In addition studies have shown that socio-demographic parameters such as maternal age, parity and education

are determinants of adverse birth outcomes for SGA and preterm births (Fraser et al., 1995; Muthayya et al., 2006).

In this PhD Thesis, the term intrauterine growth retardation (IUGR) is replaced with small for gestational age (SGA). IUGR refers to the condition resulting in growth retardation at birth and used often where there is evidence of abnormal genetic or environmental influences affecting the fetal growth. These infants are less than the third percentile (1 in 33 children), thus all IUGR infants are SGA but not all SGA infants are IUGR. The SGA is defined as infants born with birth weight less than 10^{th} percentile for the gestational age and gender on a standard growth chart. Hence in the subsequent chapters I have referred to SGA as one of the birth outcomes.

Maternal energy and protein deficiency is associated with IUGR (WHO 1995; Kramer 2002) and studies have shown a small but significant decrease in SGA babies in mothers supplemented with energy and proteins (Kramer 2002). Studies specifically relating protein intakes to birth weight outcomes are difficult to conduct in countries like India where protein intakes are closely related to energy intakes due to largely cereal based diets. This is true of both rural as well as urban sectors and protein and energy deficiency go hand in hand. While the diet is predominantly cereal based and intakes of fish and or meat is minimal, the protein requirements depend mostly on dairy products and cereals intakes. Micronutrient deficiencies have been associated with many conditions that contribute to maternal morbidity and LBW. International trials looking at effects of individual as well as combinations of micronutrients such as iron, folic acid, vitamin A, vitamin C and B-vitamins have demonstrated improved birth weights, reduced prematurity, pre-eclampsia and maternal mortality (de Onis et al., 1998; Ceesay et al, 1997; Rasmussen 2001; West et al., 1999; Fawzi et al., 1999; Ramakrishnan et al., 1999). Moreover studies have also shown positive relationship with food group intake (dairy, meat protein, green vegetables) and birthweight (Godfrey et al., 1996; Mannion et al., 2006; Kannade et al., 2008).

Previous experimental observations from our laboratory have demonstrated that vitamin B_{12} deficiency exists in pregnant Indian women, and that the risk of

delivering a LBW baby increases with increasing vitamin B_{12} deficiency (Muthayya et al., 2006a; Muthayya et al., 2006b). Gaps in our knowledge still exist concerning the relationship between nutritional intakes and status of pregnant women and maternal and child health outcomes. These include the relatively few studies that have addressed these issues in urban mothers, and the limited number of clinical trials of nutrient supplementation among women at risk of nutritional deficiencies, especially in India. Ramakrishnan and his colleagues (1999), extensively reviewed the role of a variety of micronutrients on maternal and fetal health outcomes, and concluded that although promising data existed for folate and some minerals (zinc, calcium and magnesium) with respect to improved health outcomes, inadequate data existed to make strong conclusions about B vitamins and other micronutrients. Further, food-based, rather than nutrient-based approaches are increasingly being used in the characterization of dietary recommendations and guidelines as a means of providing dietary guidance in ways that are understandable to the pregnant women (Millward & Jackson, 2004).

Deficiency of any of vitamin B_{12}, vitamin B_6 and folate involved in the methylation cycle, causes elevation of the intermediary metabolite; Hcy which is known to cause adverse birth outcomes. Further elevated Hcy is also known to cause PIH, a morbidity occurring during the second half of pregnancy (Sorensen et al., 1999). The relationship between PIH and adverse birth outcomes such as preterm, LBW and SGA has been established (Crowther et al., 1999; Belizan et al., 1991) while studies in relation to maternal calcium intakes and PIH have shown conflicting findings (Marya et al., 1987; Belizan et al., 1983; Levine et al., 1997). Few studies have shown a beneficial effect of calcium intakes in lowering the blood pressure during pregnancy and hence increasing gestational age at birth. Calcium supplementation is known to be beneficial only for women at high risk of gestational hypertension and in communities with low dietary calcium intake (Niromanesh et al., 2001). This evidence points towards the interrelation of these B vitamins and adverse birth outcomes but also possibly increasing the risk of adverse birth outcome through PIH. Therefore this thesis along with the B vitamins and food groups rich in B vitamins has also explored calcium intakes and calcium - rich food groups in relation to PIH and adverse birth outcomes.

I have hence established a prospective cohort to unveil the potential role of maternal nutrients, food group intakes and micronutrient status in determining adverse birth outcomes. The role of dietary calcium on PIH during pregnancy and adverse birth outcomes was also explored.

1.2 HYPOTHESIS

(i) Pregnant women with low dietary intakes of B vitamins particularly vitamin B_{12}, vitamin B_6 and folate, are at higher risk of developing adverse birth outcomes such as low birth weight, SGA and preterm births.

(ii) Low dietary calcium intake will relate to PIH, and in turn increase risk for adverse birth outcomes.

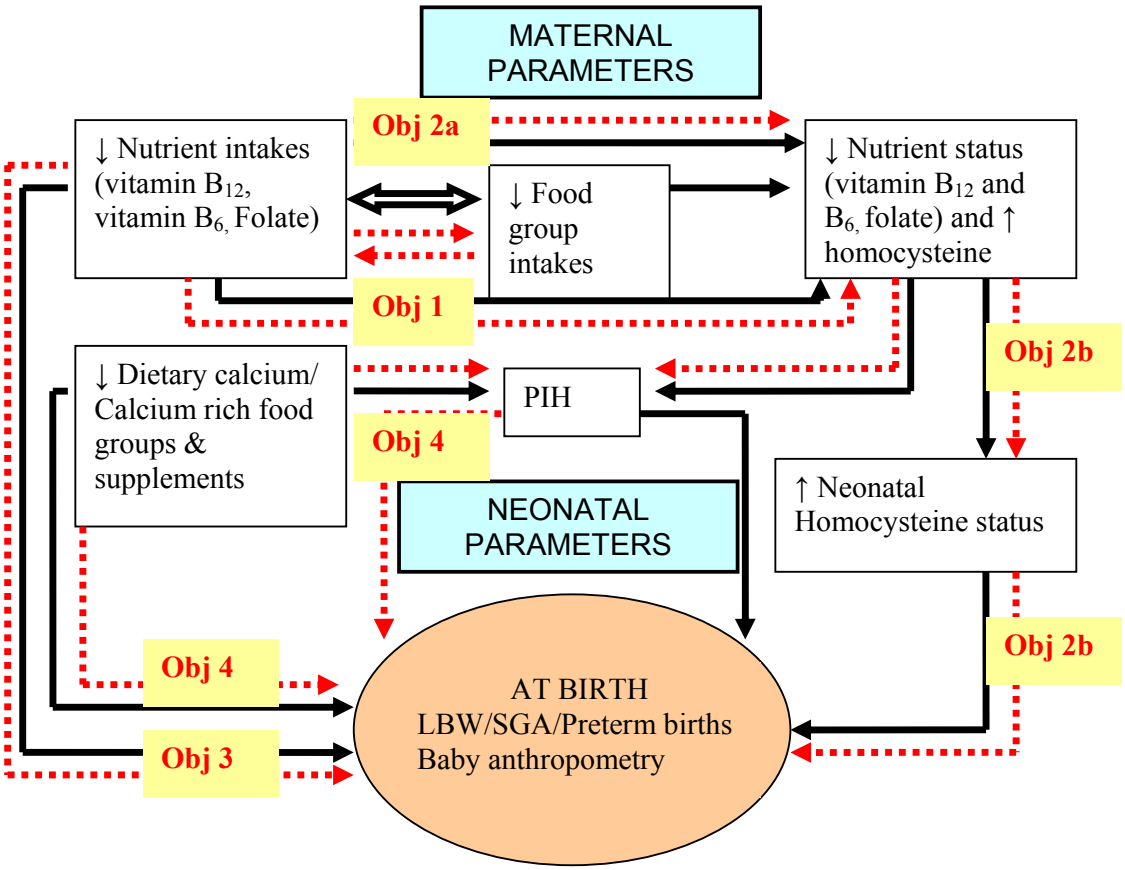


Figure 2. The conceptual framework of this PhD

Explanation

Indicate the associations from previous findings

Indicate the objectives of this PhD Thesis

1.3 OBJECTIVES

OBJECTIVE 1

To validate a semi quantitative food frequency questionnaire in the dietary assessment of south Indian pregnant women.

OBJECTIVE 2

To demonstrate the relationship between these specific B vitamin and food group intakes, and adverse birth outcomes.

OBJECTIVE 3

3a). To validate the relationship of maternal micronutrient status of vitamin B_{12}, folate & vitamin B_6 on hyperhomocysteinemia and

3b). To explore the association between maternal Hcy status on preterm birth, SGA babies and birth weight.

OBJECTIVE 4

To evaluate the influence of dietary calcium intake on the proportion of preterm and SGA babies.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Birth outcomes in India and general introduction to the subsequent sections.

2.1.1 *Introduction*

2.1.2 *Problems*

2.1.3 *Maternal parameters and adverse birth outcomes*

2.1.4 *Maternal morbidity and adverse birth outcome*

2.2 Importance of macronutrients and select nutrients such as folate, B_{12}, B_6 and calcium during pregnancy.

2.2.1 *Importance of macronutrients during pregnancy*

2.2.2 **VITAMIN B_{12}**

2.2.2.1 *Introduction*

2.2.2.2 *Vitamin B_{12} in diet*

2.2.2.3 *Role of vitamin B_{12} in methylation cycle*

2.3.2 **FOLATE / FOLIC ACID**

2.2.3.1 *Introduction*

2.2.3.2 *Folates in diet*

2.2.3.3 *The folate cycle*

2.2.4 **VITAMIN B_6**

2.2.4.1 *Introduction*

2.2.4.2 *Vitamin B_6 in diet*

2.2.4.3 *Role of vitamin B_6 in methylation cycle*

2.2.5 **CALCIUM**

2.2.5.1 *Introduction*

2.2.5.2 *Calcium in diet*

2.2.4.3 *Role of calcium in morbidity such as pregnancy induced hypertension (PIH)*

2.2.4.4 *Role of calcium in adverse birth outcomes*

2.3 Importance of food groups in relation to birth outcome (Table 2)

2.4 Interaction of select nutrients and birth outcomes

2.4.1 *Vitamin B_{12}* **(Table 3)**

2.4.2 *Folate* **(Table 4)**

2.4.3 *Vitamin B_6* **(Table 5)**

2.4.4 *Interrelationship between B vitamins and homocysteine and birth outcomes* **(Table 6)**

2.5 Importance of select nutrients in the pathogenesis of PIH

2.5.1 *Hyperhomocysteinemia and PIH* **(Table 7)**

2.5.2 *Calcium intake / supplements and PIH* **(Table 8)**

2.6 Methods of dietary assessment.

I have focused on the published literature over the last 20 years but have included some landmark papers from earlier years in the literature review section for this PhD thesis. The principal outcome was to capture the link between diet and the spectrum of birth outcomes that included the birth weight and categories of adverse birth outcomes. I have included studies employing diverse methodology and research designs (observational, prospective longitudinal, retrospective, randomized controlled trials as well as case control studies). The search included English articles on humans, without country specificity and involved searching for publications using electronic Database (MEDLINE; Pub Med, High Wire and the Cochrane Library). The key words used in the general search were: maternal vitamin B_{12}, vitamin B_6, folate intakes and birth outcomes, vitamin B_{12}, vitamin B_6 and folate status and birth outcomes, trimester, pregnancy, low birth weight, small for gestational age, preterm births, homocysteine, PIH, calcium intake, dietary methods, 24 hours dietary recall, food frequency questionnaire (FFQ).

2.1 Birth outcomes in India and general introduction to the subsequent sections

Subheadings:

Introduction, problems, factors affecting birth outcome (maternal parameters and maternal morbidity).

2.1.1 *Introduction*

Pregnancy is a critical period during which good maternal nutrition is a key factor influencing the health of both mother and child. Since this period requires additional nutrient input due to the growing fetus and placenta, as well as the metabolic and physiologic demand (King 2000) occurring within the mother. Nutrient intake during pregnancy and nutritional status before conception are both of utmost importance as they affect pregnancy outcomes (Cuco et al., 2003; Forsum et al., 2003). There are many pregnancy outcomes and these include length of gestation, fetal growth, birth defects, pre-eclampsia, and of childhood, such as cognitive development, blood pressure, adiposity and atopic disease (Botto et al., 1999; Bucher et al., 1996; Barker 1995; IOM 1990).

Maternal nutrition affects not only the mother in terms of morbidity but growth and development of the baby. This has been linked to the 'fetal origins' of adult diseases (FOAD) implying that the fetal programming for the adult diseases takes place in *utero* (Barker 1995). The evolution of this theory has been since 1960's with Neel's definition of the 'Thrifty genotype' (Neel 1962), Lucas (1991) definition of 'programming' (a process whereby a stimulus or insult (nutrient imbalance) applied at a critical or sensitive period of development results in long term or permanent changes in the structure or function of the organism) and the 'Thrifty phenotype'. The 'thrifty phenotype' was defined since it was traditionally believed that genetic susceptibility and adult faulty lifestyle leads to type 2 diabetes, a chronic non-communicable disease. The conventional model suggests a genetic predisposition which is thought to have originated in the hunter-gatherer days ('thrifty gene') and modern day lifestyle factors (abundant food and physical inactivity) which bring on the disease (Yajnik et al., 2004)

The FOAD concept is based on the finding that there is an inverse relationship between birth weight (or ponderal index, as a measure of 'thinness') and prevalence of diabetes, insulin resistance syndrome and coronary heart disease in later life implying that nutrition in fetal life is central to 'programming' or 'imprinting' of susceptibility to adult diseases (Hales & Barker 1992). This focused attention on the importance of intrauterine life as a determinant of later health. Although this association has been confirmed in many other populations including different ethnic groups in the USA, Chinese, Afro Caribbean, in South Africa, not all studies in India and elsewhere have agreed with this association. A study in south India showed that the risk of diabetes was not related to birth weight but to shorter length and higher ponderal index (Fall et al., 1998). Furthermore, a 'U' shaped relationship was observed in Pima Indians, suggesting the contribution of large birth weight presumably reflecting the effects of maternal diabetes (McCance et al., 1994). A number of terms like FOAD, 'small baby syndrome' were used in addition to the 'thrifty phenotype' to describe these findings. The 'Developmental Origins of Health and Disease' (DOHaD) model proposes that the susceptibility to type 2 diabetes originates in the intrauterine life by environmental fetal programming, further exaggerated by rapid childhood growth, i.e. a biphasic nutritional insult. Both fetal under nutrition (sometimes manifested as low birth weight) and over nutrition (the baby of a diabetic mother) increase the risk of future diabetes. DOHaD represents a paradigm shift in the model for prevention of the chronic non-communicable diseases.

A developing system (embryo and fetus) is plastic i.e. capable of taking any of the many diverse routes and forms, but the intrauterine environment 'programs' it along certain pathways which help the fetus survive and develop. This affects structure and function, sometimes reflected in altered growth, body composition (adiposity), beta cell function, tissue response to hormones, and vascular reactivity. 'Programming' restricts the options for the fetus; if the environment in later life does not match the programming environment, the capabilities of the system are exceeded, and result in 'disease'. Hence the 'predictive adaptive response' is induced by the environmental factors acting in early life. These responses may not induce an immediate physiological adaptation, but act through plasticity to modify the

phenotype so that it is matched to the environment predicted to be experienced at a later phase.

The current concept of DOHaD proposes that maternal characteristics before and during pregnancy influence fetal survival, growth, size and body composition, and function of various systems. Some of these effects are demonstrable at birth.

The incidence of poor fetal growth (both low birth weight (LBW) defined as birthweight < 2500 g, and small for gestational age babies (SGA) defined as birthweight <10th % for gestational age) is quite high in developing countries (Agarwal et al., 2002). Due to both high birth rates and a higher incidence of poor fetal growth, countries in South Asia including India have the highest rates of LBW in the world. Of all LBW infants born each year, 75% are born in Asia, 20% in Africa, and 5% in Latin America. In India, LBW nearly affects 30% of infants and the majority of LBW in India and in most developing countries are a result of intrauterine growth restriction (ACC/SCN 2000). A baby's low birth weight is either the consequence of preterm birth (baby born before 37 weeks of gestation) or due to intrauterine growth restriction (IUGR). The latter condition is akin to malnutrition and genetic disorders which may be present in both term and preterm infants. The term IUGR is a clinical term and the diagnosis is usually based on small size for gestational age at birth (SGA), the term SGA is used in this thesis.

2.1.2 *Problems*

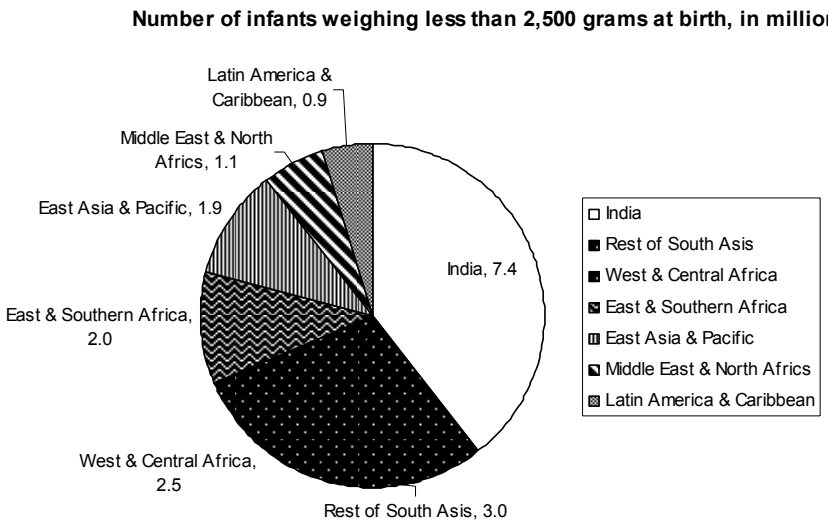


Figure 3. Number of infants weighing less than 2,500 grams at birth, in millions, 2005–2009.

Source: *Adapted from UNICEF global databases 2010. highlight: 19 million newborns weigh less than 2,500 grams in the developing world, more than half in South Asia, which has the highest incidence of low birthweight by far, at 27 per cent. India has the highest number of low-birthweight babies each year: 7.4 million.*

LBW has been associated with significantly higher perinatal and infant mortality (Ashworth A 1998) and with higher risk of hospitalization and diarrhoea (Barros et al., 1992) and pneumonia (Chandra 1975) in the first two years of life. The postnatal growth of these babies is also impaired (Barros et al., 1992) and such children remain small at 4 years of age (Gluckman 1993). They may also have developmental disabilities, including sensorineural and learning problems as well as limited cognitive development in childhood (Sorensen et al., 1997). Increasing evidence also has identified LBW as a risk factor for chronic diseases including cardiovascular diseases in industrialized (Barker 1994 & 1996) and developing (Stein et al., 1996) countries. Studies have shown that SGA babies are more likely to suffer complications including cold stress and hypoglycaemia and it is important that these infants are identified and managed appropriately at birth (Arora et al., 1987).

2.1.3 *Maternal parameters and adverse birth outcomes*

Several maternal factors are known to cause LBW, preterm and SGA babies, these are socio-demographic characteristics, maternal anthropometry; pre-pregnancy weight, body mass index (BMI), gestational weight gain; morbidity and malnutrition during pregnancy. Maternal low pre-pregnant body weight or BMI is known risk factor for preterm births and SGA (Siega-Riz et al., 1994; Kramer et al., 1995; Muthayya et al., 2006). Similarly gestational weight gain is seen to have an effect on pregnancy outcomes particularly with preterm births (Abrams & Selvin 1995; Spinillo et al., 1998). Pregnant women with low gestational weight gain during pregnancy have higher risk of delivering LBW/ Preterm or SGA babies. Muthayya et al (2006) have also observed that pregnant women with low gestational weight gain during their 2nd trimester have higher odds of delivering an IUGR baby. For a successful pregnancy it is essential for the pregnant women to gain adequate weight during pregnancy and this depends on the pre-pregnant weight. As per the WHO/FAO/UNU, gestational weight gain for Indian pregnant mothers varies from 8-18 kg depending on the women classified as low BMI or normal pre-pregnancy BMI.

Maternal age, parity and education also have an effect on adverse birth outcomes. Yang et al (2006) in a study using the natality file data from 1980 through 2000 in USA, have shown that changes in age- and parity-specific rates were the main contributors to an increase in very LBW rates among both White and Black women, as well as in LBW rates among non-Hispanic White and Hispanic women during the study period. Similarly, Frazer et al (1995) have shown a 'U' shaped relationship between maternal age and LBW with advanced maternal age most frequently seen as a cause for chromosomal anomalies such as Down's syndrome. In addition, studies have shown some association between low maternal education and LBW (Dičkutė et al., 2004). In addition, a study conducted by Dičkutė and colleagues in Eastern Europe and Lou et al (2006) in Canada has reported that pregnant women with low education had approximately twice the odds of having an IUGR baby when compared to those with higher education. These studies also identified maternal education as the single most important socio-demographic characteristic influencing IUGR. A similar study in Bangalore, India showed an adjusted odd of 1.96 for the risk of an SGA baby for a poorly educated pregnant woman (*Thomas T, Personal communication)*. In general, maternal education is most

often used as a surrogate marker of socio-demography since it implies that educated women have better access to nutrition, health care and medical facilities thereby with better birth outcomes.

Other factors such as low attendance for prenatal care, excessive physical work (Dwarkanath et al., 2007); cigarette smoking, tobacco use, frequent pregnancies, and multiple pregnancies have also been associated with LBW (Agarwal et al., 2002; Arora et al., 1987; Mavalankar et al., 1994; Bhatia et al., 1984). The chewing or smoking of tobacco, or chewing of beetle nut leaves is common in the rural population compared to urban pregnant Indian women and needs to be considered as well.

Since prenatal nutrition lays the foundation of the growing fetus, nutrition in the preconception period is important. Maternal nutritional deficiency in the 1st trimester or pre-pregnant nutritional deficiency may affect organogenesis while in the 2nd trimester, the fetal lean tissue growth is greater and thus poor maternal nutritional status may affect the length or baby size. Similarly 3rd trimester is when the maximum weight gain of the fetus takes place, thus deficiency of nutrients may lead to adverse birth outcomes. These postulates are based on the milestones of fetal growth and development at each trimester. While studies have also shown exposure to famine during gestation and life-long effects on health; these effects vary depending on the timing of exposure as well as evolution of the recovery period (Kyle & Pichard 2006). A number of nutrients play an important role in determining the pregnancy outcome. Studies have shown association between energy and protein intakes and birth size (WHO 1995; Kramer 2002). Similarly, a positive association between protein intake during late pregnancy and placental and birth weight (Godfrey et al., 1996) has been established. Currently the focus has moved from macro to micronutrients and several lines of evidences show positive associations with micronutrient supplements either single or in combination such as iron, folic acid, vitamin A, vitamin C and B-vitamins and improved birth weight, reduced prematurity, pre-eclampsia and maternal mortality (Rasmussen & Stoltzfus 2003, de Onis et al., 1998; Ceesay et al., 1997; West et al., 1999; Ramakrishnan et al., 1999). In addition to the macronutrients and micronutrients, the relationship between intakes of food groups and birth outcomes has become a focus. Nutrient and food group

intakes (green leafy vegetables, fruits, milk and meat) have shown positive effects with respect to birth outcomes.

2.1.4 *Maternal morbidity and adverse birth outcome*

Maternal morbidity during pregnancy not only affects the health of the mother but also has a profound impact on the unborn fetus. Maternal morbidity such as anemia, malaria, infections, and hypertension, pre-eclampsia or gestational diabetes are also known to cause adverse birth outcomes. Anemia is quite a common disorder in pregnancy. WHO has estimated the prevalence of anemia in pregnant women to be ~15 per cent in developed and 51 per cent in developing countries and 65-75 percent in India (DeMayer & Tegman 1998). In India, the District Nutrition Survey conducted by Indian Council of Medical Research (ICMR), in National sample (1998-2000) found that 85% pregnant women had nutritional anemia with 9.9% having severe anemia (Kapur et al., 2002). Causes of anemia range from dietary deficiency of iron and other nutrients (i.e. folic acid, vitamin B_{12}), loss of blood, chronic infections, malaria, intestinal parasites and hemoglobinopathies. Maternal anemia has been said to lead to increased risks of IUGR, LBW, preterm birth, perinatal mortality, and maternal mortality (Fleming 1989a; Fleming1989b). However, it is believed that the adverse effects of anemia are limited unless the anemia is severe and/or complicated by other conditions, e.g. pre-eclampsia and abruptio placentae.

A large number of viruses and bacteria can be passed on by so-called *vertical transmission* from mother to child during pregnancy or birth with several modes of transmission; transplacental, ascending from the maternal genital tract, and intrapartum. The maternal infection could also affect the fetus indirectly, e.g. as a result of fever and metabolic derangements. Organisms and infections that are proven or strongly suspected to affect pregnancy outcome and fetal and neonatal health include: rubella, cytomegalovirus (CMV), herpes simplex, varicella-zoster-virus (chickenpox), HIV, hepatitis B, syphilis, toxoplasmosis, and Chlamydia. There are variations in the effects that maternal infection during pregnancy can have on the fetus and the new borne. Consequences depend on the timing of in-utero exposure, the type of infection, the 'dose', and immune status of the mother. The adverse outcomes for fetus and neonate include; congenital anomalies (e.g. microcephaly,

hydrocephalus, mental retardation), IUGR, perinatal asphyxia, fetal mortality, preterm birth, LBW, neonatal morbidity (e.g. congenital infection, neonatal infection, conjunctivitis, respiratory distress), and neonatal mortality. Mothers with urinary tract infections (UTIs) have had problems ranging from morbidity to life-threatening situations for both mother and the fetus. Evidences suggest that urinary tract infection plays a role in the pathogenesis of preterm labour. While up to 27% of preterm births are associated with clinical forms of UTI (Kaul et al., 1999; *Nayak S. personal communication)*, it affects premature labour directly through the development of amnionitis (Conolly & Thorp 1999). Another study conducted in Egypt showed that the chances of having a preterm or a LBW were significantly higher among those women who experienced UTI's during pregnancy (Dimetry et al., 2007).

During the course of pregnancy, morbidity, particularly PIH, pre-eclampsia and gestational diabetes mellitus (GDM) has been on the increase in developing countries (Yajnik & Deshmukh 2008). GDM is the most frequent metabolic disorder occurring during pregnancy and is associated with adverse affects on pregnancy and the vascular system (Tarim et al., 2006). The adverse effects associated with gestational diabetes include; pre-eclampsia, hydramnios, macrosomia, major congenital anomalies (e.g. cardiovascular anomalies, anomalies of the central nervous system), unexplained late intrauterine death or still birth, complications at birth (e.g. shoulder dystocia), caesarean section and neonatal morbidity. Its prevalence may range from 1 to 16% of all pregnancies depending on the type of population and the diagnostic criteria used (Jime´nez-Moleo´n et al., 2000). Similarly, a number of studies have shown an association with PIH and increased risk of LBW due to an increased rate of preterm birth, as well as SGA babies (Xiong et al., 1999 & 2000). This PhD thesis has focused on PIH as one condition during pregnancy, and has explored the role of dietary calcium and calcium rich food groups in relation to PIH and adverse birth outcomes (*Chapter 7*)

2.2 Importance of macronutrients and selected nutrients namely folate, B_{12}, B_6 and calcium during pregnancy.

2.2.1 *Importance of macronutrients during pregnancy*

Malnutrition during pregnancy is known to be a major determinant of birth outcome. Studies in the past have shown that maternal energy and protein deficiency are also clearly associated with adverse birth outcomes such as IUGR (Kramer 2002; WHO 1995). However, attempts to increase birth weight by giving mothers high-density protein supplements have consistently reduced fetal growth (Rush 1989). Balanced energy and protein supplements have led to increased birth weight but effects have been disappointingly small (Kramer 2002). In rural Gambia, a 5 year randomised controlled trial showed that chronically undernourished pregnant women when given a high-energy groundnut snack showed better weight gain and a lower incidence of LBW than mothers not receiving the supplement (Cessay et al., 1997). These finding were supported in 2004, in Australia by Moore et al (2004) in a prospective observational study in healthy Caucasians, where the birth weight was positively associated with increased energy due to dairy protein. A case-control study suggested that carbohydrate rich food in early pregnancy reduced the risk of SGA (Mitchell et al, 2004), while, a prospective observational study by Godfrey (1996) and colleagues in Southampton, UK showed a decrease in fetal size at birth with increase in carbohydrate intake in early pregnancy. The same study also showed a decrease in fetal size associated with decrease in meat protein in late trimester. However, others have found no relation between maternal intakes of macronutrients and infant birth size among well nourished women (Mathews et al., 1999; Lagiou et al., 2004), but instead a stronger relationship with dietary intake of micronutrient-rich foods (Rao et al., 2001). There is evidence of the beneficial effects of macronutrient supplements during pregnancy and on birth outcomes.

Recent interest has turned to micronutrients as possible limiting factors for fetal growth as some micronutrients are structural components of body tissues, others essential for the processes of growth, including energy and protein metabolism, gene transcription, endocrine function and nutrient transport (Villar et al., 2003). In United States, in a low income urban woman, the use of a micronutrient/multi-mineral supplement was associated with a substantial decrease in the incidence of LBW and

preterm births (Scholl et al., 1997). Similarly, in a trial of more than 20,000 pregnant women in Nepal, weekly supplementation with vitamin A or beta carotene was associated with a 40 or 49%, respectively, reduction in pregnancy - related maternal mortality compared to placebo (West et al., 1999). Again in Nepal, a recent trial of maternal multiple micronutrient supplementation that included vitamin B_{12}, the birthweight was significantly increased and the frequency of LBW was reduced by 25% (Orsin et al., 2005). Similarly, multiple micronutrient supplementation (B vitamins including B_{12}, plus vitamins C and E) in HIV-positive Tanzanian women showed a significant reduction in preterm birth, fetal loss, and LBW (Fawzi et al., 1998). Another double-blind, randomized, placebo-controlled trial conducted by Gupta et al (2007) in East Delhi, India showed that, compared with iron and folic acid supplementation, the administration of multi-micronutrients to undernourished pregnant women reduced the incidence of low birth weight and early neonatal morbidity. These findings support the potential role of vitamin and mineral supplements during pregnancy and pregnancy on birth outcomes and may operate independently or in combination.

The select B vitamins (vitamin B_{12}, folate and vitamin B_6) have important role in methylation cycle.

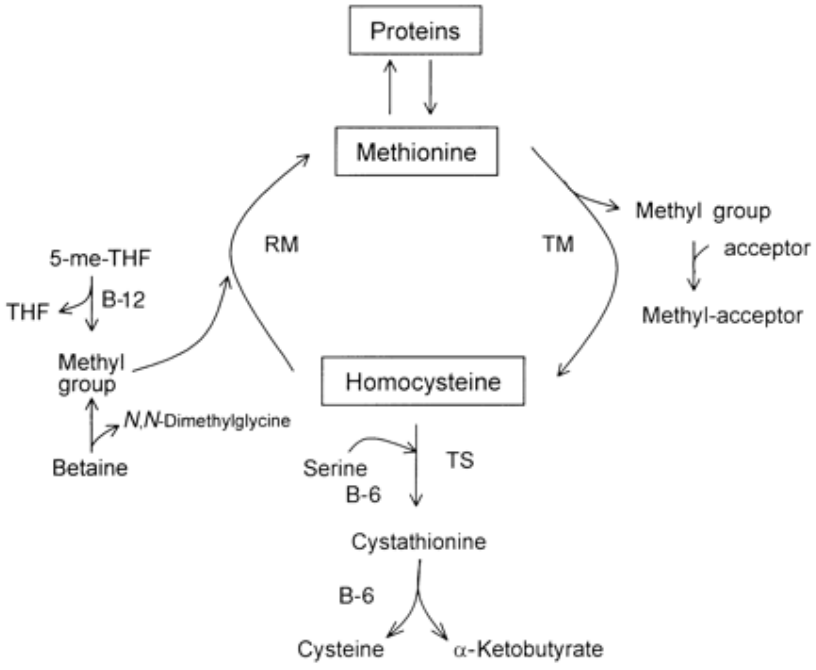


Figure 4. The Methylation cycle

RM-remethylation, TM-Transmethylation, TS-transsulfuration

Explanation in brief:

Hcy is a non-protein–forming sulfur amino acid whose metabolism is at the intersection of two metabolic pathways: remethylation and transsulfuration. It is formed from methionine as a product of numerous S-adenosylmethionine (AdoMet; SAM)-dependent transmethylation reactions (Mudd et al., 1995) are either directed to the transsulfuration pathway which irreversibly converts Hcy to cysteine or is remethylated to methionine (*Refer Fig 4*). During this process the reaction is catalysed by methionine synthase that uses vitamin B_{12} as a cofactor and methyltetrahydrofolate (methyl-THF) as a substrate (Finkelstein 1990). The elimination of both Hcy and methyl-THF formation are under strict regulation of AdoMet (SAM) and therefore the methionine level is also controlled (Finkelstein, 1990). When AdoMet (SAM) is in excess, the transsulfuration pathway is activated, leading to elimination of methionine, while in situations of low AdoMet (SAM), MTHFR is stimulated, and that directs the folates from DNA and RNA synthesis to methionine conservation (Scott & Weir 1981). Both folate and vitamin B_{12} deficiency leads to reduced methionine formation, and if provision of methionine is limited, the MTHFR activity gets stimulated, thereby providing more methyl-THF for methionine synthase.

During pregnancy there is net gain of protein by both the mother and the fetus, increasing the demand for amino acids including methionine. These amino acids are derived from the turnover of maternal proteins as well as from the diet. Any excess amino acids derived from the diet are diverted into catabolic pathways. In presence of excess methionine, there is an increase in the flux through theHcy pool and an increase in the plasma concentration. The elevated plasma Hcy concentrations are associated with common pregnancy complications and adverse outcomes, including preeclampsia, spontaneous abortion, placental abruption, and recurrent pregnancy loss (Vollset et al., 2000). Extreme elevation of Hcy may also be associated with neural tube defects (van der Put et al., 2001).

In addition, glycine, an amino acid is a vital component of sulphur amino acid metabolic pathway and it aids regulation of methionine and Hcy levels (Baglet & Stipanuk 1995). It is also needed for synthesis of fetal DNA, collagen and serine. On a low-protein diet, the endogenous formation of the amino acid glycine is thought to

become constrained. Glycine may become conditionally essential, as its rate of endogenous formation is inadequate to meet metabolic needs, and may be limiting for the normal development of the fetus (Jackson et al., 2002; Thame et al., 2010).

2.2.2 **VITAMIN B_{12}**

2.2.2.1 *Introduction*

Vitamin B_{12} also called as cyanocobalamin is essential for normal DNA and RNA biosynthesis, maintenance of normal erythropoiesis and also required for one-carbon metabolism i.e. Hcy metabolism. Overt deficiency may cause a variety of disorders including hyperhomocysteinemia, megaloblastic anemia and neurological disease (Wagner 1995; Carmel et al., 2003). Low cobalamin status - like low folate status - may impair the development of the neural tube, causing neural tubes defects in the newborn (Botto et al., 1999).

2.2.2.2 *Vitamin B_{12} in diet*

Vitamin B_{12} occurs in substantial amounts only in foods derived from animals such as fish, liver, meat and eggs. Milk and milk products such as butter, cheese and curd also contain considerable amounts of vitamin B_{12}. All types of bacteria and algae, with a few exceptions, synthesize the vitamin, which enters the human food chain by being incorporated into food of animal origin.

The recommended allowances by WHO of 1 µg/d allows enough margins of safety for cooking losses, uncertainties in absorption and a small amount for storage in adults while recent Indian Council of Medical Research (ICMR 2010) guidelines for recommended dietary allowances for Indians has suggested 1.2 µg/d and Food and agricultural organization of the United Nations / World Health Organization (FAO/WHO 2004) on B_{12} requirements recommended an intake of 2.6 µg/d during pregnancy.

2.2.2.3 *Role of vitamin B_{12} in methylation cycle*

The vital role of vitamin B_{12} is as the co-factor in the remethylation pathway of the methionine cycle and hence plays an important role in maintaining the Hcy levels formed during transmethylation of methionine (*Refer Fig 4*). Generally it is seen that it is unlikely to have folate levels below the lower reference limit since it is available in non-vegetarian as well as vegetarian diets unlike vitamin B_{12}. Therefore,

vitamin B_{12} deficiency becomes a main factor influencing the homocysteine level. Vitamin B_{12} is a cofactor for methionine synthase enzyme and in case of deficiency, an inhibition in the remethylation of homocysteine to methionine occurs. Folate in the form of 5-methyltetrahydrofolate becomes trapped because the transfer of the methyl group is inhibited. Under this condition, higher levels of folate are required in order to transfer a sufficient amount of methyl groups from 5-methyltetrahydrofolate to Hcy, preventing hyperhomocysteinemia. However, due to folate trap causing relative folate shortage, a substantial portion of vegetarians develops hyperhomocysteinemia (Herrmann et al., 2001).

2.3.2 **FOLATE / FOLIC ACID**

2.2.3.1 *Introduction*

Folate, a group of inter-convertible co-enzymes, differing by their oxidation state, number of glutamic acid moieties and one carbon substitutions are involved in amino acid metabolism, purine and pyrimidine synthesis and methylation of a large number of nucleic acids, proteins and lipids. During gestation, marginal folate nutriture can impair cellular growth and replication in the fetus or placenta. Folate deficiency can occur because dietary folate intake is low or because the metabolic requirement for folate is increased by a particular genetic defect or defects or because of a vitamin B_{12} deficiency. During pregnancy, low concentrations of dietary and circulating folate are associated with increased risks of preterm delivery, LBW, and SGA. Since folate is critically important for fetal development, once absorbed, it acts as a cofactor for many essential cellular reactions including transferring of single-carbon units; it is required for cell division because of its role in DNA synthesis. Folate is also a substrate for a variety of reactions that affect the metabolism of several amino acids, including the transmethylation and transsulfuration pathways in the methylation cycle (*Refer fig 4*).

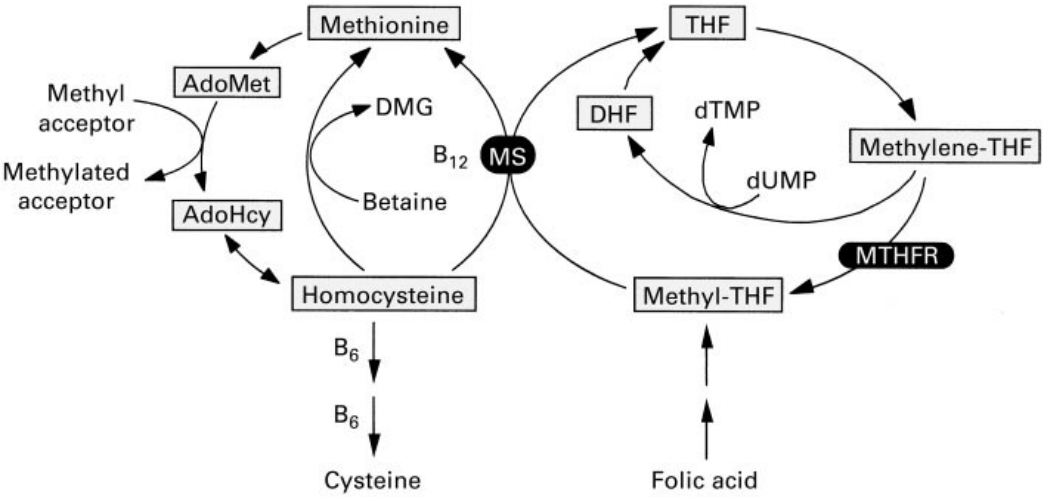
2.2.3.2 *Folates in diet*

Naturally occurring folates, called food folate mostly (polyglutamyl chain) are present in both animal and plant foods. Organ meats such as liver and egg yolk are fairly good sources of folate and in the Indian context the major sources are legumes and green leafy vegetables. Other foods such as orange juice and white bread are also of lower folate density. The intake of folate based on the National

Nutrition Monitoring Bureau India (NNMB 1999), suggested that as per the existing recommendations more than 60% of folate nutrition is met from cereals and pulses.

The recommended folate intake through diet is 500 µg/d and 400 µg/d as suggested by ICMR (2010) AND FAO/WHO (2004) guidelines.

2.2.3.3 Interrelation between vitamin B_{12}, folate and Hcy metabolism



Interrelation between folate, vitamin B_{12} and homocysteine metabolism. AdoHcy, adenosylhomocysteine (SAH); Aldomet, adenosylmethionine (SAM); DHF, dihydrofolate; DMG, dimethyl glycine; MS, methionine synthase; MTHFR, methylenetetrahydrofolate reductase; THF, tetrahydrofolate.

Ref source: Adapted from Refsum 2001.

Figure 5 The folate cycle.

In severe folate deficiency, cell division is impaired, and a characteristic morphologic picture arises, i.e. the megaloblastic changes (Wickramasinghe 1999). It is also believed that the cause of megaloblastosis is the reduction of folate dependent formation of dTMP from dUMP and this possibly inhibit purine synthesis (*Refer fig 5*). However, evidence suggests that under conditions of low folate, uracil frequently gets incorporated into DNA instead of thymine, and the normal repair processes to remove the mis-incorporated uracil often fail. This ultimately leads to double strand break and chromosome instability which promotes apoptosis (Koury et al., 1997). It is seen that chromosomal damage also occurs in subjects without clinical symptoms but with low folate or vitamin B_{12} status or increased total Hcy (Blount et al., 1997; Fenech et al., 1998).

The congenital malformation involving folate is related to inhibited folate dependent dTMP formations and is related to the common C677T polymorphism in the MTHFR gene. The NTDs; are spina bifida (an incomplete closure of the spinal cord and spinal column), anencephaly (severe underdevelopment of the brain), and encephalocele (when brain tissue protrudes out to the skin from an abnormal opening in the skull) caused in the perinatal period occur during the first 28 days of pregnancy; even before the pregnancy is confirmed (Czeizel 2004). Homozygosity for this polymorphism, the TT genotype, is associated with neural tube defects (NTD) (Shields et al., 1999). This enzyme variant has low activity, and the affected subjects frequently have hyperhomocysteinemia. Notably, TT subjects usually have a normal or even high folate content in the cell, but their methyl-THF level is low (Bagley & Selhub 1998). This is also possible due to the impaired methionine synthase function or a disturbed ratio between methionine and homocysteine as the cause of major malformations. It is also seen that the common A66G polymorphism in methionine synthase reductase, the enzyme that activates cobalamin-dependent methionine synthase, increases NTD risk when cobalamin status is also low (Wilson et al., 1999).

2.2.4 **VITAMIN B_6**

2.2.4.1 *Introduction*

Vitamin B_6 is a family of water-soluble compounds that includes pyridoxine, pyridoxamine, pyridoxal and their phosphorylated derivatives. Pyridoxal phosphate (PLP) is the coenzyme for a variety of enzymes for eg, aminotransferases, decarboxylases, and side chain cleaving enzymes. It is needed for important pathways including gluconeogenesis, synthesis of neurotransmitters serotonin, dopamine, taurine, -amino butyric acid, norepinephrine and histamine. Vitamin B_6 is an essential cofactor in the developing central nervous system and may influence brain development and cognitive function. Vitamin B_6 deficiency can also result in anemia as it can preclude the initial enzymatic step of heme synthesis and thus the incorporation of iron in erythropoietic cells (IOM 1998; Awad et al., 2006; Chaney et al., 2002). Vitamin B_6 deficiency in general has been associated with impairment of enzymes involved in determining the structural integrity of the arterial wall (Levene & Murray 1977) in altering platelet function (Lancet 1981) and in interfering with cholesterol mechanism (Chi 1984). Along with folate, vitamin B_{12} and riboflavin,

vitamin B_6 is needed for the metabolism of homocysteine, in immune system and nucleic acid metabolism.

Other roles of vitamin B_6 during pregnancy

Recent work in rats suggests that vitamin B_6 deficiency during gestation and lactation alters the function of N-methyl-D-aspartate receptors, a subtype of receptors of the glutamatergic neurotransmitter system thought to play an important role in learning and memory (Guilarte 1993). In addition, the supply of vitamin B_6 to the fetus and infant is known to be vital to DNA synthesis and to the formation of cerebrosides needed in myelination of the central nervous system. The increased requirement for vitamin B_6 in pregnancy may result from estrogen induction of vitamin B_6 requiring enzymes, increased amino acid turnover, and fetal demand for the vitamin. Vitamin B_6 deficiency impairs pancreatic insulin production and supplementation during the second and third trimester of pregnancy may improve glucose intolerance in women with gestational diabetes (Jovanovic-Peterson & Peterson 1996). Supplementation with vitamin B_6 has been associated with some benefits in non-randomised studies, such as higher *APGAR* scores, higher birth weights, and reduced incidence of pre-eclampsia (de la Calle et al., 2003) preterm birth (Ronnenberg et al., 2002) and pregnancy loss (Ronnenberg et al., 2007).

2.2.4.2 *Vitamin B_6 in diet*

Meat, fish, poultry, pulses, nuts and wheat are known to be rich sources of the vitamin, while other cereals, potato and banana are moderate sources. Pyridoxine is the predominant form of the vitamin present in the plant foods, whereas in the animal foods the major form is pyridoxal and pyridoxal phosphate. Considerable amounts of pyridoxal and pyridoxal phosphate are lost during cooking, whereas pyridoxine content of food is not affected.

Pyridoxine requirement is linked to protein content of the diet. Various guidelines suggest vitamin B_6 intakes during pregnancy to be around 2.5and 1.9 mg/d (ICMR 2010; FAO/WHO 2004).

2.2.4.3 *Role of vitamin B_6 in methylation cycle*

Vitamin B_6 is one of the essential co-factors in the transsulfuration process i.e. conversion of Hcy to cysteine (*Refer fig 4*). Cystathionine is an intermediate product of Hcy transsulfuration which increases during vitamin B_6 deficiency.

Micronutrients such as folate, vitamin B_{12} and vitamin B_6 are interrelated such that deficiency of either one leads to hyperhomocysteinemia, a condition that is known to cause adverse birth outcomes. Though these micronutrients have multiple functions, it is important to explore the interrelationships between each other during pregnancy and thereby leading to adverse birth outcomes. Moreover the Indian diet is predominantly cereal based which places the pregnant women at a higher risk of vitamin B_{12} deficiency.

2.2.5 **CALCIUM**

2.2.5.1 *Introduction*

Calcium (Ca) is the most abundant cation and a major element of the human body. Bone and teeth contain 99% of the calcium for their strength and structure while 1% of the body's calcium is distributed between the extracellular fluids and various soft tissues, where it performs a variety of regulatory functions. Though small in quantity, non-skeletal Ca has the other important functions like neuromuscular excitation, blood coagulation, membrane permeability and others. The importance of Ca in these functions is reflected in the precision with which plasma Ca level is regulated. Blood calcium level is maintained within narrow limits by the interplay of vitamin D; 1,25-hydroxyvitamin D [25(OH)D] and several hormones, like parathyroid hormone (PTH), thyrocalcitonin, cortisol and gender steroids by controlling absorption, excretion and bone turnover. Generally the body is able to maintain a delicate balance between calcium and phosphate and usually calcium deficiency does not occur. When this balance is upset, typically calcium levels in the body falls and phosphorous levels rise, the body starts utilizing calcium from bones and teeth so that it can return the calcium-phosphate balance.

Human pregnancy is associated with major changes in calcium and bone metabolism before and after gestation. The changes are compatible with the uptake and mobilization of calcium from the maternal skeleton to meet the high requirement

for fetal growth. In the final trimester of pregnancy, about 200 and 300 mg of calcium is deposited daily in the skeleton of a developing human fetus from the mother. Since 98% of calcium in the body is contained in the inorganic matrix of bone, if the diet of a pregnant woman does not provide sufficient calcium for fetal development, either the growth of the baby during gestation would be adversely affected, or calcium would be released from the maternal skeleton, with possible short- or long- term effects on the mother's health.

2.2.5.2 *Calcium in diet*

Studies among the Western population, whose habitual diets contain high levels of calcium from generous amounts of milk, have indicated a requirement of 1g/d calcium. Population groups in many developing countries subsist on a much lower calcium intake of about 500 mg. For the Indian pregnant women, based on the factorial method, the calcium intake needed to maintain calcium adequacy during pregnancy is estimated to be 1200 mg/d. It is also suggested that to achieve this level of intake, a minimum of 200 ml of milk/d would be essential on a cereal-legume diet (ICMR 2010). From an Indian context, the sources of calcium are predominantly milk products while the moderate sources are egg yolk and fish. Other rich sources of calcium among plant foods are the millet ragi (*Eleusine Coracana*), soybeans, turnip greens and mustard greens, broccoli and kale, various roots and tubers, rajkeera (*Amaranthus*), the green leafy vegetables all plant seeds such as sesame are rich in calcium content. It is also found in seaweed, sardines, and canned salmon.

2.2.4.3 *Role of calcium in morbidity such as pregnancy induced hypertension (PIH)*

Calcium is thought to play an important role in the aetiology, prevention and treatment of PIH although contradictions persist in clinical trials of calcium supplementation and prevention of PIH.

The mechanism of action of calcium on PIH is unclear. However, an increase in intracellular calcium in vascular smooth muscle cells during pregnancy is consistent with development of vasoconstriction and resultant hypertension. Analogously, an increase in intracellular calcium in uterine smooth muscle cells is consistent with induction of preterm labour. Alternatively, it has been hypothesized that calcium affects smooth muscle cell contractility indirectly by influencing the production of other vasoactive agents such as nitric oxide, prostacyclins, or angiotensin (via the

renin-angiotensin-aldosterone metabolic pathway) (Repke & Villar 1991; Myatt 1992). Though the biochemical mechanism responsible for the possible increase in intracellular calcium and concomitant decrease in extracellular calcium is unclear, it has been suggested that parathyroid hormone plays a crucial role in influencing cation transport (Myatt 1992).

2.2.4.4 *Role of calcium in adverse birth outcomes*

There is paucity of information of direct relationship between dietary calcium intakes and adverse birth outcomes. However there is evidence showing relationship between dietary calcium intakes and PIH; which in turn is a known cause of adverse birth outcomes such as preterm birth, LBW as well as IUGR or SGA (Xiong & Frazer 2004). Still births can also occur if the preeclampsia is not treated.

A prevailing pathogenesis theory of pre-eclampsia is the 'ischaemic model'. It is hypothesised that reduced uteroplacental perfusion is the primary step and the point of convergence of diverse patho-physiological processes in the development of pre-eclampsia (Friedman et al., 1991; Freidman & Lindheimer 1999). Studies on Canadian pregnancies have shown that preeclampsia increases the risk of LBW mainly through increasing preterm birth (as delivery of the baby is the only effective treatment for pre-eclampsia in cases where the disorder is severe) and decreasing fetal growth as well (Xiong et al., 2000). This may reflect the fact that most of the LBW babies in developing countries result from IUGR; the main reason for LBW in developed countries is preterm birth (Kramer 1987). Low calcium intake may cause high blood pressure by stimulating either parathyroid hormone or renin release, thereby increasing intracellular calcium in vascular smooth muscle (Belizan et al., 1988) and leading to vasoconstriction. A similar mechanism when calcium is supplemented would reduce uterine smooth muscle contractility and may prevent preterm labour and delivery (Repke et al., 1989).

The table below summarises the recommended dietary allowances for the select micronutrients and calcium as well as the cut off to define the deficiency during pregnancy.

Table 1 Recommended dietary allowances.

Nutrients	Dietary RDA	Dietary RDA reference source	Deficiency cut off (status)
Folate	500 µg/d, 400 µg/d	ICMR 2010, FAO/WHO 2004	<283 nmol/L (Red cell folate)
Vitamin B_{12}	1.2, 2.6 µg /d	ICMR 2010, FAO/WHO 2004	< 150 pmol/L (Cobalamin)
Vitamin B_6	2.5, 1.9 mg/d	ICMR 2010, FAO/WHO 2004	<20 nmol/L (pyridoxine)
Calcium	1000-1200 mg/d, 1200 mg/d	ICMR 2010	-

Micronutrient status reference (folate, vitamin B_{12}, vitamin B_6) source; Refsum et al., 2004

2.3 Importance of food groups in relation to birth outcome

Intakes of macronutrients as well as single nutrients and combination of nutrients during pregnancy have shown positive effects on birth outcomes. While there have also been controversial findings, few studies have shown association between food group intakes, birth weight and size. A few authors suggest this relationship is mediated through the nutrients involved. Studies conducted in Western part of India have shown a positive association between green leafy vegetables (GLV) and fruit intakes and birth size at 28 weeks gestation after adjusting for the potential confounders such as gender of the baby, gestational age and parity. Fruits are a rich source of vitamin C and other antioxidants whereas milk provides high quality proteins, fat, calcium, riboflavin and vitamin A. It is possible that these micronutrients in combination may be involved in the relationship between fruits, milk and birth outcomes. A study conducted in Danish lean pregnant women also had findings consistent to the Indian study regarding fruit and vegetable intakes being positively related to birth weight. This was a retrospective study and the information was collected by telephonic interviews (Mikkelsen et al., 2006). In contrast, there was no clear pattern with regard to increasing fruit consumption during antenatal period and birth weight, length or SGA (weight) in the Spanish women although the highest proportion of SGA (length) was in the lowest quintile of

fruits intake. Moreover, Ramon et al did find a linear relationship between vegetable consumption and SGA (weight) and SGA (length) baby in the 1st as well as 3rd trimester of pregnancy (Ramon et al., 2009). While fruits and vegetables have been the focus for the cereal based-vegetarian population, studies have also shown promising results with respect to milk intakes during pregnancy and birth outcomes. Milk is considered as the source of energy and high quality protein for especially the vegetarian population.

Milk and milk products as a food group intake during antenatal period have been beneficial to birth outcomes. Milk consumption in mid pregnancy (~18 weeks) has shown to be associated with birth size (Rao et al., 2001). Prenatal dairy intake in adolescent pregnant women had a significant effect on fetal femur growth after adjusting for gestational age, biparietal diameter, maternal age and height and pre-pregnancy body mass index. The fetal femur length was significantly lower in the lower dairy intake group (<2 servings/d) than in the highest dairy-intake group (>3 servings/d) suggesting that the consumption of low dairy products may negatively affect fetal bone development by limiting the amount of calcium provided to the fetus. The relationship could be very well related to calcium content of milk products (Chang et al., 2003). A study conducted in Canada has also shown that women consuming less than quarter litre milk per day gave birth to infants who weighed less than those born to women who consumed more (3410 versus 3530g, birth weight, $p<0.07$). Moreover, each additional cup of milk daily was associated with a 41g increase in birth weight (95% CI; 14.0-75.1g) and with each additional μg of vitamin D, an increase of 11g in birth weight observed (95% CI; 1.2-20.7g). It was also noted that neither protein, riboflavin or calcium intakes predicted the birth weight implying vitamin D to be the limiting micronutrient (Mannion et al., 2006). Recently birth outcomes have been the focus since babies born LBW, SGA or preterm have higher risk of morbidity during infancy and early childhood life. Hence studies have also looked at adverse birth outcomes as an outcome measure rather than absolute measurements such as birth weight, length and the size. In a Swedish population of pregnant women, low milk intakes during pregnancy was associated with increased risk of IUGR after adjusting for the confounders (Ludvigsson & Ludvigsson 2004). Similar findings with milk intake associated with higher birthweight for gestational age and lower risk of SGA in Danish subjects was

observed. The birth weight in this population was related to intake of protein, but not fat derived from milk (Olsen et al., 2007). The later study attributed this to the protein intake but not fat derived from the milk.

Though non vegetarian food group intake is known to be associated with birth outcomes, not many studies have shown consistent results. A study in Danish pregnant women showed that intakes of red and processed meat products were associated with increased risk for having a SGA baby. The maternal diet rich in vegetables, fruits, fish and poultry was associated with higher birth weight compared with a diet high in animal fat and processed meat. These findings were attributed to the subjects with highest intakes of red and processed meat, butter, lard and high-fat dairy products to high intake of saturated fats and trans fatty acid (Knudsen et al., 2008). The findings from the various study indicates that the micronutrients may be the limiting factors for the fetal growth.

Table 2 Food group intakes during pregnancy and birth outcome.

Sno	Author	Study design	Inclusion/Exclusion Criteria	Results	Conclusion
1	Ramón et al., 2009 Spain	Observational prospective study (n=787)	Healthy pregnant women with singleton pregnancy recruitment within 1st trimester and no assisted conception	Women in the lowest quintile of vegetable intake in the 1st trimester had higher odds of having a SGA (weight) baby than women in the highest quintile and similarly for SGA (length) baby in the 3rd trimester.	Higher intakes of vegetables during pregnancy were associated with higher birth weight and length as well as with a decreased risk of having a SGA (weight and length) baby.
2	Kanade et al., 2008 India	Observational prospective study (n=869)	633 Rural, 236 urban healthy pregnant women with no complications	↑ Intakes of fruits at 18 weeks gest, ↑birth length. Milk intake at 28 weeks associated with ↑ triceps in urban. Intakes of green leafy vegetables and milk products in rural women better neonatal size.	Intakes of fruits, green leafy vegetables and milk during pregnancy ↑ birth size in Indian mothers.
3	Knudsen et al., 2008 Denmark	Prospective cohort study (n=44 612)	Data only for those delivered a live born, full-term, singleton child.	The women in Western Diet class (red meat, high energy, smokers) had significantly higher Odds Ratio of having SGA infants compared with the women in Health Conscious classes.	Intakes of red and processed meat and high-fat diary, was associated with increased risk for SGA.
4	Olsen et al., 2007 Denmark (Danish study)	Prospective study (n=50117)	All pregnant women included	Milk intake associate with ↓SGA, ↑LGA, ↑mean birth weight and other body circumference after adjusting for gestational age.	↑ milk intakes ↓ SGA, ↑ LGA and mean birth weight
5	Mikkelsen et al., 2006 Denmark	Retrospective observational cohort study (n=43,585)	Women who were able to fill in questionnaires and to participate in interviews were included.	There was a strong association seen for the fruit intake in the univariate model, where the difference from the first to fifth quintile of intake was 95.6 g in birth weight.	Fruit and vegetable consumption in pregnancy was positively associated with birth weight in well-nourished Danish lean women.
6	Mannion et al., 2006 Alberta	Observational study (n=2091) (307 restricted milk intake)	Healthy women, non smokers, well educated with singleton pregnancy included.	3 cups of milk /d ↑ birthweight by 123g. A positive relation between vitamin D intake and birth weight. For each 1-µg increase of dietary vitamin D, birth weight increased by 11g	Milk and vitamin D intakes during pregnancy associated with infant birth weight, independently of other risk factors.
7	Ludvigsson & Ludvigsson 2004 Sweden	Retrospective study (n=14000)	All pregnant women	The mean birth weight ↑with ↑milk consumption. Risk of IUGR ↑with ↓milk intake but when adjusted for gestation the association disappeared.	↓ IUGR with ↑ milk intake during pregnancy.

8	Chang et al., 2003 Baltimore	10 year retrospective study (n=1120)	Adolescent pregnant women ≤ 17 years	Dairy intakes during pregnancy had a significant positive effect on fetal femur growth after adjusting for various parameters.	↑ Intakes of dairy products, ↑ fetal femur length.
9	Richardson et al., 1995 Texas, USA	Observational study (n=9291).	No complications (7,104 white, 2,187 black women pregnant). Those who had delivered a single live or still born infant at a gestational age of >140 days, not diagnosed with pre-existing hypertension.	Two glasses of milk per day had the lowest risk of preeclampsia. High risk for preeclampsia exists for women with <1 glass and those with 4 or > glasses per day.	U-shaped distribution of preeclampsia risk in relation to milk and milk plus supplement intake.
10	Rao et al., 2001 India	Observational study in rural India	797 pregnant women with singleton pregnancy and recruited < 21 weeks gestation from 6 villages	In the rural pregnant women, higher fat intake in the 18 weeks was associated with neonatal length, birth weight and triceps skin fold thickness. Birth size was strongly associated with milk at 18 weeks and GLV and fruits at 28 weeks of gestation.	Intakes of foods rich in micronutrients are suggestive of micronutrients as important limiting factors for fetal growth in undernourished community.

2.4 Interaction of selected nutrients and birth outcomes

Deficiencies of either of these nutrients (vitamin B_{12}, folate and vitamin B_6) may cause poor birth outcomes. Their role may be independent or they are unanimously involved in the important processes such as methylation cycle; and hence their functions cannot be segregated but are entwined with each other.

Since these micronutrients are known to play a vital role in the methylation cycle, their deficiency may not lead to adverse birth outcomes directly but through the elevation of an intermediary metabolite called Hcy. The body of evidence has shown elevated Hcy (hyperhomocysteinemia) to cause pregnancy complications such as; PIH, preeclampsia, abruptio-placentae as well as birth outcomes particularly; LBW, preterm births and SGA (Ronnenberg et al., 2007). Peri - conceptional intake of folic acid reduces the incidence of neural tube defects (NTD), one of the most common birth defects, by more than 50% (MRC-Vitamin Study Research Group, 1991; Botto et al., 1999), other malformations and also possibly, pregnancy complications. Folic acid is proven to have a beneficial effect on other malformations and pregnancy complications as well (Neggers et al., 1997). Studies also suggest that impaired vitamin B_{12} status (Wilson et al. 1999) and elevated blood Hcy (Ray & Laskin 1999; Vollset et al., 2000; Yajnik et al., 2005; Acılmıs et al., 2011) together may also be associated with birth defects and common pregnancy complications such as spontaneous abortions, placental abruption, pre-eclampsia and LBW.

2.4.1 *Vitamin B_{12}*

While evidence also suggest the beneficial effect of folate that may be related to improved function of methionine synthase, a vitamin B_{12}-dependent enzyme that converts homocystine to methionine, deficiency of vitamin B_{12} may still lead to hyperhomocysteinemia due to a phenomenon called the 'folate trap', thereby not making the folates available during the methylation cycle. In India, the majority of the population adheres to a vegetarian diet known to be deficient in vitamin B_{12}. In this kind of population, increased folate intake may offer minimal protection against birth defects, whereas vitamin B_{12} administration may prove beneficial to birth outcomes. Recently Molloy et al (2009) has shown that deficiency or inadequate maternal vitamin B_{12} status in Irish pregnant women was associated with a significantly increased risk for neural tube defects. The findings suggested that the

vitamin B_{12} status >300 ng/L in pre-pregnancy would reduce the risk of NTD. During the analysis in a logistic regression model, the women who started pregnancy with serum vitamin B_{12} concentrations of <300 ng/L (221 pmol/L) were at significantly higher risk for NTDs. Though the interaction between folate and vitamin B_{12} affecting the NTD is not clear, several mechanisms are possible; explained in the previous sections. In brief, as cofactor to the enzyme methionine synthase, vitamin B_{12} influences both the incorporation of folates into the cellular pool and the flux of folate derived 1-carbon units destined for DNA synthesis or for the methylation reactions. DNA synthesis being an essential feature of embryonic development, other factors also trigger the developmental changes such as cell-signalling events that lead to differential gene expression that are partially controlled by methylation reactions. Impairment of either of these systems could be involved in folate or vitamin B_{12}-responsive NTDs (Molloy et al., 2009).

Vitamin B_{12} has been discussed in length by many researchers as an important nutrient in 1-C metabolism particularly in methionine cycle and hence it's effect on adverse birth outcomes. The deficiency of vitamin B_{12} is rampant in developing countries and common in population adhering to vegetarian diets. Nearly half (49%) of the pregnant Nepali women had serum cobalamin <150 pmol/L which is the accepted cut off to define deficiency (Bondevik et al., 2001). Similar prevalence rate was observed in Indian pregnant women using the same cut offs (Muthayya et al., 2006). There are also evidences suggesting that vitamin B_{12} may be the sole determinant of NTD's. The prevalence of vitamin B_{12} deficiency (<125 pmol/L) among Ontarian women who underwent concomitant testing of serum bhCG and vitamin B_{12}, 9 years after the implementation of Canadian folic acid flour fortification suggested that almost 1 in 20 women were vitamin B_{12} deficit in the critical period of closure of the embryonic neural tube (Ray et al., 2008). Studies have also looked at the intake to status relationship of vitamin B_{12} during pregnancy. Muthayya et al have shown the translation of maternal vitamin B_{12} intakes to status and further maternal status to neonatal status, implying that mothers with low vitamin B_{12} intakes have low vitamin B_{12} concentrations during all 3 trimesters of pregnancy ($r=0.298$, $p=0.005$; $r=0.543$, $p=0.000$ and $r=0.557$, $p=0.000$ respectively) and may hence have babies with low concentrations too (Muthayya et al., 2006 & 2006). Takimoto et al have shown this relationship only in the later half of the

pregnancy i.e. 2nd and 3rd trimester (r=0.32 and 0.24, p<0.05 respectively), while Monsen et al (2001) has shown that in the neonates, serum cobalmin, but not folate was inversely associated with MMA and total homocysteine, with maternal low serum cobalamin as the strongest predictor of impaired cobalamin function (defined as low cobalamin, high total Hcy or high MMA levels) in newborns.

Hcy is also known to have a role in adverse birth outcomes. Study conducted in Japan has shown that maternal vitamin B_{12} status decreased and Hcy concentration significant increased from 1st to 3rd trimester of pregnancy. Further the increase in 1.0 μmol/L concentration of Hcy particularly in the 3rd trimester of pregnancy corresponded to a 151g decrease in birth weight (p<0.01) after controlling for maternal age, parity and pre-pregnancy BMI (Takimoto et al., 2001). Similarly, study conducted in rural population of Western India has attributed vitamin B_{12} deficiency to reflect in Hcy concentration further contributing to small size babies (Yajnik et al., 2005) such that higher maternal plasma tHcy concentration significantly associated with lower offspring birth weight (r=-0.28, p<0.05 adjusting for maternal weight, height, gestation at delivery and the baby's gender). Vitamin B_{12} status as a determinant of IUGR has been established earlier (Muthayya et al., 2006), and even in relation to preterm, such that vitamin B_{12} deficiency in the Chinese population tended to be more common in women with preterm deliveries than in control subjects (31.0% and 17.8%, respectively; p=0.08) (Ronnenberg et al., 2002). Similarly, this group postulated that elevated Hcy concentration and suboptimal vitamin B_{12} and vitamin B_6 status may increase the risk of preterm births, mainly due to the Hcy effect. This study showed that elevated Hcy (≥ 12.4 μmol/L) was associated with a nearly 4 fold risk of preterm birth (OR:3.6; 95% CI:1.3, 10.0; p<0.05), while folate status was not associated with preterm birth, and Hcy and B vitamin status were not associated with LBW or SGA status in the Chinese population. While number of studies have shown independent relationship of elevated Hcy and fetal growth restriction (*discussed in the later section*), few studies do not show relationship with birth outcomes.

Table 3. Role of maternal vitamin B_{12} intake / status and birth outcomes.

Sno	Author	Study design	Inclusion/ Exclusion Criteria	Results	Conclusion
1	Molloy et al., 2009 Ireland	Nested case control study (n=1179). Group 1 (cases=95, controls 265), Group 2 (cases 107, controls 414), group 3 (76 cases, controls 222)	Group 1 & 3 included pregnant women during a neural tube defect (affected pregnancy (NTD-AP) while group 2 who had previous pregnancy with NTD.	The pregnant women with vitamin B_{12} concentrations of < 200ng/L were at 3 times greater risk of NTD than those with levels of >400ng/L	Deficiency or inadequate maternal vitamin B_{12} status is associated with a significantly increased risk for neural tube defects. The pre-pregnant vitamin B_{12} status of >221pmol/L would reduce the further risk of NTDs.
2	Takimoto et al., 2007 Japan	Observational study (n=94)	Women with no complications recruited at 1^{st} or 2^{nd} trimester of pregnancy.	Folate and vitamin B_6 intakes were low during pregnancy. Vitamin B_{12} neither folate conc. showed any association with birthweight in all 3 trimesters. High plasma total Hcy in the 3^{rd} trimester corresponded to 151g ↓ in birthweight.	↑ Hcy in 3^{rd} trimester, ↓ birthweight.
3	Muthayya et al., 2006 India	Observational study (n=112)	< 13 weeks, no complications.	Positive correlation between neonatal vitamin B_{12} status and birth weight in the term babies' upto 40 weeks of gestation.	↓ Maternal vitamin B_{12} status reflected in ↓ cord vitamin B_{12} levels, ↓ Cord vitamin B_{12} status was associated with ↓ birthweight.
4	Muthayya et al., 2006 India	Prospective observational study (n=154)	< 13 weeks, no complications.	Mother's belonging to the lowest tertile of vitamin B_{12} status in the 1^{st} , 2^{nd} and 3^{rd} trimester of pregnancy had 5.98, 9.28 higher risk of having an IUGR baby.	↓ Maternal vitamin B_{12} status during pregnancy, ↑risk for IUGR.
5	Yajnik et al., 2005 India	Observational study (n=80)	30 SGA, 50 AGA babies	Mothers of SGA babies were lighter, shorter, had higher Hcy concentration as compared to mothers of AGA babies. Maternal total Hcy concentration was inversely related to plasma vitamin B_{12}.	Maternal vitamin B_{12} deficiency reflected in plasma Hcy concentration and contributes to small size of Indian babies i.e. ↓ Vitamin B_{12} status, ↑ Hcy plasma levels contribute to low birth weight
6	Ronnenberg et al., 2002 China	Case control study of preterm births (29 preterm, 405	Primiparous, with no complications, non smokers, textile workers, matched for	Preterm birth was associated with both ↑ plasma Hcy and ↓ vitamin B_{12}, not folate, before conception.	Elevated Hcy precedes the occurrence of preterm birth.

		controls), LBW (33 LBW, 390 not LBW) or SGA (65 SGA, 358 controls).	age and education.		
7	Bondevik et al., 2001 Nepal	Cross sectional study (n=328)	No supplements before conception, pregnant women without complications included.	↑ Hcy associated with significant ↓ in gestational age at delivery while a non significant inverse association with birthweight and *APGAR* score. Women with elevated serum Hcy values had higher risks of pre-eclampsia, preterm delivery and LBW though non significant.	The ↑ Hcy values were most likely caused by an impaired vitamin B_{12} status. ~50% of the population was vitamin B_{12} deficit, so Vitamin B_{12} supple is essential in population rather than only folate for better pregnancy outcomes.

2.4.2 *Folate*

Folate has a dual role in methylation, in folate cycle (*mentioned in the previous section*) as well as in pregnancy and birth outcomes. The importance of folate during pregnancy was addressed 40 years ago by Bryan Hibbard in one of his study of folate status in 1984 among low-income obstetric patients from Liverpool (postulating that there is an increased risk of placental abruption in gravidas with abnormal formiminoglutamic acid (FIGLU) excretion and attributed it to a defect in folate metabolism).

A review on folic acid in pregnancy and fetal outcomes revealed the importance of prenatal folic acid supplementation such that the risk of NTD, oral clefts, congenital heart defects and other congenital malformations were reduced implying that folic acid is essential periconceptionally for fetal development (Goh & Koren 2008). Tamura et al (1992) in one of the studies showed that women who had high (above the median) concentration of serum folates in the early 3rd trimester of pregnancy (30 weeks of gestation) had significantly heavier infants as compared with those with serum folate < 44 nmol/L. Similarly in a study conducted in mixed population of white and black multiparous women, use of folate during pregnancy was related to a lower risk of fetal growth retardation and increased birth weight (Goldenberg et al., 1992). Evidence suggests that parity can be a confounding parameter with regards to addressing birth weight. Kloosterman (1970), in the past suggested that multiparous women offer, through remodelling of the maternal vascular structure in former pregnancies, a more favourable environment for placental development and function in subsequent pregnancies. From this respect it can be hypothesized that peri conception folic acid supplementation interacts with these vascular remodelling processes in multiparous women, thereby affecting placental and subsequent fetal growth (Kloosterman 1970). Since the role of folate in DNA synthesis and thus cell replication suggests that folate can influence fetal growth, folate deficiency also interferes with growth of the conceptus, maternal erythropoiesis, growth of the uterus and mammary gland, and growth of the placenta (IOM 1990).

In a developing country in Pakistan, an association between decreased levels of folate in the materno-fetal circulation during the third trimester and increased

occurrence of IUGR (Lindblad et al., 2005) was observed. Since the vitamin B_{12} levels did not differ between IUGR and pregnancies with normal outcome in the non vegetarian population, this observation suggested a specific role for folate deficiency in late pregnancy complicated by IUGR. Moreover the findings of the total disappearance of the normal correlation between cord and maternal folate levels in IUGR supported the assumption of a placental dysfunction in folate deficiency with hyperhomocysteinemia (Lindblad et al., 2005). The influence of dietary and circulating folate on preterm delivery and infant low birth weight was studied in 832 women from the Camden Study (Scholl et al., 1996). Similarly Relton et al (2005) in a prospective observational study concluded that maternal RBC folate is a predictor of birth weight after controlling for gender and gestational age. Low intakes of folate from diet and supplements were associated with maternal characteristics reflecting poor nutritional status, including low energy intake, low rate of gestational weight gain, and a high frequency of iron deficiency anemia at entry to prenatal care. A significant positive relation between dietary folate intake and serum folate at week 28 (r=0.17) was observed such that the low folate intake (< 240 ng folate/d) was associated with a > 3-fold increase in risk of LBW and of preterm delivery, after controlling for confounding factors (maternal age, parity, ethnicity, smoking, gestational weight gain, and intake of energy and other nutrients (zinc, fiber, and vitamin B_{12}). Similarly circulating folate at week 28 was also associated with risk; the adjusted odds ratio for LBW increased by 1.5% and the odds ratio for preterm delivery increased by 1.6% per unit (nmol/L) for each unit decrease in serum folate at week 28 indicating that the lower concentrations of serum folate at week 28 were associated with a greater risk of preterm delivery and LBW (Scholl et al., 1996).

Studies conducted by Tamura et al (1992) and Goldenberg et al (1992) have shown that the folates from diet and supplements correlated with serum folate (r=0.25) at weeks 18, mid pregnancy and (r=0.12) at early 3rd trimester (30 weeks of gestation). Higher serum folate at 30 weeks of gestation predicted higher infant birth weight (2.1 g birth weight per unit serum folate, $p<0.05$) and decreased SGA ($p<0.005$). Similarly in an observational study, Malinow et al (1998) showed that maternal folate positively correlated with neonatal weight. In a case-control study, positive bivariate correlation (at week 32 gestation) was observed between maternal red cell folate and infant birth weight (r=0.48, $p<0.02$) suggestive of folate status in

the 3rd trimester of pregnancy as a predictor of birth weight (Frelut et al., 1995), while Lauzikiene et al (2003) showed that folate supplementation in early gestation would reduce spontaneous abortion. The study showed that women with spontaneous abortions had lower folate levels. Many observational studies of folate during pregnancy also suggest a potential benefit of good folate status and an improvement in birth weight and gestation.

Though folic acid supplementation has been a part of the routine antenatal care, number of studies has shown a positive effect of the use of folic acid in the peri-conceptional period on birth outcomes other than NTD. Preconception folic acid supplementation was associated with a 68 g higher birth weight and 13g higher placental weight when compared to those who did not use folic acid. Folic acid supplementation after pregnancy confirmation was associated with a reduced risk of LBW (OR 0.61, 95% CI 0.40, 0.94). Similarly, a reduced risk of LBW and SGA was observed for the women who started supplementation preconceptionally, compared to those who did not use folic acid (OR 0.43, 95% CI 0.28, 0.69 and OR 0.40, 95% CI 0.22, 0.72) (Timmermans et al., 2009). Despite studies showing a beneficial affects of folates, there have also been contradictory findings. Recently in a large population based prospective study of well nourished Norwegian pregnant women, the dietary folate and plasma folate during the 2nd trimester of pregnancy were not identified as the risk factors for infant birth size though there was non significant weak tendency for increased risk of SGA at lower folate values ($p=0.49$). In this study there was a strong association of maternal smoking with SGA before and after adjusting for total dietary folate intake ($p<0.001$) but not with gestational age at birth implying that the other confounding factors such as socio-economic status, life style and habits cannot be ruled out. Moreover almost (53% of the women had a total dietary folate intakes of ≥ 400 μg/d (Nilsen et al., 2010).

Folate deficiencies have been linked to increased concentration of intermediary metabolite, Hcy. Hyperhomocysteinemia, a marker for folate deficiency or metabolic abnormality, has been associated with serious complications such as PIH, preeclampsia and placental abruption (Dekker et al., 1995; Rajkovic et al., 1997). The interrelation between the folates and Hcy is addressed in the following session (*2.4.4*).

Table 4. Role of maternal folate intakes / status and birth outcomes.

Sno	Author	Study design	Inclusion/ Exclusion Criteria	Results	Conclusion
1	Nilsen et al., 2010 Norway	Population based prospective study (n=2934)	Pregnant women registered in the Medical Birth Registry of Norway with singleton pregnancy. Blood sample collected and FFQ administered in the 2nd trimester.	The dietary folate intake, supplemental folic acid use and maternal folate concentrations, measured at 2nd trimester were not associated with gestational age, infant birth weight, head circumference or crown heel length. There was a non significant tendency for increased SGA risk at lower folate values.	In well nourished Norwegian pregnant women, the dietary folate and plasma folate during the second trimester are not a risk factor for infant birth size.
2	Timmermans et al., 2009 Netherlands	Population based prospective pregnancy cohort. The generation R Study (n=6353)	Low risk singleton pregnancies. Enrolment <18 weeks gestation until delivery	Preconception folic acid supplementation was associated with 68g higher birth weight, 13 g higher placental weight compared to those who did not use folic acid.	Peri-conception folic acid supplementation is associated with increased fetal growth resulting in higher placenta and birth weight, and decreased risks of LBW and SGA.
3	Relton et al 2005 United Kingdom	Prospective observational study (n=998)	Smoking mothers included	Maternal RBC folate is a predictor of birth weight after controlling for gender and gestational age.	↑ RBC folate in the early pregnancy ↑ birth weight
4	Lindblad et al., 2005 Pakistan	Prospective observational study (n=128)	Low socio-economic subjects	IUGR increased with low maternal and cord folate concentration and with high plasma Hcy concentration.	↑ IUGR with ↓ maternal and cord folate and ↑ Hcy levels.
5	Dalia et al., 2003 Lithuania	Case control study (n=55). 27 had abortion at 1st trimester, 28 had elective abortion	Women with vitamin B supplements or oral contraceptive 3 month prior to conception were excluded, non smokers	Women with spontaneous abortion had lower folate concentration and higher Hcy as compared to the ones with elective abortion.	Folate supplementation early gestation may reduce spontaneous abortion.
6	Zeng et al., 2001 China (rural)	Double blind cluster randomised controlled trial (n=5828). 3 groups (folic acid, iron-folic acid and Multi-micronutrient, 15 mineral or vitamin.	<28 weeks gestation and no other supplements	Group with iron-folic acid supplements had longer gestational age. Multi-micronutrient supplement group showed a modest increase in the birth weight.	Iron folic acid supplement is associated with longer gestational age at birth and reduce preterm delivery.

7	Scholl & Johnson 2000, The Camden study, USA	Review (n=832)	Pregnant women <18 years of age also included	↓ Folate intake (< 240 ng folate/d) was associated with > 3-fold ↑ in risk of infant low birth weight and preterm delivery, after controlling for confounders (maternal age, parity, ethnicity, smoking, gestational weight gain, and intake of energy and other nutrients; zinc, fiber, and vitamin B_{12})	↓ Folate intake associated with ↑ LBW, preterm births and fetal retardation. A metabolic effect of folate deficiency is an elevation of blood Hcy.
8	Malinow et al., 1998 Ohio	Observational study (n=35)	Nulliparous healthy pregnant women	Low vitamin B_{12} and folate not associated with Hcy. However, neonatal weight negatively correlated with maternal levels of Hcy ($r = -0.36$; $p = 0.036$) and positively with folate ($r = 0.46$; $p= 0.006$)	Maternal folate positively correlated with neonatal weight. Maternal Hcy correlated negatively with gest age at birth and birth weight
9	Neggers et al., 1997 Birmingham, USA	Prospective study (n=289)	Women with no complication	Small positive association with folate and adjusted birth weight (beta = 0.05, $p = 0.03$)	Folate intake was significantly associated with birth weight (weak predictor)
10	Scholl et al., 1996 USA	Prospective observational study (n=832) (The Camden study)	Uncomplicated pregnancy. Information obtained at entry to prenatal care, at 28 weeks and 36 weeks gestation.	Women with low folate intake (< 240 mg/d) had > 3 times greater preterm delivery and infant low birth weight than women with folate intake > 240 µg/d ($P < 0.05$). Risk of preterm delivery without premature rupture of membranes increased 3 times ($P <0.05$). Odds of preterm delivery increased 1.5% per unit decrease in serum folate ($P < 0.05$).	Low folate associated with Low birth weight and preterm
11	Frelut et al., 1995 France	Case-control (n=21)	IUGR=8, non IUGR=13 at 3rd trimester	No relation with birth weight and folate at delivery, but with birthweight and folate in the 3rd trimester	Folate status in the 3rd trimester predicts birth weight
12	Czeizel et al., 1994 Hungary	RCT (N=5502) (Hungarian trial) (no true placebo used). Supplemented 28 days before conception with 0.8mg folic acid & trace element	12 vitamins and trace elements	No significant effect seen in birth weight and gestational age after the peri-conceptional multi vitamin supplementation	Peri-conceptional multivitamin supplement increases fertility and prevents NTD and some other major defects.
13	Tamura et al., 1992 Birmingham	Large scale study (n=285)	Data on term infants only	↑ folate concentration during pregnancy, ↓ the risk of fetal growth	Folic acid supple had favorable effects on birthweight and

				retardation	reduced fetal growth retardation.
14	Goldenberg et al, 1992 Birmingham, USA	Longitudinal study (n=289)	Mixed white & black multiparous included	Birth weight was higher in women supplemented with folate during pregnancy	Use of folate was related to a lower risk of fetal growth retardation and increase birth weight.

2.4.3 *Vitamin B_6*

Vitamin B_6 is one of the important nutrients in the methionine cycle. Numbers of studies indicate the importance of vitamin B_6 in the growth and development of the fetus. The greatest growth of the fetus and the placenta occurs between the 30^{th} week of pregnancy and term, and the placenta also has its own requirements of nutrition. In the same gestational period a rapid decrease of plasma pyridoxal 5'-phosphate (PLP) level occurs. Since PLP is involved in myelination and other aspects of the development of the fetal nervous system, it is important for the maternal vitamin B_6 to be adequate (Schuster et al., 1984).

There have been contradictory findings with regards to vitamin B_6 supplements and their effect on birth outcomes. Recent Cochrane reviews have not shown promising findings with vitamin B_6 supplementation during pregnancy but studies in the past have shown beneficial effects. One of the Cochrane reviews conducted by Mohamed & Gulmezoglu (2003), that included RCT of pyridoxine administration compared to the control groups showed only a decrease in the incidence of dental decay in pregnancy but not enough evidence on beneficial role of vitamin B_6 in birth outcomes. Another Cochrane review by Thavera et al (2006) did not find any statistically significant differences in the risk of eclampsia, pre-eclampsia or low Apgar scores at birth at one or five minutes, between supplemented and non-supplemented women. However, on contrary, a small trial showed reduced mean birth weights with vitamin B_6 supplementation (weighted mean difference -0.23 kg; 95% CI -0.42 to -0.04; n=33; one trial).

Though maternal vitamin B_6 status is not directly linked to adverse birth outcomes in terms of birth weight or gestational age, deficiency is known to affect the *APGAR* score of the baby at birth. Roepke et al (1979) have shown that mothers with low serum vitamin B_6 (mean value 7.8 ng/ml) at delivery had infants with *APGAR* score < 7 at 1 min as compared to the mother's who had higher serum vitamin B_6 (mean value 15.5 ng/ml) and their babies had *APGAR* scores of ≥ 7. Intervention studies with 2 mg/d of pyridoxine supplements have shown higher birth weights in babies of supplemented mothers group and lower *APGAR* score in babies of non supplemented group. The authors suggested that a daily supplement of 2 mg pyridoxine HCl to the healthy pregnant women provided the adequacy of maternal

and neonatal vitamin B_6 status and the satisfactory neonatal growth (Chang et al., 1999). Moreover, it is also seen that the women with very low vitamin B_6 show signs of toxaemia, which could be attributed to the high Hcy levels, since vitamin B_6 is the cofactor involved in the transsulfuration pathway. While these findings were seen in the 5^{th} month of gestation, which precedes the peak of hemodilution that is known to alter the concentration of the vitamin in serum; this time also precedes the most rapid growth in the central nervous system when vitamin B_6 is crucial for normal development (Roepke et al., 1979).

A study conducted in Japan has shown that vitamin B_6 deficiency is one of the common causes of nutritional anemia in pregnancy (Hisano et al., 2010). It is known that anemia during pregnancy has adverse affects on the pregnancy as well as the birth outcomes. The authors found that in this interventional study, supplementing pregnant women with 75gm of vitamin B_6 improved the hemoglobin concentration. The transfer of maternal vitamin B_6 to the fetus i.e. in the cord blood was seen in a study conducted in Indiana (Lumeng et al., 1976) and similar findings were also observed recently (Hisano et al., 2010) implying the placental transfer of vitamin B_6 to the fetus from the mother. Based on the plasma PLP measurements, Lumeng and his colleagues have suggested that more than 4 mg of pyridoxine is required to restore vitamin B_6 nutrition in the mother during pregnancy while Schustner et al (1984) have shown that a dose of 5 mg PN-HCl was adequate to maintain the PLP plasma levels at 30 weeks of gestation and a dose > 7.5 mg was positively correlated with *APGAR* scores at birth.

Several studies in the past have shown relationships between low vitamin B_6 and birth outcomes. Kubler (1981), Reinken and Dapunt (1978) reported positive relationships between maternal vitamin B_6 status during pregnancy and infant birth weight., while Brophy and Siiteri (1975) found an inverse association between the vitamin B_6 concentration in cord blood and the risk of preeclampsia, which is itself a risk factor for preterm births (Ananth et al., 1997). More recent findings from a case-control study of preterm births, LBW and SGA in a Chinese population led the authors to postulate that an elevated Hcy and suboptimal vitamin B_6 and vitamin B_{12} may increase the risk of preterm births (Ronnenberg et al., 2002). In a logistic regression analysis adjusting for the potential confounders (age, BMI, hemoglobin

concentration), the risk of preterm birth tended to be ~50% lower among women with adequate vitamin B_6 status (pyridoxal 5' phosphate ≥ 30 nmol/L) compared with women with vitamin B_6 deficiency, although this association was not statistically significant (p=0.09). In addition, a positive significant correlation between plasma Hcy and vitamin B_6 was observed. In another study conducted by Ronnenberg et al (2007), the higher preconception plasma vitamin B_6 concentrations were associated with reduced odds of early pregnancy losses and higher probabilities of achieving conception and clinical pregnancy. Moreover the risk of clinical spontaneous abortion was more than twice as high among women in the lowest vitamin B_6 quintile compared with those in the highest. Though the mechanism by which Hcy and vitamin B_{12} and B_6 influence preterm births is not fully known, body of evidences suggests a possible role of vitamin B_6 in preeclampsia (Rajkovic et al., 1999; Sorensen et al., 1999), elevated Hcy may also compromise pregnancy outcomes by interfering with connective tissue integrity, thereby increasing the risk of preterm premature rupture of membrane (Ferguson 2001), which could affect implantation and early placental development. Moreover vitamin B_6 serves as a coenzyme in >100 reactions, including many involved in amino acid and neurotransmitter synthesis and those necessary to form collagen cross links (Masse 1990). Therefore it is plausible that the deficiencies of either vitamin B_{12} or vitamin B_6 could contribute to chorionic, hormonal or other abnormalities resulting in preterm births. Elevated Hcy is linked to reduced nitric oxide concentration and glutathione peroxidase activity (Keaney 1997) and it is possible that such disruptions could affect the length of the gestation.

Table 5. Role of maternal vitamin B_6 intake / status and birth outcomes; baby parameters.

Sno	Author	Study design	Inclusion/ Exclusion Criteria	Results	Conclusion
1	Hisano et al., 2010 Japan	Interventional study (n=56)	Anemics and vitamin B_6 deficient pregnant women.	Anemia improved with vitamin B_6 (pyridoxine hydrochloride, 75gm) supplementation.	Vitamin B_6 deficiency is common during pregnancy and presumably contributes to the high prevalence of anemia.
2	Ronnenberg et al., 2007 China	Prospective observational study (n=364)	Married women, who conceived at least once during the prospective observation, had adequate daily diary maintained, hCG data and preconception vitamin and homocysteine concentration information available.	Low Vitamin B_6 was related to early loss of pregnancy	Vitamin B_6 status may influence the reproductive events throughout the entire course of pregnancy, from the time of conception through delivery such as low vitamin B_6 status linked to decreased probability of conception and contributing to the risk of early pregnancy loss.
3	Thaver et al., 2005	Cochrane review. Cochrane Pregnancy and Childbirth Group Trials Register (n=1646)	RCT, (placebos, no supplementations, or supplements not containing vitamin B_6) (5 trials included)	A small trial showed reduced mean birth weights with vitamin B_6 supplementation	No enough evidence to detect clinical benefits of vitamin B_6 supple in pregnancy and/or labor
4	Ronnenberg et al., 2002 China	Case control study of preterm, LBW and SGA (n=423 total); preterm 29, control 405.	Primiparous pregnant women matched for the employment, age and education, never smoked.	Total Hcy positively correlated with vitamin B_6 concentration during pregnancy. Vitamin B_6 deficiency ~50% common in preterm cases. The risk of preterm birth tended to be ~50% lower among the women with adequate vitamin B_6 status (≥30 nmol/L) compared with women with vitamin B_6 deficiency in a logistic regression analysis.	Sub optimal vitamin B_6 levels as well as vitamin B_{12} along with elevated Hcy concentrations may increase the risk of preterm births.
5	Mohamad et al., 2000	Cochrane review. Cochrane Pregnancy	RCT of pyridoxine (oral or lozenges) administration	Vitamin B_6 supple was associated with decreased	No enough evidence to detect clinical benefits of vitamin B_6 supplementation

		and Childbirth Group Trials Register (n=371)	compared to a control group. (uncomplicated pregnancy)	incidence of dental decay in pregnant women but no evidence for birth outcomes.	during pregnancy and/or labor
6	Chang et al., 1999 Japan	Randomized controlled trial (N=209)	Uncomplicated pregnant women	Higher birth weight in mothers with 2mg/d pyridoxine supple and lower *APGAR* score at 1min in mothers who were not supplemented.	A daily supplement of 2 mg pyridoxine HCl provides the adequacy of maternal and neonatal vitamin B_6 status and the satisfactory growth of neonates at birth.
7	Schuster et al., 1984 USA	Double blind randomized study (n=196).	Pregnant women and control group of non pregnant women <40 years within 22 weeks of gestation and divided into 6 groups. Each group received varying dose of vitamin B6; 2.6, 5, 7.5, 10, 15 and 20mg of PN-HCl.	Maternal plasma PLP levels positively correlated with vitamin B6 supplementation at 30 weeks of gestation. The decrease in plasma PLP levels after 30 weeks of gestation was seen in groups supplemented with 7.5 mg and more PN-HCl. *APGAR* scores at 1 min after birth was significantly higher in the group receiving supplement >7.5mg while the birth weight, length or placental weights were not significantly different.	Only 55% of the pregnant women met the RDA for vitamin B_6. Supplementation of vitamin B_6 more than 7.5 mg beneficial in terms of PLP levels as well as *APGAR* scores.
8	Roepke et al., 1979 Indiana	Observational study (n=102)	Healthy pregnant women, without pre-existing diabetes or hypertension disease.	Mother's with low serum vitamin B_6 during 5^{th} and 7^{th} month of pregnancy had low serum cord vitamin B_6 status. The mother's with low serum vitamin B_6 also had babies with *APGAR* scores <7 at 1min after birth.	Translation of maternal serum vitamin B_6 levels to cord serum levels (surrogate of fetal levels). Low maternal serum vitamin B_6 affects the *APGAR* score.
9	Lumeng et al., 1976 Indiana	Observational study, women grouped into 3 groups based on the vitamin B_6 amounts.	Healthy pregnant women	Highly significant correlation between maternal and cord plasma PLP levels.	Maternal vitamin B_6 nutrition affects the plasma level of PLP in the fetus.
10	Heller et al., 1973 Switzerland	Observational study (n=493)	Uncomplicated pregnancy	$1/3^{rd}$ of the subjects were vitamin B_6 deficit.	No clinical sequele seen with vitamin B_6 deficiency for the mother or fetus during pregnancy.

2.4.4 *Interrelationship between B vitamins and homocysteine and birth outcomes*

Vitamin B_{12}, vitamin B_6 and folates are involved in the methylation cycle hence their role in birth outcome may be interrelated and may also mediate through Hcy.

In a study in Japan, negative correlations between total Hcy and maternal vitamin B_{12} and folates was observed implying the relationships of vitamin B_{12} and folate deficiencies leading to hyperhomocysteinemia. Moreover higher plasma total Hcy in the 3rd trimester was a predictor of low birth weight (Takimoto et al., 2007). In a Brazilian population, maternal total Hcy level was inversely correlated with maternal serum folate, whereas neonatal total Hcy levels were directly affected by maternal total Hcy levels and inversely affected by neonatal B_{12} level. These findings support the conclusion that low vitamin B_{12} and folate levels are associated with hyperhomocysteinemia in pregnant women and new born babies (Guerra-Shinohara et al., 2002). While vitamin B_{12} and folate status are inversely correlated to Hcy concentration, study conducted in rural, India showed that higher plasma total Hcy concentrations were significantly associated with lower birth weight ($r=-0.28$, $p<0.05$ adjusting for maternal height, weight, gestational age at delivery and the baby's gender. The group also confirmed on the inverse association between maternal total Hcy concentration and the size of the offspring at birth. Elevated Hcy may impair fetal growth by affecting maternal cardiovascular adjustments in pregnancy as well as the placental circulations.

In a study conducted in Peru suggested that hyperhomocysteinemia during pregnancy was a risk factor for preeclampsia but not associated with low plasma vitamin B_{12} concentration. The women in the highest quartile of Hcy concentration experienced a three-fold increased risk of preeclampsia as compared with women in the lowest tertile. After adjustment for potential confounding factors, the relative risk between extreme quartiles increased slightly (OR=4.0; 95% CI: 1.8, 8.9). Similar findings with Hcy concentrations were shown by Murphy (2004), such that; the women supplemented with folates had lower Hcy levels during labour and the neonates born to mothers with elevated Hcy had lower birth weight babies. Wallace and his colleagues also found folate to be a significant predictor of the total Hcy concentration during pregnancy (Wallace et al., 1999). A negative correlation between RBC folate and total Hcy was seen in a cross sectional study conducted in

Ottawa and in these subjects supplementation of folic acid increased the serum folate levels with decreased Hcy levels confirming the inverse relation between folate and Hcy (Walker et al., 1999). A randomised controlled trial showed that the group receiving folic acid for a short duration during pregnancy had increasing plasma Hcy concentration as the pregnancy progressed while the group that had folic acid supplementation throughout pregnancy had no significant change in the fasting plasma Hcy concentrations implying that intervention with folic acid may be effective in reducing plasma Hcy levels and the vascular events (Ellison et al., 2004). Akin to the above findings, Holmes et al observed that plasma total Hcy levels were lower in pregnant women taking folic acid supplements than in those women not taking a supplement, an effect that was statistically significant at 35 wks ($p<0.001$; ratio=0.78; CI, 0.72 - 0.86) further concluding that beyond 12 weeks of gestation, continuing folic acid supplements may prevent late pregnancy complications attributed to hyperhomocysteinemia, such as preeclampsia (Holmes et al., 2005). Although folate status was not associated with preterm birth, and Hcy and B vitamin status were not associated with LBW or SGA status in a study by Ronnenberg et al (2002), the authors postulated that elevated Hcy and suboptimal vitamin B_{12} and B_6 status may increase the risk of preterm birth. It is thought that elevated Hcy may compromise pregnancy outcomes by interfering with connective tissue integrity, thereby increasing the risk of preterm premature rupture of membranes (Ferguson et al., 2001). Further elevated Hcy is also known to be linked to reduced nitric oxide concentration and glutathione peroxidase activity (Keaney et al., 1997), and it is possible that such disruptions could affect the length of gestation.

Further studies have looked at associations between Hcy and birth outcomes independently. In a study conducted in Norway, plasma Hcy was significantly related to IUGR while a weaker association with LBW was seen suggesting total Hcy as a maker of pregnancy complications and adverse birth outcomes (Vollset et al., 2000). The important pathways and the enzymes involved in the methylation cycle are well known. Cystathionine β-synthase requires vitamin B_6, methionine synthase requires folate and vitamin B_{12}, and methylenetetrahydrofolate reductase (MTHFR) requires folate for its normal function. In addition it is also known that Hcy metabolism can be affected by variants of enzyme genes, particularly the thermolabile 677C→T variant of MTHFR, which results in reduced enzyme activity. Homozygosity of this

variant, expressed as TT genotype is associated with elevated Hcy and low folate levels. Along these lines, a study from a Korean population showed that serum Hcy negatively correlated with serum folate in all MTHFR genotypes implying that increased serum folate, vitamin B_2 and vitamin B_{12} concentration may decrease the MTHFR genotypic effect on serum Hcy levels (Kim et al., 2004).

Table 6. Interrelationship between B vitamins and homocysteine and birth outcomes

Sno	Author	Study design	Inclusion/ Exclusion Criteria	Results	Conclusion
1	Wallace 2008 Seychelles	Observational study (n=276)	Healthy pregnant women, blood samples at recruitment, 28 weeks and at del	Folate was a significant predictor of Hcy during pregnancy. Betaine was only a significant predictor of maternal Hcy when the essential amino acid methionine was low	Fetal requirements for folate are paramount, such that cord blood folate status is maintained, even when maternal status is low. Folate was the strongest predictor of total Hcy.
2	Takimoto et al., 2007 Japan	Observational study (n=94) with assessment of Vitamin B_{12}, serum folate & RBC Folate	Women wit no complications included	Significant negative correlation between plasma Hcy and serum folate concentration in 1^{st} tri, in 2^{nd} tri plasma Hcy showed negative associations with serum B_{12}, and serum and RBC folate conc. In the 3^{rd} tri, Hcy and serum folate correlated negatively and serum B_{12} correlated significantly with serum folate	Higher plasma Hcy in the third trimester, a predictor of lower birth weight
3	Yajnik et al., 2005 India	Subset from the prospective population study (PMNS) (n=80). Mother's of SGA babies (n=30) and mother's of AGA babies (n=50)	Healthy pregnant women recruited < 18 weeks of gestation.	Mother's of SGA babies were lighter, shorter and had higher total Hcy concentration as compared to mother's of AGA babies.	Maternal vitamin B_{12} deficiency reflected in plasma total Hcy concentration. Higher maternal plasma Hcy concentration was significantly associated with lower offspring birth weight.
4	Holmes et al., 2005 United Kingdom	Longitudinal study (n=120). Healthy pregnant women and age-matched control individuals	Pregnant women at ~12 weeks of gestation with age-matched non pregnant controls randomly recruited at the same time. Study participants had no previous history of thrombosis to other chronic illness.	Plasma Hcy was lower in omen with folic acid supplementation than the women not taking supplements. The Hcy lowering effect of folic acid supplementation in the 3^{rd} trimester was observed irrespective of the women reported history of miscarriage.	Folic acid supplements throughout pregnancy have the potential role in reducing pregnancy complications associated with hyperhomocysteinemia.
5	Ellison et al., 2004 United Kingdom	Randomized controlled trial; one group to	Healthy singleton pregnant women.	Hcy increased in the control group as the pregnancy progressed while	Folic acid supplementation may be effective in reducing plasma Hcy

		receive folic acid (400μg) once daily until 16 weeks and other group to take folic acid until delivery (n=15 each).	Overnight venous blood collected at 3 time points;(14-19 weeks, 26-32 and 34-37 weeks of gestation)	no significant change in fasting plasma Hcy seen those who continued folic acid until delivery.	levels and the vascular events.
6	Murphy et al., 2004 Spain	Longitudinal study (n=93)	Pre-pregnancy blood samples also collected	Folate Supplemented mothers had lower Hcy at labor. Neonates of mothers in the highest tertile of Hcy weighed less.	Neonates born to mothers with ↑ Hcy had lower birthweight.
7	Kim 2004 Korea	Cross sectional study (n=177)	No complication, no medication, and free of pregravid chronic diseases.	↑ Hcy in women with the T/T genotype than those with the C/T or C/C of the MTHFR gene. Serum Hcy negatively correlated with serum folate in all MTHFR genotypes. ↑ Hcy in the subjects with the T/T MTHFR genotype when the serum folate was below the median level.	Serum Hcy levels in pregnant women varied significantly with MTHFR genotype and the serum B vitamin status. ↑ Serum folate, vitamin B_2 & vitamin B_{12} concentration may decrease the MTHFR genotypic effect on serum Hcy levels.
8	Guerra-Shinohara 2002 Brazil	Prospective observational study (n=69)	Healthy pregnant women. Subjects with clinical diagnosis of metabolic disease, multiple deliveries, and complications during delivery including the birth of am immature baby, or a baby with congenital malformation, and anoxia were excluded.	High correlation between maternal vitamin B_{12} and cord vitamin b12 ($r=0.68$, $P<0.01$). Significant correlation between neonatal vitamin B_{12} and neonatal total Hcy levels ($r=-0.53$, $P<0.01$) and a strong correlation between maternal and neonatal total Hcy levels ($r=0.76$, $P<0.01$).	Low vitamin B_{12} and folate levels are associated with hyperhomocysteinemia in pregnant women and new born babies.
9	Ronnenberg et al., 2002 China	Case control study of preterm, LBW and SGA (n=423 total);	Primiparous pregnant women matched for the employment, age and education, never smoked.	Total Hcy positively correlated with vitamin B_6 concentration during pregnancy. Vitamin B_6 deficiency ~50% common in preterm cases. The risk of preterm birth tended to be ~50% lower among the women with adequate vitamin B6 status (≥30 nmol/L) compared with	Sub optimal vitamin B_6 levels as well as vitamin B_{12} along with elevated Hcy concentrations may increase the risk of preterm births.

				women with vitamin B_6 deficiency in a logistic regression analysis.	
10	Vollset et al., 2000 Norway	Population based study retrospective (n=5883)	Women with no pregnancies registered were not included in this study. Linked population-based cardiovascular survey of 40–42-y-old women to the Medical Birth Registry of Norway	Strong association between LBW Hcy conc. Plasma Hcy was significantly related to growth retardation, but the strength of the association was weaker than the associations between Hcy and low birth weight.	Total Hcy as a marker of pregnancy complications and adverse pregnancy outcomes.
11	Walker et al., 1999 Ottawa	Cross sectional study (n=155). 40 subjects (8-16 weeks), 37 in 20-28 while 50 in 36-42 wks, 28 in the control Group (non-pregnant). 84 took no vitamin supplement. 71 took prenatal vitamin supplement with 1 mg of folic acid.	Non smokers pregnant women with no complication included	Positive correlation between albumin and Hcy concentration, and negatively correlation between serum RBC folate and Hcy	Folic acid supple resulted in increase serum RBC folate and decrease Hcy levels.

2.5 Importance of selected nutrients in the pathogenesis of PIH and adverse birth outcomes.

Elevated homocysteine concentration is involved in pathogenesis of PIH as well as responsible for adverse birth outcomes. Similarly evidences show relationship between low dietary calcium intakes and PIH, and PIH in turn affecting adverse birth outcomes.

Pregnancy induced hypertension (PIH), preeclampsia

PIH which occurs in ~11 % pregnancy is a major risk factor for maternal and perinatal morbidity and mortality. PIH includes gestational hypertension as well as preeclampsia and eclampsia. Gestational hypertension is characterised by an abnormal rise in blood pressure that usually develops after the 20^{th} week of pregnancy where the diastolic blood pressure is greater than or equal to 90 mmHg at two consecutive intervals of 4 hours apart. In addition to hypertension, symptoms of preeclampsia include proteinuria (2+ by dipstick testing, greater than or equal to 300 mg / 24 hours, or greater or equal to 500 mg/L and edema. Urine protein/creatinine ratio is used increasingly as a measure of proteinuria. PIH can also result in preterm labour and delivery and LBW infants. Preeclampsia is a major risk for severe perinatal morbidity and mortality, but gestational hypertension without proteinuria also independently increases perinatal risk. If the condition progress to eclampsia, life threatening convulsions and coma can occur.

It is hypothesized that preeclampsia and some forms of intrauterine growth restriction share a common cause of placental disease described as an abnormal implantation and characterized by failure of trophoblasts to differentiate, to invade, and to remodel the spiral arteries affecting the vascular structure of the placenta or reducing blood flow to the fetus. Though preeclampsia and intrauterine growth restriction have common cause of placental dysfunction they have different clinical manifestations.

2.5.1 *Hyperhomocysteinemia and PIH*

Increased Hcy concentration (hyperhomocysteinemia) is linked to other B vitamins such as folate, vitamin B_{12} as well as vitamin B_6 that are involved in the methylation cycle. Several studies have looked at relationship between folic acid

intakes and folate blood status and plasma Hcy concentration during pregnancy. In a longitudinal study, a negative relationship was observed with serum folic acid concentration and plasma Hcy implying that low concentration during pregnancy may lead to higher concentration of Hcy (Patrick et al., 2004). A high homocysteine concentration was also seen in preeclamptic women. Similarly in another case control study of severe preeclamptic and normotensive controls, subjects with hyperhomocysteinemia in early pregnancy had higher risk of developing preeclampsia and had lower plasma folate and vitamin B_{12} levels as well as delivered smaller babies. Cotter et al (2003) showed that high Hcy in early pregnancy was associated with 4-fold increased risk for developing non severe pre-eclampsia. Findings from randomised control trial; one group that received 400 μg/d folic acid until 16 weeks of gestation while the other group received the supplements until delivery suggested that folic acid supplementation may be effective in reducing the plasma Hcy levels and thereby the vascular events (Ellison et al., 2004).

While folic acid supplements have shown a relationship with Hcy, studies have also looked at vitamin B_{12} status. Sanchez et al (2001) in a case control study of preeclamptic and normotensive subjects have shown that subjects with low plasma vitamin B_{12} had lower risk of preeclampsia and the relative risk of preeclampsia increased with increasing quartiles of maternal Hcy concentration. In addition plasma folate was inversely related to Hcy concentration. Evidence indicates that Hcy has a role in the pathogenesis of preeclampsia. A case-control study conducted by Rajkovic et al (1997) of preeclamptic and normotensive women during delivery showed an increased plasma Hcy concentration and early delivery in the cases compared to the normotensive women suggesting that in nulliparous with preeclampsia, elevated Hcy concentration injures the vascular endothelium, thereby contributing to the pathogenesis of preeclampsia. Looking at the specific trimester, a prospective nested case control study in the United States; among 52 preeclamptic women and 56 normotensive women throughout pregnancy revealed that an association of increased Hcy and preeclampsia existed in patients with Hcy in the highest deciles of the distribution. After adjusting for the potential confounders (maternal age, parity and body mass index), a second trimester elevation of Hcy was associated with 3.2 fold increased risk of preeclampsia (AOR 3.2; 95% CI 1.1-9.2; p=0.030) (Sorensen et al., 1999). In a prospective observational study, a positive

correlation between maternal Hcy and neonatal Hcy was observed. Moreover the women belonging to the highest tertile of Hcy concentration had babies weighing significantly less (228 g) as compared to the babies born to mothers belonging to the low and medium Hcy tertiles (Murphy et al., 2004). A population - based observational study conducted in Norway (*called The Hordaland Study*) showed that there was a significant association of maternal plasma Hcy with premature delivery both of <37 weeks and <32 weeks of gestation. The adjusted odds ratio, after comparing the upper with the lower Hcy quartile for premature delivery (<32 weeks gestation) was 1.93 (95% CI: 0.96, 3.93; p=0.03) implying that although maternal plasma total Hcy was significantly related to growth retardation, the strength of the association with very LBW was the strongest (Vollest et al., 2000).

Although maternal plasma Hcy is thought to have a role in adverse birth outcome, a study conducted by Hogg et al (2000) showed a contradictory finding in that the second trimester plasma Hcy concentrations did not predict the subsequent development of PIH, preeclampsia, and IUGR. The reason attributed to the findings was due to the similar mean Hcy levels in women with PIH and preeclampsia to those of control subjects at 26 weeks of gestation. In addition the Hcy levels were similar between women with pregnancies complicated by IUGR and control subjects at both time points.

Table 7. Role of nutrients in relation to blood pressure and birth outcomes

Sno	Author	Study design	Inclusion/Exclusion Criteria	Results with respect to PIH/birth outcome	Conclusion
NUTRIENTS					
1	Patrick et al., 2004 Pittsburgh, PA	Longitudinal study (n=78). Recruited at the time of admission to the labour ward for delivery	Nulliparous with no history of complication. At recruitment some were coded hypertensive and normal	↓Folic acid correlated with ↑ Hcy in black women with preeclampsia	↑ Hcy concentration in preeclamptic women, ↓ folic acid correlated with ↑ Hcy
2	Ellison et al., 2004 United Kingdom	RCT (n=30). One group received folic acid (400µg/d) until 16 weeks, other group got until del	20-35 years. No complications	In control group, ↑ in Hcy and ↓ of folate with increasing gestational age.	Folic acid may be effective in reducing plasma Hcy levels.
3	Murphy et al., 2004 Spain	Prospective observational study (n=93)	Homocysteine levels screened at pre-pregnant period, 8, 20, 32 weeks of gestation and in cord blood.	Folic acid supplemented women had lower Hcy concentration. Neonates of mothers in the highest Hcy tertile at labour weighed, on average, 227.98 g less than those of mothers in the low and medium tertiles (P =0.014).	Neonates of mothers in the highest tertile of Hcy weighed less.
4	Cotter et al., 2003 Dublin	Case control study (n= 213). 71 cases of non severe preeclampsia, 142 controls normotensive.	Controls had no complications & matched for parity, gestational age and date of sample collection.	Subjects with ↑Hcy in early pregnancy had higher risk of developing pre-eclampsia, had ↓plasma folate and B12 levels and delivered smaller babies.	↑Hcy in early pregnancy is associated with a 4-fold ↑ risk for the development of non severe pre-eclampsia
5	Sanchez et al., 2001 Peru	Case control study (n=304). Pre-eclampsia (n=125), normotensive (n=179)	Cases to be diagnosed as pre-eclampsia, control no complications	No increased risk of preeclampsia associated with low Vitamin B12 concentrations. Relative risk of pre-	Though elevated Hcy concentration was associated with increased risk of preeclampsia, folic acid and other B vitamins may be

				eclampsia ↑ with increasing quartiles of maternal Hcy concentration ($p<0.0001$)	important in the pathogenesis of preeclampsia.
6	Hogg et al., 2000 Birmingham, USA	Observational study (n=437). Pregnant women screened based on their zinc status at recruitment for a randomized control trial for a different purpose.	Subjects recruited <16 weeks, non fasting blood samples collected at 26 and 37 weeks of gestation.	Hcy concentrations were not significantly different among the groups at 26 weeks of gestation but at 37 weeks, the Hcy levels were higher in the PIH and preeclampsia group women	There was no difference in Hcy concentration at 26 and 37 weeks gestation between mothers with infants with IUGR and those with normally growth infants.
7	Vollset et al., 2000 Norway	Population based observational study. Hordaland study (n=5883)	Pregnant women registered by the medical Birth Registry of Norway > 16 weeks of gestation were included in the study from 1967-1996.	The adjusted risk for preeclampsia was 32% higher that for prematurity was 38% higher and that for very low birth weight was 101% higher when the upper quartile of total plasma Hcy compared to the lowest quartile.	Maternal plasma Hcy concentration was associated with premature delivery both of <37 weeks and < 32 weeks of gestation. Plasma total Hcy was significantly related to growth retardation, but the strength of the associations was weaker than the associations between total Hcy and LBW.
8	Sorensen 1999 USA	Prospective nested case control study. 52 women with preeclampsia and 56 normotensive throughout pregnancy.		Second trimester elevation of homocysteine was associated with a 3.2 fold increased risk of preeclampsia after adjusting for potential confounders.	An association of increased homocysteine and preeclampsia was evident in patients with homocysteine in the highest decile of the distribution.
9	Rajkovic 1997 Ohio	Case control study (n=40). 20 preeclamptic pregnant women, 20 normal pregnant control at the time of delivery	Nulliparous pregnant women included. Subjects with chronic hypertension, diabetes, o renal or metabolic disease excluded.	Preeclamptic women had higher plasma homocysteine concentration and early delivery	Elevated homocysteine concentration may cause preeclampsia in nulliparous women possibly injuring the vascular endothelium.

2.5.2 *Calcium intake / supplements and PIH*

Role of calcium in pathogenesis of PIH

Profound changes in calcium metabolism occur during pregnancy. Since the mother has to make available extra calcium for fetal requirements while ensuring that her plasma and bone calcium concentrations are maintained, studies have looked at the calcium regulating hormones namely (calcitonin, parathyroid hormone, 25-hydroxyvitamin D (25-OHD), and 1, 25-dihydroxyvitamin D (1,25-(OH),D) and their interrelations during normal pregnancy. The circulating concentrations of 1, 25-(OH) 2D and calcitonin were increased during pregnancy and this increase is thought to enable the increased physiological need for calcium to be met by enhancing the intestinal absorption of this element. The simultaneous rise in calcitonin opposes the bone-resorbing activities of 1, 25-(OH) 2D, thereby protecting the integrity of the maternal skeleton. Maternal calcium homoeostasis is thus maintained yet the requirements of the fetus are fulfilled. It is also seen that the incidence of hypertension disorders in pregnancy may be reduced by high doses of calcium salts. But raised calcium intakes may also increase the risk of kidney stones and urinary tract infections and may reduce the absorption of other minerals, particularly iron, zinc and magnesium (Hallberg et al., 1992). Therefore there are gaps in the data supporting the role of increased calcium intakes and pathogenesis of PIH.

One of the proposed hypotheses is that a low calcium intake results in increased parathyroid hormone secretion. An elevation in parathyroid hormone (PTH) causes an increase in free intracellular (cytosolic) ionized calcium which further triggers the contraction of vascular smooth muscle thereby producing vasoconstriction of the muscle and a rise in blood pressure. An inverse relation could be expected with a high calcium intake (Belizan et al., 1988).

An observational study in Mayan Indians in Guatemala, who traditionally soaked their corn in lime before cooking, had high calcium intakes and low incidence of pre-eclampsia and eclampsia. These observations were supported by other epidemiological and clinical studies and led to the hypothesis that an increase in calcium intake during pregnancy might reduce the incidence of high blood pressure and pre-eclampsia among women with low dietary calcium.

Increased dietary calcium intake has been associated with lower blood pressure among children, adults and pregnant women (Van Mierlo 2006; Carroli 1994). The effect seems to be more evident among individuals with low calcium intake (Gillman 1992 & 1995; Atallah 2002; Dwyer 1998). Some recent experimental and observational studies in humans and animals have reported an association between maternal calcium intake during pregnancy and blood pressure in the offspring (Atallah 2002; Dwyer 1998), while others have not (Van Mierlo 2006; Stary 2000). These findings follow a large body of evidence indicating that blood pressure levels in childhood and young adulthood are influenced by factors operating early in life (Stary 2000; Berenson 1998) and are associated with later cardiovascular disease and mortality (Mc Carron 2000).

A number of trials have looked at the efficacy of calcium supplements in preventing preeclampsia, gestational hypertension, and premature delivery where most of them were involved in providing supplements containing 1-2 g Ca/d during the second half of pregnancy. The outcomes of the studies were based on the PIH status, change in blood pressure and the length of the gestation. There were promising results seen in both of the parameters. In general, the results suggested that calcium salts at 1-2 g Ca/d would be helpful in reducing the incidence of some hypertensive disorders or pregnancy in high risk women. The diets during supplementation also contained moderate-to-high amounts of calcium that consequently led to higher total calcium intakes often exceeding 2000 mg/d implying that the effect was best observed with higher levels of calcium supplements and adequate dietary calcium intakes. (Belizan et al., 1983 & 1991; Lopez et al., 1989 & 1990; Marya et al., 1987; Villar et al., 1987 & 1990; Repke et al., 1989; Knight et al., 1992).

A case-control study in Canada showed that preeclampsia incidence was not related to dietary calcium intake but that women with gestational hypertension tended to have lower intakes of dairy products than controls (Marcoux et al., 1991). The findings could be due to the small amount of daily supplements provided (200 mg/d) compared to the average daily intake (1575 mg/d). As seen earlier (Marya et al., 1987) have also found the similar results of lower dietary calcium at baseline (~500 mg/d) and the women assigned the supplemental group (375 mg/d calcium and

1200 IU vitamin D) from 20-24 weeks of gestation, had lower (6%) incidence of proteinuric hypertension as compared to the non supplemented group (9%) though lacked the statistical significance (Marya et al., 1987). Another study (King et al., 1992) of women consuming 1200-2600 mg Ca/d monitored the effects of a calcium supplement containing 750 mg Ca/d on calcium metabolism during late pregnancy and 12 wk of lactation. The supplement had no effect on various indices of calcium metabolism and bone turnover, except to reduce 1, 25-dihydroxyvitamin D concentrations in the third trimester of pregnancy.

In general, calcium supplementation appears to be beneficial for women at high risk of gestational hypertension and in communities with low dietary calcium intake. In a systematic review Hofmeyr et al (2007) showed that there was no overall effect on the risk of preterm delivery, although there was a reduction in risk amongst women at high risk of hypertension where the randomised trials comparing at least one gram of calcium daily during pregnancy with placebo were considered. It is thought that calcium salts (1-2g Ca/d) may reduce hypertensive disorders in pregnancy, but the role of dietary calcium needs to be further explored. Further also concluding that calcium supplementation with at least 1 g of calcium was associated with halving in the relative risk (RR) of pre-eclampsia. The greatest reduction in risk appeared to be for women at high risk and for those with low baseline dietary calcium intake. There was also a 30% reduction in the risk of gestational hypertension, with again the greatest effect being among women at high risk and those with a low calcium intake during trial entry. There was no overall effect on the relative risk (RR) of preterm birth, although a moderate reduction associated with calcium supplementation remained.

In a trial conducted by Levine et al (1997), called the Trial of Calcium for Preeclampsia Prevention, a number of studies have been summarised that focused on the calcium supplemented and the percent preeclampsia in the control groups. It was seen that only for the subjects those had dietary calcium of > 600 mg/d and additional calcium supplements with 2g/d had lower % preeclampsia (3.2 & 3.9% preeclampsia) (Villar & Repke 1990; Belizan et al., 1991). In agreement to these findings, Crowther et al (1999) in a randomised controlled trial have shown a decrease in the prevalence of preeclampsia and preterm delivery when supplemented

1.8g of calcium /day. Similarly number of studies has shown the beneficial affects of calcium supplements of about ~2g/d either by decreasing the blood pressure or in the prevalence of preeclampsia and preterm births (Niromanesh et al., 2001; Puwar et al., 1996; Sanchez-Ramos et al., 1994). Contradictory findings from the United States have shown no effect in lowering blood pressure or the gestational age at birth (Knight et al., 1992).

Further, studies have quantified the amount of calcium ingested by the women during pregnancy through diet as well as in the form of supplements or consumption of antacids. Harville et al (2004) in a mixed population of White and African-American pregnant women in the United States showed that racial differences were not significant and the overall calcium intakes were similar except that the white women were more likely to use antacids later in pregnancy. Dairy products were the major calcium source providers.

A number of studies have shown a potential role of calcium in hypertensive disorders during pregnancy but there is paucity of data on the dietary calcium intake and birth outcomes. Since calcium has shown no direct effect on the preterm births or still births, it is postulated to have a strong relation to hypertensive disorders of pregnancy. Hypertension disorders particularly PIH is known cause of placental abruptio, pre-eclampsia, preterm births as well as fetal loss.

Table 8. Role of calcium in relation to blood pressure and birth outcomes

Sno	Author	Study design	Inclusion/Exclusion Criteria	Results with respect to PIH/birth outcome	Conclusion
CALCIUM					
1	Hofmeyr et al., 2007	Systematic review (n=15528). 12 studies with RCT included	<35 wks gest, at least 1g calcium supple compared to placebo.	With supple, ↓ in BP Women with ↓ calcium intake had ↑ risk of preeclampsia.	No effect on the risk of preterm births. Ca supple reduces the risk of preeclampsia
2	Villar et al., 2006 WHO 2006 (multicentric)	Randomised double blind placebo controlled trial (n=8325). 1.5 g elemental calcium (3 tab of 500mg each/day) vs. Placebo	Primi <20 weeks, no complication with median calcium intake<600mg/day	Preterm del tended to reduce in women ≤ 20 years	1.5g/day calcium supple did not prevent pre-eclampsia but ↓ severity. ↓ preterm del in young women
3	Niromanesh et al., 2001 Iran	Double blind placebo controlled trial. 2g/d calcium (500mg 6 hourly) or placebo	Women at high risk of preeclampsia, 28-32 weeks pregnancy	7 fold ↓ in occurrence of preeclampsia and infants weighed 552g more in supple group. Gestational age was also greater.	Calcium supplements are beneficial for preventing preeclampsia among Iranian women at high risk of developing pre-eclampsia.
4	Crowther et al., 1999 Australia	Randomized controlled double blind trial (n=456). 1.8g/d calcium or placebo, 24 weeks until term	Healthy nulliparous women with singleton pregnancy	↓ Preeclampsia and preterm delivery. No significant difference in PIH among the 2 groups	1.8g/d calcium supple during pregnancy ↓ the risk of preeclampsia and preterm birth in nulliparous women
5	Levine et al., 1997 (CPEP trial, USA)	Randomized double blind placebo controlled trial (n=4589). 2g/d calcium carbonate (n=2295) or placebo (n=2294)	Nulliparous 18-40 yrs, 13-21 wks, average intake of calcium 1100mg/d	No benefit to subjects with > 422mg of calcium in diet, no decrease in incidence of PIH, or adverse birth outcomes. No effect in women with adequate calcium intakes	Calcium supple did not prevent preeclampsia. No effect on BP or incidence of preeclampsia or PIH with 2g/d calcium supplement
6	Purwar et al., 1996 India	Randomized double blind placebo control trial (n=201). 2g/d calcium (n=103), identical placebo (n=98) from 20 wks	Healthy nulliparous	Rate of PIH lower and ↓ incidence of preeclampsia in calcium supple group	Calcium supple reduced the incidence of PIH in nulliparous women

		gestation			
7	Sanchez-Ramos et al., 1994 Florida	Randomized double blind placebo control trial (n=281)	Normotensive, nulliparous. Angiotensin-sensitive women were given 2 g/day of oral elemental calcium or placebo.	↓% of preeclampsia and hypertension in supple group as compared to the placebo group.	Calcium supple to high risk nulliparous ↓ the incidence of PIH
8	Knight et al., 1992 USA	Randomised controlled trial (no placebo) (n=30). 1g calcium/day from 12-32 weeks of gestation	18-28 years, no complications	No significant effect on the BP and gestational age	No effects on BP
9	Belizan et al., 1991 Argentina	Randomised double blind placebo controlled trial (n=1194), Multi-centric trial (593 calcium supple vs. 601 placebo). 2g calcium, as 500mg calcium carbonate tab from 20 weeks until term	Nulliparous, 20-35 yrs, <20wks, BP<140/90mmHg, no complications	↓ PIH and BP but no effect on preterm births. No difference in the gestational age at birth seen in both groups	↓ PIH and BP
10	Marcoux et al., 1991 Canada	Case control study. (172 preeclampsia, 251 gestational hypertension and 505 controls).	Primiparous subjects with no history of high blood pressure before pregnancy and no sign of hypertension during the first 20 weeks of gestation.	Higher dietary calcium intake, at least during the first 20 weeks of pregnancy, may be related to lower occurrence of PIH	Calcium intake maybe related to gestational hypertension but not to preeclampsia.
11	Lopez-Jaramillo et al., 1990 Ecuador	Randomized double blind placebo controlled trial (n=56). 2g calcium/day from 28-32 weeks until term, placebo starch tab	Healthy nulliparous <20 years old, recruited at 28-32 weeks gestation	There was a decrease in incidence of PIH. But not significant in BP. A positive association with supple and gestational age at birth.	↓ PIH, The gestational age at birth ↑
12	Villar & Repke 1990 USA	Randomised double blind placebo controlled trial (n=162). 2g calcium/day from 20 wks until term versus placebo tab	≤ 17years, primi, singleton pregnancy	The effect of supplement on BP not clear and no effect on PIH. But ↑ in length of gestation.	No effect on PIH but positive association with gestational age at birth
13	Repke JT et al., 1989 USA	Randomised double blind placebo controlled trial (n=34). 1.5g calcium/day from 24-26 weeks until term versus placebo tab	17-30 years, nulli or primiparous, singleton pregnancy.	↓ BP in the supple group. Effect on birth gestation not determined.	↓ BP

14	Lopez-Jaramillo et al., l989 Ecuador	Randomized double blind placebo controlled trial (n=106). 2g calcium/day (4 tab) from 23 weeks until term versus placebo	Nulliparous women ≤ 25 years, normotensive, no medications	Dietary calcium intakes were very low, 292mg/d. There was a decrease in blood pressure and PIH. A positive association with supple and gestational age at birth.	↓ PIH and BP. The gestational age at birth ↑
15	Marya et al., 1987 India	Randomised controlled trial (no placebo) (n=400). 375mg calcium/day from 20-24 wks	Women with any gravida, 20-35 years	The mean dietary calcium intakes at baseline were ~500mg/d. The incidence of protein uric hypertension was lower among the supplemented subjects (6%) than among the non supplemented subjects (9%) though not significant.	No effect on PIH, but ↓ BP
16	Villar et al., 1987 USA	Randomised double blind placebo controlled trial (n=52). 1.5g calcium/day from 26 weeks until term versus placebo tab	18-30 years, nulli or primiparous, singleton pregnancy	↓ BP and PIH in women with low calcium intake.	↓ BP but no effect on PIH.
17	Belizan et al., 1983 Gautemela	Randomised placebo controlled trial (n=36). 1000mg calcium/day from <15 weeks	Women with any gravida, 20-35 years		No effect on PIH, but ↓ BP

2.6 **Methods of dietary assessment.**

Inadequate intakes of specific nutrients in pregnancy have been reported to lead to a variety of poor maternal and infant outcomes such as length of gestation, fetal growth, birth defects, pre-eclampsia, and of childhood such as cognitive development, blood pressure, adiposity and atopic disease (Botto et al., 1999; Hurley et al., 1996; Goldenberg et al., 1995; Olsen et al., 1992; Bucher et al., 1996). Diet in the 1st trimester may be important to development and differentiation of various organs, whereas diet later in pregnancy may be important for overall fetal growth as well as for brain development (IOM 1990). Women might change their dietary intake patterns during pregnancy after they learn that they are pregnant, after they receive counselling at their initial prenatal visit, or because nausea or vomiting tends to resolve after the 1st trimester. It is equally important that maternal diet during pregnancy should be sufficient and varied to provide enough energy and nutrients to meet the mother's usual requirements, as well as the needs for fetal growth and development, along with alterations in maternal tissues and metabolism (King 2000).

The dietary intake assessment and methodology to assess the diet-disease relationship is one of the most challenging areas of epidemiology due to difficulties in accurately quantifying food intake of a population. The area of dietary intake methodology is expansive and a research topic in its own right. Various methods such as single or multiple 24 - hour dietary recalls (24-HDR), weighed diet records, self reports of diet history and FFQ have been used to assess dietary intakes in populations (Xu et al., 2006; Jakobsen et al., 2004; Soinio et al., 2003). None of these methods has particularly high accuracy in determining food intake (Livingstone et al., 2003; Porrini et al., 1995). Studies may have measurement errors from random misreporting or systematic reporting bias (Paul et al., 2005; Bingham et al., 2003; Schatzkin et al., 2003).

Nevertheless, FFQs have been used as the method for long term diet assessment in most large-scale epidemiological studies (Willet 1998; Song et al., 2006; Liese et al., 2007; Drogan et al., 2006; Tucker et al., 2006). Benefits of using FFQs over other dietary methods are that they are relatively simple in construct and easy to administer. They also have a lower responder burden when capturing long term habitual intakes in epidemiological studies (Cade et al., 2002).

The FFQ describes the habitual dietary intake over a relatively long reference period and is thus more relevant than measurements of short-term intake in the evaluation of study hypotheses. The FFQ structure has as its backbone, a food list containing foods that are eaten reasonably often by an appreciable part of the population and which contain substantial amounts of the nutrients of interest, the intake of which can vary from individual to individual.

In many parts of the world, FFQs have been shown to be robust in measuring energy and macronutrient intakes and to be a reasonably good measure of micronutrient intakes (Willett et al., 1985; Bingham et al., 1994; Hernandez-Avila et al., 1998; Egami et al., 1999). Earlier FFQs developed in India have been used in the states of Gujarat (Hebert et al., 1999a) and Kerala (Hebert et al., 1999b) in cancer epidemiological studies, and in Delhi for chronic disease epidemiology (Umesh et al., 2003). There are no FFQs that include foods consumed in the southern states, particularly in relation to diabetes and other metabolic disorders. As compared with northern India, there are wide differences in the choice of foods eaten in four southern states of India (Tamil Nadu, Karnataka, Kerala and Andhra Pradesh), which comprise a population of over 222 million people (Census of India 2001). The foods consumed are fairly similar in these states. Using a FFQ for collecting dietary data in India was especially attractive due to: relatively large inter-relative to intra- (mainly day-to-day) person variability; a shorter questionnaire (under 100 food items) could make querying respondents easier (Rao 1987), smaller chance of response bias due to prior knowledge of diet-disease relationships. Also, in another study it was established that rural Indians were more accurate in estimating weights and volumes of food items than were more highly educated individuals in the West (Hebert et al., 1999b). The performance of an FFQ is sensitive to the culture and ethnic background of the study population. In India, evaluation of the food lists of published FFQs developed for regions such as Kerala, Gujarat and Lucknow show that less than 20% of foods are similar across the FFQs due to regional variations in food habits (Hebert et al., 1998; Hebert et al., 1999; Pandey et al., 2005). Hence in India, a single FFQ is not likely to capture the variations in dietary intakes in populations with different dietary habits, unless a very long food list is used which becomes impractical. However, in order to be culturally sensitive, FFQs need to be developed and validated specifically for each region, and should reflect the prevailing food culture (Cassidy 1994). FFQs are usually validated against multiple (24-HDR) (Subar et al., 2001). In addition,

non-dietary methods such as the ratio between energy intake (EI) and the estimates of basal metabolic rate (BMR) (FAO/WHO/UNU 1985) may also be used as a tool for testing the validity of dietary energy intake (Jackson et al., 2001). Studies on the validity and reproducibility of FFQs usually focus on nutrients rather than food groups; similar studies on food groups have been performed in Denmark, German, Spain, and Sweden with 121, 104, 101, and 195 subjects, respectively. (Johansson et al., 2002; Ockr et al., 1997; Bohlscheild-Thomas et al., 1997). A study conducted by Esfahani in Tehran found that the FFQ overestimated dairy products, nuts and seeds, and liquid oil consumption and underestimated soft drink and meat consumption, as compared with 24-hour dietary records (DRs) (Esfahani et al., 2010). However, other FFQ validity studies have shown both over- and underestimation of the same food groups (Ocke et al., 1997; Goldbohm et al., 1994; Salvini et al., 1989). Usually, overestimation occurs for foods that are perceived as healthy, and underestimation occurs regarding socially unacceptable foods. Comparing the mean food group intakes from the first and second FFQ revealed that for most food groups there was no significant difference between the FFQs (Esfahani et al., 2010)

Since FFQs are used for ranking individuals based on their habitual intakes of foods and nutrients, accurate estimation of intakes is crucial (Kaaks et al., 1997). As in other dietary assessment methods, random and systematic errors can arise in FFQ estimates, which may not represent the "actual" usual diet (Marks et al., 2006). Random errors that occur in FFQs can attenuate the associations in epidemiological studies (Kipnis et al., 2003; Schatzkin et al., 2003). To prevent incorrect estimations of dietary intakes, which may lead to misunderstandings of the relationship between dietary factors and diseases, the reproducibility and validity of a FFQ, is assessed (Cade et al., 2002). Hence, surveys using an FFQ as their dietary assessment tool must validate their measurement method for nutrients, foods, and food groups (Feskanich et al., 1993; Date et al., 2005; Johansson et al., 2002; Ocke et al., 1997; Malekshah et al., 2006).

Reference methods such as multiple 24-h dietary recalls (24-HDR) (Kaaks et al., 1997) or multiple-day food records (Rosner et al., 1989) are used to adjust for attenuation. Moreover to reduce the chance variation of the diet intake reported, multiple (24-HDR) are administered and the average intake is recorded. An optimal comparison method to assess the validity and reproducibility of the FFQ is the (24-HDR) method that are commonly employed in nutritional

epidemiology to evaluate the performance of FFQs primarily because it doe not require subject literacy and produce high levels of specificity. Moreover to reduce the chance variation of the diet intake reported, multiple 24HRs are administered and the average intake is recorded. However, because the corrected RR is biased when the sources of error for the reference method and the tested tool are similar, (Kipnis et al., 2003; Wacholder et al., 1993) the addition of biomarkers can determine the lower limit for the validity coefficient (VC). Kaaks and Ocke (Kaaks et al., 1997; Ocke et al., 1997) suggested using the "method of triads," by which correlation (validity coefficient) of the dietary assessment method and "true" long-term intake could be estimated from 3 pairwise correlations between the FFQ, the reference method (24HRs or diaries), and the biomarker. This technique is an application of a factor analysis model and corrects for bias due to correlated errors in the repeated measurements from the reference method. Although the performance of FFQs in estimating intake of individual nutrients has been evaluated widely in European countries and in the United States, (Subar et al., 2003; Kipnis et al., 2003; Andersen et al., 1999; Willet et al 1985), little is known about its performance in other populations, such as Mediterranean countries in the Middle East, compared with that of 24HRs. A study conducted by Shai et al in an Israeli population (non pregnant ≥35 years) using the "method of triads", evaluated the performance of the urine and blood biomarkers in association with the "true intake" compared with the dietary assessment tools (Shai 2005). During pregnancy, authors have used various reference methods. Brown (1996) and his collegues have used food records as a reference method whereas Forsythe (1994) have used the (24-HDR) in validating the FFQ. Few studies have focused on the duration of administration of the questionnaire depending on their objective of the study such as; Erkkola (2001) validated the FFQ in the 8th month (3rd trimester) of pregnancy as they were looking at the development and determinants of type I diabetes (Erkkola et al., 2001). Similarly, Cheng et al also focused on validation and reproducibility of FFQ again in the beginning and the end of the 3rd trimester of pregnancy (Cheng et al., 2008). A single study with dietary method validated in all 3 trimesters is still lacking. Therefore in this thesis the dietary intakes during pregnancy are assessed by the FFQ and are validated against the multiple (24-HDR) in each trimester. In addition the use of selected biomarkers for micronutrients; vitamin B_{12}, vitamin B_6 and folates in the 1st trimester and mid pregnancy were also used to validate the FFQ.

There have been studies conducted in the past in the field of maternal nutrition and pregnancy outcomes including the validation of the dietary tool. Gaps in the knowledge still exist in data pertaining to the tools used in assessing the dietary intakes and number of studies conducted in developed countries. Evidences reflect information based on the retrospective data also which is likely to have a bias in retrieving the dietary information. Moreover understanding the relationship of B vitamins and birth outcomes is trimester specific too. Therefore this PhD thesis work has tried to clarify the relationship between the select maternal B vitamin intakes and status, effect on homocysteine and the adverse birth outcomes such as LBW, SGA and preterm births in a prospective observational study in a developing country, India. In addition to the dietary intakes a range of food group intakes during all three trimesters of pregnancy has been explored. The relationship between calcium and calcium rich food groups in the pathogenesis of PIH, a morbidity that is also a known cause for adverse birth outcomes is also examined.

CHAPTER 3
METHODOLOGY

The study was conducted at St. John's Research Institute and St. John's Medical College Hospital (SJMCH), Bangalore, India. This 1200-bed tertiary hospital draws patients of diverse socioeconomic status, from urban slums to high-income residential areas. Institutional ethical review board of SJMCH Bangalore approved all study procedures and a written and signed consent was obtained from each study participant at enrolment. The family member or the companion of the study participant was the witnesses and co-signed the consent form. The approval from Human Research Ethics Committee, Curtin University of Technology, Perth, WA was also obtained (HR 98/2008).

Training and standardization of research team

I have been a part of the research team at St. John's Research Institute for many years prior to my PhD and have assisted with several similar studies in pregnancy. This required being trained by senior anthropometrists and nutritionist in all aspects of study methodology, both quantitative and questionnaire based.

During the initial phase of the PhD work, I performed the following training and standardization for my research team,

- Recruitment and information gathering from study participants
- Anthropometric and blood pressure measurements
- Dietary intakes using a FFQ and 24-hour dietary recall (24HDR)
- Calculation of macro- and micro-nutrients from the dietary intake data

A period of 3 months was required for setting up the study, training and standardization of techniques. Since the data set and sample sizes involved in the study were very large, this required a team of 3 research assistants to assist in the work. The work of the PhD candidate included training and validation of data collected from all members of the group. The standardization of the dietary questionnaire was done by repeating the administration of the questionnaire on a subject by the research assistants. The mean values of the macronutrients computed from the dietary method were compared with that from each of the research assistants and the trainer (PhD candidate). This procedure was repeated with other patients till no clinically

significant differences were observed between the trainer and research assistants. The team was also trained for anthropometric measurements. The inter and intra observer variation was tested and the inter-rater variability was within 5% for all research assistants. The instruments used during the data collection such as the weighing scale, blood pressure instrument, the skinfold callipers as well as the infant weighing scale was calibrated regularly. The blood sample for various micronutrient analyses was collected by a trained phlebotomist, processed and stored. To ensure quality of data collected and to tackle logistic problems during the study, bimonthly meetings with the medical consultants, phlebotomist and the research assistants were held throughout the program. Data collected was entered on daily basis. I randomly conducted a check on 10% of information collected each month to ensure validity and accuracy of data entry.

3.1 STUDY DESIGN

This study was a prospective cohort study. All pregnant women aged 17–40 years and at <13 weeks of gestation, registered for antenatal screening at the Department of Obstetrics and Gynaecology at St John's Medical College Hospital, were eligible for the study. Women willing to participate were recruited as early in their pregnancy as possible and were followed until delivery. Women with multiple pregnancies, those with a clinical diagnosis of chronic illness such as diabetes mellitus, hypertension, heart disease and thyroid disease, those who tested positive for HbSAg / HIV / VDRL infection or who anticipated moving out of the city before delivery were excluded. Socio-demographic details were collected at baseline (11.3 ± 2.6 weeks of gestation). Information on maternal anthropometry, dietary intake, clinical status and blood biochemistry was collected at baseline, second trimester (24.2 ± 1.6 weeks) and the third trimester (34.1 ± 1.5 weeks) of pregnancy. Of 788 women who were contacted at the antenatal clinic, 714 consented to participate and were recruited. Seventy four subjects discontinued; 64 subjects refused to be part of the study while 10 subjects were excluded as per the study protocol. Of the 10 subjects; 3 were with twin pregnancies, confirmed after a late ultrasonography scan, one subject was diabetic, confirmed with random glucose test at recruitment. Two subjects were diagnosed to be HIV positive while the other two HbsAg positive. One subject was VDRL positive and another subject had false pregnancy test positive; hence non pregnant. The 714 subjects consented continued to be part of the study, but during the course of pregnancy 77 subjects dropped out of the study. The common reason was that they moved out of the study site

for various reasons. Hence, remaining 637 subjects completed the study with a known pregnancy outcomes. It is also observed that since St. John's Medical college-Hospital is functioned by the Catholic Bishops, the family planning is not encouraged. Therefore the study participants who were multiparous and wished to undergo tubectomy (family planning) at the time of delivery were also included in the number of subjects who consented and then dropped out of study or were lost to follow up.

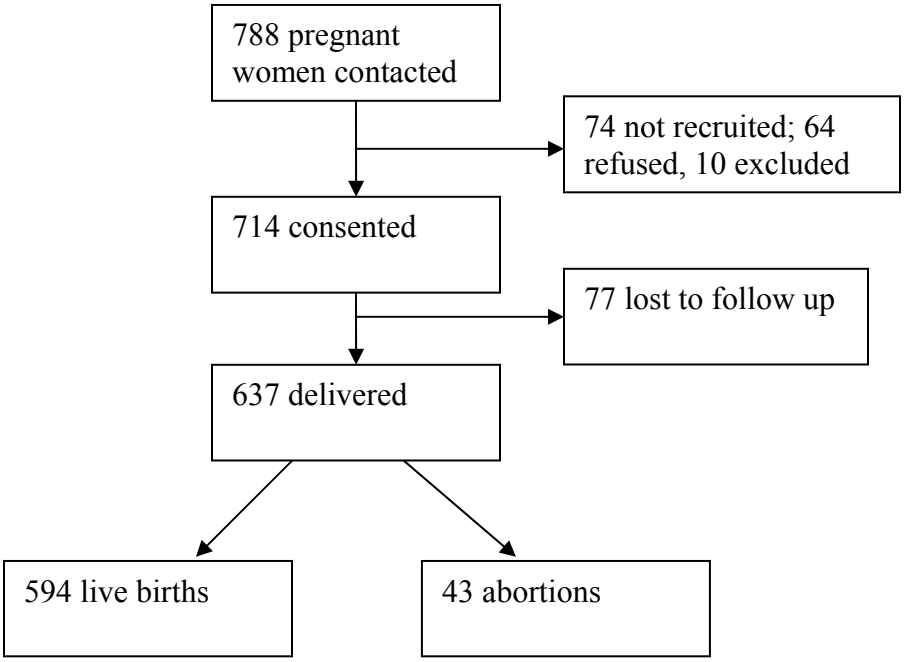


Figure 6. Prospective cohort flow chart

Baseline data available among the 151 women (19.2%) who were lost to follow up and dropped off the study were similar to those included in the present analysis: age (24.9 ± 3.6 versus 24.8 ± 3.9 years; p=0.77, unpaired t-test), parity (primiparous, 62.7 % versus 60.8%; p=0.66, chi-square test), education (up to high school; 35.3% versus 33.2%, PUC / diploma; 18.0% versus 22.4%, University degree and above 46.7% versus 44.4%, p=0.28, chi-square test of significance). The energy intakes at baseline were also not significantly different among the subjects in the present study and the ones lost to follow up (Energy intakes; 1899 ± 497 versus 1942 ± 481 kcal/d, p=0.35, unpaired t-test).

Table 9 ASSESSMENTS DURING PREGNANCY AND AT BIRTH

Parameters	**At Recruitment (12±1 week)**	**Trimester 2 (24±1 week)**	**Trimester 3 (34±1 week)**	**At Delivery**
Screening (inclusion/exclusion)	✓			
Ultrasound sonography scan	✓	✓	✓	✓ **(before delivery optional)**
Antenatal blood investigation				
• HIV, VDRL, HBsAg	✓			
• Hemoglobin	✓	✓	✓	
• Random glucose	✓			
• OGCT/ GTT		✓		
Routine urine screening (dip test)	✓✓	✓✓✓	✓✓✓	
Demographic information	✓			
Maternal anthropometry	✓✓	✓✓✓	✓✓✓	
Dietary information FFQ	✓	✓	✓	
Multiple 24 hr diet recall	✓✓	✓✓✓	✓✓✓	
Micronutrient status (blood sample; plasma vitamin B_{12}, vitamin B_6, homocysteine and red cell folate,)	✓	✓		✓
Blood pressure monitoring	✓✓	✓✓✓	✓✓✓	✓
Physical activity record	✓	✓	✓	
Delivery details and baby anthropometry				✓

The number of ticks indicates number of assessments in each trimester.

3.2 SOCIO-DEMOGRAPHIC AND ANTHROPOMETRIC INFORMATION

At the baseline visit, trained research assistants interviewed the study subjects to obtain information on age, obstetric history, family composition and socioeconomic status. A standard living Index questionnaire (Parsuraman 1998) was administered to grade the subjects into categories of low, middle and high income group. The obstetric history of the study participant was recorded to know the parity. The medical conditions and complications during the previous pregnancy were recorded. Women with multiple abortions or recurrent miscarriages, bad obstetric history were not included in the study. Gestational age (in weeks) was calculated from the reported first day of the last menstrual period (LMP). Subsequent ultrasonographic measurements done within 2 weeks of the initial visit and again closer to the time of delivery were used to confirm gestational age calculated by LMP. A digital balance (Soehnle, Germany) was used to record the weights of all mothers to the nearest 100 g. The digital weighing scale was standardised using the standard weights once every month. Measurements of height were made using a stadiometer to the nearest 1 cm. Mid-upper arm circumference (MUAC) was measured to the nearest 0.1cm using a plastic measuring tape and skinfolds were measured at three sites (biceps, triceps and subscapular) using the Holtain Caliper (Crosswell Crymych Pembrokeshire, UK) for the assessment of body composition using prediction equations (Durnin & Womersley 1974).

The research assistants were trained for anthropometric measurements. The inter and intra observer variation was tested and the inter-rater variability was within 5% for all research assistants. Weekly maternal weight gain during the second trimester was calculated as the difference between the body weight at baseline and the weight measured at second trimester, divided by the difference between the gestational age at baseline and the gestational age at second trimester. Similarly, weekly maternal weight gain during the third trimester was calculated as the difference between the body weight at second and the weight measured at third trimester, divided by the difference between the gestational age at the second and the third trimester respectively. The total weight gain during pregnancy was not available for all study participants. For only (40.3%) of subject, the total gestational weight gain was estimated since they had their last antenatal visit at the Obstetric and Gynaecology department and with us at least one week prior to the delivery. The weight of the mother before delivery was obtained

either at the antenatal visit or at the labor room. Maternal body mass index (BMI) (kg/m^2) was calculated using weight and height Subjects were categorized as undernourished based on their BMI (BMI<19.5 kg/m^2). Almost 16% of the subjects were anaemic in the 1st trimester of pregnancy.

Antenatal care

Each study participants were screened for the routine antenatal tests before enrolling in the study. The antenatal care included screening for blood borne diseases such as HIV, VDRL and HBsAg. Random blood glucose and hemoglobin concentration were estimated. Subjects with random blood glucose value >140 mg/dl were not recruited for the study. An ultrasonography scan at recruitment was conducted to confirm on the viability of the fetus, singleton pregnancy and the gestational age, based on which the subjects' were recruited. Tetanus toxoid injections were prescribed twice during the pregnancy as per the routine antenatal care. Similarly, antenatal supplements such as folic acid, iron and calcium supplements were prescribed by the obstetrician as per the antenatal schedule. Supplement compliance were recorded during the course of pregnancy in the form of tablet count. All subjects were prescribed 5 mg of folic acid per day in the 1st trimester with ferrous sulphate 150 mg (equivalent to 45 mg iron), folic 0.5 mg and 1000 mg calcium, each per day from 2nd trimester until delivery. The 1000 mg calcium prescribed per day was consumed as two tablets, each of 1250 mg calcium carbonate (equivalent to 500 mg elemental calcium) with vitamin D_3, IP 250 I.U. None of the subjects were prescribed multi - vitamins or multi - minerals.

3.3 DIETARY INTAKE

The dietary intake during pregnancy was assessed using 2 techniques. The food frequency questionnaire used to capture the longer term intakes for a period of 3 months while repeated 24-HDR was administered to record the previous day intake at each monthly visit in a trimester.

Food frequency questionnaire

A food frequency questionnaire (FFQ) was administered at each trimester visit to obtain information on the habitual dietary intake for the preceding 3 months. The FFQ was adapted from that developed for the urban middle class residing in South India (Vaz et al., 2009). The food frequency questionnaire (FFQ) was interviewer administered by a trained research assistant. This questionnaire has a food list of 108 items, derived from a food database developed over a period of several years from studies at the Division of Nutrition, St John's Medical College. It consists of four frequency categories (daily, weekly, monthly and yearly). Standard measures were placed before the respondent to quantify the portion size of each food item when administering the questionnaire. Recipes for the food items were tested in the laboratory and raw ingredients for each recipe were weighed, and volume to weight conversions measured for each cooked food item. Nutrient composition of the food item was calculated using standard food conversion tables for the ingredients (Gopalan 1996). Wherever available, Indian data was used. However, for some nutrients, for which Indian data was not available, USDA data in the public domain (USDA ARS; http://www.nal.usda.gov/fnic/foodcomp/search/) was used.

Administration of a 24 hours diet recall

The 24-hours diet recall (24-HDR), is a recording of the intake over the past 24-hour period. We based ours on an interview conducted by the trained research assistants that takes 10 -15 minutes to administer. The interview is open-ended with key prompts to help the respondent in recalling the dietary intakes. The questionnaire was structured to facilitate the women in recalling the food consumed during the previous day for morning meal, breakfast, mid meal, lunch, supper and dinner and bed time. The most common use of 24-HDR in nutritional epidemiology is to assess the validity of a FFQ that is used as the primary dietary data collection tool. During the routine antenatal check up at each monthly visit a 24-HDR was administered and hence on an average 2 or 3, 24 hours diet recall was used to validate FFQ during each trimester.

Nutrient calculation

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. (Gopalan 1996; USDA ARS; http://www.nal.usda.gov/fnic/foodcomp/search/).

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. Nutrient information was obtained for 27 macro- and micro-nutrients.

3.4 BIOCHEMICAL ANALYSIS

Blood was drawn from subjects during their visit as per the protocol scheduled in the early and mid pregnancy (12±1, 24±1 weeks of gestation) by venepuncture using trained personnel and collected in both ethylene diaminetetraacetate (EDTA) and plain vacutainers (Beckton Dickinson). Whole blood was used for the measurement of hemoglobin and hematocrit (HCT). Hemoglobin was estimated on ABX Pentra 60 C^+ that uses the modified Drabkin method (cyanmethemoglobin). The measuring range was between 8-18 g/dl with the within run precision of <1.0%. For HCT, the measuring range was between 34-58% with the within run precision of < 2.0%.

The whole blood was treated with freshly prepared 1% ascorbic acid and the hemolysate was stored at -80°C until analysis. Red cell Folate was measured by chemiluminescence on ADVIA Centaur (Bayer Diagnostics, Tarrytown, USA).

The RBC folate was calculated from the measured folate and hematocrit values.

$$\text{RBC folate (ng/mL)} = \frac{\text{(Folate result for hemolysate, ng/mL) x 21 x 1.3 x 100}}{\text{Hematocrit}}$$

The samples were diluted 21 times during hemolysate preparation and corrected by a factor of 1.3 for the matrix difference between calibrators and RBC hemolysate.

Serum samples were used to estimate vitamin B_{12} status. Vitamin B_{12} was measured by the electrochemilumenesence method using the principle of Competition on Elecsys 2010 (Roche Diagnostics Mannheim, USA).The measuring range was 22 – 1476 pmol/L and the within run precision was <10%. Plasma samples were used to estimate $B_{6,}$ and homocysteine (Hcy). The active form of Vitamin B_6 (Pyridoxal phosphate, PLP) was analyzed using HPLC (Shimadzu Corporation, Kyoto,Japan) method using fluorescence detector that involves the principle of Ion Exchange Chromatography. The signal from the column was detected by fluorescence detector

and the within run precision was <10%. The measurement of total homocysteine (tHcy) in the plasma was estimated using an isotope-dilution gas chromatography–mass spectrometry (GC-MS) method (Windelberg et al., 2005), using methylchloroformate and toulene for derivatization. During delivery, the cord blood was collected, processed and stored for the similar analysis as that of antenatal period.

Routine blood sugars were recorded as per the antenatal care. A random glucose test was administered at the 1st trimester and if the subject had an elevated level (>140 mg/dl), then a fasted and post prandial sample was tested for the glucose levels in the blood. Similarly a screening test, oral glucose challenge test (OGCT) for gestational diabetes mellitus (GDM) was done at 20 weeks of gestation by giving a load of 75g anhydrous glucose. If the glucose levels were greater than 140 mg/dl, the OGCT was repeated or the subjects were advised by the obstetrician to perform a glucose tolerance test (GTT), with repeated blood measurements of a fasted, 1 hour as well as 2 hours samples. The subject with high glucose levels at 1st trimester was excluded, while once recruited and then diagnosed to have GDM in the 2nd trimester they continued to be part of the study.

3.5 PHYSICAL ACTIVITY

A physical activity questionnaire was administered to know the activity level during pregnancy at each trimester. Information was collected for activities in 5 domains – occupational activity outside the house, discretionary exercise, household chores, sedentary activities, hobbies and sleep. Physical activity data were expressed as the duration (minutes/day), or as the product of the intensity (PAR) and duration (PAR-min). A composite measure of daily physical activity, the physical activity level (PAL), was also calculated as the ratio of the total energy expenditure (TEE; kJ/day) and the basal metabolic rate (BMR; kJ/day). Basal metabolic rate was predicted using standard WHO equations (WHO 1985) based on weight and height.

3.6 BLOOD PRESSURE MONITORING

A random blood pressure was recorded in duplicate after 10 min seating at each antenatal visit with a recommended automated blood pressure monitor (OMRON Intelli Sense, Model HEM-757, and Tokyo Japan) using appropriate adult size cuff. The elevated blood pressure

(≥140/90 mmHg) on two occasion's ≥ 6 hours apart was indicative of hypertension during pregnancy commonly known as PIH; pregnancy induced hypertension. PIH is observed in the 2nd trimester or later, generally after 20 weeks of gestation. Severe PIH was defined as systolic blood pressure ≥160 mmHg or diastolic blood pressure ≥110 mmHg. In addition, subjects with PIH and proteinuria or edema were categorized as preeclampsia and eclampsia as preeclampsia associated with seizure (National high blood pressure education program working group). OMRON Intelli Sense, Model was calibrated once in 2 months using a mercury sphygmomanometer.

3.7 MEDICATIONS AND HOSPITALISATION

The acute or chronic health problems experienced by the pregnant women were recorded. The information and medication prescribed by the obstetrician during the course of pregnancy were recorded. Every effort was made to note medicines and antibiotics even if the subject had consulted physician other than doctors at St. John's Medical College Hospital. Similarly information on hospitalization, duration, medications and causes for hospitalisation were also recorded.

3.8 DELIVERY AND BIRTH INFORMATION

At delivery the information pertaining to mother and the baby's health was recorded. The cord blood was collected that represents the baby's blood. Serum and plasma samples were analysed for the same micronutrients as that of antenatal blood micronutrients (vitamin B_{12}, vitamin B_6, folate and Hcy). To protect the samples from sunlight, the collection tubes were covered with aluminium foil until blood processing and storage of the serum/plasma at -80° C for micronutrient status analysis.

The delivery information was recorded from the medical chart pertaining to the mode of delivery, time of birth, gender of the baby, placental weight and medical condition of the mother and the baby. The information of transfer of the baby to the neonatal intensive care unit (NICU), reason for the transfer and the condition was recorded. The baby anthropometric measurements included (birthweight, gm; length, cm; circumferences (mid upper arm, head and chest, cm) and skinfold thickness (biceps, triceps, subscapular and suprailiac; mm). Infants were weighed to the nearest 10 g on an electronic weighing scale (Salter Housewares 914 Electronic Baby and

Toddler Scale, NY, USA) immediately after birth. The length of the baby was measured using Infantometer (Seca 416, NY, USA). LBW was defined as a birth weight <2500 g, small for gestational age (SGA) as birth weight less than 10th percentile for gestational age and preterm delivery as delivery before 37 weeks of gestation (WHO 1995). The babies in the NICU were also measured depending on their medical condition. Of the 637 women who had a known pregnancy outcome, there were 43 spontaneous abortions (6.8%) and 594 live births (93.2%). Twenty two women (3.5%) developed diabetes during the course of pregnancy, and 55 (8.8%) were diagnosed to have mild PIH, PIH or eclampsia. The prevalence of LBW, SGA and preterm births was 19.0% (n=121), 26.8% (n=171) and 9.4% (n=60), respectively.

3.9 STATISTICAL ANALYSIS

The data used for this PhD thesis was collected by me and the research assistants who I have trained. I was involved in quality checks, cleaning the data and the preliminary analysis. All analyses were done using the SPSS program (version 16.0, SPSS, Chicago, IL, USA). The normality distribution, the descriptive analysis, correlations, univariate and bivariate analysis, Chi square test of significance as well as the detailed analysis involving the logistic regression model was also performed. The analysis was carried out based on the conceptual framework provided. The odds ratio (OR) 95% confidence intervals (CIs) were reported. Two-sided P-values < 0.05 were considered statistically significant. The statistical analysis for each chapter is explained in detail in the respective chapters.

All statistical analysis was cross checked by the Head of the Biostatistics Department (Dr Tinku Thomas) at St. John's Research Institute. Dr Thomas is also the Statistical consultant for this PhD thesis.

CHAPTER 4

This chapter addresses objective 1 of the thesis, namely to validate the semi quantitative food frequency questionnaire for the dietary assessment of South Indian pregnant women.

Manuscript in press:

Food frequency questionnaire is one of the valid tools to estimate dietary habits of South Indian pregnant women. Asia Pacific Journal of Public Health, 2012 *(in press*).

Pratibha Dwarkanath, Mario J Soares, Tinku Thomas, Mario Vaz, Sumathi Swaminathan and Anura V Kurpad

The tables in this chapter are numbered sequentially beginning from the previous chapter.
Since the chapter was submitted for a publication, additional data are added in the Appendix A (Tables 1& 2)

Abstract

Food frequency questionnaire (FFQ) was validated against multiple 24-hours dietary recalls (24-HDR) and for few blood biomarkers in 154 pregnant women at obstetrics and gynecology department of St. John's Medical-College Hospital, Bangalore, India. Absolute nutrient intakes from FFQ correlated positively with the average 24-HDR during pregnancy. Energy adjusted nutrients from FFQ in all trimesters except proteins, carbohydrate, folate intakes and vitamin B_6 in the 3rd trimester correlated positively with average 24-HDR. Overestimation by FFQ compared to 24-HDR ranged from 9% to 41%. Vitamin B_{12} status in 1st and 2nd trimesters positively correlated with energy adjusted and absolute vitamin B_{12} intakes from FFQ. The Bland Altman plots showed a pattern such that a trend was seen towards underreporting of intakes through FFQ with increasing mean intakes of the two methods, considering 24-HDR as the reference tool. We conclude that FFQ is a valid tool to measure dietary intakes during pregnancy.

Key words: Food frequency questionnaire, micronutrient status, pregnancy, 24 hours diet recall.

Introduction

One third of infants born in India are low birth weight (LBW; < 2500g) and the majority of these are the result of intrauterine growth retardation (IUGR)[1,2]. Maternal and fetal adverse outcomes during pregnancy have been largely attributed to widespread maternal malnutrition[3,4]. Maternal intakes of green leafy vegetables and fruits in the 2nd trimester have shown a strong association with birth weight[5]. We have previously shown that vitamin B_{12} deficiency in pregnant Indian women results in a greater risk of delivering a LBW baby[6,7]. Diets low in animal products, augment the risk for B_{12} deficiency[8].

Appropriate methods are needed to assess the diet during pregnancy. The semi-quantitative food frequency questionnaire (FFQ) currently used for assessing food intake for population-based studies[9], is designed to assess long term eating habits and for ranking individuals according to their nutrient intakes. An optimal comparison method employed in nutritional epidemiology to assess the relative validity of the FFQ is the 24 hour diet recall; 24-HDR method. This method is however prone to chance variation in the diet, and therefore needs to be repeated several times in order to obtain an accurate estimate of the diet[10]. Studies have used urinary or blood biomarkers as a tool to validate the dietary method [11, 12]. In pregnancy, fluctuations in appetite due to nausea are a potential problem of using recall methods to evaluate dietary intake in the 1st trimester. Since the 1st trimester is the transition period during which embryogenesis takes place and the 2nd trimester is associated with major metabolic changes, serum levels of selected biomarkers such as plasma vitamin B_{12} and vitamin B_6 and red cell folate were analyzed during these two trimesters. Though the validity of FFQs have been assessed in a number of studies in India[13], FFQs are culture specific and can perform differently among various demographic groups and subcultures within a population[14], as well as in varying physiological states. Given these issues, this study was undertaken to validate a FFQ to assess the dietary intakes of pregnant women in an urban South Indian population across the three trimesters of pregnancy using multiple 24 HDR as the reference method.

Methods

Study population

This study was a part of an ongoing observational prospective cohort study that focused on maternal factors influencing pregnancy and birth outcomes at the obstetrics and gynecology

department of St. John's Medical-College Hospital, Bangalore India. This hospital caters to patients of diverse socio-economic status. The Institutional Ethical Review Boards at St. John's Medical College Hospital and Curtin University of Technology, Perth, WA, approved all study procedures. The study was explained to the subjects in their local language and signed informed consent was obtained before enrollment. Pregnant women attending the routine antenatal clinic (< 13 weeks of gestation) at the out patient department of Obstetrics and Gynecology and planning to deliver at the hospital were recruited and followed until delivery Women with multiple pregnancies, those with a clinical diagnosis of chronic illness (for example, diabetes mellitus, hypertension, cardiac disease, thyroid disease, and epilepsy), and those who tested positive for hepatitis B surface antigen, HIV, or syphilis were excluded. Maternal age, parity and education were obtained at enrollment. Anthropometric measures, antenatal nutritional intake recordings and micronutrient status through a blood draw were obtained once in each trimester. A total of 419 pregnant women were enrolled from 2004 until 2006. Biomarkers of plasma vitamin B_{12}, vitamin B_6 and red cell folate (RCF) were analyzed for a sub-set of 154 subjects at the 1st and 2nd trimester of pregnancy. A single FFQ and two to three 24-HDR including at least one weekday and one weekend/holiday, were administered at each trimester of pregnancy. Both the FFQs and 24-HDR were interviewer-based and administered by trained research assistants. The standardization of the dietary questionnaire (FFQ and 24-HDR) was done by repeating the administration of the questionnaire on a subject by the research assistants. The mean values of the macronutrients computed from the dietary method were compared with that from each of the research assistants and the trainer (nutritionist). This procedure was repeated with volunteers till no clinically significant differences were observed between the trainer and research assistants. An average of two 24-hours dietary recall was used to validate the FFQ in the 1st trimester while average of three 24-hours dietary recall was used to validate the FFQ administered in the 2nd and 3rd trimester of pregnancy.

A sample size of 154 subjects was selected for the dietary validation, since fewer subjects are needed for a validation study with high degrees of validity[9].

Administration of semi quantitative food frequency questionnaire (FFQ).

The FFQ used in this study had a food list of 108 items, derived from a food database developed over a period of several years at the Division of Nutrition, St John's Medical College[15]. It was

administered at each trimester to obtain information on the habitual dietary intake for the preceding 3 months. The FFQ consisted of frequency categories (daily, weekly, monthly and yearly) and the portion size consumed using standard measures. Recipes for the food items were tested in the laboratory, raw ingredients for each recipe were weighed, and volume to weight conversions measured for each cooked food item. The use of routine antenatal supplements including the iron, folate and calcium supplements during pregnancy was recorded.

Administration of a 24 hours diet recall; *24-HDR*

The 24-HDR was administered when pregnant women visited the clinic for antenatal check up. This was interviewer administered using standard portion size models and using probes to help the respondent recall the intake. The standard portion sizes used were similar to that used for the FFQ.

Supplements during pregnancy

All study participants were uniformly prescribed the routine antenatal supplements that included 5 mg of folic acid in the 1st trimester. From the 2nd trimester until delivery, 0.5 mg folic acid, 60 mg elemental iron and 1000 mg of calcium prescribed. The study subjects were compliant to the prescribed supplements as the compliance was recorded during the subsequent routine antenatal visits.

Nutrient calculation

The nutrient composition of the food item was calculated using standard food conversion tables for the ingredients[16,17]. Nutrient information was obtained for 27 macro- and micro-nutrients.

Biochemical analysis

Venous blood was drawn from subjects at the 1st and 2nd trimester of pregnancy and plasma samples were stored at -80°C for vitamin B_{12} and B_6 analysis. For red cell folate (RBC Folate) analysis, whole blood was treated with freshly prepared 1% ascorbic acid and the hemolysate stored at -80°C until analysis.

Plasma vitamin B_{12} was measured by the electrochemilumenesence method using the principle of Competition on Elecsys 2010 (Roche Diagnostics Mannheim, USA). The measuring range was 22 – 1476 pmol/L and the within run precision was <10%.

The signal from the column was detected by fluorescence detector and the within run precision was <10%. Plasma samples were used to estimate $B_{6.}$ The active form of Vitamin B_6 (Pyridoxal phosphate, PLP) was analyzed using HPLC (Shimadzu Corporation, Kyoto,Japan) method using fluorescence detector that involves the principle of Ion Exchange Chromatography. The signal from the column was detected by fluorescence detector and the within run precision was <10%.

Red cell folate measurement was made by chemiluminescence on ADVIA Centaur (Bayer Diagnostics, Tarrytown, USA).

The RBC folate was calculated from the measured folate and hematocrit values.

$$\text{RBC folate (ng/mL)} = \frac{\text{(Folate result for hemolysate, ng/mL) x 21 x 1.3 x 100}}{\text{Hematocrit}}$$

The samples were diluted 21 times during hemolysate preparation and corrected by a factor of 1.3 for the matrix difference between calibrators and RBC haemolycate.

Statistical analysis

All analyses were carried out using SPSS (version 13.0, SPSS, Chicago, IL, USA). Nutrient intakes were expressed as the mean (95% CI) and food group data as median (lower and upper quartile) since the data was non-normally distributed. Nutrients were energy adjusted to remove variation due to energy, using the residual method[9]. Both nutrients and food groups were assessed in this relative validation of the FFQ against multiple 24-HDR.

The FFQ was validated against multiple 24-HDR during each trimester for all macro-nutrients and select micro-nutrients (Vitamin B_6, B_{12}, folate) and calcium. The intakes from FFQ and multiple 24-HDR were compared using one sample t test. Spearman Rank correlations were used to assess the association between the nutrients measured through the FFQ and the average 24-HDR. Since the FFQ primarily ranks individuals, the agreement between the FFQ and the multiple 24-HDR was also examined by evaluating the proportion of subjects who fell within the same quintile of the nutrient distribution for both the methods. Further assessment of relative validity was accomplished by computing Spearman Correlations between nutrient intakes and biomarkers of their status.

The Bland Altman method was used to assess the agreement and bias between the two methods across the range of intakes[18]. A stepwise linear regression of the differences between

the two methods (bias) with maternal factors such as age, body weight, educational status and parity was performed to identify the factors that influenced the bias.

Results

Table 10 shows the baseline characteristics of the study participants. The average gestational age at which women were recruited was towards the end of the third month of pregnancy. While 40% of the women had an education that was less than or at high school, about a third had an university education.

The relative validity of the FFQ is shown in Table 11. In the 1st and 2nd trimesters, the FFQ overestimated nutrient intake compared to average 24-HDR by 21-40% whereas in the 3rd trimester the overestimation was of the order of 9-28%. The food group intakes with the FFQ were also higher than the intakes estimated by the 24-HDR during pregnancy in all trimesters (Table 12).

Cross tabulations of energy adjusted nutrient intakes assessed by the FFQs and average 24-HDR into quintiles showed that between 16 to 55% of nutrient intakes in the 1st trimester, 26% to 52% in the 2nd trimester and 17% to 44% in the 3rd trimester respectively fell into the same quintiles for both methods. However, the relative intake agreement was approximately 60% of (selected) nutrients with exact or within ± 1 quintile difference assessed for biomarkers and multiple 24-HDR and the FFQ. The relative agreement with exact or within ± 1 quintile was similar when assessed between the 2 dietary methods (*Data shown in table 1, Appendix A*).

Only plasma vitamin B_{12} positively correlated with energy adjusted dietary vitamin B_{12} intake assessed by the FFQ in the 1st and 2nd trimester of pregnancy while no correlation was seen between plasma vitamin B_6 and RCF biochemical status (biomarkers) with the intakes. While RCF status correlated positively with average 24-HDR only in the 1st trimester, there was no correlation between plasma vitamin B_{12} and vitamin B_6 biochemical status and the dietary intake from the average 24-HDR (Table 13).

The Bland Altman plots for the select nutrients except energy, in all 3 trimesters showed that there was systematic bias such that with increasing nutrient intakes (mean of the 2 dietary methods) there was a trend towards underreporting of intakes (Fig 7). The limits of agreement (LOA) were smallest for vitamin B_{12} in the 1st trimester (mean 0.46 g, LOA -1.75 g to 2.67 g).

A stepwise linear regression of the differences between the two methods (bias) and potential predictors indicated that maternal education was a significant predictor for most of the nutrients except energy and vitamin B_{12} in the 1st, 2nd and 3rd trimester of pregnancy. Education explained 7.5, 4.6 and 2.7% variance of the bias for vitamin B_6, folate and calcium, respectively, in the 1st trimester. Similarly the percentage of variance of the bias contributed by education was 7.4, 9.8, 6.4 in the 2nd trimester and 4.6, 7.6 and 4.7 in the 3rd trimester for same nutrients (*Data shown in table 2, Appendix A*).

Discussion

This study examines the relative validity of 108 item FFQ against the multiple 24-HDR and select biomarkers in an urban, South Indian pregnant women. The multiple 24-HDR in each trimester served as the reference method.

The assessment of the dietary intake of pregnant women is complicated because of various factors depending on the period of pregnancy. Poor correlation between instruments may be partly explained by appetite fluctuations and nausea, which may influence the long- term diet reports[19]. Number of validation studies with pregnant women has been performed[20, 21]. They are difficult to compare with our study and with each other since they cover various periods of pregnancy and differ in the reference method used as well as the number of days of dietary recording. Other studies[21] have used the food record as a reference method or the 24 hour diet recall[21,22,23].

In our study, different statistical approaches, like comparing mean and median intakes, correlation analysis and quintile categories and Bland Altman methods were used to asses the relative validity of the FFQ administered at each trimester of pregnancy. The mean daily intakes of nutrients and median intakes of food groups estimated from FFQs were in general higher than that obtained from multiple 24-HDR. Even though some differences among the two methods were not statistically significant; the findings of overestimation by FFQ are consistent with other dietary methods reported by other authors[24, 25]. Despite this overestimation of the energy adjusted intakes, on an average 30 to 35% of the nutrients fell into the same quintile as against the 8 to 13% in the opposite quintile during pregnancy indicating the nutrient intake measured by the FFQ was appropriate to rank the pregnant women by level of nutrient intake. Though studies conducted in pregnant women have shown a higher percentage of participants classified in the

same quintiles, they also have greatest misclassification into extreme quintiles for select food groups and nutrients; >15 percent upto 23 percent in the highest quintile on FFQ and lowest quintile on food records[22]. The correlation coefficients between absolute nutrients from FFQs and the average of multiple (24-HDR) ranged from 0.179 to 0.589 for nutrients in the 1st trimester, 0.355 to 0.568 in the 2nd trimester and 0.335 to 0.536 in the 3rd trimester respectively. Although energy adjustment removes nutrient variation due to variation in energy intakes, it also reduces the range of between-person variance, which may decrease the correlation[26]. Similar findings were seen in our study as the correlation coefficient of mean energy adjusted FFQ and average of multiple 24-HDR decreased after energy adjustment.

The mean intakes for most nutrients in the 2nd and 3rd trimester were greater than the 1st trimester. This result is similar to the findings from the Finnish pregnant women study where intakes of all nutrients increased with the 2nd FFQ[22]. In our study the mean coefficient correlation between the FFQ and the multiple 24-HDR was lower as compared to the other studies designed for use in women though non pregnant[27, 28]. One of the reasons suggestive of low correlation coefficient may be the selection of few macro and micronutrients for analyses as these were the nutrients of focus in the larger study. It is also observed that the correlation coefficient between the two dietary methods were lower for micronutrients as compared to the macronutrients[29]. Hence our mean correlation coefficient being low could be due to the inclusion of select macro and micronutrients together. We also examined the difference in intake after adjusting for the food items and observed that it did not alter the significance. This finding suggested that the quantity of intakes reported by the two methods may contribute to the differences rather than the number of food items.

The Bland Altman plots showed a trend towards underreporting of intakes through FFQ with increasing means intakes of the two methods with 24-HDR as the reference tool. A wide LOA indicates the potential for large differences and thus poor agreement between methods[30]. Since, this analysis tended to show a systematic error, further linear regression was performed of the differences between the two methods with potential predictors such as maternal age, education, parity and body weight at recruitment. Evidences have shown that the validity of the FFQ increased with years of maternal education and income[31,32] as well as race and ethnicity[32].

In our study too, maternal education, a marker of the socio-economic status of the subject was found to be a significant predictor for most of the nutrients with bias decreasing with increasing

maternal education. While studies have also highlighted the role of maternal age playing a vital role in utilization of health care services[33], Lacerte and colleagues through quantitative and qualitative studies pertaining to adherence to routine antenatal supplements have suggested that maternal education and knowledge are important factors influencing the use of antenatal services and consumption of supplements[34]. Moreover our study subjects were prescribed with folic acid in the 1st trimester and later from 2nd trimester onwards supplemented with folic acid and elemental iron until delivery. The strength of our study is that we have looked into all three trimester of pregnancy for the validation of the dietary tool and in addition considered the biomarkers; for the assessment of the micronutrient status in the early and mid pregnancy[11]. Our findings have also indicated that the pregnant women are consistent in receiving their antenatal care which is recognized as an important component of maternal health[33]. This study was a part of the larger study that focused on the relationship of intakes of B vitamins particularly (vitamin B_{12}, vitamin B_6 and folate) with the birth outcomes. FFQ is a tool commonly used in large epidemiological studies in different contexts, groups and populations and its validation depends on the use of the reference method and the most commonly used reference methods are 24-HDR, weighed dietary records or use of biomarker. The use of nutrient biomarker to validate a measure of intake is based on the assumption that the biochemical indicators are responsive to intake in a dose-dependent manner. No biochemical indicator will provide a perfect measure of intake because many factors other than diet influence the circulating nutrient concentrations. Control of circulating nutrient concentrations by factors other than diet tends to reduce correlations between intake and the biochemical indicators of nutrient status. The observed associations therefore reflect the lower bound of questionnaire validity. The advantage of using both the 24-HDR as well as the biomarker in this study is that, the errors in the estimation of nutrient status from dietary and biochemical measures (biomarkers) are much more likely to be independent[11]. While the correlation trends between the nutrients assessed by the FFQ, the 24-HDR and the biomarkers (micronutrient status) vary, these findings may be indicative of greater 'demand' of the micronutrients as the pregnancy progressed and the physiological changes during pregnancy.

Study Limitations

The choice of referent method such as food records (weighed or by diary) over 24-HDR is also preferred in epidemiological studies. Compared to using (24-HDR) as the comparison methods,

weighed or by diary records usually represent an optimal comparison method because sources of error are largely independent of error associated with a FFQ[9]. As our subjects were pregnant women attending the obstetrics and gynecology out patient department, use of weighed food records or diary records was not feasible, hence we administered (24-HDR) at each visit to have at least two (24-HDR) per trimester. We have considered only few of the macro and micronutrients analysis those were considered important as a part of the ongoing study.

Conclusions

The study findings suggest that compared to the average 24-HDR, the FFQ tends to overestimate a number of nutrients at the lower intakes and underestimates at the higher intakes. However, relative nutrient intake agreement was fair to moderate when assessed by the quintile method. Moreover, a fair correlation was seen with serum vitamin B_{12} levels with FFQ at both the trimesters. The FFQ has acceptable validity for ranking within a group – as the Bland Altman plots illustrate the agreement between methods for individuals that could also be wide. The strength of this tool is its advantages over the other methods such as; applicable to large cohorts, ease of administration, allows for repeat measurements to capture changes in diet overtime, provides information on large number of foods, relatively cheap to analyze and calculate nutrients, and moderate reliability and validity.

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None of the authors have any conflict of interest.

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Legend to the Figure 7.

Bland Altman plot-assessing agreement between the FFQ in the 1st trimester and multiple 24 hours diet recall in Panel A (vitamin B_{12}), Panel B (vitamin B_6), Panel C (folate) and Panel D (calcium) intakes. The values on the X axis represent the mean of nutrient between the 2 methods and the Y axis represents the nutrient difference between the 2 methods on the Y axis.

Table 10. Baseline characteristics of the study participants

Variables (n=154)	**Mean**	**SD**
Age (years)	24.5	4.3
Gestational age at recruitment (weeks)	11.6	2.4
Weight in the 1st trimester (kg)	52.7	9.7
Height (m)	1.55	0.06
BMI (kg/m^2)	22.03	4.08
Weight in the 2nd trimester (kg) (n=137)	57.6	10.8
	Number	**Percentage**
Parity[†]		
Primiparous	89	57.8
Multipaorus	65	42.2
Education[†]		
High school or lower	63	40.9
Diploma/PUC	36	23.4
University and above	55	35.7

[†] Values represent number, Percentage

Table 11. Comparison and correlation of daily nutrient intakes estimated using the food frequency questionnaire and multiple 24-hour recalls

Nutrients	FFQ		Average diet recall		Percentage overestimation[§]	Spearman correlation	
	Mean	95% CI	Mean	95% CI		Energy unadjusted	Energy adjusted
Trimester 1							
Energy (KJ/d)	8255	(7949, 8556)	6492	(6179, 6806)	27	0.37**	
Protein (g/d)	56.2	(55.5, 56.9)	43.6	(41.1, 46.1)	29	0.38**	0.13
Fat (g/d)	50.4	(48.9, 51.9)	39.0	(36.2, 41.8)	29	0.45**	0.35**
Carbohydrate (g/d)	323.3	(311.5, 335.1)	256.4	(244.4, 268.5)	26	0.34**	-0.08
Vit B_6 (mg/d)	1.72	(1.65, 1.79)	1.33	(1.25, 1.41)	29	0.31**	0.20*
Vit B_{12} (mcg/d)	1.87	(1.73, 2.02)	1.41	(1.25, 1.57)	33	0.42**	0.25**
Folate (mcg/d)	277.4	(265.2, 289.6)	197.4	(182.7, 212.2)	41	0.18*	0.11
Calcium (g/d)	936.9	(882.4, 991.5)	721.6	(671.5, 771.6)	30	0.59**	0.40**
Trimester 2							
Energy (KJ/d)	9146	(8807, 9481)	7334	(7053, 7614)	25	0.52**	
Protein (g/d)	63.7	(61.1, 66.2)	50.0	(47.9, 52.0)	27	0.43**	-0.09
Fat (g/d)	59.1	(56.3, 61.9)	44.4	(42.1, 46.7)	33	0.41**	0.19*
Carbohydrate (g/d)	349.6	(336.6, 362.7)	288.1	(277.0, 299.1)	21	0.53**	0.06
Vit B_6 (mg/d)	1.93	(1.84, 2.01)	1.55	(1.47, 1.63)	25	0.50**	0.27**

Vit B_{12} (mcg/d)	2.39	(2.20, 2.59)	1.89	(1.67, 2.11)	26	0.37**	0.26**
Folate (mcg/d)	305.0	(292.2, 317.8)	235.3	(221.9, 248.8)	30	0.36**	0.05
Calcium (mg/d)	1099.4	(1036.9, 1161.8)	843.8	(793.0, 894.5)	30	0.57**	0.38**
Trimester 3							
Energy (KJ/d)	8803	(8435, 9176)	7987	(7690, 8284)	10	0.51**	
Protein (g/d)	61.0	(58.2, 63.7)	54.7	(52.5, 56.8)	12	0.46**	0.05
Fat (g/d)	55.6	(52.6, 58.6)	48.6	(46.1, 51.1)	14	0.43**	0.18*
Carbohydrate (g/d)	339.8	(325.4, 354.1)	312.9	(301.0, 324.7)	09	0.54**	0.08
Vit B_6 (mg/d)	1.86	(1.77, 1.95)	1.67	(1.59, 1.75)	11	0.45**	0.15
Vit B_{12} (mcg/d)	2.54	(2.22, 2.87)	1.99	(1.78, 2.21)	28	0.34**	0.19*
Folate (mcg/d)	289.2	(275.8, 302.6)	254.0	(240.1, 267.9)	14	0.36**	0.01
Calcium (mg/d)	1081.2	(1016.0, 1146.4)	958.8	(900.7, 1016.9)	13	0.49**	0.20*

Nutrients obtained from FFQ are energy adjusted.

*Percentage overestimation; (FFQ/24 hr diet recall*100)*

[§] *All values compared to 100 using one sample t test were statistically significance at p<005.*

**P<0.05; **P<0.01 Test of significance*

Table 12. Comparison and correlation of daily food group intakes estimated using the food frequency questionnaire and multiple 24-hour recalls.

Food groups (g/day)	**FFQ Median (Q1, Q3)**	**Average diet recall Median (Q1, Q3)**	**Validity Spearman correlation**
	Trimester 1		
Cereals	264.2 (203.6, 321.0)	201.5 (159.4, 260.8)	0.41**
Pulses	17.1 (11.0, 33.1)	13.0 (0.00, 25.9)	0.09
Fish	0.57 (0.00, 3.39)	0.00 (0.00, 0.00)	0.20*
Milk Products	169.2 (46.7, 354.5)	120.0 (0.00, 293.3)	0.71**
Total vegetables	93.1 (68.4, 123.3)	24.48 (0.00, 95.49)	0.16
Total fruits	86.7 (46.1, 134.9)	100.0 (0.00, 175.1)	0.38**
	Trimester 2		
Cereals	263.6 (215.8, 325.7)	222.9 (188.1, 264.9)	0.54**
Pulses	19.8 (11.0, 34.8)	19.4 (11.9, 28.3)	0.16
Fish	1.58 (0.00, 4.08)	0.00 (0.00, 0.00)	0.31**
Milk Products	300.0 (135.4, 486.9)	204.3 (75.3, 326.3)	0.74**
Total vegetables	100.3 (78.5, 141.5)	43.2 (0.00, 112.9)	0.33**
Total fruits	107.8 (60.3, 146.3)	125.0 (67.3, 200.0)	0.31**
	Trimester 3		
Cereals	258.5 (224.1, 298.2)	235.6 (199.8, 281.8)	0.60**
Pulses	18.2 (9.4, 30.9)	19.4 (7.07, 31.9)	0.05
Fish	1.88 (0.00, 4.16)	0.00 (0.00, 0.00)	0.30**
Milk Products	285.1 (150.0, 479.9)	240.0 (112.5, 384.6)	0.65**
Total vegetables	101.7 (72.0, 123.3)	49.0 (9.1, 119.5)	0.35**
Total fruits	109.4 (65.3, 144.1)	125.0 (72.0, 200.0)	0.41**

Values for food groups represent median (lower, upper quartile)

**P<0.05; **P<0.01 Test of significance, Spearman correlations*

Table 13. Bivariate correlation of micronutrients status with energy adjusted intake from FFQ and multiple 24 hours diet recall during pregnancy.

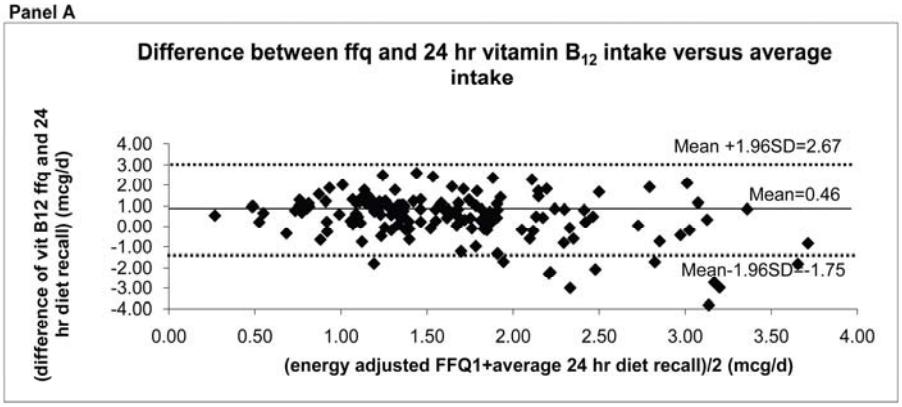
Nutrients	Micronutrient status		FFQ				24 hr diet recall		
	Mean	95% CI	Mean	95% CI	Correlation, r (p value) Energy adjusted	Energy unadjusted	Mean	95% CI	Correlation, r (p value)
Trimester 1									
Vitamin B_{12}	186.3 (n=154)	(162.6, 210.5)	1.72	(1.76,1.98)	0.343 (<0.001)	0.295 (<0.001)	1.41	(1.27, 1.56)	0.047 (0.566)
Vitamin B_6	35.8 (n=151)	(29.9, 41.7)	1.87	(1.68,1.76)	-0.087 (0.289)	-0.082 (0.314)	1.33	(1.27, 1.39)	0.146 (0.075)
Folate	526.5 (n=153)	(486.1,566.9)	277.4	(269.2,285.6)	-0.026 (0.749)	0.012 (0.879)	197.4	(185.5, 205.1)	0.175 (0.030)
Trimester 2									
Vitamin B_{12}	147.6 (n=134)	(135.2,160.0)	2.39	(2.24,2.54)	0.201 (0.022)	0.261 (0.003)	1.89	(1.67, 2.10)	0.113 (0.195)
Vitamin B_6	29.7 (n=131)	(25.8,33.6)	1.93	(1.88,1.98)	-0.061 (0.497)	-0.106 (0.261)	1.55	(1.50, 1.67)	-0.120 (0.173)
Folate	632.1 (n=135)	(587.1,677.2)	305.0	(296.7,313.3)	-0.031 (0.723)	-0.006 (0.946)	23.3	(225.2, 245.4)	0.025 (0.778)

Values represent correlation coefficient and p value using Spearman correlations

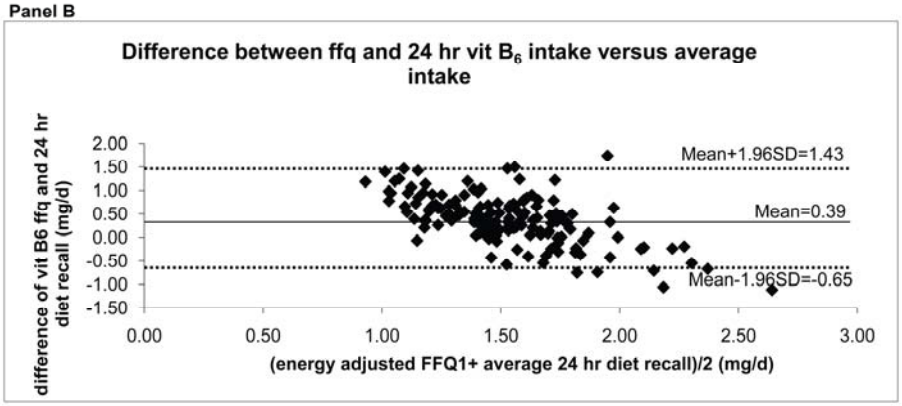
Units for micronutrient status: vitamin B_{12} (pmol/L), vitamin B_6 (nmol/L) and folate (nmol/L)

Units for micronutrient intake through FFQ and 24 hours diet recall: vitamin B_{12} (mcg/d), vitamin B_6 (mg/d) and folate (mcg/d)

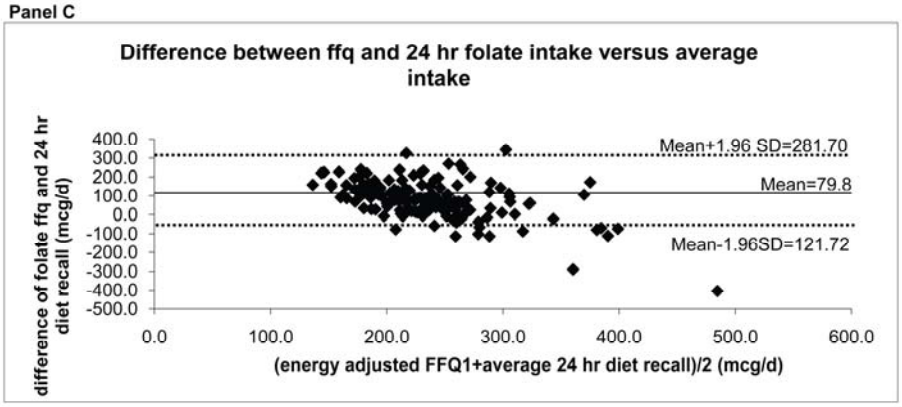
Panel A



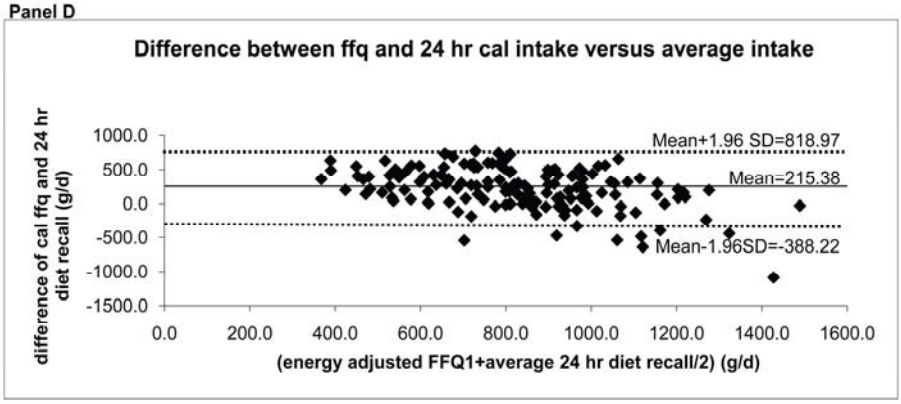
Panel B



Panel C



Panel D



CHAPTER 5

INFLUENCE OF MATERNAL DIETARY INTAKE OF B VITAMINS (VITAMIN B_{12}, VITAMIN B_6, FOLATE) AND FOOD GROUP INTAKES ON BIRTH WEIGHT, SMALL FOR GESTATIONAL AGE BABIES AND PRETERM BIRTHS

5.1 INTRODUCTION

The period of intrauterine growth and development is one of the most vulnerable periods in the human life-cycle. Fetal growth depends on the uptake of nutrients, which occurs at the end of a complex maternal supply line that begins with the mother's intake. Maternal diet is the modifiable factor but the complex maternal supply line depends on various factors such as maternal, placental and fetal metabolic and endocrine status. The transfer of nutrients from mother to the placenta and from placenta to the fetus through the uterine blood flow is also important. Placenta is an organ that transfers nutrients to the growing fetus. Any insult at the placenta may affect the transport of nutrients. Hence, alterations in either of these stages may lead to adverse birth outcomes. The weight of the infant at birth is a powerful predictor of infant growth and survival and is dependent on prior maternal health and nutrition during pregnancy.

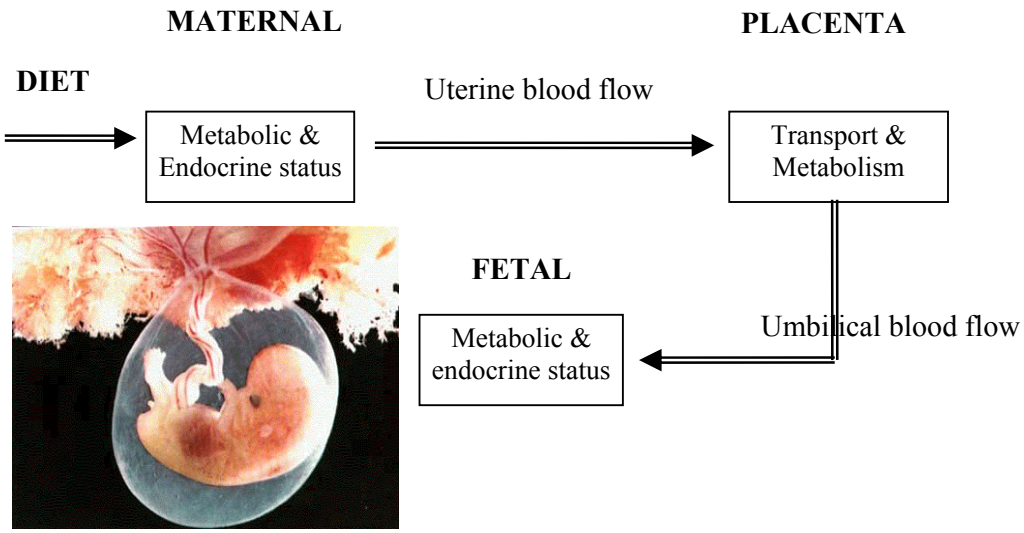


Figure 8. The fetal supply line.

Kind courtesy of Soares MJ

Babies born small in size in developing countries and in India were ascribed to the small size and 'chronic undernutrition' of Indian mothers (Gopalan 1994). LBW babies in developing countries, and in India (a newly industrialised country), comprise 30% of the babies born and majority of these are a result of SGA. The babies born LBW have been associated with significantly higher perinatal and infant morbidity (Barros et al., 1992; Chandra, 1975) and mortality (Ashworth, 1998) in the first two years of life. Increasing evidence also has identified LBW as a risk factor for chronic diseases (Stein et al, 1996). LBW is a strong predictor for size in later life because SGA infants seldom catch-up to normal size during childhood. The fetal origins of adult disease suggests that term-infants who are SGA have increased susceptibility to chronic disease in adulthood as a consequence of physiologic adaptations to under-nutrition during fetal life. The cause of LBW/SGA being multi-factorial, a number of nutrients have a critical role in contributing to an optimal birth outcome.

Vitamin B_{12}, vitamin B_6 and folate play an important role in determining birth outcomes and are involved in methylation cycle. Vitamin B_{12} (a co-factor) in presence of folate (substrate) is essential in the remethylation pathway; converting homocysteine to methionine. Vitamin B_6 (co-factor) is important in the transsulfuration pathway converting Hcy to cysteine. In general, 1-C (methyl) metabolism plays a key role in fetal programming (Yajnik 2008). A study in India has shown that almost 70% of the pregnant women are vitamin B_{12} deficient while folate deficiency is relatively rare Yajnik et al., 2005). Further studies have shown that children born to strict vegetarian mothers are also vitamin B_{12} deficit (Jadhav et al., 1962, Yajnik & Deshmukh 2008).

Maternal nutrition is an important factor from a public health point of view because it is modifiable and therefore susceptible to public health interventions. In elucidating the potential effect of maternal diet on fetal growth, both diet compositions and single foods and nutrients have been examined with varying outcomes. Although energy and protein are believed to be the major macronutrients that are associated with birth size (WHO, 1995; Kramer, 2002), intervention studies have shown borderline decrease in SGA babies in the mothers supplemented with energy and proteins (Kramer, 2002). Protein intake during the late pregnancy in

normal BMI mothers has also shown positive relationship with placental and birth weight (Godfrey et al., 1996). However, others have found no relation between maternal intakes of macronutrients and infant birth size among well nourished women (Mathews et al., 1999; Lagiou et al., 2004). Trials conducted in the past have shown positive effects of individual micronutrients as well as combinations such as iron, folic acid, vitamin A, vitamin C and B-vitamins and improved birth weight, reduced prematurity, pre-eclampsia and maternal mortality (Rasmussen & Stoltzfus 2003, de Onis et al., 1998; Ceesay et al., 1997; West et al., 1999; Ramakrishnan et al., 1999). Previously, we have demonstrated that vitamin B_{12} deficiency exists in pregnant Indian women, and that the risk of delivering a LBW baby increases with increasing vitamin B_{12} deficiency (Muthayya et al., 2006). Moreover, studies have shown a stronger relationship with dietary intake of micronutrient-rich foods (fruits and vegetables) in the second trimester of pregnancy with birth weight (Rao et al., 2001). Similarly observations were also made in Danish population (Mikkelsen et al., 2006) with regards to fruits and vegetable intakes and birth weight. Study conducted in Norwegian pregnant women have shown a beneficial effects of milk-based probiotics consumption and lower risk to preeclampsia in primiparous pregnant women (Brantsaeter et al., 2011) and preterm delivery (Myhre et al., 2011). While intakes of dairy, meat protein have also shown positive relationship with birthweight (Godfrey et al., 1996; Mannion et al., 2006; Kannade et al., 2008), Knudsen and colleagues have shown high intakes of red and processed meat and high fat diary intakes associated with increased risk of SGA (Knudsen et al., 2008). In the population that consumes predominantly cereal based diet, it is essential to characterise the food groups that may be involved in determining adverse birth outcomes.

Gaps in our knowledge still exist concerning the relationship between food group intakes, of pregnant women and birth outcomes. It is likely that single nutrient deficiency is rare and nutrients may cluster together, it can be postulated that the role of nutrients may be mediated through food group intakes. If combination of nutrients as seen in food groups found true, these data lend themselves to future guidelines for healthy eating practices, and possibly translate into operational procedures for individual counselling and population-based health programs. Therefore I also aimed

to study the relationship of food group intakes during pregnancy and adverse birth outcomes.

5.2 METHODS

Apparent healthy subjects in the reproductive age were recruited within 1st trimester of pregnancy and followed until delivery. The study procedures, selection of study subjects, inclusion, exclusion criteria, methodology and the study protocol is mentioned in the previous chapter. All study subjects received routine antenatal care during pregnancy. The anthropometric measurements and dietary information during pregnancy and birth details at delivery was collected as per the study protocol.

5.3 STATISTICAL ANALYSIS

All analyses were carried out with the SPSS program (version 16.0, SPSS, Chicago, IL, USA). Data was assessed for the normality of distribution. The maternal baseline characteristics were normally distributed and represented as Mean ± SD while the nutrient intakes through FFQ were expressed as the median (lower and upper quartile ranges). Nutrients were energy adjusted to remove variation due to kilo joules (KJ) intake, using the residual method. The food group intakes from FFQ were also represented as median (lower and upper quartile ranges). Spearman Rank correlations were used to assess the relationship between the dietary nutrient and food group intakes to birth parameters. Chi square test was performed to find the association between the tertiles of nutrient and food group intakes and birth outcomes; such as SGA, LBW and preterm births. Since there were significant differences observed in nutrients and food group intakes of mothers of SGA and non SGA in the 2nd trimester of pregnancy, further analysis was carried out in these pregnant women.

Repeated measure ANOVA was performed to observe the food group intakes (cereals and milk product) during the 3 trimesters of pregnancy with respect to SGA as the birth outcome and the trimester by SGA status interaction effect was considered. Multivariate logistic regression adjusting for maternal demographic characteristics such as age, parity, education, maternal weight and any effects of nutrients significant in the univariate analysis were examined. The model consisted of various steps such that, the 1st row of odds ratio refered to the univariate analysis

of SGA with cereal/milk product intakes. The effect of these intakes on SGA could be confounded with a set of socio-demographic variables and dietary intakes. In order to understand the effect of cereals/milk product intakes on SGA in the presence of these variables separately, I choose to include the confounding variables at different stages into the logistic regression analysis. Of these confounding variables, in the Multiple Logistic Regression analysis, protein intake was of particular interest. In the 1st step I examined the effect of cereals/milk product intakes on SGA while adjusting for the socio-demographic characteristics. Further, added maternal weight at recruitment, energy and so on until the last step where all other dietary variables such as fat, carbohydrates, folate, vitamin B_{12}, vitamin B_6 intakes which were found to be associated with SGA in univariate analysis were included into the model to examine the effect of cereal /milk product intakes on SGA. The odds ratio (OR) 95% confidence intervals (CIs) are reported, with two sided P values < 0.05 being considered statistically significant. In addition, total proteins from milk products and cereals were computed as well as the contribution of protein from milk products and cereals to total protein intakes in the 2nd trimester of pregnancy and compared across the two groups of SGA and AGA mothers.

5.4 RESULTS

The mean age of the pregnant women was around 25 years. Almost 60 percent of the subjects were pregnant for the 1st time. With regards to the education of the participants, it was observed that almost 33% had education upto high school while ~44% had education of university level and above. Moreover, almost 28% of the women were employed outside their house (**Table 14**).

Table 14. Maternal baseline characteristics

Parameters (n=637)	Values Number (percentage)
Age[a]	24.8 ± 3.9
Parity	
Primiparous	387 (60.8)
Multiparous	250 (39.2)
Education	
Upto High School	211 (33.1)
Pre-university /diploma	143 (22.4)
University & Above	283 (44.4)
Employment	
Working outside the house	177 (27.8)
Home makers	460 (72.2)

[a] values represent mean ± SD

Many subjects had anemia with the proportion being 16%, ~28% and 40% in the 1st, 2nd and 3rd trimester respectively. The weight gain during pregnancy was calculated as the weight difference between the 1st trimester and weight measured during their last antenatal visit. The mean weight of the subjects before delivery was 63.9 ± 10.2 kg. The weight gain in the 2nd and 3rd trimester of pregnancy was also calculated similarly such that for the 2nd trimester, the weight difference between weights measured at 12 ± 1 week of gestation and 24 ± 1 week of gestation was considered. For the 3rd trimester too, the weight difference between the 24 ± 1 week of gestation and 34 ± 1 week of gestation gave the gestational weight gain in the 3rd trimester of pregnancy. The weight gain per week was calculated as the weight difference calculated between trimesters divided by the difference in the gestational age at the time of measurements. Hence the total weight gain was around 11.0 ± 3.9 kg with 0.49 ± 0.21 kg per week during the course of pregnancy. The maternal weight was significantly different between the 1st and 2nd, 2nd and 3rd and 1st and 3rd trimester of pregnancy. Similar trend was seen with BMI and fat mass. Physical activity reduced from 1st to 3rd trimester as well as the hemoglobin levels (**Table 15**).

Table 15. Maternal anthropometry during pregnancy

Parameters	**Tri 1 (n=637)**	**Tri 2 (n=423)**	**Tri 3 (n=379)**
Gestational age (weeks)	11.3 ± 2.6	24.2 ± 1.60[1,2]	34.15 ± 1.55[3]
Height (cm)	155.2 ± 6.0	-	-
Body weight (Kg)	53.5 ± 9.6	58.4 ± 10.3[1,2]	62.5 ± 10.5[3]
BMI ($kg.m^{-2}$)	22.2 ± 3.8	24.3 ± 4.0[1,2]	26.3 ± 4.8[3]
Undernourished subjects[a]	157 (24.6)	44 (10.4)	10 (2.6)
Physical activity level	1.48 ± 0.15	1.47 ± 0.14	1.46 ± 0.13[3]
Percent fat	28.6 ± 5.3 (n=635)	29.1 ± 5.1[1]	28.8 ± 5.1[3]
Fat mass (kg)	15.7 ± 5.3 (n=635)	17.5 ± 5.7[1,2]	18.4 ± 5.8[3]
Fat free mass (kg)	37.7 ± 5.0 (n=635)	40.8 ± 5.7[2]	44.2 ± 5.5[3]
Hemoglobin (($mg.dl^{-1}$)	12.1 ± 1.3 (n=593)	11.3 ± 1.5[1](n=254)	11.2 ± 1.4[3] (n=352)
Anemia[b]	95 (16.0)	70 (27.6)	24 (40.6)
Weight gain (kg)	-	5.0 ± 2.3 (n=422)	4.9 ± 2.2 (n=324)
Wt gain per week (kg)	-	0.43 ± 0.15[1,2]	0.40 ± 0.19[3]

Values indicate Mean ± SD except for [a] & [b] as number and percentage

[a] BMI <19.5 $kg.m^{-2}$,

[b]Anemia classified as Hb < 11 gm% in the 1st and 2nd trimester Hb <10.5 gm% in the 3rd trimester of pregnancy

[1] Significant difference between trimester 1 and 2 (paired t test)

[2] Significant differences between trimester 2 and 3 (paired t test)

[3] Significant differences between trimester 1 and 3 (paired t test)

The **table 16** describes the dietary nutrient and food group intakes across the 3 trimesters of pregnancy. It was observed that the nutrient intakes were significantly higher in the 2nd and 3rd trimester as compared to the 1st trimester, while there was no significant increase in the intakes from the 2nd to the 3rd trimester of pregnancy. Similar trend was observed with food group intakes as well except for intakes of pulses and eggs.

Of the pregnant women, 69% of the women had normal delivery and 48% gave birth to male babies. The mean birth weight was around 2.9 kg born at term (~39 weeks gestation). With regards to the birth outcome categories, ~20% were

born LBW; ~27% SGA while 9% preterm births. Around 7% of the fetal losses occurred during pregnancy which included abortions and IUD (**Table 17**).

Table 16. Maternal dietary nutrient and food group intakes during pregnancy as assessed by FFQ

Nutrients	Trimester 1 (n= 636)	Trimester 2 (n=417)	Trimester 3 (n=370)
Energy ($kcal.d^{-1}$)	1895 (1602, 2246)	2140 (1829, 2544)[1]	2096 (1765, 2576)[3]
Protein ($g.d^{-1}$)	56.2 (53.0, 59.3)	63.6 (60.7, 67.2)[1]	63.7 (61.0, 66.6)[3]
Fat ($g.d^{-1}$)	51.3 (45.8, 57.6)	50.1 (53.4, 65.7)[1]	58.8 (53.7, 64.9)[3]
Carbohydrate ($g.d^{-1}$)	314.3 (297.6, 327.4)	350.7 (337.0, 368.8)[1]	351.5 (336.1, 364.6)[3]
Vitamin B_{12} ($mcg.d^{-1}$)	1.98 (1.51, 2.60)	2.47 (1.91, 3.16)[1]	2.49 (1.94, 3.04)[3]
Vitamin B_6 ($mg.d^{-1}$)	1.66 (1.51, 1.85)	1.88 (1.69, 2.11)[1]	1.90 (1.71, 2.10)[3]
Folate ($mcg.d^{-1}$)	269.6 (241.8, 301.4)	301.0 (275.5, 335.8)[1]	300.2 (269.1, 330.9)[3]
Calcium ($mg.d^{-1}$)	937.1 (791.8, 1084.4)	1091.6 (960.0, 1236.1)[1]	1124.9 (979.8, 1271.6)[3]
Food groups			
Cereals ($g.d^{-1}$)	244 (198, 303)	263 (215, 319)[1]	262 (216, 318)[3]
Pulses ($g.d^{-1}$)	16.6 (8.8, 33.4)	19.0 (10.3, 35.3)	17.7 (9.6, 31.2)
Fish ($g.d^{-1}$)	1.1 (0.0, 4.5)	1.9 (0.0, 5.5)[1]	1.9 (0.0, 5.3)[3]
Poultry ($g.d^{-1}$)	6.3 (0.0, 12.9)	6.8 (0.0, 14.8)[1]	6.3 (0.0, 14.0)[3]
Eggs ($g.d^{-1}$)	10.2 (1.8, 19.3)	11.6 (1.8, 19.3)	9.9 (0.0,18.0)
Meat ($g.d^{-1}$)	17.4 (6.0, 26.7)	19.5 (6.4, 32.3)[1]	19.5 (7.0, 29.4)[3]
Milk products ($g.d^{-1}$)	217.1 (71.2, 378.3)	290.6 (150.3, 474.2)[1]	296.5 (173.0, 485.0)[3]
Green leafy vegetables ($g.d^{-1}$)	19.3 (12.3, 30.7)	21.9 (14.0, 36.9)[1]	24.6 (18.4, 37.7)[3]
Vegetables ($g.d^{-1}$)	96.5 (73.8, 126.3)	103.7 (79.6, 142.6)[1]	105.2 (78.1, 141.1)[3]
Fruits ($g.d^{-1}$)	87.1 (49.6, 129.6)	104.0 (60.3, 145.4)[1]	109.4 (65.1, 145.4)[3]

Values represent median with lower and upper quartile ranges

[1] Significant difference between trimester 1 and 2 (paired t test)

[3] Significant differences between trimester 1 and 3 (paired t test)

Table 17. Baby characteristic at birth

Parameters	**Values (Mean ± SD)**
Gestational age at birth (weeks) (n=637)	37.0 ± 6.6
Birth weight (g) (n=594)	2860 ± 503
Placental weight (g) (n=389)	524 ± 96
Baby anthropometry	
Length (cm) (n=423)	49.6 ± 2.4
MUAC (cm) (n=418)	9.7 ± 0.9
Head Circumference (cm) (n=426)	33.5 ± 1.4
Chest Circumference (cm) (n=419)	31.3 ± 2.0
Biceps (mm) (n=415)	2.8 ± 0.6
Triceps (mm) (n=415)	3.7 ±0.8
Subscapular (mm) (n=415)	3.6 ± 0.9
Suprailiac (mm) (n=207)	2.7 ±0.6
Sum of 3 skinfolds	10.1 ± 2.1
Sum of 4 skinfolds	13.0 ± 2.8
Baby body composition	
Arm muscle area (cm^2) (AMA)	5.9 ± 1.0
Arm muscle index (AMI)	77.5 ± 3.3
Cross section of the arm (MAA)	0.60 ± 0.05
Arm fat area (cm^2) (AFA)	1.68 ± 0.15
Arm fat index (AFI)	22.5 ± 3.3
	Number (percentage)
Gender (n=594)	
Male	307 (48.2)
Female	287 (45.1)
Type of Delivery (n=637**)**	
Normal	439 (68.9)
C-Section	198 (31.1)
Birth Outcomes	
SGA	171 (26.8)
LBW	121 (19)
Preterm	60 (9.4)
Fetal Loss	44 (6.9)

The maternal vitamin B_{12}, vitamin B_6 and folate intakes were correlated with baby parameters. Vitamin B_{12} intakes in the 1st and 2nd trimester of pregnancy correlated positively with birth weight and vitamin B_6 intakes in the 3rd trimester with gestational age at birth. There was a positive correlation observed between the gestational weight gain in the 2nd trimester and the birth weight ($r=0.138$, $p=0.004$).

Our preliminary analysis showed a significant difference in the maternal anthropometry, dietary intake and food groups and birth outcomes among SGA and AGA babies. The demographic characteristics of the pregnant women of the SGA and AGA babies at recruitment are shown in **Table 18**. Pregnant women were recruited at ~11 weeks of gestation and almost 63% were primiparous. The mothers of the SGA babies were significantly younger and 35% of them had education greater than university. The mothers of SGA babies weighed significantly less (50.5 ± 9.0 versus 54.6 ± 9.7 kg), were shorter (154.2 ± 6.2 versus 155.5 ± 5.9 cm) and had lower BMI (21.2 ± 3.6 versus 22.5 ± 3.8 $kg.m^{-2}$) as compared to the mother's of AGA babies at recruitment.

Table 18. Maternal characteristics of SGA and AGA babies

Parameters	SGA (n=171)	AGA (n=423)
Age (years)	24.1 ± 3.8	25.0 ± 4.0*
Parity[a]		
Primiparous	112 (65.5)	255 (60.3)
Multiparous	59 (34.5)	168 (39.7)
Education[†]		
Upto high school	75 (43.9)	123 (29.1)*
Pre-University	37 (21.6)	94 (22.2)
/Diploma	59 (34.5)	206 (48.7)
University and above		
LMP (weeks)	11.7 ± 2.4	11.3 ± 2.6
Anthropometry		
Weight (kg)	50.5 ± 9.0	54.6 ± 9.7*
Height (cm)	154.2 ± 6.2	155.5 ± 5.9*
BMI ($kg.m^{-2}$)	21.2 ± 3.6	22.5 ± 3.8*
Hb (gm %)	12.0 ± 1.3	12.2 ± 1.2
	(n=164)	(n=404)

Values represent: Mean ± SD; [a] represent number (percentages)

LMP: Last menstrual period, BMI: Body mass index, Hb: Hemoglobin concentration

[*] Student's t test of significance, $p < 0.05$

Dietary intakes of the macronutrients and vitamin $B_{12,}$ folate and vitamin B_6 during the three trimesters of pregnancy across the groups of mothers of SGA, AGA; LBW, normal weight babies and preterm and term babies were examined. With regards to mothers of SGA and AGA babies, in the 1st trimester the fat and carbohydrate intakes were significantly lower ($p < 0.05$) in the mother's of SGA babies. Similarly in the 2nd trimester, intakes of protein, fat, carbohydrate, folate and vitamin B_{12} were significantly lower ($p < 0.05$) in the SGA mother's as compared to the mother's of AGA babies. There were no differences in the intakes in the 3rd trimester as the pregnancy progressed (**Table 19a**). With respect to the mothers of LBW and normal weight babies, significantly low intakes of protein and vitamin B_{12} in the 1st trimester in mothers of LBW babies was observed and vitamin B_{12} also remained to be lower in the 2nd trimester of pregnancy. As the pregnancy progressed

there was no significant difference in the dietary intakes in the 3rd trimester among the 2 groups (**Table 19b**). The mothers of preterm babies had significantly lower fat intake in the 3rd trimester (~ 3.6 $g.d^{-1}$) while no differences were seen in the other nutrients though a decreased trend for vitamin B_{12} in the 1st and 3rd trimester was observed (**Table 19c**).

The dietary food group intakes across the 3 birth outcome groups (SGA-AGA, preterm-term and LBW- normal birth weight) were also examined. There was no significant difference in the food group intakes among the mothers of the two groups (SGA versus AGA) at the 1st and 3rd trimester of pregnancy while the mother's of SGA babies had significantly lower median intakes of total cereals (~ 255 versus 267g) and milk products (~ 257 versus 323g) in the 2nd trimester of pregnancy (**Table 20a**). The comparison between the food group intakes between mothers of LBW and normal weight babies did not shown any significant differences (**Table 20b**). There was a significant increase in the milk product intakes in the 2nd trimester in the mothers of preterm babies ($p=0.027$) while none of the other food groups intakes showed significant differences among the mothers of preterm and term babies (**Table 20c**).

Table 19a. Dietary intakes during pregnancy among mothers of SGA and AGA babies

Nutrients	Mothers of SGA babies	Mothers of AGA babies
Trimester 1	**(n=171)**	**(n=423)**
Energy ($kcal.d^{-1}$)	1849 (1526, 2172)	1899 (1605, 2264)
Protein ($g.d^{-1}$)	56.0 (52.7, 58.4)	56.4 (53.1, 59.5)
Fat ($g.d^{-1}$)	50.4 (44.9, 54.7)	52.2 (46.5, 57.9)*
Carbohydrate ($g.d^{-1}$)	312.7 (296.7, 326.0)	316.3 (304.1, 330.4)*
Folate ($mcg.d^{-1}$)	262.2 (240.2, 293,9)	271.0 (243.1, 306.3)
Vitamin B_6 ($mg.d^{-1}$)	1.65 (1.52, 1.85)	1.67 (1.50, 1.84)
Vitamin B_{12} ($mcg.d^{-1}$)	1.85 (1.45, 2.46)	2.00 (1.60, 2.64)*
Trimester 2	**(n=124)**	**(n=292)**
Energy ($kcal.d^{-1}$)	2092 (1815, 2526)	2160 (1856, 2562)
Protein ($g.d^{-1}$)	63.1 (59.7, 66.2)	64.3 (61.1, 68.0)*
Fat ($g.d^{-1}$)	56.7 (51.8, 64.4)	60.4 (54.3, 66.1)*
Carbohydrate ($g.d^{-1}$)	348.5 (334.8, 365.8)	360.3 (340.6, 374.9)*
Folate ($mcg.d^{-1}$)	291.7 (264.3, 322.2)	307.1 (377.9, 339.5)*
Vitamin B_6 ($mg.d^{-1}$)	1.91 (1.69, 2.15)	1.87 (1.68, 2.10)
Vitamin B_{12} ($mcg.d^{-1}$)	2.37 (1.71, 2.86)	2.51 (1.97, 3.23)*
Trimester 3	**(n=112)**	**(n=257)**
Energy ($kcal.d^{-1}$)	2060 (1750, 2598)	2112 (1768, 2559)
Protein ($g.d^{-1}$)	63.9 (60.7, 66.6)	63.7 (61.0, 66.5)
Fat ($g.d^{-1}$)	58.9 (54.8, 63.1)	58.8 (53.4, 65.3)
Carbohydrate ($g.d^{-1}$)	351.2 (338.9, 362.7)	351.6 (334.5, 365.4)
Folate ($mcg.d^{-1}$)	298.6 (265.5, 323.3)	301.6 (271.3, 333.9)
Vitamin B_6 ($mg.d^{-1}$)	1.91 (1.72, 2.08)	1.89 (1.71, 2.09)
Vitamin B_{12} ($mcg.d^{-1}$)	2.50 (1.74, 3.08)	2.48 (1.96, 3.05)

Values represent Median (lower, upper quartile)

*Kruskal Wallis test of significance, $p < 0.05$

Table 19b. Dietary intakes during pregnancy among mothers of LBW and normal weight babies

Nutrients	Mothers of LBW babies	Mothers of Normal weight babies
Trimester 1	**(n=121)**	**(n=473)**
Energy ($kcal.d^{-1}$)	1905 (1553, 2279)	1884 (1597, 2223)
Protein ($g.d^{-1}$)	55.5 (52.2, 58.3)	56.4 (53.2, 59.4)*
Fat ($g.d^{-1}$)	50.6 (44.9, 55.4)	51.4 (46.1, 57.6)
Carbohydrate ($g.d^{-1}$)	316.8 (302.6, 330.6)	314.1 (297.3, 326.5)
Folate ($mcg.d^{-1}$)	269.7 (240.2, 301.7)	269.3 (243.1, 301.1)
Vitamin B_6 ($mg.d^{-1}$)	1.70 (1.55, 1.87)	1.66 (1.50, 1.85)
Vitamin B_{12} ($mcg.d^{-1}$)	1.83 (1.33, 2.46)	2.01 (1.60, 2.66)*
Trimester 2	**(n=91)**	**(n=325)**
Energy ($kcal.d^{-1}$)	2054 (1829, 2540)	2167 (1834, 2552)
Protein ($g.d^{-1}$)	64.4 (59.9, 67.3)	63.5 (60.9, 67.2)
Fat ($g.d^{-1}$)	60.5 (53.4, 65.8)	58.9 (53.5, 65.7)
Carbohydrate ($g.d^{-1}$)	349.4 (334.6, 369.6)	351.6 (337.3, 368.4)
Folate ($mcg.d^{-1}$)	309.7 (276.3, 329.8)	300.0 (275.1, 336.1)
Vitamin B_6 ($mg.d^{-1}$)	1.94 (1.71, 2.15)	1.87 (1.68, 2.10)
Vitamin B_{12} ($mcg.d^{-1}$)	2.37 (1.70, 2.81)	2.50 (1.95, 3.21)*
Trimester 3	**(n=74)**	**(n=295)**
Energy ($kcal.d^{-1}$)	2071 (1730, 2554)	2098 (1775, 2582)
Protein ($g.d^{-1}$)	63.6 (59.7, 66.4)	63.7 (61.1, 66.6)
Fat ($g.d^{-1}$)	58.1 (54.7, 63.8)	59.0 (53.4, 65.2)
Carbohydrate ($g.d^{-1}$)	354.0 (341.4, 364.0)	351.3 (334.6, 365.1)
Folate ($mcg.d^{-1}$)	303.6 (270.4, 330.7)	299.8 (268.8, 331.5)
Vitamin B_6 ($mg.d^{-1}$)	1.93 (1.74, 2.09)	1.90 (1.71, 2.10)
Vitamin B_{12} ($mcg.d^{-1}$)	2.33 (1.99, 2.91)	2.50 (1.90, 3.09)

Values represent Median (lower, upper quartile)

* Kruskal Wallis test of significance, $p < 0.05$

Table 19c. Dietary intakes during pregnancy among mothers of preterm and term babies

Nutrients	Mothers of Preterm babies	Mothers of Term babies
Trimester 1	**(n=60)**	**(n=534)**
Energy (kcal.d^{-1})	1939 (1551, 2422)	1886 (1596, 2217)
Protein (g.d^{-1})	56.1 (51.9, 59.0)	56.3 (3.0, 59.3)
Fat (g.d^{-1})	52.1 (45.3, 57.4)	51.1 (45.7, 57.2)
Carbohydrate (g.d^{-1})	313.0 (298.3, 329.8)	314.7 (298.2, 327.0)
Folate (mcg.d^{-1})	270.7 (332.3, 306.7)	268.9 (241.8, 301.0)
Vitamin B_6 (mg.d^{-1})	1.70 (1.45, 1.84)	1.66 (1.51, 1.85)
Vitamin B_{12} (mcg.d^{-1})	1.82 (1.40, 2.35)	1.99 (1.56, 2.65)
Trimester 2	**(n=43)**	**(n=373)**
Energy (kcal.d^{-1})	2160 (1863, 2540)	2140 (1826, 2552)
Protein (g.d^{-1})	64.5 (60.5, 67.6)	63.5 (60.7, 67.1)
Fat (g.d^{-1})	57.9 (50.7, 65.8)	59.3 (53.2, 65.7)
Carbohydrate (g.d^{-1})	356.4 (336.5, 370.7)	350.7 (337.0, 367.8)
Folate (mcg.d^{-1})	308.5 (275.3, 323.5)	300.8 (275.4, 337.5)
Vitamin B_6 (mg.d^{-1})	1.86 (1.71, 2.09)	1.88 (1.68, 2.12)
Vitamin B_{12} (mcg.d^{-1})	2.50 (1.92, 2.81)	2.47 (1.91, 3.18)
Trimester 3	**(n=33)**	**(n=336)**
Energy (kcal.d^{-1})	2123 (1734, 2624)	2096 (1767, 2574)
Protein (g.d^{-1})	62.8 (60.2, 66.2)	63.8 (61.0, 66.6)
Fat (g.d^{-1})	55.5 (51.6, 60.9)	59.1 (54.1, 65.2)*
Carbohydrate (g.d^{-1})	359.0 (344.8, 368.9)	351.1 (335.4, 363.7)
Folate (mcg.d^{-1})	314.5 (368.5, 328.1)	299.8 (268.9, 322.1)
Vitamin B_6 (mg.d^{-1})	1.83 (1.67, 1.99)	1.91 (1.72, 2.10)
Vitamin B_{12} (mcg.d^{-1})	2.23 (2.03, 2.70)	2.51 (1.90, 3.10)

Values represent Median (lower, upper quartile)

*Kruskal Wallis test of significance, $p < 0.05$

Table 20a. Dietary food group intakes during pregnancy among mothers of SGA and AGA babies

Food groups ($g.d^{-1}$)	**Mothers of SGA Babies**	**Mothers of AGA babies**
Trimester 1	**(n=171)**	**(n=422)**
Cereals	241.6 (186.5, 294.9)	243.2 (198.7, 306.8)
Meat	16.3 (5.0,25.3)	17.8 (6.8,27.6)
Milk products	177.6 (53.5, 369.0)	231.0 (77.6, 381.2)
Green leafy vegetables	18.4 (12.3, 28.1)	20.1 (12.3, 30.8)
Vegetables	92.0 (72.9, 121.2)	99.4 (73.9, 128.1)
Fruits	81.4 (48.0, 122.9)	91.2 (50.0, 132.9)
Trimester 2	**(n=114)**	**(n=279)**
Cereals	254.5 (204.7,305.8)	266.9 (221.6,330.1)*
Meat	20.7 (4.5,33.8)	20.5 (9.0,32.8)
Milk products	256.8 (110.0,397.7)	322.8 (165.1,498.4)*
Green leafy vegetables	24.6 (17.0, 37.4)	21.9 (12.7, 36.9)
Vegetables	103.7 (79.2, 142.5)	104.2 (79.7, 142.7)
Fruits	102.5 (64.4, 145.6)	105.7 (59.2, 146.0)
Trimester 3	**(n=101)**	**(n=247)**
Cereals	266.0 (214.7, 319.0)	261.5 (218.6, 327.5)
Meat	17.7 (3.2, 30.1)	19.9 (9.4, 29.5)
Milk products	289.9 (152.0, 498.4)	316.0 (192.5, 486.9)
Green leafy vegetables	24.6 (17.7, 37.1)	24.6 (18.4, 37.7)
Vegetables	101.7 (74.4, 138.2)	107.7 (80.6, 141.6)
Fruits	99.3 (58.4, 141.9)	112.6 (68.8, 146.5)

Median (lower, upper quartile)

*Kruskal Wallis test of significance, $p < 0.05$

Table 20b. Dietary food group intakes during pregnancy among mothers of LBW and normal weigh babies

Food groups ($g.d^{-1}$)	**Mothers of LBW babies**	**Mothers of Normal weight babies**
Trimester 1	**(n=121)**	**(n=472)**
Cereals	248.4 (200.5, 299.4)	237.8 (196.5, 305.1)
Meat	15.7 (3.9, 26.4)	17.9 (6.9, 27.2)
Milk products	202.8 (59.6, 356.9)	217.1 (69.3, 382.6)
Green leafy vegetables	19.3 (11.7, 28.1)	19.3 (12.3, 30.7)
Vegetables	93.9 (73.4, 125.0)	97.7 (73.9, 127.1)
Fruits	93.7 (49.8, 143.7)	86.5 (49.9, 128.2)
Trimester 2	**(n=81)**	**(n=312)**
Cereals	275.4 (224.8, 319.7)	261.7 (215.5, 322.0)
Meat	18.6 (0.00, 29.6)	20.6 (8.7, 33.4)
Milk products	300.0 (169.2, 480.8)	284.9 (147.9, 478.9)
Green leafy vegetables	26.3 (16.3, 37.8)	21.9 (13.1, 36.9)
Vegetables	115.0 (86.1, 145.1)	101.9 (78.6, 141.9)
Fruits	100.0 (64.6, 148.5)	106.3 (60.0, 145.4)
Trimester 3	**(n=71)**	**(n=277)**
Cereals	261.7 (233.5, 324.2)	262.5 (215.1, 325.9)
Meat	19.9 (3.2, 29.2)	19.8 (8.9, 30.0)
Milk products	323.2 (195.7, 493.8)	293.0 (176.8, 492.1)
Green leafy vegetables	27.7 (18.8, 38.6)	24.6 (16.0, 37.3)
Vegetables	105.4 (81.5, 136.5)	106.2 (78.0, 141.2)
Fruits	104.4 (72.4, 145.8)	111.5 (64.3, 145.9)

Median (lower, upper quartile)

No significant differences observed across the two groups

Table 20c. Dietary food group intakes during pregnancy among mothers of preterm and term babies

Food groups ($g.d^{-1}$)	**Mothers of Preterm babies**	**Mothers of Term babies**
Trimester 1	**(n=60)**	**(n=533)**
Cereals	253.9 (206.7, 331.1)	239.7 (196.0, 301.6)
Meat	15.1 (3.6, 27.1)	17.8 (6.4, 26.7)
Milk products	245.3 (74.1, 402.7)	211.3 (66.7, 378.7)
Green leafy vegetables	21.7 (18.4, 36.9)	19.3 (12.2, 30.7)
Vegetables	97.3 (77.2, 104.3)	96.8 (73.7, 125.7)
Fruits	102.8 (50.0, 143.1)	86.1 (49.9, 128.9)
Trimester 2	**(n=43)**	**(n=351)**
Cereals	289.2 (220.0, 339.4)	263.0 (215.8, 319.8)
Meat	21.9 (12.4, 32.0)	19.7 (6.5, 32.8)
Milk products	349.3 (262.9, 502.4)	278.9 (147.7, 473.0)*
Green leafy vegetables	21.9 (13.5, 32.5)	21.9 (13.9, 36.9)
Vegetables	107.6 (89.4, 144.3)	103.9 (78.9, 142.0)
Fruits	90.2 (63.1, 161.3)	107.0 (61.5, 145.4)
Trimester 3	**(n=33)**	**(n=309)**
Cereals	258.5 (235.8, 328.7)	263.3 (215.5, 319.0)
Meat	21.8 (12.5, 29.4)	19.5 (7.1, 29.8)
Milk products	323.2 (186.5, 479.9)	296.6 (178.9, 497.4)
Green leafy vegetables	24.6 (18.8, 37.7)	24.6 (16.0, 37.7)
Vegetables	105.4 (75.6, 146.7)	106.1 (79.9, 140.6)
Fruits	104.4 (69.6, 136.7)	111.0 (64.8, 146.9)

Median (lower, upper quartile)

*Kruskal Wallis test of significance, $p < 0.05$

Repeated measure ANOVA was performed to observe the food group intakes during the 3 trimesters of pregnancy with respect to SGA and AGA outcome. There was no significant interaction effect between the trimesters and SGA for cereals or milk product intakes (p=0.105, p=0.099 respectively). Further it was observed that milk product intake in the 2nd trimester showed small but significant correlation with the gestational weight gain (r=0.116, p=0.044).

The individual baby parameters (birth weight, length of the baby, mid upper arm circumference, head circumference and gestational age at birth) were also correlated with the dietary nutrient and food group intakes. Energy adjusted folate intake in the 1st trimester of pregnancy was positively correlated with baby length (r=0.10, p=0.035) while vitamin B_{12} intakes in the 3rd trimester positively significantly correlated with gestational age at birth (r=0.107, p=0.039). Vitamin B_{12} intakes in the 1st and 2nd trimester of pregnancy correlated positively with birth weight (r=0.124, p=0.002 and r=0.143, p=0.003 respectively). With regards to food group intakes, total green leafy vegetable intakes in the 1st and 2nd trimester of pregnancy showed negative correlation with length of the baby (r=-0.120, p=0.014; r=-0.125, p=0.036 respectively) (*data shown in table 3, Appendix A*).

Mothers of SGA babies had significantly lower intakes of total cereals and milk products in the 2nd trimester of pregnancy as compared to the mothers of AGA babies. The distribution of percent SGA among the tertiles of total milk product and cereal intakes in the 2nd trimester of pregnancy showed that the women who belonged to the lowest tertile of milk product intakes i.e. intakes approx < 105 g/d had higher percent of SGA babies as compared to the women who belonged to the highest tertile constituting around 580 g/d of milk product intakes (p<0.05) (**Figure 9a**). Similar results were observed for the cereal intakes too, indicating higher percent of SGA babies born to women with < 190 g/d as compared to the women with >370 g/d of cereal intakes in the 2nd trimester (**Figure 9b**). Since protein intakes in the 2nd trimester of pregnancy was also lower in the mothers of SGA babies and could be one of the major nutrient contributor in the food group such as cereals and milk, I also assessed the percent SGA distribution among tertiles of protein intakes. Similar results were seen with protein intakes such that women with low protein intakes had higher percent of SGA babies (**Figure 9c**).

The computed variables of total proteins from milk products and cereals in the 2nd trimester were significantly lower in the mothers of SGA babies as compared to the mothers of AGA babies (9.0 ± 6.3 g versus 11.2 ± 7.4 g, p=0.004 and 22.4 ± 6.5 g versus 24.8 ± 8.0 g, p=0.005 respectively). Further the contribution of proteins from milk products and cereals to the total protein intakes in the 2nd trimester showed that SGA mothers had significantly lower milk protein contributing from milk

products as compared to the mothers of AGA babies (9.0 ± 6.3 g versus 11.2 ± 7.4 g, p=0.004) respectively.

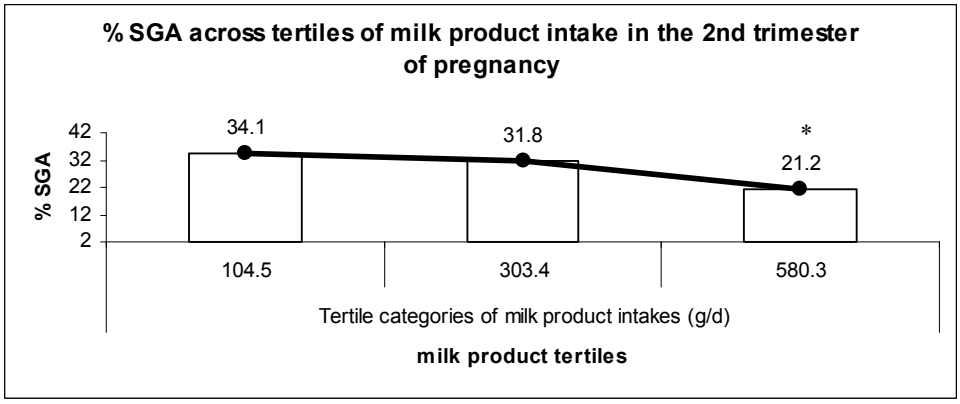


Figure 9a Distribution of percent SGA babies among tertile categories of milk product intakes in the 2nd trimester of pregnancy. (* p<0.05 from Tertile 1)

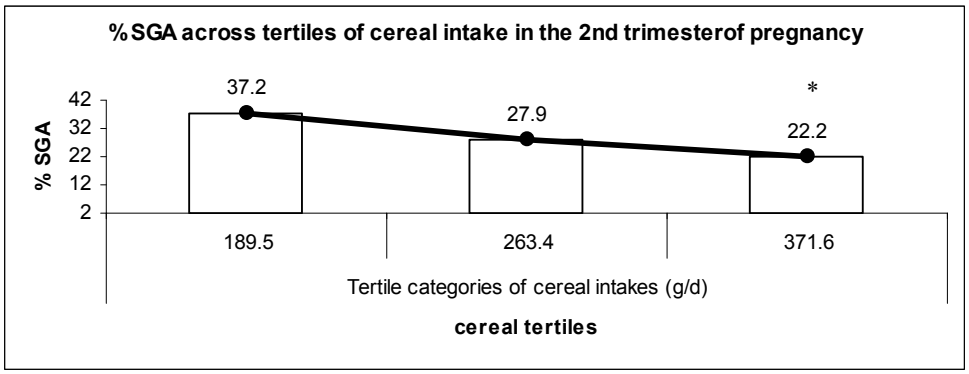


Figure 9b Distribution of percent SGA babies among tertile categories of cereal intakes in the 2nd trimester of pregnancy. (* p<0.05 from Tertile 1)

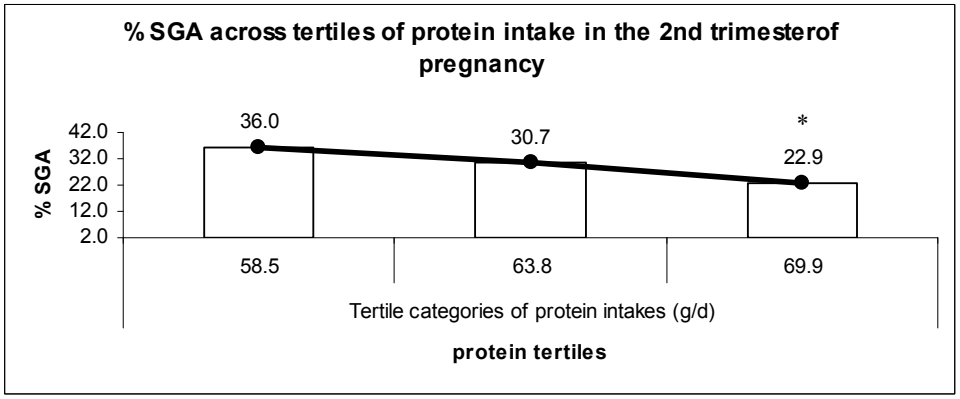


Figure 9c Distribution of percent SGA babies among the tertiles of energy adjusted protein intakes in the 2nd trimester. (* p<0.05 from Tertile 1)

Lower maternal weight, age, parity, carbohydrate, protein, fat, folate, vitamin B_{12} intakes, total cereals and milk product consumption in the second trimester of pregnancy were associated with significantly higher odds of developing a SGA in univariate analyses. Low intakes of cereals and milk products in the second trimester of pregnancy were associated with a significantly higher risk of SGA after multivariate adjustments **(Table 21, 22).** When compared to women who belonged to the highest tertile of cereal intakes in the second trimester of pregnancy, the adjusted ORs were significantly higher among women in the lowest tertile of cereal intake for each maternal demographic parameters (AOR: 2.37; CI: 1.22, 4.60; p=0.011), with additional energy adjustment (AOR: 2.34; CI: 1.19, 4.59; p=0.013), with protein (AOR: 2.46; CI: 1.23, 4.90; p=0.011) and with other significant nutrients adjustment (AOR: 2.64; CI: 1.29, 5.44; p=0.008) respectively **(Table 21).** Similar trend of association between the milk product intakes and SGA was seen in the second trimester of pregnancy **(Table 22)**. This association was marginal after adjusting for the maternal demographic characteristics and energy intake (AOR: 1.82; 95% CI: 0.94, 3.50; p=0.074) while with additional adjustments of protein and other nutrients, the statistical significance disappeared (AOR: 1.68; 95% CI: 0.86, 3.28; p=0.130 and AOR: 1.65; 95% CI: 0.84, 3.25; p=0.145) respectively. I also explored the relationship of milk product intakes in mothers of preterm and term babies. Though milk product intakes were higher in the mothers of preterm babies in the 2nd trimester of pregnancy, further logistic regression adjusting for the similar confounding factors did not show any association with milk products intakes and preterm births.

Table 21. Incidence of SGA and odds ratio by maternal cereal intakes during the 2nd trimester of pregnancy

Cereal intake					
Median (IQR)	Tertile 1 (n=138)	P value	Tertile 2 (n=139)	P value	Tertile 3 (n=139)
	196.2 (167.1, 215.1)		263.2 (245.3, 279.4)		354.2 (318.8, 392.2)
No. SGA / total no.	48/129		36/129		30/135
Univariate OR (95%CI)	2.07 (1.21-3.56)	0.008	1.36 (0.78-2.07)	0.287	1.0
Adjusted OR (95%CI)[1]	2.37 (1.22-4.60)	0.011	1.41 (0.72-2.76)	0.313	1.0
Adjusted OR (95%CI)[2]	2.34 (1.19-4.59)	0.013	1.51 (0.76-2.99)	0.237	1.0
Adjusted OR (95%CI)[3]	2.46 (1.23-4.90)	0.011	1.50 (0.75-3.00)	0.249	1.0
Adjusted OR (95%CI)[4]	2.62 (1.28-5.38)	0.009	1.63 (0.80-3.33)	0.182	1.0
Adjusted OR (95% CI)[5]	2.64 (1.29-5.44)	0.008	1.65 (0.80, 3.3)	0.174	1.0

Model 1 [1] Adjusted odds ratio from a logistic regression model controlling for maternal age, education and parity.

Model 2 [2] Adjusted odds ratio from a logistic regression model controlling for variables in model 1 + maternal weight and energy intakes in the 2nd trimester.

Model 3 [3] Adjusted odds ratio from a logistic regression model controlling for variables in model 2 + tertiles of energy adjusted protein in the 2nd trimester.

Model 4 [4] Adjusted odds ratio from a logistic regression model controlling for variables in model 3 + intakes of energy adjusted fat, carbohydrate, folate, vitamin B_{12} and vitamin B_6 in the 2nd trimester.

Model 5 [5] Adjusted odds ratio from a logistic regression model controlling for variables in model 4 + energy adjusted calcium in the 2nd trimester.

Table 22. Incidence of SGA and odds ratio by maternal milk product intakes during the 2nd trimester of pregnancy

Milk Products					
Median (IQR)	Tertile 1 (n=141)	P value	Tertile 2 (n=137)	P value	Tertile 3 (n=138)
	116.7 (52.7, 151.2)		292.2 (260.8, 347.7)		534.7 (474.2, 646.6)
No. SGA / total no.	45/132		41/129		28/132
Univariate OR (95%CI)	1.92 (1.11-3.33)	0.020	1.73 (0.99-3.02)	0.054	1.0
Adjusted OR (95%CI)[1]	1.89 (0.99-3.63)	0.054	1.59 (0.82-3.09)	0.168	1.0
Adjusted OR (95%CI)[2]	1.82 (0.94-3.50)	0.074	1.63 (0.84-3.16)	0.153	1.0
Adjusted OR (95%CI)[3]	1.68 (0.86-3.28)	0.130	1.43 (0.72-2.84)	0.302	1.0
Adjusted OR (95%CI)[4]	1.65 (0.84-3.25)	0.145	1.50 (0.84-3.25)	0.256	1.0

[1] Adjusted odds ratio from a logistic regression model controlling for maternal age, education, parity, weight in the 2nd trimester.

[2] Adjusted odds ratio from a logistic regression model controlling for maternal age, education, parity, weight and energy intakes in the 2nd trimester.

[3] Adjusted odds ratio from a logistic regression model controlling for maternal age, education, parity, weight and tertiles of protein in the 2nd trimester.

[4] Adjusted odds ratio from a logistic regression model controlling for maternal age, education, parity, weight, fat, carbohydrates, folate and vitamin B_{12} intakes and intakes of tertiles of proteins in the 2nd trimester.

5.5 DISCUSSION

Inadequate nutrition during pregnancy has serious consequences for pregnant women in terms of morbidity, mortality and with birth outcome. Further, infants born SGA at term have increased susceptibility to chronic disease in adulthood (Moore et al., 2004). While alterations to maternal and fetal well being have been documented for macronutrients (both energy and protein), there is increasing awareness that micronutrient deficiencies also have important implications for fetal growth and outcome (de Onis et al., 1998).

In this study I found that around 20% of the babies born were LBW and ~27% were born SGA, though the overall mean birth weight was ~ 2.8 kg. My findings are similar to the studies conducted in other parts of India those showing high prevalence of LBW (30%) in spite of mean birth weight being 2.7 kg (Gopalan 1994; Kulkarni et al., 2006; UNICEF 2004; Yajnik, 2001). With regards to the maternal characteristics, I observed that 45% of the women had education of university or more. When level of education was compared between the two groups of mothers of AGA and SGA, it was seen that almost 50% of mothers of AGA babies were educated upto university degree and only 30% had the least education. On the other hand, only 35% had the highest education of university degree and almost 44% mothers of SGA babies had the least education (upto high school). Further it was seen that women with education greater than university had significantly lower percent of SGA babies born as compared to the women with lower education (22.3 versus 34.0 SGA babies, p=0.001). The overall weight gain during pregnancy was around 11 kg.

The dietary intakes and food group intakes increased significantly from 1st to 2nd trimester and from 1st to 3rd trimester, but there was no significant change between 2nd to 3rd trimester of pregnancy for most of the nutrients and food groups. The intake of vitamin B_{12} in the 1st and 2nd trimester of pregnancy correlated positively with birth weight. Our previous findings from the observational studies have shown that maternal low vitamin B_{12} status is a strong determinant for SGA babies (Muthayya et al., 2006). In the present study I have explored the relationship between the dietary B vitamin (vitamin B_{12}, vitamin B_6 and folate) rich food group intakes with that of SGA versus AGA, LBW versus normal weight babies and

preterm versus term babies. I observed that vitamin B_{12} intake in the 1st and 2nd trimester of pregnancy was significantly lower in the mothers of SGA and LBW babies and is in concurrence with our previous findings of vitamin B_{12}; the dietary vitamin B_{12} translating to maternal serum vitamin B_{12} (Muthayya et al., 2006). Further vitamin B_{12} intakes in the 1st and 2nd trimester significantly correlated with the vitamin B_{12} status and negatively correlated with Hcy concentration (*the micronutrient status is discussed in the next chapter*). Folate intake was seen to be significantly lower in mothers of SGA and LBW babies in the 2nd trimester. The pregnant women in this population were prescribed routinely with folic acid and iron during pregnancy. Studies have shown inverse relation of vitamin B_{12} status and Hcy (an intermediary metabolite of methylation cycle) concentration (Yajnik et al., 2005), similarly with folate status and Hcy concentration (Takimoto et al., 2007). Further elevated Hcy during pregnancy is also known to cause adverse birth outcomes (Vollset et al., 2000). These findings suggest that the deficiency of these micronutrients involved in 1-C (methyl) metabolism could lead to adverse birth outcomes, may be through elevated Hcy levels.

Food groups rich in micronutrients have been the focus. Evidences show varying relationship between nutrient and food group intakes at different trimesters and birth outcomes. The frequency of consumption of milk and green leafy vegetables was positively associated with birth parameters of the baby (Rao et al., 2001) while Kanade et al (2008) have shown a positive relationship with vegetables intakes at week 28 and length of the baby ($p<0.01$) in the rural pregnant women and fruit intakes at weeks 18 associated with birth weight ($p<0.05$) in the urban women. At the same time there are contradictory findings also such that Ramon et al (2009) have shown a positive effect of vegetable intake but not with fruits on fetal growth such that women in the lowest quintile of vegetable intake during the first trimester had higher odds of having a SGA (weight) baby than women in the highest quintile [odds ratio (OR), 3.7; 95% CI: 1.5–8.9; P-trend < 0.001] and had a higher odds of having an SGA (length) baby in the third trimester (OR, 5.5; 95% CI: 1.7–17.7; P-trend = 0.04) in multivariate analysis. In our data we found significant differences with regards to intakes of cereals and milk products among mothers of SGA and AGA babies. A study among African-American pregnant adolescents showed a positive effect on fetal femur growth with the dairy product intakes. The findings

were attributed to the calcium content of dairy foods (Chang et al., 2003). Mannion et al (2006) have shown that each additional cup of milk daily was associated with a 41 g increase in birth weight; however this was attributed to the beneficial effect of vitamin D rather than protein, riboflavin or calcium. Similarly a study conducted in a Swedish population also showed that low milk intake during pregnancy was associated with an increased risk of IUGR after adjusting for the confounders (Ludvigsson & Ludvigsson 2004). While studies have compared birth outcomes among women those who consumed almost 6 glasses of milk per day against 0 glasses or no milk showed that milk intake was associated with higher birth weight for gestational age, lower risk of SGA and higher risk of LGA (large for gestational age) baby, also concluding that the birthweight was related to intake of protein, but not of fat, derived from milk (Olsen et al., 2007). Our data also supports a similar finding such that the women of SGA babies had significantly lower milk protein contributing to the total protein in the diet when compared to the mothers of AGA babies implying that milk protein would be the major contributor to the overall effect seen. Moreover, the univariate logistic regression showed that women with low protein intakes in the 2^{nd} trimester had higher odds of delivering a SGA baby and this finding is also reflected in the figure 2c. Therefore the role of various nutrients such as vitamin D, protein, calcium or combination of nutrients may be important in these birth outcomes.

In the present study it was observed that mothers of preterm babies had higher milk product intakes in the 2^{nd} trimester. The other nutrients (major contributors of milk products; such as proteins, vitamin B_{12}, calcium) were not significantly higher in the mothers of preterm babies. Therefore the high milk product intake in the mothers of preterm babies could be due to the few babies who were AGA. Alternative explanation is that the milk product results in AGA babies who deliver early. It is not clear on the mechanism of action but milk product nutrient (calcium?) may be responsible. Evidence show that ionised calcium (Ca^{2+}) with oxytocin may facilitate early delivery (Arthur et al., 2007). Further analysis on this group of women who delivered preterm babies did not show any significant findings. Number of studies has shown beneficial effects of probiotic food intakes during pregnancy and risk of preeclampsia and preterm births (Brantsaeter et al., 2011; Myhre et al., 2011). While Myhre et al (2011) have shown a beneficial effect

of probiotic dairy products during pregnancy and reduced risk of spontaneous delivery, it is essential to know that the FFQ used to obtain the dietary information was administered retrospectively only once during pregnancy. Moreover the dietary pattern during each trimester changes. The intakes of these probiotics were categorised into none, low and high consumption for the analysis. On the other hand, in our study we have considered the milk product intake as a continuous variable and hence have a range of intakes. The strength of our study is also that, the study participants were followed from 1st trimester until delivery and the dietary information was obtained at each trimester of pregnancy.

A number of studies have also shown correlations between meat/fish intake and birth weight (Godfrey et al., 1996; Mitchell et al., 2004, Olsen et al., 1990 & 2002). Even among the pregnant women those who consume animal protein, the mean intake of poultry and meat were low (~ 7 g/d and ~ 18.0 g/d respectively in comparison to the mean cereal and milk product intakes, ~ 254 g/d and ~ 217 g/d). The typical dietary profile of Indian population is cereal based with low intake of fish and or meat. The cereal consumed in this group contained predominantly rice, ragi (Indian millet; *Eleusina coracana*, and commonly called as finger millet) and to a lesser extent wheat, bajra (*Pennisetum glaucum*, and commonly called as pearl millet) and jowar (Sudan grass; *sorghum bicolor*). It is important to note the method of cooking since ragi has low glycemic index than rice. The mixture of millet (ragi) is cooked in water to make a homogenous paste and then rolled into a ball. Studies have shown that infants born to mothers who consumed low-glycemic index carbohydrate foods during pregnancy were of normal size but were smaller and had less body fat than did the women whose dietary glycemic index did not change during pregnancy (Moses et al., 2006). Since birth weight predicts long-term risk of obesity and chronic disease (Krassas & Tzotzas 2004), a low-glycemic index diet in pregnancy may favourably influence long-term outcomes. Similarly, a study by Scholl et al (2001) also showed that, low dietary glycemic index was associated with reduced infant birth weight and an increased risk of fetal growth restriction (Scholl et al., 2001). This could also be one of the reasons for having high prevalence of SGA in our population. While I have explored the other nutrients in the cereals in a logistic regression model that could contribute to SGA, further analysis with respect to fibre is warranted. The habitual Indian diet is based predominantly on unrefined

cereals and plant foods which is rich in fiber and depending on the different socioeconomic groups and the type of cereal, the fiber consumed varies from 60 to 70 g/day. Dietary fiber intakes in wheat- or millet-based diets are generally higher than in a rice-based diet (Rao 2003). Therefore, it is equally important to also understand the different ways the food items are cooked and thereby their bio-availability.

Women who delivered SGA babies were found to consume low intakes of milk products throughout their pregnancy although this reached the level of statistical significance only at the 2nd trimester of pregnancy which is in agreement with few studies that have also seen effects of food groups during mid pregnancy. There are also several studies pointing towards a correlation between balanced energy/protein supplementation in pregnancy and infant growth (de Onis et al., 1998; Kramer 2000). Evidence also supports correlation between milk intake and SGA. Since milk and cereals are the source of energy and protein especially in vegetarian diet, it is possible that protein might have contributed to the overall effect in the current study. It was observed that women belonging to the lowest tertile of protein intakes in the 2nd trimester had significantly higher odds 1.90 (CI: 1.12, 3.21) of having a SGA baby, $p<0.05$ *(Data shown in table 4, Appendix A).* Further it was seen that the women who consumed normal protein in the 2nd trimester and later low protein intakes in the 3rd trimester also had higher odds of having SGA babies though was not statistically significant. Further women with low protein in the 2nd and 3rd trimesters had ~32% SGA babies *(data shown in table 5, Appendix A).* These findings suggest that protein intakes in the 2nd trimester may be playing a key role in determining birth outcomes either through cereal or milk product intakes.

I also explored the relationship between protein requirements during pregnancy and the birth outcomes and assessed whether these women met the protein requirements at each trimester. Though the pregnant women started their pregnancy with adequate protein intakes, they did not meet the requirements in the 2nd and 3rd trimester of pregnancy. Association between these parameters and birth outcomes were not statistically significant. Maternal weight has been a strong determinant for adverse birth outcomes. I also found that milk product intake was significantly correlated with gestational weight gain which was further positively correlated with

birth weight. While gestational weight gain is also a strong determinant of birth weight, the association of milk product with birth weight could be mediated through gestational weight gain during pregnancy and hence milk product intakes may be beneficial to the pregnant women and the baby. Evidences also suggest that modern cow milk, because of the way it is produced, may have a high content of estrogen and probably other sex steroids (Ganmaa et al., 2001). Estrogen is known to have stimulating effect on fetal growth (Mucci et al., 2003). Another potential candidate is the peptide hormone, IGF-I, that may be the underlying factor accounting for the association between milk and birth weight (Olsen et al., 2007).

The main contribution of the two food groups; cereals and or milk products to the nutrient intakes are protein, fat, folate, vitamin B_{12}, vitamin B_6, calcium and vitamin D. From my findings, it is clear that none of these individual nutrients demonstrated a clear difference among mother of SGA and AGA babies consistent in all trimesters. This lead me to hypothesize that the contribution of food groups to maternal nutrition and birth outcome goes beyond the sum of its individual nutrients. This may be similar to the observations of Mannion et al (2006) where the individual milk nutrients did not have any association with birth weight. Another study conducted in rural and urban pregnant women showed a positive effect of milk intake and birth size but not with any of the macronutrients (Rao et al., 2001). While milk is thought to be available in abundance, contains about 335 kcal energy and 16 gm protein in 500 ml and is easier to deliver during pregnancy to meet the requirements of proteins (Kurpad & Soares 2010). Hence, it can be suggestive that those complete foods, a mixture of macronutrients and micronutrients (such as cereals and milk) have a beneficial effect on the birth outcome compared to individual nutrients. Similarly in population consuming cereals as the staple diet, the quality and quantity may also enhance the effect on the birth outcomes.

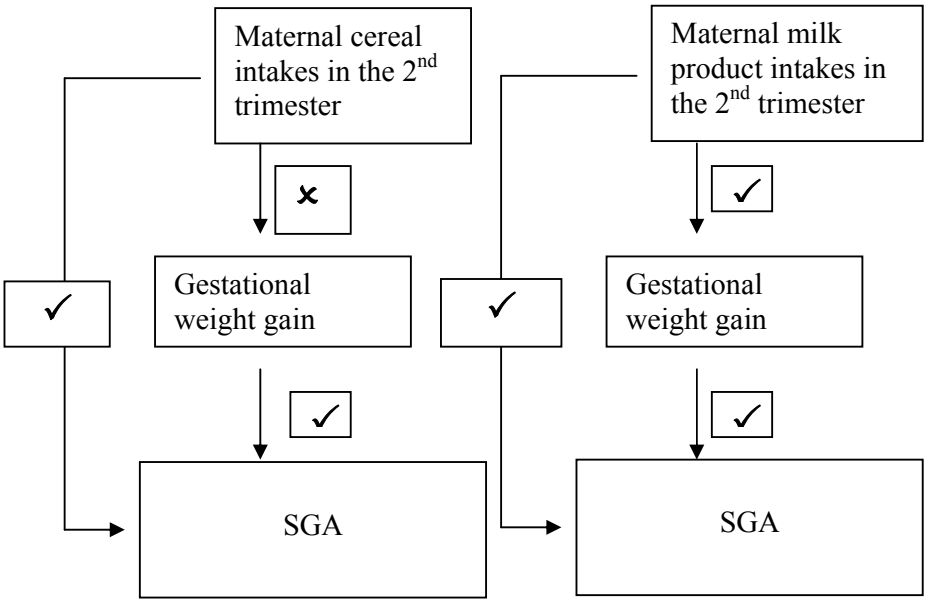


Figure 10. Highlight of the objective 2.

5.6 Conclusion

My findings of association between cereal and milk product intakes in the second trimester of pregnancy and SGA may also reflect the nutrient-nutrient or nutrient-food group interaction. In a community it is important to focus on the food-base approach, rather than the nutrient-base, since it is easier to characterize the dietary recommendations and provide dietary guidance in ways that are understandable to the pregnant women.

CHAPTER 6

RELATIONSHIP OF MATERNAL BIOMARKERS OF B VITAMINS (VITAMIN B_{12}, VITAMIN B_6, FOLATE) ON HOMOCYSTEINE AND ITS EFFECTS ON BIRTH WEIGHT AND BIRTH OUTCOMES (SMALL FOR GESTATIONAL AGE BABIES AND PRETERM BIRTHS)

6.1 INTRODUCTION

Vitamin B_{12}, vitamin B_6 and folate play an important role in determining birth outcomes. The importance of these micronutrients has been described in the previous chapter. 1-C methyl metabolism plays a key role in fetal programming (Yajnik 2008). Babies born small in size in developing countries and in India is usually ascribed to the small size and 'chronic undernutrition' of Indian mothers (Gopalan 1994) though there is no information on specific nutrients that may be responsible. Although energy and protein intakes are believed to be the major macronutrients that are associated with birth size, worldwide studies of supplementation of these nutrients during pregnancy have produced variable and sometimes conflicting results (Kramer 2000). Reported studies on pregnant women in India (Pathak & Kapil 2004, Gomber et al., 2002) have examined micronutrients supplementation with folate, iron and zinc in pregnancy and have shown beneficial impact on fetal growth. However, there are limited data from controlled trials examining effects of other micronutrients on fetal growth.

Recently, interest has turned to specific micronutrients as possible limiting factors for fetal growth. A study conducted in rural West India showed a strong relationship between the maternal intake of green leafy vegetables and fruits in the second trimester of pregnancy and birth weight (Rao et al., 2001) attributing this relationship to folate and vitamin C micronutrients that are rich in green leafy vegetables and fruits. Similarly, the effect of micronutrients in significantly decreasing the risk of LBW has also been demonstrated in randomized trials of multiple micronutrient supplementation of HIV-infected pregnant women in Tanzania (Fawzi et al., 1998) and in pregnant mothers in Nepal (Osrin et al., 2005).

Evidences have shown that Indians have high plasma total Hcy concentrations due to low vitamin B_{12} status (Refsum et al., 2001). Moreover in a

study conducted on rural and urban pregnant women have shown almost 70% women to be vitamin B_{12} deficit while folate deficiency being relatively rare (Yajnik et al., 2005). There are several reports on vitamin B_{12} deficiency in children of mothers who are strict vegetarians (Jadhav et al., 1962; Higginbottom et al., 1978; Yajnik & Deskmukh 2008). The propensity of infants who are born to mothers with low vitamin B_{12} intake to become deficient (Jadhav et al., 1962; Baker et al., 1962; Muthayya et al., 2006) suggests that vitamin B_{12} status during infancy is critically dependent on fetal vitamin B_{12} accumulation and, thereby, maternal vitamin B_{12} status in pregnancy.

Studies have also shown low vitamin B_{12} deficiency reflected to elevated Hcy concentration further contributing to small size babies (Yajnik et al., 2005) while Ronnenberg and his colleagues showed that elevated Hcy level was related to preterm births (Ronnenberg et al., 2002). It is postulated that Hcy concentrations are tightly regulated by 2 main enzymatic pathways *(refer fig 4)*. Hcy can be remethylated to methionine by a pathway requiring folic acid as a methyl donor. In addition to adequate folic acid, the pathway requires vitamin B_{12} as an important cofactor. Alternatively, Hcy can be removed by transsulfuration, a pathway dependant on the cofactor vitamin B_6. Enzymatic defects in either of these pathway results in increased Hcy, as does deficiency of vitamin B_{12}, folic acid, or vitamin B_6.

Studies describe the relationship between the B vitamins and Hcy status to be negatively correlated implying the deficiency of vitamin B_{12} and folate leading to hyperhomocysteinemia during pregnancy (Takimoto et al., 2007; Walker et al., 1999) as well as in the new born babies (Guerra-Shinohara et al., 2004). In regards to the relationship of B vitamins and Hcy status, evidences further have indicated a negative relationship with circulating maternal Hcy concentration as a predictor of low birth weight (Takimoto *et al*., 2007; Yajnik et al., 2005; Murphy et al., 2004). Elevated Hcy is known to cause preterm deliveries (Ellison et al., 2004). Ronnenberg in one of his studies has shown that though folate status was not associated with preterm birth, and Hcy and B vitamin status were not associated with LBW or SGA status, postulated that elevated Hcy and suboptimal vitamin B_{12} and B_6 status may increase the risk of preterm birth (Ronnenberg et al., 2002).

The current chapter aimed to investigate the interrelationship of select micronutrients namely B vitamins (vitamin B_{12}, vitamin B_6, folate) and Hcy in 1st and 2nd trimester of pregnancy and with neonatal micronutrient status. I hypothesized that vitamin B_{12}, folate and vitamin B_6 deficiencies may cause elevated Hcy leading to adverse birth outcomes such as LBW, SGA or preterm births.

6.2 METHODS

The selection of study subjects and baseline information, their dietary intakes, anthropometric information and delivery details are mentioned in the previous chapter. The subjects received routine antenatal care during pregnancy. The biochemical analysis included analysis of plasma vitamin B_{12}, vitamin B_6 and red cell folate in the 1st and 2nd trimester of pregnancy and in cord blood. The deficiencies of each B vitamin micronutrient status were defined (Refsum et al., 2004). The cut off for Vitamin B_{12} deficiency was below 150 pmol/L, for vitamin B_6 <20 nmol/L, <283 nmol/L for folate deficiency and ≥10 µM/L for high Hcy levels during pregnancy (Refsum et al., 2004). Another cut off (≥12.4 µM/L) to define hyperhomocysteinemia was also used (Ronnenberg et al., 2002) in the analysis.

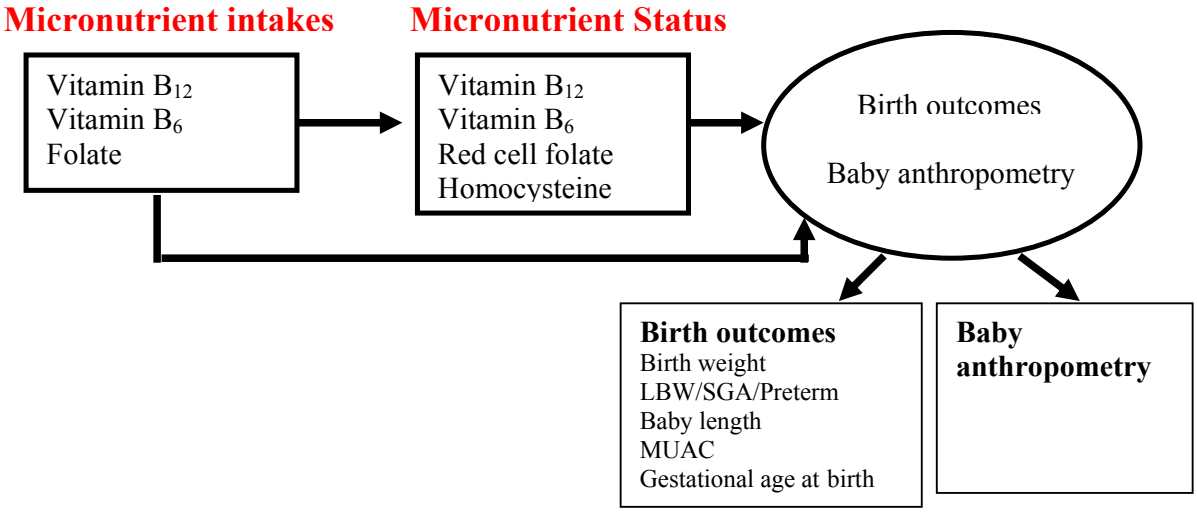


Figure 11. The conceptual framework underpinning objective 3

6.3 STATISTICAL ANALYSIS

Results are represented as median with lower and upper quartile since the micronutrient status was non-normal continuous variables. All analyses were done

using the SPSS program (version 16.0, SPSS, Chicago, IL, USA). Categorical data was represented as number and percentages.

Wilcoxon signed Rank test was used to compare the antenatal micronutrients across the trimester and the neonatal status. Spearman Rank Correlations were used to assess the association between the micronutrient B status and homocysteine within each trimester and in the cord blood. The correlation coefficient between maternal B vitamins and cord blood was also examined. Further the B vitamins were correlated with the baby anthropometric parameters at birth such as birth weight, baby length, mid upper arm circumference (MUAC), skinfold thickness, arm muscle area (AMA), arm fat area (AFA) and the gestational age at birth. The maternal and the neonatal micronutrient status was compared between the outcome groups (LBW, SGA and preterm births) using Mann Whitney U test. For the Hcy status in the 1^{st} and 2^{nd} trimester, a binomial category of normal and hyperhomocysteinemia was made using the reference cut offs and the baby parameters were compared across the two categories. The proportions of adverse birth outcomes in these Hcy categories and in categories of vitamin $B_{12,}$ Vitamin B_6 and folate deficiency were also explored using Chi square test of significance. Further using cut off of 12.4 μM/L for maternal Hcy status in the 1^{st} and 2^{nd} trimester of pregnancy, the relationship between adverse birth outcomes and Hcy categories were explored. Multivariate logistic regression model of LBW with this Hcy category adjusting for maternal demographic characteristics such as age, parity, education, maternal weight and any micronutrient status known to be determinant of the outcome was performed. The odds ratio (OR) 95% confidence intervals (CIs) were reported. Two-sided P-values < 0.05 were considered statistically significant.

6.4 RESULTS

The antenatal and the neonatal (cord blood) micronutrient status is shown in **table 23**. Plasma vitamin B_{12}, vitamin B_6 and Hcy levels decreased significantly from 1^{st} trimester to 2^{nd} trimester ($p<0.001$) as the pregnancy progressed, while the folate status increased from the 1^{st} to 2^{nd} trimester of pregnancy. Neonatal status of vitamin B_{12}, vitamin B_6 and Hcy levels were higher as compared to the maternal status at both trimesters of pregnancy ($p<0.05$).

Table 23. Maternal and neonatal micronutrient status

Micronutrient Status	Trimester 1	Trimester 2	Neonatal
Vitamin B_{12} ($pmol.L^{-1}$)	157.2 (109.1, 222.5)[1]	131.0 (100.4, 180.1)[2]	195.8 (129.9, 293.4)[3]
Vitamin B_6 ($nmol.L^{-1}$)	27.8 (17.7, 41.9)[1]	22.9 (15.0, 36.1)[2]	41.9 (31.0, 58.6)[3]
Folate ($nmol.L^{-1}$)	525.5 (375.3, 634.7)[1]	619.7 (441.3, 801.3)[2]	368.3 (242.5, 578.4)[3]
Homocysteine ($\mu M.L^{-1}$)	7.8 (6.1, 10.2)[1]	6.4 (4.9, 8.6)[2]	13.7 (9.8, 20.0)[3]

Values represent median with lower and upper quartile range. [1,2,3] Values that do not share the same superscript are significantly different from each other, $p < 0.05$

[1] Significant difference between the 1st and the 2nd trimester

[2] Significant differences between 2nd trimester and neonatal status

[3] Significant differences between 1st trimester and neonatal status

Wilcoxon signed Rank paired t test of significance, $p < 0.05$

In the 1st trimester of pregnancy, approximately half (48.1%) of the study subjects had vitamin B_{12} deficiency and ~ 30% subjects were vitamin B_6 deficit. Folate deficiency was seen in only 12% subjects and approximately 30% had hyperhomocysteinemia. As the pregnancy progressed, the vitamin B_{12} and vitamin B_6 deficiency deepened such that almost 62% and 40% had vitamin B_{12} and vitamin B_6 deficiency. The folate and hyperhomocysteinemia deficiency decreased to ~ 10% and 16% in the 2nd trimester of pregnancy (**Table 24**).

Table 24. Percentage deficiency of B vitamins and hyperhomocysteinemia during pregnancy

Micronutrient status	Deficiency cut off	Trimester 1	Trimester 2
Vitamin B_{12} (pmol/L)	< 150 pmol/L	48.1	61.9
Vitamin B_6 (nmol/L)	< 20 nmol/L	31.1	40.5
Red cell Folate (nmol/L)	< 283 nmol/L	12.4	10.4
Homocysteine (μM/L)	> 10 μM/L	28.6	16.4
	> 12.4 μM/L	19	8

Values represent percentage deficiency for vitamin B_{12}, vitamin B_6 and folate while for homocysteine; values represent hyperhomocysteinemia.

The correlations between the micronutrients status in the respective trimesters were examined. Vitamin B_{12} correlated negatively with Hcy status in both trimesters of pregnancy. This negative significance remained even after adjusting for the other micronutrient status (vitamin B_6 and folate) and the energy adjusted protein intakes in that trimester. Vitamin B_6 negatively correlated with Hcy status in the 1st trimester of pregnancy (**Table 25**). Positive correlation between vitamin B_{12} and vitamin B_6 in the 1st, 2nd trimesters and in cord blood was seen *(Data shown in table 6, Appendix A)*.

Table 25. Correlation between B vitamin status and Hcy concentration in the 1st and 2nd trimester of pregnancy

Maternal B vitamin status	Maternal Hcy status	
	Trimester 1	**Trimester 2**
Vitamin B_{12}		
Unadjusted	-0.331***	-0.196*
Adjusted *(energy adjusted dietary protein, vitamin B_6 and folate status in that trimester)*	-0.228**	-0.270**
Vitamin B_6		
Unadjusted	-0.206*	0.082
Adjusted *(energy adjusted dietary protein, vitamin B_{12} and folate status in that trimester)*	-0.052	0.119
Red Cell Folate		
Unadjusted	-0.121	-0.069
Adjusted *(energy adjusted dietary protein, vitamin B_{12} and vitamin B_6 status in that trimester)*	-0.058	-0.067

Values represent coefficient correlation

Signifiance indicates; *** p< 0.001, ** p<0.005, * p<0.05

No significant correlation observed between folate and Hcy status

Units for micronutrient status: vitamin B_{12} (pmol/L), vitamin B_6 (nmol/L), folate (nmol/L) and Hcy (μM/L)

Maternal micronutrient statuses were also correlated with the neonatal micronutrient status. Maternal vitamin B_{12}, vitamin B_6 and Hcy status in the 1st and 2nd trimester of pregnancy correlated positively with the neonatal vitamin B_{12}, vitamin B_6 and Hcy concentration respectively. The significant negative correlation between maternal Vitamin B_{12} in the 1st trimester and neonatal Hcy disappeared after adjusting for the neonatal vitamin B_{12} status. Maternal folate status in the 1st trimester though did not significantly correlate with neonatal Hcy status, after adjusting for neonatal vitamin B_{12} and vitamin B_6, a trend towards significance was observed. The cord blood micronutrient status between the different birth outcomes

was also explored and showed no significant differences between the micronutrient status cord blood and birth outcomes such as LBW, preterm and SGA babies. Maternal Hcy in the 1st trimester also showed a trend towards significance with neonatal Hcy after adjusting for neonatal vitamin B_{12} and vitamin B_6 status. In the 2nd trimester vitamin B_{12} status remained significantly correlated with neonatal Hcy status even after adjusting for neonatal vitamin B_{12} status. Similar observations were made with regards to maternal Hcy status in the 2nd trimester of pregnancy and neonatal Hcy status (**Table 26**). Further maternal vitamin B_{12} and vitamin B_6 in both the trimester were negatively correlated with neonatal Hcy levels (Data shown in *table 6, Appendix A*).

Baby characteristics are mentioned in the chapter 5. Correlations between antenatal micronutrient status and baby anthropometric parameters were performed. Maternal Hcy status in the 1st and 2nd trimester of pregnancy showed trend of negative correlation with birthweight, mid upper arm circumference, skinfold thicknesses and arm muscle area. Vitamin B_6 in the 1st trimester showed a significant negative correlation with duration of gestation ($p<0.05$), birthweight and baby length in the 2nd trimester of pregnancy (Data shown in *table 7, Appendix A*). Similarly the micronutrient statuses during pregnancy were compared across the birth outcome groups such as LBW, SGA and preterm births. Vitamin B_{12} showed a decreased trend in all adverse birth outcomes; SGA, LBW and preterm births at both trimesters (median values; 148.8 versus 157.3; 136.4 versus 160.3 and 148.5 versus 157.6 pmol/L in the 1st trimester while 117.9 versus 133.4; 121.7 versus 137.4 and 126.6 versus 133.5 pmol/L in the 2nd trimester of pregnancy respectively). Hcy status on the other hand showed higher levels in all adverse birth outcomes of SGA, LBW and preterm births at both trimester (median values; 7.9 versus 7.8; 8.2 versus 7.7 and 8.2 versus 7.8 μM/L in the 1st trimester while 7.0 versus 6.4, 6.7 versus 6.4 and 6.7 versus 6.4 in the 2nd trimester of pregnancy respectively). Vitamin B_6 and folate did not show a consistent trend during pregnancy across the adverse birth outcomes (Data shown in *table 8, Appendix A)*.

Table 26. Contribution of maternal micronutrient status in the 1st and 2nd trimester of pregnancy to neonatal micronutrient status and Hcy status

Maternal	**Neonatal micronutrient status →**			
Micronutrient status ↓	**Vitamin B_{12}**	**Vitamin B_6**	**Folate**	**Hcy**
Trimester 1				
Vitamin B_{12} (pmol/L)				
Unadjusted	0.651***			-0.163
Adjusted				-0.134
Vitamin B_6 (nmol/L)				
Unadjusted		0.373***		-0.035
Adjusted				0.093
Folate (nmol/L)				
Unadjusted			0.117	0.085
Adjusted				0.159
Hcy (μM/L)				
Unadjusted				0.201**
Adjusted				0.169§
Trimester 2				
Vitamin B_{12} (pmol/L)				
Unadjusted	0.646***			-0.271**
Adjusted				-0.196*
Vitamin B_6 (nmol/L)				
Unadjusted		0.299***		-0.114
Adjusted				-0.014
Folate (nmol/L)				
Unadjusted			0.039	-0.006
Adjusted				-0.022
Hcy (μM/L)				
Unadjusted				0.198†
Adjusted				0.199‡

Values represent- correlation coefficient

Values *** $p<0.001$, ** $p<0.005$

§ $p=0.064$ after adjusting for neonatal vitamin B_{12}, vitamin B_6 and folate status

* $p=0.035$ after adjusting for neonatal vitamin B_{12} status

‡ $p=0.036$ after adjusting for neonatal vitamin B_{12}, vitamin B_6 and folate status

The distribution of adverse birth outcomes across the categories of deficiency and sufficiency of each micronutrient in both trimester based on their cut offs was examined. It was seen that the percentage of LBW, SGA and preterm births were higher in the vitamin B_{12} deplete group at both trimesters of pregnancy (35.6% versus 23.8%; 35.6% versus 32.5% and 16.4% versus 12.5% for LBW, SGA and preterm births in the 1st trimester while 31.3% versus 15.7%; 33.7% versus 29.4%

and 15.7% versus 5.9% in the 2nd trimester respectively). The hyperhomocysteinemia group in the 1st trimester showed greater percent of LBW, SGA and preterm births (39.5% versus 25.5%; 41.9% versus 30.9% and 16.3% versus 13.6% respectively) (**Table 27**).

A trend of high proportion of LBW in the hyperhomocysteinemia group was seen using both the cut offs (>10.0 and >12.4 μM/L) for defining hyperhomocysteinemia. I explored the relationship of LBW with elevated Hcy cut off of >12.4 μM/L, since 50% of the LBW babies were born to mothers with hyperhomocysteinemia using this cut off while 39.5% of LBW babies were born to mothers with hyperhomocysteinemia using cut off of >10.0 μM/L. In the univariate analysis, women grouped as hyperhomocysteinemia (Hcy status > 12.4 μM/L) in the 1st trimester of pregnancy had higher odds of delivering a LBW baby. Further even after adjusting for the potential confounders in the multivariate analysis, hyperhomocysteinemia remained a significant determinant for LBW. When compared to women with normal Hcy in the 1st trimester, the adjusted ORs were significantly higher among women in the hyperhomocysteinemia for each maternal demographic characteristics (AOR: 2.78; CI: 0.98, 7.92; p=0.055), with additional maternal weight adjustment (AOR: 3.19; CI: 1.08, 9.39; p=0.035), with gestational age at birth (AOR: 4.90; CI: 1.17, 20.64; p=0.030) and with vitamin B_{12} status adjustment (AOR: 4.89; CI: 1.12, 21.4; p=0.035) respectively **(Table 28).** Similar trend of higher odds of LBW in hyperhomocysteinemia mothers with cut off >10 μM/L in the univariate analysis (AOR: 1.92; CI: 0.91, 4.04; p=0.088) was observed though lacked statistical significance.

Table 27. Birth outcome categories across the categories of maternal micronutrient status at the 1st and 2nd trimester of pregnancy.

	Vitamin B_{12}		Folate		Vitamin B_6				Hcy	
	<150 pmol/L	**>150 pmol/L**	**<283 nmol/L**	**>283 nmol/L**	**<30 nmol/L**	**>30 nmol/L**	**>10 µM/L**	**<10 µM/L**	**>12.4 µM/L**	**< 12.4 µM/L**
Trimester 1										
LBW	35.6 (n=26)	23.8* (n=19)	26.8 (n=5)	25.6 (n=40)	27.7 (n=13)	31.1 (n=32)	39.5 (n=17)	25.5† (n=28)	50.0¥ (n=9)	26.7 (n=36)
SGA	35.6 (n=26)	32.5 (n=26)	36.8 (n=7)	33.6 (n=45)	31.9 (n=15)	35.9 (n=37)	41.9 (n=18)	30.9 (n=34)	44.0 (n=8)	32.6 (n=44)
Preterm	16.4 (n=12)	12.5 (n=10)	10.5 (n=2)	14.9 (n=20)	12.8 (n=6)	15.5 (n=16)	16.3 (n=7)	13.6 (n=15)	22.2 (n=4)	13.3 (n=18)
Trimester 2										
LBW	31.3 (n=26)	15.7§ (n=8)	28.6 (n=4)	25.6 (n=31)	18.9 (n=10)	30.8 (n=24)	22.7 (n=5)	25.9 (n=29)	37.5 (n=3)	24.6 (n=31)
SGA	33.7 (n=28)	29.4 (n=15)	28.6 (n=4)	33.1 (n=40)	24.5 (n=13)	38.5 (n=30)	31.8 (n=7)	32.1 (n=36)	62.5 (n=5)	30.2 (n=38)
Preterm	15.7 (n=13)	5.9‡ (n=3)	14.3 (n=2)	11.6 (n=14)	11.3 (n=6)	12.8 (n=10)	9.1 (n=2)	12.5 (n=14)	12.5 (n=1)	11.9 (n=15)

Values represent percentage distribution of adverse birth outcome in the category of deficiency and sufficiency of micronutrient statuses.

Chi Square test of Significances are reported as;

* p=0.076,

† p=0.066,

§ p=0.033

‡ p=0.074

¥ p=0.042

Table 28. Incidence of LBW and odds ratio by maternal Hcy cut offs in the 1st trimester of pregnancy

	Category 1 (>12.4) (n=19)	**P value**	**Category 2 (<12.4) (n=135)**
Median (IQR)	17.4 (13.1, 20.3)		7.6 (5.8, 8.8)
No. LBW / total no*	9/18		36/135
Univariate OR (95%CI)	2.75 (1.01 - 7.47)	0.047	1.00
Adjusted OR (95%CI)[a]	2.78 (0.98 - 7.92)	0.055	1.00
Adjusted OR (95%CI)[b]	3.19 (1.08 - 9.39)	0.035	1.00
Adjusted OR (95%CI)[c]	4.90 (1.17 - 20.64)	0.030	1.00
Adjusted OR (95%CI)[d]	4.89 (1.12- 21.40)	0.035	1.00

***** Chi square test of significance, $p<0.05$

[a]Adjusted odds ratio from a logistic regression model controlling for maternal age, education, parity.

[b] Adjusted odds ratio from a logistic regression model controlling for maternal age, education, parity, and weight in the 1st trimester.

[c] Adjusted odds ratio from a logistic regression model controlling for maternal age, education, parity, weight in the 1st trimester and gestational age at birth

[d] Adjusted odds ratio from a logistic regression model controlling for maternal age, education, parity, weight, vitamin B_{12} status in the 1st trimester and gestational age at birth.

6.5 DISCUSSION

Vitamins related to Hcy metabolism, such as vitamin B_{12}, folate and vitamin B_6 are essential for adequate fetal growth (Tamura & Picciano 2006, Relton et al., 2005, Schuster et al., 1984). There are reports suggesting that high maternal plasma tHcy is related to fetal growth restriction (Murphy et al., 2004; Vollset et al., 2000; Steegers- Theunissen et al., 2004) but other reports do not (Pagan et al., 2002; Ronnenberg et al., 2002). Yajnik (2005) in a study conducted in rural and urban pregnant women have shown almost 70% women to have vitamin B_{12} deficiency while folate deficiency being relatively rare. Further the important role of these nutrients is attributed in nucleic acid metabolism, cell growth and proliferation and thereby important determinants of fetal growth (Allen 1994).

In the light of high prevalence of vitamin B_{12} deficiency during pregnancy in a population that consumes predominantly cereal based diet (Refsum et al., 2001), it was important to characterise the status of B vitamins (vitamin B_{12}, vitamin B_6 and folate) and the intermediary metabolite of the methylation cycle, Hcy concentration during pregnancy and its relationship with neonatal micronutrient status and birth outcomes. Our findings showed decrease in micronutrient status of plasma vitamin B_{12}, vitamin B_6 and Hcy levels as the pregnancy progressed. This finding is in agreement with many other studies that indicate the reduction in micronutrient concentrations during pregnancy due to the physiological hemodilution, maternal hormonal changes (Andersson et al, 1992; Kang et al, 1986), and an increased remethylation of Hcy due to increased demands for methionine by the growing fetus (Bonnette et al, 1998). Our pregnant women also showed high prevalence of vitamin B_{12} deficiency, almost 50% and later 62% in the 1st and 2nd trimester of pregnancy. The antenatal regime in India recommends supplementation of folic acid (5 mg) in the 1st trimester of pregnancy and later 0.5 mg, folic acid and 60 mg elemental iron from 2nd trimester of pregnancy until delivery and could have hence reflected on the folate status. Low folate deficiency and low prevalence of hyperhomocysteinemia in the 2nd trimester could be due to the prenatal supplementation program (Walker et al., 1999).

The negative significant correlation observed between plasma vitamin B_{12}, vitamin B_6 and folate status with Hcy concentration in the 1st trimester of pregnancy is in agreement with findings from other studies (Yajnik et al., 2005; Bondevik et al., 2001) where plasma vitamin B_{12} and folate status negatively correlated with Hcy levels ($r = \sim -0.5$, $p<0.01$, both). Studies have also shown that vitamin B_{12} deficiency traps folate as tetrahydrofolate (THF) and makes it unavailable for metabolic use which could create a functional folate deficiency despite normal circulating levels and may have a direct effect on fetal growth (Shane & Stokstad 1985). The negative significant correlation between maternal vitamin B_{12} status and Hcy at both trimester after adjusting for protein intakes, vitamin B_6 and folate status indicate that the vitamin B_{12} is directly related to Hcy status. Further though vitamin B_6 was negatively correlated with Hcy status in the 1st trimester, after adjusting for other micronutrient status lost its significance indicating that the micronutrients are interrelated.

The maternal vitamin B_{12}, vitamin B_6 and Hcy status correlated positively with neonatal status implying that maternal micronutrients status could translate into neonatal status which is in consistent with our earlier findings of vitamin B_{12} status (Muthayya et al., 2006). While maternal vitamin B_{12} correlated negatively with the neonatal Hcy status, further adjusting for neonatal micronutrients status (vitamin B_6 and folate) indicated a trend towards significance. Maternal Hcy also correlated with the neonatal Hcy. Therefore it could be thought that maternal vitamin B_{12} interacts with its Hcy status, further together affecting the Hcy status of the neonate.

The mean birthweight was around 2.8 kg and babies were born at term i.e. 37 weeks gestation. The prevalence of 30% babies born SGA could also be due to many of them being term SGA. Almost 19% babies were born LBW and 10% as preterm births defined as per the standard World Health Organization classification (WHO 1995). Our findings suggest high prevalence of adverse birth outcomes (SGA, LBW and preterm births) in women with vitamin B_{12} deficiency and in women with hyperhomocysteinemia is similar to the findings by Vollset et al (2000). This group has found high prevalence of preterm births in mothers who had elevated Hcy concentration. We did not find any uniform results with regards to folate or vitamin B_6 status during pregnancy. There is paucity of data pertaining to antenatal vitamin B_6 status and birth outcomes or baby birth parameters, rather in one of the studies the group supplemented with vitamin B_6 had lower birth weight as compared to the birthweight of the babies born to the mothers supplemented with placebo (Schuster 1984). In a small trial reported in a Cochrane review showed reduced mean birth weights with vitamin B_6 supplementation (weighted mean difference -0.23 kg; 95% CI -0.42 to -0.04; n=33) but due to small sample size this study was not referred further (Thaver et al., 2006). Evidences have also shown relationship with baby APGAR score at birth such that that maternal serum vitamin B_6 levels were lower in mothers whose infants had Apgar scores of less than 7 at 1 minute after birth than in those whose infants scored 7 or better (Roepke & Kirksey 1979) and anemia during pregnancy (Hisano et al., 2010).

Folate levels in our study also have shown differences in relationship with birth outcomes such as LBW and preterm births. Folate status were significantly higher in mothers of normal weight babies as compared to the mothers of LBW

babies but this trend was not seen when analyzed for the preterm birth as an outcome in the 2nd trimester. These conflicting results are suggestive of micronutrients not related directly to birth outcome but may be through Hcy. Further recent study has shown that in folate-replete population, vitamin B_{12} is a major determinant of high Hcy (MacFarlane et al., 2011). This can be correlated with our study since our study subjects were prescribed with folic acid and iron during pregnancy and a negative significant correlation was observed between vitamin B_{12} status and Hcy concentration. The beneficial affects of folate on birth outcome is thought to be through reduction of Hcy concentration (Sorensen et al., 1999). We have further also explored the relationship of Hcy at different cut off with LBW. Studies in Asian pregnant women have suggested hyperhomocysteinemia cut off to be at Hcy concentration > 12.4 μM/L (Ronnenberg et al., 2002). In addition, Murphy (2004) and his colleagues have shown that pre-pregnancy homocysteine and Hcy concentration in the 1st trimester are better biomarkers for determining birth outcomes. I observed that in the 1st trimester of pregnancy after adjusting for the confounding parameters in the logistic regression model, mothers with hyperhomocysteinemia had higher odds of delivering a LBW.

Since number of maternal factors such as maternal age, parity, education and weight at recruitment are determinants of LBW (ACC/SCN 2000) and maternal vitamin B_{12} status (Muthayya et al., 2006); it is possible that they have a combined effect on the outcome. Studies have also seen physical activity during pregnancy as a confounder for birth outcomes. Manual physical activity during pregnancy is seen to be associated with SGA babies (Launer et al., 1990) and strenuous physical work to be associated with increased rates of abortion and pre-term delivery (Teitelmann et al., 1990). Further studies in non pregnant adults suggest that exercise appears to decrease the vitamin B group status of trained and untrained individuals. For example, riboflavin status deteriorated during a short period of increased physical activity in individuals whose riboflavin was marginal to start with, indicating increased demand for that vitamin for the selective biochemical functions during exercise (Soares et al., 1993). This seems to be true for thiamine and vitamin B_6 as well, though effects on vitamin B_{12} and folate are expected but not yet studied (Manore, 2000). I have observed that women belonging to the highest tertile of physical activity level (PAL) in the 1st trimester had significantly higher odds of

having a baby in the lowest tertile of birth weight ($p<0.05$). This significant association continued even when adjusted for maternal weight and energy intakes indicating that high physical activity (mean PAL>1.66) in the 1st trimester of pregnancy during the organogenesis period was related to higher risk of having a LBW baby (Dwarkanath et al., 2007). However in the current study, the mean PAL of the pregnant women in the 1st trimester of pregnancy was sedentary to moderate (PAL 1.47 ± 0.16). There was no association between maternal PAL during the three trimesters of pregnancy and LBW. Almost 21.6%, 17.1% and 22.3% of LBW babies belonged to the 1st, 2nd and 3rd tertiles of mean PAL in the 1st trimester of pregnancy respectively.

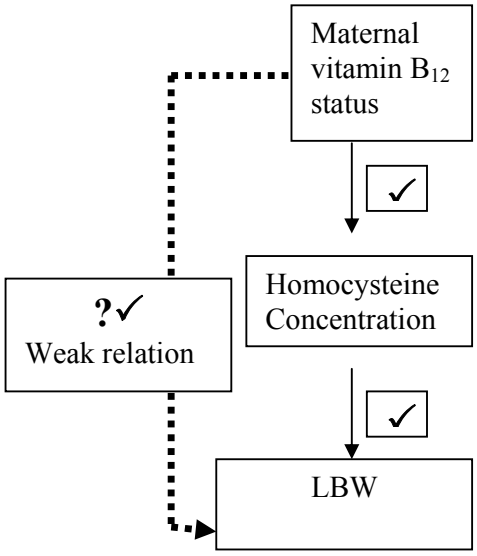


Figure 12. The highlights of the objective 3

In brief, the metabolism of Hcy, a by-product of the essential amino acid methionine, involves either transsulfuration to cysteine or remethylation to methionine (*refer fig 4*). The transsulfuration pathway requires vitamin B_6 and the enzyme cystathionine-β-synthase (CBS). Defect in CBS gene result in the newborn error of metabolism homocysteinuria (Mudd et al., 1985). The major pathway of remethylation requires methylene tetrahydrofolate reductase (MTHFR), methionine synthase, vitamin B_2, vitamin B_{12} and folate (Moghadasian et al., 1997). Mutations in MTHFR genes or dietary deficiencies of vitamin B_6, vitamin B_{12} and folate results in elevation of Hcy concentration and vascular disease (Frosst et al.,1995). Moreover, 677 C-T mutations in the MTHFR gene only causes increased Hcy when folate

concentrations are low (Jacques et al., 1996). Hyperhomocysteinemia can be usually corrected by supplementation with B vitamins including folic acid (Tucker et al., 1996). It is also to be noted that the elevation of Hcy levels during pregnancy could be due to various other factors such as conditions related to vascular disease, PIH and preeclampsia.

6.6 CONCLUSION

My findings suggest that it is important to consider B vitamins together rather than in isolation in understanding their role in determining adverse birth outcomes. I could not explore the relation of subjects with concurrent deficiency of vitamin B_{12}, folate, vitamin B_6 and hyperhomocysteinemia with birth outcomes due to small percentage of subjects. It is important to examine these relationships in larger sample size and to explore the nutrients that are known to be involved in pathogenesis of PIH which may further affect birth outcomes.

CHAPTER 7

INFLUENCE OF MATERNAL DIETARY CALCIUM INTAKE ON PREGNANCY INDUCED HYPERTENSION, SMALL FOR GESTATIONAL AGE BABIES AND PRETERM BIRTHS

7.1 INTRODUCTION

The prevalence of adverse birth outcomes; LBW, SGA and preterm births is high in developing countries including India (WHO 1995, ACC/SCN 2000). The cause of these outcomes is multi- factorial, and a number of nutrients may have critical roles in this process. Several lines of evidence show that calcium and calcium rich food groups play an important role in determining preterm births (Repke & Villar 1991; Hardy et al., 1987). It is also postulated that preterm birth is mediated through a potentially serious morbidity; pregnancy induced hypertension (PIH). PIH indicates an elevated blood pressure observed after 20 weeks of gestation for the 1st time. PIH is clinically defined as an increase in the systolic blood pressure >30 mmHg or diastolic blood pressure ≥ 15 mmHg or blood pressure ≥ 140/90 mmHg on two consecutive occasions ≥ 6 hours apart (National high blood pressure education program working group 2000).

In presence of routine antenatal care, it is thought that calcium supplements would decrease the incidence of PIH and other forms of gestational hypertension (pre-eclampsia and eclampsia), which are known as major risk factors for maternal and perinatal morbidity and mortality. A study on adolescent pregnant women has suggested that calcium supplementation has a significant impact in reducing the incidence of prematurity (Repke & Villar 1991). In another study, it was seen that in a group of pregnant women who showed high compliance in taking calcium supplements, no case of premature delivery was reported, as opposed to a higher incidence of prematurity in the placebo group (Hardy et al., 1987).

While the patho-physiology of PIH is not clearly known, it is possible that PIH is characterized by an increased vascular sensitivity to the pressor effects of angiotensin II. A series of experiments by Grant et al (1973) showed angiotensin II sensitivity, locally-produced prostaglandins, and cyclic AMP to be related to PIH.

Additionally it is also known that these substances exert their effect through a final common pathway mediated via changes in intracellular ionized calcium (Everett et al., 1978). Similarly, results from epidemiologic studies and clinical trials of non pregnant adults suggest that dietary calcium may play a role in the etiology, prevention, and treatment of primary hypertension (Hamet 1995). Moreover calcium supplementation appears to affect circulating concentrations of parathyroid hormone and renin, which may modulate intracellular ionized calcium, resulting in smooth-muscle relaxation (Repke et al., 1989; Belizan et al., 1988). In pregnant women too, it is suggested that calcium supplementation may reduce the blood pressure thereby reducing the risk of PIH and preterm delivery. The mechanism of preterm delivery is likely to be similar via smooth muscle relaxation mediated in turn by alterations in parathyroid hormone and plasma renin concentrations. The incidence of preeclampsia may also be reduced by calcium supplementation.

Calcium is an active participant in the final common pathway of virtually all physiologic reactions. The effect of nutritional calcium intake on outcomes of hypertension, preeclampsia and LBW, preterm births would therefore appear real. In addition, elevation of Hcy, an intermediary metabolite of 1-carbon metabolism is also thought to cause hypertensive disorders and thereby PIH. Hyperhomocysteinemia, a condition defined as an increase in total Hcy concentration could also be due to deficiency of B vitamins namely, vitamin B_{12}, folate or vitamin B_6. Therefore the combination of nutrients may also be responsible for pathogenesis of PIH.

With regards to pathogenesis of preeclampsia, a prevailing hypothesis is the "ischemic model". Preeclampsia is a pregnancy-specific syndrome of reduced organ perfusion related to vasospasm and activation of the coagulation cascade. Decreased uteroplacental perfusion is thought to be the primary step and the point of convergence of diverse pathogenic processes in the development of preeclampsia (Friedman et al., 1991; van Beek & Peeters 1998). It is intuitive that reduced placental blood flow should result in decreased fetal growth, with an increased risk of SGA and LBW. However, epidemiologic studies have not conclusively established an association between preeclampsia or gestational hypertension and poor fetal growth (Misra 1996). Birth weight is determined both by duration of

gestation as well as rate of fetal growth. Preeclampsia significantly increases the risk of iatrogenic preterm birth (delivery) for maternal indications. Preeclampsia of early onset (≤37 weeks) may be more likely to be severe, more likely to have a detrimental effect on fetal growth while preeclampsia that is of late onset (>37 weeks) may be more likely to be mild and less likely to lead to "iatrogenic" premature delivery. Uteroplacental hypo perfusion, if present, may be of too short a duration to affect fetal size (Xiong et al., 2000).

This relationship of calcium to blood pressure has been the key factor in understanding the mechanism in reducing PIH. A number of studies relate the beneficial effects of dietary and supplemental calcium on blood pressure, PIH, pre-eclampsia as well as duration of gestation. However, there are also a few contradictory findings. There are very few studies conducted with respect to calcium supplements in Indian pregnant women. The table below illustrates few of the conflicting findings with varying concentrations of calcium supplements during pregnancy.

Table 29. List of studies of calcium and PIH and birth outcomes (selected)

S. No	Author	Study design	Inclusion / Exclusion Criteria	Findings
1	Hofmeyr et al 2007	Systematic review (n=15528). 12 studies with Randomized controlled trial	Pregnant women <35 weeks of gestation, with at least 1g calcium supplementation compared to placebo.	No effect of calcium supplementation on the risk of preterm births. Calcium supplementation reduces the risk of preeclampsia
2	Villar et al 2006 WHO 2006 (multicentric)	Randomized double blind placebo controlled trial (n=8325). 1.5 g elemental calcium (3 tab of 500mg each/day) versus placebo	Healthy pregnant primiparous women <20 weeks with median calcium intake of <600 mg/day	1.5 g/day calcium supple did not prevent pre-eclampsia but decreased the severity and the preterm deliveries.
4	Crowther et al 1999	Randomized controlled double blind trial (n=456). 1.8 g/d calcium or placebo from 24 weeks until term	Healthy nulliparous women with singleton pregnant	1.8 g/d calcium supplements during pregnancy decreased the risk of preeclampsia and preterm birth in nulliparous women
5	Levine et al 1997 (CPEP trial)	Randomized double blind placebo controlled trial (n=4589). 2 g/d calcium carbonate (n=2295) or placebo (n=2294).	Nulliparous pregnant women 18-40 yrs within 13-21 weeks gestation and average intake of calcium 1100 mg/d	Calcium supple did not prevent preeclampsia. No effect on Blood Pressure or incidence of preeclampsia or PIH with 2g/d calcium supplements.
6	Purwar et al 1996	Randomized double blind placebo control trial (n=201). 2 g/d calcium (n=103), placebo (n=98) from 20 wks gestation	Healthy nulliparous	Calcium supplements reduced the incidence of PIH and preeclampsia in nulliparous women
7	Sanchez-Ramos et al 1994	Randomized double blind placebo control trial (n=281) 2g/d calcium or	Nulliparous pregnant women	Calcium supple to high risk nulliparous decreased the incidence of PIH

		placebo.		
8	Knight et al 1992	Randomized controlled trial (control group and calcium supplemented) (n=30 normotensive and n= 20 hypertensive). 1g calcium/day.	18-28 years, no complications	No significant effect on the Blood Pressure and duration of gestational age.
9	Belizan et al 1991	Randomized double blind placebo controlled trial (n=1194), Multicentric trial (593 calcium supple versus 601 placebo). 2g calcium, as 500mg calcium carbonate tab from 20 weeks until term	Nulliparous healthy pregnant women, 20-35 yrs old and <20wks of gestation. Normotensive at recruitment.	Decrease in PIH and Blood pressure but no effect on preterm births and duration of gestational age.
10	Lopez-Jaramillo et al 1990	Randomized double blind placebo controlled trial (n=56). 2g calcium/day	Healthy nulliparous <20 years old, recruited at 28-32 weeks gestation	Decrease in incidence of PIH, but not significant decrease in BP. A positive association with supplements and gestational age at birth.

Calcium intake during pregnancy:

From these studies it is evident that the effects of calcium supplementation are either on PIH or on the length of gestation at delivery. Of the studies reported in the table, two studies did not show promising effects of calcium on blood pressure, incidence of preeclampsia or duration of gestational age at birth with 2 g calcium/d or 1 g calcium/d, while 6 studies have shown a decrease in the incidence of PIH or preeclampsia and 3 studies have shown an increase in the duration of gestational age at birth.

While this is encouraging, it is essential to realize that recommended dietary calcium during pregnancy varies across the countries studied. Recommendations for calcium intake during pregnancy differ by more than twofold, from 600 to 1425 mg/d; for an increase in intake relative to those of non-pregnant, non-lactating women, recommendations range from 0 to 825 mg calcium/d across the world (Trichopoulou & Vassilakou 1990, Truswell et al., 1983). The habitual intakes of 200-500 mg/d are typical in African and Asian societies where the consumption of animal milk is low (Prentice 1991), while the average supply of calcium in Northern Europe, North America, and Australasia is in excess of 1000 mg/d. Similar differences exist within countries. Diets derived principally from plant sources, rather than from milk, may contain components that interfere with calcium retention thus reducing calcium availability (Allen 1992). The variability in recommended calcium intake lies in the present inadequate state of knowledge about calcium requirements for human pregnancy and lactation. In general, a recommendation for pregnant women is derived by adding an increment to the figure for non pregnant to cover the calcium costs of fetal growth.

However, it is possible that alterations in calcium absorption and excretion, mediated by changes in metabolism, could compensate for these extra needs without necessitating a change in diet. Approximately 200 mg calcium/d is deposited in the fetal skeleton during the third trimester of gestation. More than 98% of calcium in the body is contained in the inorganic matrix of bone. If the diet of a pregnant woman does not provide sufficient calcium for fetal development, the growth of the baby during gestation would be adversely affected, or calcium would be released from the maternal skeleton, with possible short- or long- term effects on the mother's

health. However, because, in general, only about one-third of dietary calcium is absorbed, while losses into urine and gastrointestinal fluids are sizable, it is possible that changes in calcium absorption, metabolism, and excretion may ensure that sufficient calcium is supplied to the placenta without drawing on the maternal skeleton or necessitating an increase in calcium intake. The capacity of the human body to make such adjustments and the threshold calcium intake below which adverse outcomes might be expected are not known (Prentice 1991).

Calcium supplementation during pregnancy is therefore of concern especially in countries with low to modest dietary calcium intakes. With contradictory findings on calcium supplementation and its effects on PIH and preterm births, we aimed to explore the relationship between dietary calcium, supplemental calcium and calcium rich food groups with adverse birth outcomes particularly the preterm births. A secondary aspect of the study was to characterize the prevalence of PIH, another cause of preterm in a population that is routinely prescribed with calcium supplements.

7.2 METHODOLOGY

The selection of study subjects and baseline information, their dietary intakes, anthropometric information and delivery details is mentioned in the previous chapter. The subjects received routine antenatal care during pregnancy. They were prescribed with 5 mg folic acid/d until the end of the first trimester followed with 0.5 mg/d until delivery. From the 2nd trimester (~13 weeks of gestation) onwards, one tablet of dried ferrous sulphate 150 mg (equivalent 45 mg elemental iron) and two tablets of 1250 mg calcium carbonate (equivalent to 500 mg elemental calcium) with vitamin D_3, IP 250 I.U were prescribed uniformly for all subjects until delivery. At each monthly follow up visit, information pertaining to calcium supplements ingested during the previous month was asked. The total calcium ingested during each trimester was calculated. The expected weeks of supplementation in the 2nd trimester were considered from 13 weeks until the 26th week of gestation, while in the last trimester; it was from week 26 until delivery. The difference between the compliance recorded (computed as daily mg of calcium multiplied by the number of days supplements taken in the previous month) during the monthly follow up and the expected weeks of ingestion in that month (mg of calcium multiplied by the number of days in that

month) was calculated. This computation was suggestive of total calcium ingested per day after taking into account the days for which the supplements were missed. A composite intake of calcium was computed i.e. the sum of the absolute calcium intakes through FFQ and the supplemental calcium during each trimester.

Blood pressure monitoring

A random blood pressure was recorded in duplicate after 10 min seating position at each antenatal visit with a recommended automated blood pressure monitor (OMRON Intelli Sense, Model HEM-757, and Tokyo Japan) using appropriate adult size cuff. The cuff was fitted closely to the arm and centered over the branchial artery. The instrument was calibrated every 2 months against a mercury sphygmomanometer. The subjects were classified into categories of hypertension disorders by the clinicians. The classification was based on the guidelines defined by the American College of Obstetricians and Gynecologists (National high blood pressure education program working group 2000).

Hypertension defined as an increase in the SBP (systolic blood pressure) ≥ 30mmHg or increase in the DBP (diastolic blood pressure) ≥ 15 mmHg or blood pressure ≥ 140/90 mmHg on two occasion ≥ 6 hours apart.

Gestational hypertension defined as hypertension only (also named as 'transient hypertension' or mild PIH) observed for the 1st time after 20 weeks of gestation.

***Severe hypertension*;** PIH defined as gestational hypertension with SBP ≥ 160mmHg or DBP ≥ 110 mmHg.

Proteinuria *defined* as protein ≥ 0.3 g/24 hours or ≥ 30 mg/dl (1+ dipstick) on two occasion's ≥ 6 hours apart.

Severe proteinuria defined as protein ≥ 0.3 g/24 hours or ≥ 2+ dipsticks.

Preeclampsia defined as hypertension + proteinuria or edema.

Eclampsia defined as preeclampsia + seizure.

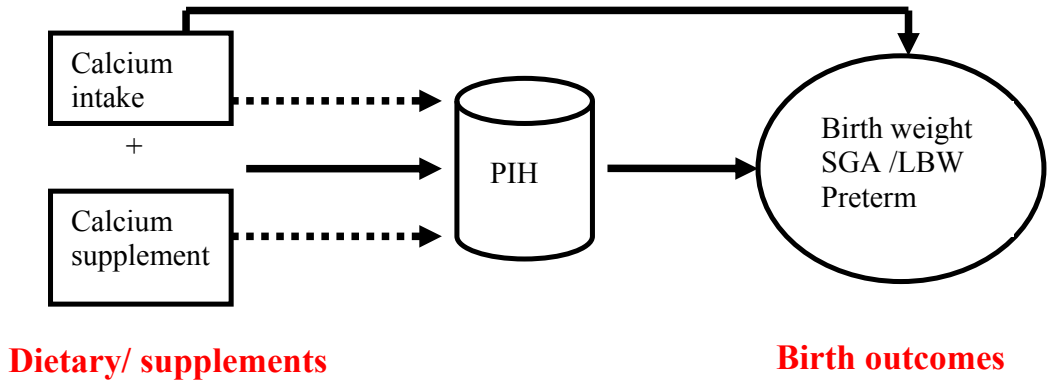


Figure 13. The conceptual framework underpinning objective 4

7.3 STATISTICAL ANALYSIS

All analyses were carried out with the SPSS program (version 16.0, SPSS, Chicago, IL, USA). Data was assessed for the normality distribution. The maternal baseline characteristics were normally distributed and represented as Mean ± SD while the nutrient and food group intakes through FFQ were expressed as the median with lower and upper quartiles. Nutrients were energy adjusted to remove variation due to energy, using the residual method. Spearman Rank correlations were used to assess the relationships between dietary nutrients, food groups, and birthweight and gestational age at birth. All categories of hypertensive subjects (mild PIH, PIH and preeclampsia) were classified as PIH. Mann Whitney U test was used to compare nutrients such as the calcium, vitamin B_{12}, vitamin B_6, folate and calcium rich food group across the birth outcomes; LBW, SGA and preterm babies. A similar analysis was done to compare with presence of PIH. Since calcium was the nutrient of interest in exploring the relationship with PIH and birth outcomes, absolute as well as energy adjusted values were considered.

A composite intake was computed i.e. the sum of the absolute calcium intake and the intake of calcium supplement during each trimester. Calcium - rich food groups (milk products, cereals, green leafy vegetables, total vegetables and total fruits) were also similarly summed to obtain a composite of their intakes in (g/d). The absolute, energy adjusted calcium intakes as well as the composite intakes were divided into tertiles for further analysis. Chi square test was performed to find the

association of tertiles of calcium intake with PIH and birth outcomes; LBW, SGA and preterm births. I also explored the role of B vitamins in the pathogenesis of PIH as well as preterm births. Preterm as the birth outcome was explored further since evidences have shown relationships between PIH and duration of gestational age.

7.4 RESULTS

The characteristics of the mothers of the babies born preterm and term are shown below. The mothers of both, preterm and term babies had similar baseline characteristics with respect to age, parity and education. The mean age of the study participants was 24.8 ± 3.9 years, 60% of the women were primiparous and were recruited at 11 weeks gestation during their first visit. Almost 36% of the subjects had education upto high school level. The women weighed 53.5 ± 9.6 kg , were 1.55 ± 0.06 meters tall with a normal BMI of 22.2 ± 3.8 $kg.m^{-2}$ and a mean blood pressure of 115/74 mmHg. All chronic hypertensive subjects were excluded from the study at enrolment (**Table 30**).

During the course of pregnancy, 13.5% of the women developed gestational hypertension and were classified as mild PIH (7.1 %), PIH (5.5 %) and preeclampsia (0.9 %) (**Figure 14**). Higher percent of women with PIH underwent caesarean delivery as against normal delivery, p=0.004 (19.2 % versus 10.9%) (*Data shown in table 9, Appendix A*). This trend was not observed in mother of SGA or LBW babies. None of the study participants developed eclampsia. Nineteen subjects (22.1%) were prescribed medications and 21 (24.4%) were hospitalised for the management of the hypertension disorder.

Table 30. Baseline characteristics of mothers who delivered preterm and term babies

Parameters	Preterm (n=60)	Term (n=534)
Age (years)	24.9 ± 4.3	24.7 ±3.9
Parity[a]		
Primiparous	36 (60)	331 (62)
Multiparous	24 (40)	203 (38)
Education[a]		
Upto high school	23 (38.3)	175 (32.8)
PUC/Diploma	12 (20)	119 (22.3)
University and above	25 (41.7)	240 (44.9)
LMP (weeks)	11.3 ± 2.2	11.5 ± 2.6
Anthropometry		
Weight (kg)	52.1 ± 9.0	53.5 ± 9.8
Height (cm)	153.4 ± 6.2	155.4 ± 5.9
BMI ($kg.m^{-2}$)	22.2 ± 3.6	22.2 ± 3.8
Blood pressure		
SBP (mmHg)	115 ± 10	116 ± 9
DBP (mmHg)	74 ± 7	74 ± 6

Values represent: Mean ± SD; [a] represent number (percentages)

LMP: Last menstrual period, BMI: Body mass index

Student's t test of significance used to compare the parameters in the 2 groups.

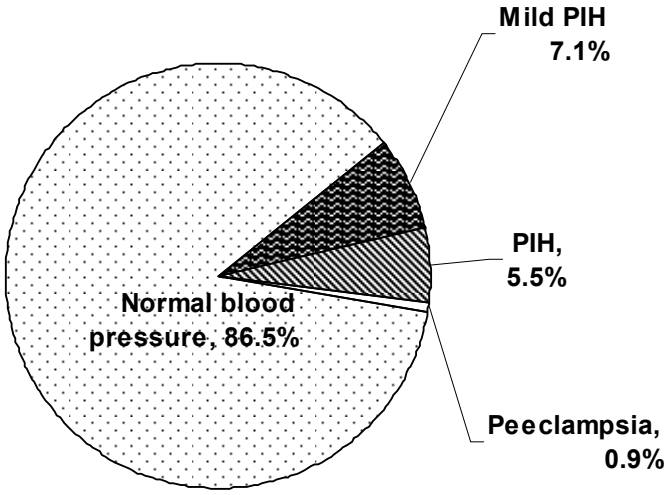


Figure 14. Distribution of women with categories of hypertension during pregnancy

Mild PIH- mild pregnancy induced hypertension; PIH-pregnancy induced hypertension

Baby parameters at birth were examined. The binary variables defined for birth outcomes showed that ~20 % of the babies were born LBW (birth weight <2500 g), 30% were born SGA (birth weight < 10^{th} percentile for the gestational age) while 10% were born preterm (babies born <37 weeks of gestation). Preterm babies, as expected, had anthropometric measures at birth that were significantly lower as compared to the full-term babies. Higher percent of preterm babies were delivered by caesarean sections as compared to the term babies (45% versus 31.8%, p=0.030) (*Data shown in table 10, Appendix A*). This trend of high proportion of caesarean section delivery was not seen in those mothers who delivered SGA or LBW babies. Spearman correlations of dietary energy adjusted calcium, composite as well as calcium - rich food groups with gestational age at birth and birth weight showed no significant correlation with these parameters (*Data shown in table 11, Appendix A*).

The dietary intakes of the study participants during the 3 trimesters of pregnancy were assessed by a FFQ and compared across birth outcomes (term babies versus preterm, LBW versus normal weight babies and SGA versus AGA). The macronutrient intakes during pregnancy were not significantly different between mothers of preterm and term babies. The sources of calcium through diet as well as

oral supplements were examined. The mothers of preterm babies had slightly lower intakes of composite calcium and calcium - rich food groups except the energy adjusted calcium intake in the 2nd trimester of pregnancy, though they were no statistically significant. In the 3rd trimester of pregnancy there was decreased calcium intakes in the mother's of preterm babies, with significantly low intakes of energy adjusted calcium ~ 87 mg/d (p=0.033) and calcium - rich food groups ~141 g/d (p=0.012) when compared to the mothers of term babies (**Table 31a**). The calcium intakes and sources of calcium were compared among the mothers of LBW versus normal weight babies and SGA versus AGA babies. Similar trend of low calcium - rich food groups (789 versus 868 $g.d^{-1}$) was observed in the mothers of LBW as compared to mothers of normal weight babies (**Table 31b**). There were no significant differences observed in the mothers of SGA as compared to the mothers of non SGA babies (**Table 31c)**.

Table 31a. Dietary calcium intake of mothers with preterm babies.

Calcium intakes (mg/d) **Trimester 2**	**Mothers of preterm babies (n=43)**	**Mothers of term babies (n-373)**
Energy adjusted habitual intake	1103 (941, 1206)	1091 (962, 1241)
Composite intake[1]	1872 (1700, 2099)	1957 (1683, 2230)
Calcium rich food groups (g/d)	834 (721, 990)	828 (645, 1061)
Trimester 3	**(n=33)**	**(n=336)**
Energy adjusted habitual intake	1044 (936, 1163)	1131 (987, 1283)*
Composite intake[1]	1923 (1710, 2226)	2034 (1758, 2247)
Calcium rich food group (g/d)	726 (641, 937)	867 (689, 1081)*

Values represent median with lower and upper quartile ranges

[1]Composite calcium intake (absolute dietary calcium + supplemented calcium)

Calcium rich food group intake (daily intakes of milk products + cereals + green leafy vegetables + total vegetables + total fruits)

* Mann Whitney U test of significance, $p< 0.05$.

Table 31b. Dietary calcium intake of mothers with LBW babies.

Calcium intakes (mg/d) **Trimester 2**	**Mothers of LBW babies (n=91)**	**Mothers of normal weight babies (n=325)**
Energy adjusted habitual intake	1112 (941, 1227)	1083 (962, 1241)
Composite intake[1]	1926 (1696, 2088)	1957 (1683, 2244)
Calcium rich food groups (g/d)	776 (668, 1046)	840 (646, 1064)
Trimester 3	**(n=74)**	**(n=295)**
Energy adjusted habitual intake	1116 (1007, 1269)	1126 (977, 1278)
Composite intake[1]	2026 (1756, 2227)	2020 (1757, 2254)
Calcium rich food group (g/d)	789 (631, 1055)	868 (695, 1075)*

Values represent median with lower and upper quartile ranges

[1]Composite calcium intake (absolute dietary calcium + supplemented calcium)

Calcium rich food group intake (daily intakes of milk products + cereals + green leafy vegetables + total vegetables + total fruits)

* Mann Whitney U test of significance, $p < 0.05$.

Table 31c. Dietary calcium intake of mothers with SGA babies.

Calcium intakes (mg/d) **Trimester 2**	**Mothers of SGA babies (n=124)**	**Mothers of AGA babies (n=292)**
Energy adjusted habitual intake	1101 (941, 1218)	1087 (962, 1247)
Composite intake[1]	1930 (1669, 2147)	1965 (1701, 2241)
Calcium rich food groups (g/d)	771 (620, 1057)	845 (672, 1054)
Trimester 3	**(n=112)**	**(n=257)**
Energy adjusted habitual intake	1168 (1022, 1287)	1114 (965, 1255)
Composite intake[1]	2025 (1786, 2257)	2020 (1752, 2231)
Calcium rich food group (g/d)	855 (611, 1086)	843 (699, 1063)

Values represent median with lower and upper quartile ranges

[1]Composite calcium intake (absolute dietary calcium + supplemented calcium)

Calcium rich food group intake (daily intakes of milk products + cereals + green leafy vegetables + total vegetables + total fruits)

While the women with PIH gave birth to significantly higher percentage of preterm babies (27.1% as compared to 7.3%; $p<0.000$ in non PIH mothers), the

distribution of preterm births in mothers of PIH categories showed high proportion of preterm births as compared to the women with normal blood pressure (**Figure 15**). This indicates that the two conditions coexist and hence it is important to examine the relation between calcium intake and PIH. The association between PIH and LBW and SGA were also examined. Though no significant association were observed with PIH and SGA versus AGA group, PIH mothers gave birth to significantly higher LBW babies (18.5% as compared to 31.8%, p=0.005 in the non PIH group).

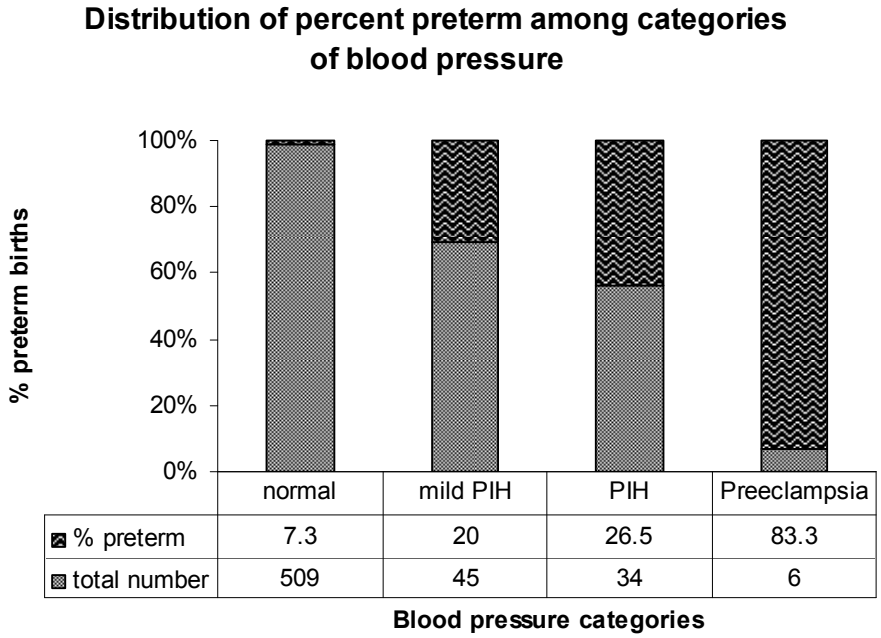


Figure 15. Distribution of percent preterm across the blood pressure categories

Mild PIH- mild pregnancy induced hypertension

PIH- Pregnancy induced hypertension

The intakes of calcium were compared between the two groups of PIH showed that though the mothers with PIH had slightly lower intakes of energy adjusted calcium, composite calcium as well as calcium rich food groups in the 2nd and 3rd trimester, there was no statistical differences observed (**Table 32**).

Table 32. Dietary calcium, supplement and calcium rich food group intakes among PIH and non PIH women.

Calcium intake (mg/d)	PIH	Non PIH
	Trimester 2	
	(n=62)	**(n=355)**
Energy adjusted	1077 (1008,1190)	1101 (946,1249)
Composite calcium intake[1]	1851 (1697, 2116)	1964 (1690, 2231)
Calcium rich food group (g/d)	795 (647, 1009)	840 (650, 1055)
	Trimester 3	
	(n=58)	**(n=312)**
Energy adjusted	1118 (1004, 1256)	1126 (976, 1276)
Composite calcium intake[1]	1983 (1748, 2229)	2031 (1761, 2241)
Calcium rich food group (g/d)	805 (657, 1018)	857 (685, 1073)

Values represent median with lower and upper quartile ranges

[1]Composite calcium intake (absolute dietary calcium + supplemented calcium)

Calcium rich food group intake (daily intakes of milk products + cereals + green leafy vegetables + total vegetables + total fruits)

Spearman correlation was performed. No significant differences observed among the groups.

Tertiles of energy adjusted calcium, composite and calcium - rich food group intakes in the 2nd and 3rd trimester of pregnancy were performed and compared with PIH and preterm in order to examine the effect of the lowest tertile (lowest intake) with these outcomes. The proportion of PIH and preterm was slightly higher in the lowest tertile of all the intake parameters in the 2nd and 3rd trimester except PIH for energy adjusted calcium intakes. However, in the 3rd trimester alone, women in the lowest tertile of energy adjusted calcium intakes had slightly higher proportion of preterm births as compared to the women in the highest tertile (12.1% versus 3.3%, p=0.008, OR 4.09; CI: 1.32, 12.71) and the remaining associations were not statistically significant (**Table 33**). The wide confidence intervals of the OR indicated a poor goodness of fit attributed to the small sample number of preterm (15 subjects versus 4 in the lowest to highest energy adjusted calcium intakes). Therefore the multivariate model was not performed. The distribution of LBW across tertiles of calcium intakes was also examined. Though not significant, the trend of high

percentage of LBW with low calcium intakes was not consistent in all categories of calcium intakes (absolute calcium, energy adjusted, composite or calcium - rich food group).

Table 33. Percentage of women with PIH and preterm births across the lowest and the highest tertile of calcium intakes in the 2nd and 3rd trimester of pregnancy

Variable	Trimester 2		Trimester 3	
	Lowest tertile (n=138)	Highest tertile (n=140)	Lowest tertile (n=123)	Highest tertile (n=124)
	Absolute habitual calcium intake			
% PIH	22 (15.9)	19 (13.6)	19 (15.4)	17 (13.7)
% Preterm	14 (10.2)	9 (6.4)	13 (10.6)	7 (5.6)
	Energy adjusted habitual calcium intake			
% PIH	15 (24.2)	17 (27.4)	18 (31.0)	18 (31.0)
% Preterm	16 (11.7)	12 (8.6)	15 (12.1)	4 (3.3)*
	Calcium rich food groups			
% PIH	21 (15.1)	17 (12.2)	21 (17.1)	18 (14.5)
% Preterm	11 (8.0)	12 (8.6)	17 (13.8)	8 (6.5)
	Composite calcium intake (absolute calcium + supplemental calcium)			
% PIH	20 (14.5)	16 (11.6)	22 (17.7)	17 (13.7)
% Preterm	14 (10.2)	9 (6.5)	13 (10.5)	10 (8.1)

Values represent- number (percentages)

* Test of significance, $p < 0.05$, Chi square test of significance.

The calcium rich food groups during pregnancy was associated with preterm births and these food groups are also rich sources of vitamin B_{12}, therefore the effect of vitamin B_{12} was also examined with preterm births and PIH. Though there was no significant difference observed with vitamin B_{12} intakes and mothers of preterm and term groups, energy adjusted vitamin B_{12} median intakes in the 3rd trimester was significantly lower in the women with PIH as compared to non PIH women (2.23 versus 2.52 mcg/d; p=0.022 respectively).

7.5 DISCUSSION

This study focused mainly on calcium intakes (both diet as well as oral supplements) during pregnancy and its effects on LBW, SGA and preterm births. The *a priori* hypothesis was to look at the beneficial effects of calcium on birth outcomes. However with evidences showing a relationship between dietary and or supplemental calcium and PIH, it was also essential to explore the intermediary outcome i.e. PIH in this population. During the course of pregnancy, hypertension has been estimated to complicate 5% of all pregnancies and 11% of first pregnancies (Villar 2004), while half of the women with hypertension tend to have pre-eclampsia. In contrast, in my data, almost 13.5% of the pregnant women were diagnosed to have hypertension in any form; mild PIH, PIH or preeclampsia as per the standard classification, with only 0.9% to have preeclampsia (National High Blood Pressure Education Program Working Group, 2000). The women with PIH had lower intakes of dietary and supplemental calcium as compared to the non PIH women.

In addition, women who had PIH also had significantly higher proportion of preterm births and LBW babies as compared to non-PIH women. A trend of low energy adjusted calcium as well as supplemental calcium intakes in the mothers of preterm babies indicate that calcium might play a role in pathogenesis of PIH and preterm births. This association was however not seen for LBW. A number of studies relate the association of calcium and PIH with preterm births rather than LBW. Our findings of significant higher percentage of LBW born to PIH could be explained based on the birthweight, since LBW babies could be the result of preterm births. Moreover, findings with calcium intakes and LBW were not consistent. In general a higher percentage of women with PIH, and a higher incidence of preterm births were seen in the group belonging to the lowest tertile of calcium in any form (energy adjusted, composite or calcium rich food groups) as compared to the women in the highest tertile.

I could not establish a strong relationship between low calcium intake and the risk of PIH, since the median intake of dietary calcium in both PIH and non PIH mothers was quite high (~1000 mg/d). Moreover the blood pressure and the dietary calcium intakes in the mothers of preterm babies were not significantly different when compared to the mothers of full-term babies at recruitment which indicated that there

were no women with chronic hypertensive disorder when they were confirmed to be pregnant.

Several well conducted trials have studied the efficacy of calcium supplements in preventing preeclampsia, gestational hypertension, and premature delivery (Belizan et al., 1991, Marya et al., 1987, Knight & Keith 1992). In Argentina, where the dietary calcium intake was 644 mg/d, calcium supplements of 2000 mg/d resulted in an overall lowering of blood pressure and a decrease in incidence of PIH (Belizan et al., 1991). However, a randomized trial conducted in 400 women in India showed no difference in the incidence of PIH, blood pressure or the length of the gestation at birth (Marya et al., 1987) when supplemented with calcium. In this population the dietary calcium intake was 500 mg/d with a calcium supplement of 375 mg/d. A study conducted in the United States by Knight & Keith (1992) showed marginal effect on lowering the blood pressure effect due to calcium, but not on the incidence of PIH or the length of the gestation when supplemented with 1000 mg/d of calcium. The calcium intake in this population was 580 mg/d. These findings suggest that reduction in the incidence of PIH or lowering of blood pressure is observed mainly when the supplemental dose was beyond 1000 mg/d. Moreover the studies lacked association between calcium and length of the gestational age at delivery; hence relationships with calcium intakes and preterm births are not clearly established.

In another randomized controlled trial conducted in India, Purwar et al (1996) showed that supplementation with 2 g of calcium per day decreased the incidence of PIH but there was no difference in the preterm births. Another classic observation was that Mayan Indians in Gautemala, who traditionally soaked their corn in lime before cooking, had a high calcium intake and a low incidence of preeclampsia and eclampsia (Villar et al., 1983). In a systematic review on calcium supplementation for prevention of preeclampsia and related problems by Hofmeyr et al (2007), it was suggested that calcium supplementation in the second half of pregnancy reduced blood pressure and thus the diagnosis and clinical manifestations of preeclampsia, without having any significant effect on the underlying pathologies. Even in my population, the routine antenatal care included oral supplementation of calcium 1000 mg/d from 2nd trimester onwards until delivery. Contradictory findings exist, but a

number of studies have also suggested calcium supplementation having a beneficial role in reducing preterm delivery (Villar & Repke 1990, Sanchez-Ramos et al., 1991).

I further examined the calcium rich food group intakes in PIH and non PIH mothers. A similar trend with calcium intakes was seen, i.e. directionally lower intakes of calcium - rich food group in PIH group against the non PIH. In the current study, the main food group rich in calcium was dairy products, which is also the main source of protein, vitamin B_{12} and calcium. The role of B vitamins in pathogenesis of PIH also exists through elevation of Hcy (Yajnik et al., 2005; Takimoto et al., 2001). I did not find correlation between Hcy concentration and calcium intakes during pregnancy. My results demonstrating low intakes of calcium and vitamin B_{12} in women with PIH indicates that the role of milk and dairy products in PIH cannot be ruled out. This could very well relate to the case-control study in Canada that assessed the relationship between calcium intakes from dairy products and hypertensive disorders in pregnancy, where the incidence of preeclampsia was not related to dietary calcium intake, but women with gestational hypertension tended to have lower intake of dairy products than controls (Marcoux et al., 1991).

In this population, 40% of the subjects were educated upto high school and most of them belonged to urban middle class group. The dietary calcium intakes of our study population were about 1000 mg/d with additional oral calcium supplements of 1000 mg/d. With this information as the backdrop, my findings are in line with findings of several well-conducted trials looking at the efficacy of calcium supplements in preventing preeclampsia, gestational hypertension, and premature delivery. The authors have not found a definite association between the calcium intakes and or supplementation since the diets contained moderate-to-high amounts of calcium and over and above were supplemented with 1-2 g calcium/d. Consequently, total calcium intakes during supplementation were very high, often exceeding 2000 mg/d (Levine et al., 1997). The results, although not clear-cut, suggest that calcium salts at 1-2 g/d may be helpful in reducing the incidence of some hypertensive disorders of pregnancy in high risk women.

Evidences suggest the mechanism relating calcium and preterm may be through hypertensive disorders. Jose et al (1988) have shown that low calcium intake may cause high blood pressure by stimulating the parathyroid hormone secretion. A possible mode of action for calcium supplementation is that it reduces parathyroid release and intracellular ionized calcium and so reduces smooth muscle contractility. Calcium supplements prevent preterm labour and delivery by reducing the uterine smooth muscle contractility (Villar et al., 1990). The blood-pressure-lowering effect of calcium supplementation was demonstrated in populations with mild to moderate hypertension (McCarron & Morris 1985) while studies have shown beneficial effects of calcium supplementation in reduction of preeclampsia (Villar J 1987, Villar J & Repke JT 1990, Kawasaki N 1985, Lopez-Jaramillo 1987). In the present study, only 0.9% subjects were diagnosed with pre-eclampsia and 67% of them were diagnosed to have PIH >37 weeks of gestation. This suggests that in this study sample, the routine supplementation of calcium may have minimized the incidence of preeclampsia.

There are contradictory findings with regards to the time of supplementation during pregnancy. Few studies suggest that the calcium supplements should begin early in pregnancy (1st trimester) when the alterations in calcium homeostasis are already beginning to occur (Ritchie et al., 2000 & 1998) as opposed to the mid pregnancy when most clinical trials begin supplementation. In addition whether increased consumption of dairy foods has a larger impact than does supplemental calcium alone is not clear.

Calcium and vitamin D are thought to modulate postprandial endothelial function. Acute increases in dietary calcium and vitamin D showed a dose dependant suppression of PTH and inverse increase in vascular tone (Soares et al., 2010) in young adults. PTH also regulates feto-placetal blood flow in an autocrine and paracrine function (Macgill et al., 1997). It is also observed that calcium accelerates weight reduction on a calorie-restricted diet and attenuates weight gain when added to a diet without calorie restriction (Cummings 2006; Soares et al., 2011). Since BMI, elevated Hcy are related to hypertensive disorders, it is essential to explore this combination as well. Studies have also shown dietary calcium deficiency leading to secondary vitamin D deficiency (Hypovitaminosis D) (Clements et al., 1987) which

in turn during pregnancy has important consequences for the newborn, including fetal hypovitaminosis D, neonatal rickets and tetany, and infantile rickets (Delvin et al., 1986). Moreover, it is observed that approximately 85% of urban and rural pregnant women in North India are vitamin D deficit (Sachan et al., 2005) indicating that vitamin D, a key regulator of calcium metabolism, is also of concern.

The strength of my study is that this is the only prospective observational study conducted in India that has followed pregnant women receiving the routine antenatal care until delivery. The low incidence of preeclampsia and gestational hypertension disorder at term or during labor (0.9 %) might be due to the protective effect of calcium supplementation during pregnancy. Despite this constraint, I could prospectively relate lower calcium intakes in PIH women and mothers of preterm babies though I could not show statistical significance. Evidence however supports the beneficial effect of dietary calcium in the prevention and treatment of PIH and high-risk groups, such as pregnant teens (Hardy et al., 1987). Populations with inadequate calcium intake, and women at risk of developing PIH, may also benefit from consuming additional dietary calcium.

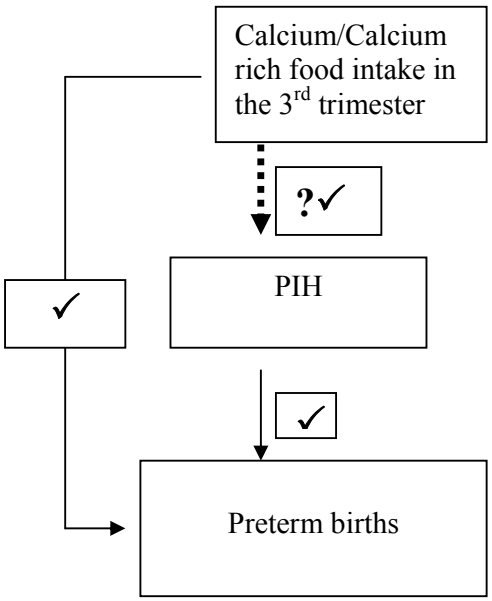


Figure 16. Highlights of the objective 4

7.6 CONCLUSION

My findings are suggestive of possible association between calcium, calcium - rich food group intakes and preterm births. While the antenatal regime indicates calcium supplementation from the 2nd trimester of pregnancy, further studies examining the effect of supplementation in early pregnancy is warranted when the alterations in calcium homeostasis are already beginning to occur. To substantiate these findings, large interventional studies in supplemented mothers are warranted.

CHAPTER 8
SUMMARY

Pregnancy has long been recognized as a vulnerable period during which the health and well-being of both the unborn child and mother are at risk. Micronutrient deficiencies during pregnancy have shown to have serious implications on the developing fetus. LBW remains a significant public health problem in many developing countries and is associated with a range of both short- and long- term adverse consequences. In elucidating the potential effect of maternal diet on fetal growth, both diet compositions and single food and nutrients have been examined.

This PhD program was conducted to address the role of micronutrients involved in the methylation cycle and their relationship to poor birth outcomes. This prospective cohort of pregnant women, were followed antenatally until delivery. I validated the FFQ for selective B group intake against multiple 24-HDR and their respective micronutrient status. The dietary vitamin B_{12} assessed by FFQ (absolute as well as energy adjusted) correlated positively with the reference method and the biomarker (vitamin B_{12} status) at both trimesters of pregnancy, vitamin B_6 intakes were positively correlated with the 24-HDR. This gave me some confidence in the analysis of data.

Women with vitamin B_{12} deficiency and with elevated Hcy concentration in the 1st trimester of pregnancy had higher proportion of adverse birth outcomes (LBW, SGA and preterm births) implying that the insult occurring during organogenesis period. Pregnant women with low vitamin B_{12} had elevated homocysteine concentration and higher risk of LBW; indicating that maternal vitamin B_{12} would affect the birth outcome possibly through Hcy. In this cohort, the study participants were supplemented with folic acid throughout pregnancy and with elemental iron from 2nd trimester until delivery. Hence folate deficiency was not very high and B_{12} rather than folate influenced Hcy.

Single nutrient deficiencies are not as common as the clustering of micronutrient deficiency. It was important to explore the role of these B vitamin rich food group intakes in relation to adverse birth outcomes. The mothers of SGA babies

had lower intakes of cereals and milk products in the 2nd trimester of pregnancy. Univariate analysis also showed a decrease in intakes of protein, fat, vitamin B_{12} and folate in mothers of SGA babies. These nutrients could be linked to food group (milk products) as rich sources; mainly of proteins, vitamin B_{12} and calcium. This is important particularly in this population that consumed predominantly cereal based diet. While considering the protein requirements during pregnancy, it was observed that the pregnant women though had adequate protein intakes in the 1st trimester did not meet the requirements as the pregnancy progressed indicating that these micronutrient deficiency in the 2nd trimester enhances the chance of adverse birth outcomes. The cereal most commonly consumed in this population is rice and ragi (*Eleusine Coracaca*, millet) followed by wheat and jowar (Sudan grass; *sorghum bicolor*) which are generally ingested with green leafy vegetables or a lentil curry. Ragi is also rich in calcium. The findings of low cereals with high risk of SGA continued even after adjusting for potential micronutrients and suggested that there could be other nutrients involved that we need to explore further. The nutrients such as vitamin B_{12}, vitamin B_6, folate and calcium were of my interest in understanding their role in determining adverse birth outcomes and hence these were considered in the analysis. Though maternal gestational weight gain in the 2nd trimester correlated positively with birth weight, cereal intakes did not show this association with gestational weight gain. The findings indicate further work in understanding the different ways the food items are cooked and thereby their bio-availability.

Our findings of low milk product in mothers of SGA babies and the significant relationship being lost after adjusting for the protein intakes indicate that the protein may be the macronutrient of interest. In addition the milk product intakes were positively correlated with gestational weight gain of the pregnant women in the 2nd trimester which was further correlated to birth weight. Therefore there is a possibility that the milk product intake could benefit the pregnant women and thereby the birth weight. These pregnant women consume cereal based diet, low in meat and fish intakes hence I presume that protein obtained is most likely from the milk products. Milk is considered as a balanced food group. On an average 500 ml of milk contains ~ 300 kcal energy and 16 g protein. Therefore in this population it may be best to suggest high intakes of milk products and cereals during pregnancy to meet the requirements and ensure a better birth outcome.

The interrelation of vitamin B_{12}, vitamin B_6 and folate status with Hcy at 1st and 2nd trimester of pregnancy and with neonatal micronutrient status was examined. Vitamin B_{12} status and plasma Hcy concentration were negatively correlated. Importantly, maternal micronutrient status determined neonatal status. Due to financial constraints the micronutrient status were only analysed in a sub-set of 154 subjects in the 1st and 2nd trimester of pregnancy and in cord blood. The micronutrient analysis for a larger sample in all 3 trimesters may indicate strong relationships. I also observed that calcium and calcium - rich food group intakes during pregnancy were lower in women with PIH and in those mothers who delivered preterm births. This association was significant in the 3rd trimester of pregnancy with preterm births. PIH is a morbidity occurring after 20 weeks of gestation and most of our subjects were diagnosed to have PIH around the time of delivery. Moreover, the mean gestational age of preterm births was ~ 35 weeks, supporting our findings of low calcium / calcium - rich food groups in the 3rd trimester of pregnancy and high proportion of women with PIH and preterm births. There is a possibility that the pathogenesis would have occurred or started to occur in the 2nd trimester of pregnancy but the effects noticed in the 3rd trimester. These findings put together aims at nutrients and combination of nutrients such as food groups (including proteins, B vitamins and calcium) driving the relationship towards morbidity and preterm births.

The association between maternal nutrition and birth outcome is very complex and is also influenced by many biologic, socioeconomic, and demographic factors, which vary widely in different populations. Moreover the adverse birth outcomes, LBW, SGA or preterm births are all categories based either on the birth weight or gestational age at birth. As seen earlier, LBW could include preterm births and SGA babies except for the term SGA babies. Therefore results pertaining to either of the birth outcome indicate that other determinants of adverse birth outcomes are also needed to be considered while addressing these critical issues. This prospective observational PhD program has indicated the potential role of these nutrients and food groups in pregnancy related morbidity and adverse birth outcomes and has tried to contribute valuable knowledge and information in public health in developing countries. Our findings endorse interventional studies in delineating the cause-effect relationship between maternal dietary/nutritional influences on birth

outcomes. In addition, some efforts to study antenatal fetal growth and development might provide fresh insights into this problem. The real challenge, however, is to improve program delivery and to integrate services. Therefore attention to socio-economic factors, specific cultural behaviours, or food intake patterns may provide the way forward for effective and sustainable prevention strategies. This would help in developing future guidelines for healthy eating practices and possibly translate into operational procedures for individual counselling and population-based health programs.

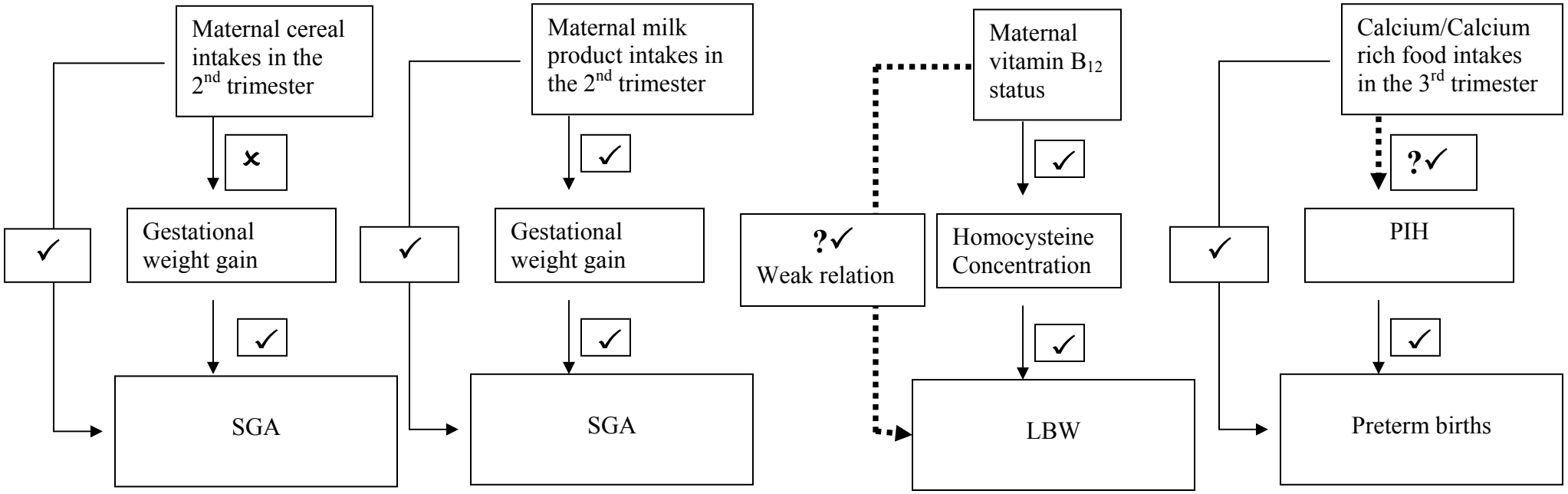


Figure 17. Overall findings of the PhD Thesis.

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APPENDIX- A

Additional Tables

Table 1 Distribution of percentage of pregnant women in the quintiles of nutrient intakes assessed through FFQ and average of multiple 24 hours dietary recall during the 3 trimesters of pregnancy.

Table 1.1. Distribution of percentage of pregnant women in the quintiles of energy intake assessed by FFQ and 24-HDR in the 1st trimester of pregnancy.

Energy intakes through 24-HDR (Quintiles)	**Energy intakes in the 1st trimester through FFQ (Quintiles) (p= 0.002)**					
	1	**2**	**3**	**4**	**5**	**TOTAL**
1	9 (30.0)	11 (36.7)	5 (16.7)	2 (6.7)	3 (10.0)	31
2	11 (35.5)	5 (16.1)	5 (16.1)	6 (19.4)	4 (12.9)	32
3	3 (9.4)	6 (18.8)	11 (34.4)	6 (18.8)	6 (18.8)	31
4	6 (19.4)	8 (25.8)	3 (9.7)	9 (29.0)	5 (16.1)	31
5	1 (3.3)	2 (6.7)	7 (23.3)	8 (26.7)	12 (40.0)	30
Total	30 (19.5)	32 (20.8)	31 (20.1)	31 (20.1)	30 (19.5)	154

Table 1.2. Distribution of percentage of pregnant women in the quintiles of protein intakes assessed by FFQ and 24-HDR in the 1st trimester of pregnancy.

Protein intakes through 24-HDR (Quintiles)	**Protein intakes in the 1st trimester through FFQ (Quintiles) (p= 0.833)**					
	1	**2**	**3**	**4**	**5**	**TOTAL**
1	8 (25.8)	7 (22.6)	7 (22.6)	5 (16.1)	4 (12.9)	27
2	9 (30.0)	6 (20.0)	5 (16.7)	3 (10.0)	7 (23.3)	27
3	4 (12.9)	6 (19.4)	8 (25.8)	8 (25.8)	5 (16.1)	27
4	7 (21.9)	7 (21.9)	5 (15.6)	7 (21.9)	6 (18.8)	26
5	3 (10.0)	5 (16.7)	6 (20.0)	8 (26.7)	8 (26.7)	28
Total	31 (20.1)	31 (20.1)	31 (20.1)	31 (20.1)	30 (19.5)	154

Table 1.3. Distribution of percentage of pregnant women in the quintiles of fat intakes assessed by FFQ and 24-HDR in the 1st trimester of pregnancy.

Fat intakes through 24-HDR (Quintiles)	**Fat intakes in the 1st trimester through FFQ (Quintiles) (p= 0.191)**					
	1	**2**	**3**	**4**	**5**	**TOTAL**
1	9 (29.0)	7 (22.6)	7 (22.6)	5 (16.1)	3 (9.7)	31
2	7 (22.6)	4 (12.9)	7 (22.6)	8 (25.8)	5 (16.1)	31
3	8 (25.8)	11 (35.5)	5 (16.1)	4 (12.9)	3 (9.7)	31
4	5 (16.7)	4 (13.3)	6 (20.0)	7 (23.3)	8 (26.7)	30
5	2 (6.5)	5 (16.1)	6 (19.4)	7 (22.6)	11 (35.5)	31
Total	31 (20.1)	31 (20.1)	31 (20.1)	31 (20.1)	30 (19.5)	154

Table 1.4. Distribution of percentage of pregnant women in the quintiles of carbohydrate intakes assessed by FFQ and 24-HDR in the 1st trimester of pregnancy.

Carbohydrate intakes through 24-HDR (Quintiles)	**Carbohydrate intakes in the 1st trimester through FFQ (Quintiles) (p= 0.299)**					
	1	**2**	**3**	**4**	**5**	**TOTAL**
1	11 (35.5)	7 (22.6)	5 (16.1)	6 (19.4)	2 (6.5)	31
2	7 (23.3)	8 (26.7)	5 (16.7)	6 (20.0)	4 (13.3)	30
3	4 (12.5)	5 (15.6)	7 (21.9)	9 (28.1)	7 (21.9)	32
4	7 (22.6)	4 (12.9)	7 (22.6)	6 (19.4)	7 (22.6)	31
5	2 (6.7)	7 (23.3)	7 (23.3)	3 (13.3)	10 (33.3)	30
Total	31 (20.1)	31 (20.1)	31 (20.1)	31 (20.1)	30 (19.5)	154

Table 1.5. Distribution of percentage of pregnant women in the quintiles of vitamin B_6 intakes assessed by FFQ and 24-HDR in the 1st trimester of pregnancy.

Vitamin B_6 intakes through 24-HDR (Quintiles)	**Vitamin B_6 intakes in the 1st trimester through FFQ (Quintiles) (p= 0.299)**					
	1	**2**	**3**	**4**	**5**	**TOTAL**
1	11 (35.5)	9 (29.0)	6 (19.4)	4 (12.9)	1 (3.2)	31
2	8 (25.8)	7 (22.6)	5 (16.1)	7 (22.6)	4 (12.9)	31
3	5 (16.1)	8 (25.8)	6 (19.4)	6 (19.4)	6 (19.4)	31
4	6 (19.4)	4 (12.9)	7 (22.6)	9 (29.0)	5 (16.5)	31
5	1 (3.3)	3 (10.0)	7 (23.3)	5 (16.7)	14 (46.7)	30
Total	31 (20.1)	31 (20.1)	31 (20.1)	31 (20.1)	30 (19.5)	154

Table 1.6. Distribution of percentage of pregnant women in the quintiles of vitamin B_{12} intakes assessed by FFQ and 24-HDR in the 1st trimester of pregnancy.

Vitamin B_{12} intakes through 24-HDR (Quintiles)	**Vitamin B_{12} intakes in the 1st trimester through FFQ (Quintiles) (p= 0.827)**					
	1	**2**	**3**	**4**	**5**	**TOTAL**
1	9 (30.0)	6 (20.0)	5 (16.7)	5 (16.7)	5 (16.7)	31
2	4 (12.9)	7 (22.6)	7 (22.6)	6 (19.4)	7 (22.6)	31
3	6 (18.8)	7 (21.9)	6 (18.8)	5 (15.6)	8 (25.0)	31
4	5 (16.7)	6 (20.0)	7 (23.3)	10 (33.3)	2 (6.7)	31
5	7 (22.6)	5 (16.1)	6 (19.4)	5 (16.1)	8 (25.8)	30
Total	31 (20.1)	31 (20.1)	31 (20.1)	31 (20.1)	30 (19.5)	154

Table 1.7. Distribution of percentage of pregnant women in the quintiles of Folate intakes assessed by FFQ and 24-HDR in the 1st trimester of pregnancy.

Folate intakes through 24-HDR (Quintiles)	**Folate intakes in the 1st trimester through FFQ (Quintiles) (p= 0.056)**					
	1	**2**	**3**	**4**	**5**	**TOTAL**
1	5 (16.1)	10 (32.3)	8 (25.8)	4 (12.9)	4 (12.9)	31
2	9 (30.0)	4 (13.3)	10 (33.3)	3 (10.0)	4 (13.3)	31
3	7 (22.6)	6 (19.4)	4 (12.9)	9 (29.0)	5 (16.1)	31
4	3 (9.4)	7 (21.9)	5 (15.6)	11 (34.4)	6 (18.8)	31
5	7 (23.3)	4 (13.3)	4 (13.3)	4 (13.3)	11 (36.7)	30
Total	31 (20.1)	31 (20.1)	31 (20.1)	31 (20.1)	30 (19.5)	154

Table 1.8. Distribution of percentage of pregnant women in the quintiles of Calcium intakes assessed by FFQ and 24-HDR in the 1st trimester of pregnancy.

Calcium intakes through 24-HDR (Quintiles)	**Calcium intakes in the 1st trimester through FFQ (Quintiles) (p= 0.000)**					
	1	**2**	**3**	**4**	**5**	**TOTAL**
1	17 (54.8)	5 (16.1)	2 (6.5)	7 (22.6)	0	31
2	9 (29.0)	8 (25.8)	5 (16.1)	6 (19.4)	3 (9.7)	31
3	2 (6.7)	7 (23.3)	8 (26.7)	7 (23.3)	6 (20.0)	31
4	1 (3.1)	6 (18.8)	10 (31.3)	4 (12.5)	11(34.4)	31
5	2 (6.7)	5 (16.7)	6 (20.0)	7 (23.3)	10 (33.3)	30
Total	31 (20.1)	31 (20.1)	31 (20.1)	31 (20.1)	30 (19.5)	154

Table 1.9. Distribution of percentage of pregnant women in the quintiles of energy intakes assessed by FFQ and 24-HDR in the 2nd trimester of pregnancy.

Energy intakes through 24-HDR (Quintiles)	**Energy intakes in the 2nd trimester through FFQ (Quintiles) (p= 0.000)**					
	1	**2**	**3**	**4**	**5**	**TOTAL**
1	10 (43.5)	6 (26.1)	5 (21.7)	1 (4.3)	1 (4.3)	23
2	9 (32.1)	7 (25.0)	4 (14.3)	6 (21.4)	2 (7.1)	28
3	6 (22.2)	7 (25.9)	5 (18.5)	6 (22.2)	3 (11.1)	27
4	1 (3.6)	6 (21.4)	7 (25.0)	8 (28.6)	6 (21.4)	28
5	1 (3.4)	1 (3.4)	6 (20.7)	6 (20.7)	15 (51.7)	29
Total	27 (20.0)	27 (20.0)	27 (20.0)	27 (20.0)	27 (20.0)	135

Table 1.10. Distribution of percentage of pregnant women in the quintiles of protein intakes assessed by FFQ and 24-HDR in the 2nd trimester of pregnancy.

Protein intakes through 24-HDR (Quintiles)	**Protein intakes in the 2nd trimester through FFQ (Quintiles) (p= 0.528)**					
	1	**2**	**3**	**4**	**5**	**TOTAL**
1	7 (25.9)	5 (18.5)	6 (22.2)	4 (14.8)	5 (18.5)	23
2	5 (18.5)	9 (33.3)	3 (11.1)	4 (14.8)	6 (22.2)	28
3	3 (11.1)	7 (25.9)	6 (22.2)	5 (18.5)	6 (22.2)	27
4	8 (30.8)	1 (3.8)	7 (26.9)	7 (26.9)	3 (11.5)	28
5	4 (14.3)	5 (17.9)	5 (17.9)	7 (25.0)	7 (25.0)	29
Total	27 (20.0)	27 (20.0)	27 (20.0)	27 (20.0)	27 (20.0)	135

Table 1.11. Distribution of percentage of pregnant women in the quintiles of fat intakes assessed by FFQ and 24-HDR in the 2nd trimester of pregnancy.

Fat intakes through 24-HDR (Quintiles)	**Fat intakes in the 2nd trimester through FFQ (Quintiles) (p= 0.096)**					
	1	**2**	**3**	**4**	**5**	**TOTAL**
1	8 (32.0)	4 (16.0)	5 (20.0)	5 (20.0)	3 (12.0)	23
2	7 (23.3)	9 (30.0)	6 (20.0)	7 (23.3)	1 (3.3)	28
3	5 (18.5)	4 (14.8)	3 (11.1)	8 (29.6)	7 (29.5)	27
4	2 (7.7)	3 (11.5)	9 (34.6)	5 (19.2)	7 (26.9)	28
5	5 (18.5)	7 (25.9)	4 (14.8)	2 (7.4)	9 (33.3)	29
Total	27 (20.0)	27 (20.0)	27 (20.0)	27 (20.0)	27 (20.0)	135

Table 1.12. Distribution of percentage of pregnant women in the quintiles of carbohydrate intakes assessed by FFQ and 24-HDR in the 2nd trimester of pregnancy.

Carbohydrate intakes through 24-HDR (Quintiles)	**Carbohydrate intakes in the 2nd trimester through FFQ (Quintiles) (p= 0.126)**					
	1	**2**	**3**	**4**	**5**	**TOTAL**
1	9 (34.6)	4 (15.4)	5 (19.2)	2 (7.7)	6 (23.1)	23
2	6 (21.4)	7 (25.0)	7 (25.0)	4 (14.3)	4 (14.3)	28
3	6 (22.2)	8 (29.6)	6 (22.2)	5 (18.5)	2 (7.4)	27
4	2 (6.9)	7 (24.1)	5 (17.2)	9 (31.0)	6 (20.7)	28
5	4 (16.0)	1 (14.0)	4 (16.0)	7 (28.0)	9 (36.0)	29
Total	27 (20.0)	27 (20.0)	27 (20.0)	27 (20.0)	27 (20.0)	135

Table 1.13. Distribution of percentage of pregnant women in the quintiles of vitamin B_6 intakes assessed by FFQ and 24-HDR in the 2nd trimester of pregnancy.

Vitamin B_6 intakes through 24-HDR (Quintiles)	**Vitamin B_6 intakes in the 2nd trimester through FFQ (Quintiles) (p= 0.007)**					
	1	**2**	**3**	**4**	**5**	**TOTAL**
1	7 (26.9)	11 (42.3)	4 (15.4)	3 (11.5)	1 (3.8)	23
2	9 (33.3)	4 (14.8)	6 (22.2)	4 (14.8)	4 (14.8)	28
3	4 (13.8)	7 (24.1)	9 (31.0)	5 (17.2)	4 (13.8)	27
4	4 (14.8)	3 (11.1)	6 (22.2)	8 (29.6)	6 (22.2)	28
5	3 (11.5)	2 (7.7)	4 (15.4)	5 (19.2)	12 (46.2)	29
Total	27 (20.0)	27 (20.0)	27 (20.0)	27 (20.0)	27 (20.0)	135

Table 1.14. Distribution of percentage of pregnant women in the quintiles of vitamin B_{12} intake assessed by FFQ and 24-HDR in the 2nd trimester of pregnancy.

Vitamin B_{12} intakes through 24-HDR (Quintiles)	**Vitamin B_{12} intakes in the 2nd trimester through FFQ (Quintiles) (p= 0.001)**					
	1	**2**	**3**	**4**	**5**	**TOTAL**
1	13 (46.7)	7 (25.0)	2 (7.1)	1 (3.6)	5 (17.9)	23
2	5 (20.0)	7 (28.0)	7 (28.0)	4 (16.0)	2 (8.0)	28
3	3 (11.5)	7 (26.9)	6 (23.1)	5 (19.2)	5 (19.2)	27
4	2 (7.7)	5 (19.2)	8 (30.8)	7 (26.9)	4 (15.4)	28
5	4 (13.3)	1 (3.3)	4 (13.3)	10 (33.3)	11 (36.7)	29
Total	27 (20.0)	27 (20.0)	27 (20.0)	27 (20.0)	27 (20.0)	135

Table 1.15. Distribution of percentage of pregnant women in the quintiles of Folate intake assessed by FFQ and 24-HDR in the 2nd trimester of pregnancy.

Folate intakes through 24-HDR (Quintiles)	**Folate intakes in the 2nd trimester through FFQ (Quintiles) (p= 0.871)**					
	1	**2**	**3**	**4**	**5**	**TOTAL**
1	7 (26.9)	4 (15.4)	6 (23.1)	4 (15.4)	5 (19.2)	23
2	7 (23.3)	9 (30.0)	5 (16.7)	6 (20.0)	3 (10.0)	28
3	6 (22.2)	3 (11.1)	5 (18.5)	5 (18.5)	8 (26.9)	27
4	5 (17.9)	6 (21.4)	5 (17.9)	7 (25.5)	5 (17.9)	28
5	2 (8.3)	5 (20.8)	6 (25.0)	5 (20.8)	6 (25.0)	29
Total	27 (20.0)	27 (20.0)	27 (20.0)	27 (20.0)	27 (20.0)	135

Table 1.16. Distribution of percentage of pregnant women in the quintile of Calcium intake assessed by FFQ and 24-HDR in the 2nd trimester of pregnancy.

Calcium intakes through 24-HDR (Quintiles)	**Calcium intakes in the 2nd trimester through FFQ (Quintiles) (p= 0.000)**					
	1	**2**	**3**	**4**	**5**	**TOTAL**
1	15 (51.7)	8 (27.6)	6 (20.7)	0	0	23
2	2 (7.7)	9 (34.6)	7 (26.7)	5 (19.2)	3 (11.5)	28
3	4 (16.0)	5 (20.0)	5 (20.0)	7 (28.0)	4 (16.0)	27
4	2 (7.1)	4 (14.3)	6 (21.4)	8 (28.6)	8 (28.6)	28
5	4 (14.8)	1 (3.7)	3 (11.1)	7 (25.9)	12 (44.4)	29
Total	27 (20.0)	27 (20.0)	27 (20.0)	27 (20.0)	27 (20.0)	135

Table 1.17. Distribution of percentage of pregnant women in the quintiles of Energy intake assessed by FFQ and 24-HDR in the 3rd trimester of pregnancy.

Energy intakes through 24-HDR (Quintiles)	**Energy intakes in the 3rd trimester through FFQ (Quintiles) (p= 0.000)**					
	1	**2**	**3**	**4**	**5**	**TOTAL**
1	12 (44.9)	6 (21.4)	6 (21.4)	3 (10.7)	1 (3.6)	28
2	3 (11.5)	6 (23.1)	11 (44.3)	3 (11.5)	3 (11.5)	26
3	7 (28.0)	8 (32.0)	3 (12.0)	5 (20.0)	2 (8.0)	25
4	3 (13.6)	2 (9.1)	3 (13.6)	3 (13.6)	11 (50.0)	22
5	1 (3.8)	3 (11.5)	2 (7.7)	11 (42.3)	9 (34.6)	26
Total	26 (20.5)	25 (19.7)	25 (19.7)	25 (19.7)	26 (20.5)	127

Table 1.18. Distribution of percentage of pregnant women in the quintiles of protein intake assessed by FFQ and 24-HDR in the 3rd trimester of pregnancy.

Protein intakes through 24-HDR (Quintiles)	**Protein intakes in the 3rd trimester through FFQ (Quintiles) (p= 0.032)**					
	1	**2**	**3**	**4**	**5**	**TOTAL**
1	6 (26.1)	4 (12.4)	6 (26.1)	1 (4.3)	6 (26.1)	23
2	9 (29.0)	9 (29.0)	5 (16.1)	5 (16.1)	3 (9.7)	31
3	9 (39.1)	4 (17.4)	3 (13.0)	6 (26.1)	1 (4.3)	23
4	1 (3.6)	5 (17.3)	6 (21.4)	8 (28.6)	8 (28.6)	28
5	1 (4.5)	3 (13.6)	6 (27.3)	5 (22.7)	7 (31.8)	22
Total	26 (20.5)	25 (19.7)	26 (20.5)	25 (19.7)	25 (19.7)	127

Table 1.19. Distribution of percentage of pregnant women in the quintiles of fat intake assessed by FFQ and 24-HDR in the 3rd trimester of pregnancy.

Fat intakes through 24-HDR (Quintiles)	**Fat intakes in the 3rd trimester through FFQ (Quintiles) (p= 0.044)**					
	1	**2**	**3**	**4**	**5**	**TOTAL**
1	10 (40.0)	5 (20.0)	4 (16.0)	5 (20.0)	1 (4.0)	25
2	8 (30.8)	5 (19.2)	5 (19.2)	4 (15.4)	4 (15.4)	26
3	2 (9.1)	7 (31.8)	6 (27.3)	4 (18.2)	3 (13.6)	22
4	5 (17.9)	3 (10.7)	6 (21.4)	8 (28.6)	6 (21.4)	28
5	1 (3.8)	5 (19.2)	5 (19.2)	4 (15.4)	11 (42.3)	26
Total	26 (20.5)	25 (19.7)	26 (20.5)	25 (19.7)	25 (19.7)	127

Table 1.20. Distribution of percentage of pregnant women in the quintiles of carbohydrate intakes assessed by FFQ and 24-HDR in the 3rd trimester of pregnancy.

Carbohydrate intakes through 24-HDR (Quintiles)	**Carbohydrate intakes in the 3rd trimester through FFQ (Quintiles) (p= 0.065)**					
	1	**2**	**3**	**4**	**5**	**TOTAL**
1	9 (34.6)	5 (19.2)	6 (23.1)	4 (15.4)	2 (7.7)	26
2	7 (28.0)	7 (28.0)	7 (28.0)	3 (12.0)	1 (4.0)	25
3	4 (14.8)	5 (18.5)	3 (11.1)	9 (33.3)	6 (22.2)	27
4	4 (17.4)	4 (17.4)	3 (13.0)	6 (26.1)	6 (26.1)	23
5	2 (7.7)	4 (15.4)	7 (26.9)	3 (11.5)	10 (38.5)	26
Total	26 (20.5)	25 (19.7)	26 (20.5)	25 (19.7)	25 (19.7)	127

Table 1.21. Distribution of percentage of pregnant women in the quintiles of vitamin B_6 intakes assessed by FFQ and 24-HDR in the 3rd trimester of pregnancy.

Vitamin B_6 intakes through 24-HDR (Quintiles)	**Vitamin B_6 intakes in the 3rd trimester through FFQ (Quintiles) (p= 0.031)**					
	1	**2**	**3**	**4**	**5**	**TOTAL**
1	8 (34.8)	6 (26.1)	4 (17.4)	3 (13.0)	2 (8.7)	23
2	7 (25.0)	8 (28.6)	4 (14.3)	5 (17.9)	4 (14.3)	28
3	5 (19.2)	6 (23.1)	9 (34.6)	5 (19.2)	1 (3.8)	26
4	5 (17.9)	4 (14.3)	5 (17.9)	5 (17.9)	9 (32.1)	28
5	1 (4.5)	1 (4.5)	4 (18.2)	7 (31.8)	9 (40.9)	28
Total	26 (20.5)	25 (19.7)	26 (20.5)	25 (19.7)	25 (19.7)	127

Table 1.22. Distribution of percentage of pregnant women in the quintiles of vitamin B_{12} intakes assessed by FFQ and 24-HDR in the 3rd trimester of pregnancy.

Vitamin B_{12} intakes through 24-HDR (Quintiles)	**Vitamin B_{12} intakes in the 3rd trimester through FFQ (Quintiles) (p= 0.145)**					
	1	**2**	**3**	**4**	**5**	**TOTAL**
1	9 (37.5)	5 (20.8)	6 (25.0)	2 (8.3)	2 (8.3)	24
2	6 (24.0)	6 (24.0)	5 (20.0)	2 (8.0)	6 (24.0)	25
3	5 (19.2)	3 (11.5)	6 (23.1)	8 (30.8)	4 (15.4)	26
4	3 (10.3)	7 (24.1)	6 (20.7)	9 (31.0)	4 (13.8)	29
5	3 (13.0)	4 (17.4)	3 (13.0)	4 (17.4)	9 (39.1)	23
Total	26 (20.5)	25 (19.7)	26 (20.5)	25 (19.7)	25 (19.7)	127

Table 1.23 Distribution of percentage of pregnant women in the quintiles of folate intakes assessed by FFQ and 24-HDR in the 3rd trimester of pregnancy.

Folate intakes through 24-HDR (Quintiles)	**Folate intakes in the 3rd trimester through FFQ (Quintiles) (p= 0.846)**					
	1	**2**	**3**	**4**	**5**	**TOTAL**
1	4 (16.7)	5 (20.8)	4 (16.7)	4 (16.7)	7 (29.2)	24
2	6 (24.0)	4 (16.0)	7 (28.0)	4 (16.0)	4 (16.0)	25
3	6 (22.2)	5 (18.5)	7 (25.9)	7 (25.9)	2 (7.4)	26
4	4 (16.7)	5 (20.8)	6 (25.0)	3 (12.5)	6 (25.0)	29
5	6 (22.2)	6 (22.2)	2 (7.4)	7 (25.9)	6 (22.2)	23
Total	26 (20.5)	25 (19.7)	26 (20.5)	25 (19.7)	25 (19.7)	127

Table 1.24 Distribution of percentage of pregnant women in the quintiles of calcium intakes assessed by FFQ and 24-HDR in the 3rd trimester of pregnancy.

Calcium intakes through 24-HDR (Quintiles)	**Calcium intakes in the 3rd trimester through FFQ (Quintiles) (p= 0.003)**					
	1	**2**	**3**	**4**	**5**	**TOTAL**
1	11 (44.0)	5 (20.0)	5 (20.0)	2 (8.0)	2 (8.0)	24
2	5 (21.7)	3 (13.0)	7 (30.4)	6 (26.1)	2 (8.7)	25
3	6 (24.0)	7 (28.0)	4 (16.0)	5 (20.0)	3 (12.0)	26
4	3 (10.7)	8 (28.6)	7 (25.0)	4 (14.3)	6 (21.4)	29
5	1 (3.8)	2 (7.7)	3 (11.5)	8 (30.8)	12 (46.2)	23
Total	26 (20.5)	25 (19.7)	26 (20.5)	25 (19.7)	25 (19.7)	127

Values represent number (percentages)

Table 2. Multivariate Linear Regression of difference between FFQ and 24 hr dietary recall for vitamin B_6, folate and calcium in the 2nd and 3rd trimester of pregnancy.

Nutrients (difference between FFQ and 24 hr diet recall)	**β coefficient**	**P value**	**% variance**
TRIMESTER 2			
Difference in vitamin B_6 intake (mg/d)			
Age	-0.067	0.460	
Parity	0.084	0.315	
Baseline wt	0.103	0.235	
Education	-0.109	0.001	7.4
Difference in folate intake (mcg/d)			
Age	-0.035	0.694	
Parity	0.083	0.316	
Baseline wt	0.087	0.312	
Education	-23.655	0.000	9.8
Difference in calcium intake (g/d)			
Age	-0.055	0.549	
Parity	0.014	0.867	
Baseline wt	0.242	0.005	
Education	-65.798	0.003	5.4
TRIMESTER 3			
Difference in vitamin B_6 intake (mg/d)			
Age	-0.131	0.157	
Parity	-0.051	0.565	
Baseline wt	0.010	0.910	
Education	-0.085	0.016	4.6
Difference in folate intake (mcg/d)			
Age	-4.479	0.036	
Parity	-0.067	0.442	
Baseline wt	0.075	0.400	
Education	-21.885	0.002	3.2
Difference in calcium intake (g/d)			

Age	-0.057	0.542	
Parity	-0.007	0.932	
Baseline wt	0.094	0.301	
Education	-63.911	0.014	4.7

Table 3 Correlations between maternal green leafy vegetable intakes during pregnancy with birth weight and baby length at birth.

Baby parameter	**Green leafy vegetable intakes (gm)**		
	TRIMESTER 1	**TRIMESTER 2**	**TRIMESTER 3**
Birth weight (g)	0.016	-0.052	-0.009
Length at birth (cm)	-0.120	-0.125**	0.073

Values represent- correlation coefficient
Significance indicates **p<0.005

Table 4 Odds ratio of SGA among tertiles of energy adjusted protein intakes in 2nd trimester of pregnancy

Tertiles of energy adjusted protein intakes (g)	**OR**	**95% Confidence Interval**		**P value**
		Lower limit	**Upper limit**	
Tertile 3 (ref group) (n=139)				
Tertile 1 (n=138)	1.90	1.12	3.21	0.017
Tertile 2 (n= 140)	1.49	0.87	2.55	0.144

Table 5 Distribution of percentage SGA among women with low protein intakes compared with 1st and 3rd tertile in the 2nd and 3rd trimester of pregnancy.

Protein intake in trimester 2	Birth category	Protein intake in trimester 3		Total	P value
		Tertile 1	Tertile 3		
Tertile 1	**AGA**	39 (68.4)	29 (60.4)	68	0.258
	SGA	18 (31.6)	19 (39.6)	37	
Tertile 3	**AGA**	26 (60.5)	117 (72.7)	143	0.088
	SGA	17 (39.5)	44 (27.3)	61	

Table 6 Correlations of maternal vitamin B_{12}, vitamin B_6, folate status and Homocysteine concentration with neonatal concentrations, during 1st and 2nd trimester of pregnancy

Neonatal status	**Maternal status**							
	TRIMESTER 1				**TRIMESTER 2**			
	Vitamin B_{12}	**Vitamin B_6**	**Folate**	**Hcy**	**Vitamin B_{12}**	**Vitamin B_6**	**Folate**	**Hcy**
Vitamin B_{12}	0.651***				0.646***			
Vitamin B_6		0.373***				0.299***		
Folate			0.117				0.039	
Hcy	-0.163*	-0.035	0.085	0.201**	-0.271**	-0.114	-0.006	0.198*

Values represent- correlation coefficient
Significance indicates *** $p< 0.001$; **$p<0.005$; * $p<0.05$
Units for micronutrient status- vitamin B_{12}- pmol/L, Vitamin B_6- nmol/L, Folate-nmol/L, Hcy- μM/L

Table 7 Correlations of maternal vitamin B_{12}, vitamin B_6, folate status and Homocysteine concentration with baby parameters.

Baby parameters	**Maternal B Vitamin status (vitamin B_{12}, Vitamin B_6, folate) and Homocysteine concentration**							
	Trimester 1				**Trimester 2**			
	Vitamin B_{12}	**Vitamin B_6**	**Folate**	**Hcy**	**Vitamin B_{12}**	**Vitamin B_6**	**Folate**	**Hcy**
	pmol/L	**nmol/L**	**nmol/L**	**µM/L**	**pmol/L**	**nmol/L**	**nmol/L**	**µM/L**
Birth weight (g)	0.091	-0.089	0.066	-0.083	0.064	-0.180*	0.072	-0.071
Gestational age at birth (weeks)	0.058	-0.164*	-0.035	0.118	0.063	-0.252**	-0.061	0.018
Birth length (cm)	0.012	-0.115	-0.011	0.070	-0.023	-0.237**	-0.042	0.057
MUAC (cm)	0.154	-0.025	0.106	-0.103	0.087	-0.190*	-0.002	-0.117
Biceps (mm)	0.001	0.053	0.022	0.026	-0.037	-0.060	0.009	0.032
Triceps (mm)	0.085	0.034	0.117	-0.005	0.054	-0.058	0.078	-0.086
Subscapular (mm)	0.149	0.038	0.053	-0.053	0.099	-0.142	0.092	-0.106
Suprailiac (mm)	-0.240	0.100	-0.105	-0.019	-0.147	0.227	-0.032	0.046

MUAC- mid upper arm circumference
Values represent correlation coefficient
Significance indicates ** $p < 0.005$; *$p < 0.05$

Table 8 Distribution of adverse LBW, SGA and Preterm babies across median levels of maternal micronutrient status and Homocysteine concentration in 1st and 2nd trimester of pregnancy

		Maternal nutrient status							
		Vitamin B_{12} pmol/L	**Vitamin B_6** nmol/L	**Folate** nmol/L	**Hcy** µM/L		**Vitamin B_{12}** pmol/L	**Vitamin B_6** nmol/L	**Folate** nmol/L	**Hcy** µM/L
Birth outcome	**N**	**TRIMESTER 1**				**N**	**TRIMESTER 2**			
SGA										
Yes	52	148.8	28.4	530.5	7.9	44	117.9	23.2	564.0	7.0
no	101	157.3	26.8	525.5	7.8	91	133.4	22.2	656.2	6.4
LBW										
Yes	45	136.4	28.6	479.8	8.2	34	121.7	30.7	641.8	6.7
no	108	160.3	26.9	539.4	7.7	100	137.4	21.6	614.7	6.4
Preterm										
Yes	22	148.5	32.1	501.6	8.2	16	126.6	29.2	776.0	6.7
no	131	157.6	27.5	526.0	7.8	118	133.5	22.5	611.9	6.4

Values represent nutrient median levels.

Table 9 Distribution of type of delivery among pregnant women with normotensive blood pressure and gestational hypertension

Type of delivery	Gestational hypertension		Total	P value
	No	Yes		
Normal	391 (89.1)	48 (10.9)	439	
Caesarean section	160 (80.8)	38 (19.2)	198	0.004
Total	551	86	637	

Values represent number (percentage)

Table 10 Distribution of type of delivery with babies born term and preterm

Type of delivery	Term babies	Preterm babies	Total	P value
Normal	364 (91.7)	33 (8.3)	397	
Caesarean section	170 (86.3)	27 (13.7)	197	0.030
Total	534	60	594	

Values represent number (percentage)

Table 11 Correlations of calcium intakes in the 2nd and 3rd trimester with baby birth weight and gestational age at birth

Calcium intakes (mg/d)	**Birth weight**	**Gestational age at birth**
TRIMESTER 2		
Energy adjusted habitual intake	0.020	0.088
Composite intake[1]	0.052	0.044
Calcium rich food groups (g/d)	0.037	-0.004
TRIMESTER 3		
Energy adjusted habitual intake	-0.020	0.069
Composite intake[1]	-0.026	-0.037
Calcium rich food group (g/d)	0.103*	0.085

[1]Composite calcium intake (absolute dietary calcium + supplemented calcium)
Calcium rich food group intake (daily intakes of milk products + cereals + green leafy vegetables + total vegetables + total fruits)
Values represent correlation coefficient
Significance indicates *$p<0.05$

APPENDIX- B

Presentations at conferences

PRESENTATIONS AT CONFERENCES

Presentations as part of this PhD thesis.

Poster presentation at the 19th International Congress of Nutrition, Bangkok, Thailand - A LONGITUDINAL ASSESSMENT OF VITAMIN B_{12} AND HOMOCYSTEINE STATUS IN PREGNANCY

P Dwarkanath[1], S Muthayya[1], T Thomas[2], M J Soares[3], A Mhaskar[4], R Mhaskar[4], A Thomas[4], M Vaz[2], P Parikh[5], R Mehra[5], A V Kurpad[1]
[1]Division of Nutrition, St John's Research Institute, Bangalore, India
[2]Division of Epidemiology and Biostatistics, St John's Research Institute, Bangalore, India
[3]School of Public Health, Curtin University of Technology, Perth, Western Australia
[4]Dept of Obstetrics & Gynecology, St John's Medical College Hospital, Bangalore, India
[5] GlaxoSmithKline Consumer Healthcare Ltd, Gurgaon, India

Background – Low vitamin B_{12} status is a determinant of IUGR in Indian pregnant women. Potential mechanisms may involve homocysteine as well as other nutrients in these adverse birth outcomes.
Objective - To determine the relationship of vitamin B_{12} intake, B_{12} status and plasma homocysteine during pregnancy.
Design – Vitamin B_{12} intakes of 419 pregnant Indian women were assessed through a validated food frequency questionnaire at each trimester. In a sub-sample, serum vitamin B_{12} and homocysteine concentrations were measured during pregnancy and in cord blood at delivery.
Outcomes – Median vitamin B_{12} intakes were low throughout pregnancy at ~2.1 µg /day with 46% of women below RDAs. Vitamin B_{12} intake and status were significantly correlated at the first and second trimesters ($r=0.36$, $p<0.001$ and $r=0.25$, $p=0.004$, respectively). Egg, meat and milk intakes contributed to 11%, 21% and 46% of total B_{12} intake. Cord blood homocysteine concentration was significantly higher than in maternal blood in the first two trimesters (13.82 vs. 7.97 and 6.38 µM/L, respectively). A weak inverse relationship between vitamin B_{12} status and plasma homocysteine was noted at the 1st trimester ($r = - 0.20$, $p=0.029$).
Conclusions- A very high percentage of South Indian women have poor vitamin B_{12} intakes related to their low intake of B_{12} rich foods. The significant rise in homocysteine during pregnancy was only partially explained by low B_{12} status.

Recipient of the International Nutrition Foundation/Kraft Short-term Fellowship to attend the 19th International Congress of Nutrition, Bangkok, Thailand, 4–9October 2009.

Oral presentation at the 43rd National Conference of the Nutrition Society of India held at the National Institute of Nutrition, ICMR, Hyderabad, India 2011

- INFLUENCE OF MATERNAL DIETARY CALCIUM INTAKE ON PREGNANCY INDUCED HYPERTENSION AND PRETERM BIRTHS

Pratibha Dwarkanath[1,2], Tinku Thomas[1], Anura V Kurpad[1] and Mario J Soares[2]
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[2] School of Public Health, Curtin Health Innovation Research Institute, Curtin University Perth, Western Australia
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Calcium intakes during pregnancy play an important role in determining preterm births (babies born <37 weeks of gestation). Preterm birth is in turn mediated through a potentially serious morbidity called pregnancy induced hypertension (PIH). The primary objective was to explore the relationship between dietary calcium, supplemental calcium and calcium rich food groups with preterm births and secondly to characterize the prevalence of PIH in a population that is routinely prescribed with calcium supplements. A cohort of 637 pregnant women was studied at each trimester until delivery. Intakes of dietary calcium, calcium rich food groups were assessed by a 3-month food frequency questionnaire. Compliance on calcium supplement intake was recorded. Presence of PIH and / or preeclampsia during pregnancy, and birth outcomes at delivery was recorded. Approximately 13.5% women were diagnosed as gestational hypertension (7.1% mild PIH, 5.5% PIH and 0.9% with preeclampsia). Mothers of preterm babies had significantly lower energy adjusted calcium intakes ~87 mg/d ($p=0.033$) and calcium rich food groups ~141 g/d ($p=0.012$) as compared to the mothers of term babies in the 3rd trimester of pregnancy. Similar trend of low calcium and calcium rich food group intakes were seen in women with PIH as compared to non PIH women. High proportion of preterm babies were born to women with PIH (27.1% versus 7.3%; $p<0.001$) as compared to normotensive mothers. Our findings are suggestive of possible association between calcium and calcium rich food group intakes with PIH and preterm births. To substantiate these findings, large interventional studies in supplemented mothers are warranted.

Key words: calcium intakes, food frequency questionnaire, Pregnancy, Pregnancy induced hypertension (PIH), preterm births.

Recipient of the RAMANATHAN PRIZE for the best oral presentation the 43rd National Conference of the Nutrition Society of India held at the National Institute of Nutrition, ICMR, Hyderabad, India 2011

APPENDIX- C

List of related publications

LIST OF RELATED PUBLICATIONS

Three manuscripts that compliment this thesis but are not from this dataset are listed.

1. Dwarkanath P, Muthayya S, Vaz M, Thomas T, Mhaskar A, Mhaskar R, Thomas A, Bhat S, Kurpad A. The relationship between maternal physical activity during pregnancy and birth weight. Asia Pac J Clin Nutr 2007;16:704-10.

   (Impact factor: 1.438)

2. Muthayya S, Dwarkanath P, Thomas T, Ramprakash S, Mehra R, Mhaskar A, Mhaskar R, Thomas A, Bhat S, Vaz M, Kurpad AV. The effect of fish and omega-3 LCPUFA intake on low birth weight in Indian pregnant women. European Journal of Clinical Nutrition 2009;63:340-6.

   (Impact factor: 2.561)

3. Anura V Kurpad, Pratibha Dwarkanath, Tinku Thomas, Arun Mhaskar, Annamma Thomas, Rita Mhaskar, and Farook Jahoor. Comparison of leucine and dispensable amino acid kinetics between Indian women with low or normal body mass indexes during pregnancy. American Journal of Clinical Nutrition 2010;92:320–9.

   (Impact factor: 6.307)

APPENDIX- D

Patient information sheet & consent form

THE RELATIONSHIP OF MATERNAL MICRONUTRIENT INTAKES OF VITAMIN B_{12}, VITAMIN B_6, FOLATE AND CALCIUM ON INTRAUTERINE GROWTH RETARDATION AND BIRTH WEIGHT: A PROSPECTIVE COHORT STUDY OF URBAN SOUTH INDIAN PREGNANT WOMEN

Study Code: **Hospital Code:** **ANC code:**

Subject Id: **Name:**

PATIENT'S INFORMATION SHEET

Thank you for volunteering your valuable time for this program. This study focuses on what a mother does during pregnancy and how it can affect her baby.

- At the start, we will ask you to fill a set of questionnaires such as your income and age. Trained research assistants will also help you in filling up a detailed history of what you ate (called a food frequency questionnaire), that covers the preceding 3 months of your pregnancy, and will also carry out few measurements such as your weight and height. During that time about two teaspoons (8ml) of blood will be taken from one of your veins by a nurse. This will be used to measure important health indicators and blood nutrients such as hemoglobin, red cell folate, vitamin B_{12}, vitamin B_6, vitamin A, erythrocyte fatty acid, serum ferritin, serum insulin and homocysteine.
- At each month during your pregnancy, your weight will be recorded and what you ate and drank the previous day will be noted. At the same time your blood pressure will also be monitored. These measurements will be performed each month until you deliver.
- At 24 weeks and at 34 weeks of your pregnancy, you will be helped to fill in a food frequency questionnaire. At these time points we will also take another blood sample for the same test as mentioned above.
- At the time of delivery, before discarding the cord, the delivering physician (obstetrician) will collect on our behalf, 3ml of the cord blood (approx 0.75 teaspoon). Similar blood analysis will be carried out on the cord blood. Your baby's weight will be recorded after delivery and baby's body proportions such as length, head, chest, mid upper arm circumferences and skin fold thicknesses will be measured.

Your participation in this study will help us to understand what a mother eats and drinks during pregnancy and how it may affect pregnancy and the baby's body proportions at birth. This will help us tell other mothers what is best to eat and drink during pregnancy.

Date: **Signature:**

Name: Pratibha Dwarkanath MSc

CONSENT FORM

Patient's Statement

I voluntarily consent to participate in the study conducted by the Division of Nutrition, St John's Research Institute at the Obstetrics and Gynecology out patient department. The nature, demands and potential hazards involved in this study has been fully explained to me. I understand that I may withdraw from this study at any time for any reason, and this will not affect the treatment that I will receive".

I consent to the release of scientific data resulting from my participation in this study to the Principal Investigator for use by him/her for scientific purposes. The Principal Investigator assures my anonymity at all stages of communicating these data. I understand that the record of this program becomes part of the Division of Nutrition's medical record system and is protected as a confidential document in a locked cupboard in a locked room. I understand that only the physicians and investigators involved with this study will have access to this information.

In the unlikely event of any physical injury such as discomfort or giddiness resulting from event such as blood draw during my participation in this research, I understand that the basic medical treatment will be provided by St John's Medical College Hospital, including first aid, emergency treatment and follow-up care as needed. However, no monetary compensation will be provided towards medical care, apart from the foregoing. I further understand that making such medical treatment available, or providing it, does not imply that such injury is the fault of the Investigator or team members. I also understand that by participating in this study, I am not waiving any legal rights. I understand that in the case of any problem or for further clarification at any time, I can contact the concerned investigators in this study or the Convener, Institutional Ethical Review Board, St. John's Medical College & Hospital by quoting IERB/1/350/06.

Date: ---------- **Signature: ----------**

Witness: ----------. **Signature: ----------**

APPENDIX- E

Questionnaires

THE RELATIONSHIP OF MATERNAL MICRONUTRIENT INTAKES OF VITAMIN B_{12}, VITAMIN B_6, FOLATE AND CALCIUM ON INTRAUTERINE GROWTH RETARDATION AND BIRTH WEIGHT: A PROSPECTIVE COHORT STUDY OF URBAN SOUTH INDIAN PREGNANT WOMEN

Study Code: **Hospital Code:** **ANC code:**

Subject Id: **Name:**

SOCIO ECONOMIC FORM

1. Subject Name:

2. Subject Id: ☐☐☐☐

3. Hospital Code: ☐☐☐☐☐☐☐

4. ANC Code: ☐☐☐☐ ☐☐

5. Date: ☐☐☐☐☐☐
d d m m y y

6. Interviewer Name: ……

7. Husband's Name:

8. Date of birth (dd.mm.yy) ☐☐☐☐☐☐

9. Age ☐☐ (years): 10. LMP ☐☐☐☐☐☐

11. Religion: a. ☐ Hinduism a. ☐ Christianity b. ☐ Islam d. ☐ Others

12. No of family members in the house:

Adults: ☐☐ Children: ☐☐

13. Type of family: ☐Nuclear family ☐Extended family ☐Joint family

14. Educational information of the subject and her husband.

14a. Subject's Education Highest level obtained	14b. Husband's Education Highest level obtained
☐1. Illiterate	☐1. Illiterate
☐2. Primary school (1-5th std)	☐2. Primary school (1-5th std)
☐3. Middle school (6-8th std)	☐3. Middle school (6-8th std)
☐4. High school (9-10th std)	☐4. High school (9-10th std)
☐5. P.U.C/ Diploma	☐5. P.U.C/ Diploma
☐6. Graduate	☐6. Graduate
☐7. Post graduate	☐7. Post graduate

15. Do you normally work outside the home? (Y/N) ☐

If yes, enter the information in the table

16. Are you currently employed? (Y/N) ☐

17. If not employed, since how many ☐☐ months?

18.Subject		18.Husband	
18a.Occupation	18a. Income	18b. Occupation	18b. Income
☐1. Unemployed ☐2. Unskilled Worker[1] ☐3. Skilled Worker[2] ☐4. Petty business, shop owner ☐5. Secretarial staff, primary school teacher ☐6.Semi-professional, high school teacher ☐7. Professional	..... .rps per months	☐1. Unemployed ☐2. Unskilled Worker[1] ☐3. Skilled Worker[2] ☐4. Petty business, shop owner ☐5. Secretarial staff, primary school teacher ☐6.Semi-professional, high school teacher ☐7. Professional	..... .rps per months

19. No of earning members in the house: | | |

20. Total monthly income of the household Rs: | | | | | | |

21. Standard of Living Index for India (Parasuraman et al. 1999)

Q No	**Household Information**	**Categories**	**Score**
21a	Type of house	Pucca (1)	**4**
		Semi-pucca(2)	**2**
		Katcha (3)	**0**
21b	Does this household own this house or any other house?	Yes	**2**
		No	**0**
21c	How much agriculture land does this household own?	5 acres or >	**4**
		2.0 – 4.9 acres	**3**
		< 2 acres or unknown acreage	**2**
		No land	**0**
21d	Out of this land, how much is irrigated?	Some	**2**
		None	**0**
21e	Does this household own any livestock?	Yes	**2**
		No	**0**
21f	Do you have a separate room which is used as kitchen?	Yes	**1**
		No	**0**
21g	What type of fuel does your household mainly use for coking?	Wood	**0**
		Crop residues	**0**
		Dung cakes	**0**
		Coal/coke/lignite	**1**
		charcoal	**1**
		kerosene	**1**
		electricity	**2**
		Liquid petroleum	**2**
		Gas	**2**
		Bio-gas	**2**
21h	What is the main source of lighting for your household?	electricity	**2**
		kerosene	**1**
		Gas	**1**
		Oil	**1**
21i	What is the main source of drinking water for members of your household?	PIPED WATER	
		Piped into Residence/Yard/Plot	**2**
		Public Tap	**1**
		GROUND WATER	
		Hand pump in residence/Yard/Plot	**2**
		Public Hand pump	**1**
		WELL WATER	
		Well in Residence/Yard/Plot	
		Covered well	**2**
		Open	**2**

		Public well		
		Covered well	**1**	
		Open well	**1**	
		SURFACE WATER		
		Spring	**0**	
		River/Stream	**0**	
		Pond/Lake	**0**	
		Dam	**0**	
		Rain water	**0**	
		Tanker truck	**0**	
21j	What kind of toilet facility does your household have?	FLUSH TOILET		
		Own Flush Toilet	**4**	
		Shared Flush Toilet	**2**	
		Public Flush Toilet	**2**	
		PIT TOILET/ LATRINE		
		Own pit toilet/latrine	**2**	
		Shared toilet/latrine	**1**	
		Public toilet/latrine	**1**	
		NO FACILITY/ BUSH/ FIELD	**0**	
21k	Does this household own any of the following?			
			Yes	No
	A Mattress		**1**	**0**
	A Pressure Cooker		**1**	**0**
	A Chair		**1**	**0**
	A Cot or Bed		**1**	**0**
	A Table		**1**	**0**
	A clock or watch		**1**	**0**
	An electric fan		**2**	**0**
	A bicycle		**2**	**0**
	A radio or transistor		**2**	**0**
	A sewing machine		**2**	**0**
	A telephone		**3**	**0**
	A refrigerator		**3**	**0**
	A black & white television		**2**	**0**
	A color television		**3**	**0**
	A moped, scooter or motorcycle		**3**	**0**
	A car		**4**	**0**
	A water pump		**2**	**0**
	A bullock cart		**2**	**0**
	A thresher		**2**	**0**
	A tractor		**4**	**0**
		Total score *SHome*		

22. Index score: Low 0 – 15 ☐ Medium 16 – 24 ☐ High 25 – 67 ☐

23. Amount spent on the food items per month?

QNo	Consumption of foods	Total amount spent (Rs. per month)
23a	Fruits	
23b	Milk	
23c	Oil	
23d	Rice	
23e	Vegetables	
23f	Sugar	
23g	Dhal	
23h	Wheat	
23i	Ragi	
23j	Fish	
23k	Meat	
23l	Chicken	
23m	Fried foods	
23n	Entire expenditure on food	

B. MEDICAL HISTORY

24. Has a physician ever diagnosed you from any of the following chronic illness?

(exclusion criteria)☐Yes, ☐No

Illness	YES	NO
Hypertension (High blood pressure)	☐	☐
Diabetes (sugar)	☐	☐
HIV	☐	☐
VDRL1 (Syphilis)	☐	☐
Hepatitis B/C	☐	☐
Heart attack/ Angina/ coronary heart disease	☐	☐
Cancer	☐	☐
Others Specify1 ……	☐	☐

25. Has a physician ever diagnosed you from any of the following chronic

illness?☐Yes,☐No

ILLNESS	YES	NO
Tuberculosis	☐	☐
Asthma	☐	☐
Epilepsy	☐	☐
Jaundice (Hepatitis A)	☐	☐
Kidney problems	☐	☐
Others Specify2 ……..	☐	☐

26. Any past complications during your previous pregnancy? ☐Yes, ☐No
(Information confirmed from reports/ discharge summary/ doctors chart)

COMPLICATIONS	YES	NO
PIH (High blood pressure)	☐	☐
GDM (sugar/ big baby)	☐	☐
intermittent spotting	☐	☐
Placental previa	☐	☐
ectopic pregnancy	☐	☐
recurrent abortion	☐	☐
recurrent UTI	☐	☐
VDRL	☐	☐
others Specify3 ……	☐	☐

C. OBSTETRICS HISTORY

27. Previous obstetrics history ☐Yes, ☐No

(Information confirmed from reports/ discharge summary/ doctors chart)

Order of children	Pregnancy outcome	Gest week	Gender of the child	Birth weight	Type of delivery	Complications
1.	a.	b.	c.	d.	e.	f.
2.	a.	b.	c.	d.	e.	f.
3.	a.	b.	c.	d.	e.	f.
4.	a.	b.	c.	d.	e.	f.

D. HABITS

28. Do you consume or consumed tobacco in any form? a.☐ yes, b.☐ no.

If yes to Q 28, fill in the table.

Type of tobacco	Current usage		Type of tobacco	Past usage	
	Frequency per week (no/week)	No of years of use		Frequency per week (no/week)	No of years of use
28a. Beedi (no)			28a. Beedi (no)		
28b. Cigarette (no)			28b. Cigarette (no)		
28c.Snuff (no of pinch)			28c.Snuff (no of pinch)		
28d. Chew tobacco (no of sachets)			28d. Chew tobacco (no of sachets)		

29. Are you exposed to other people's smoke (cigarette/beedi) for more than 5 mins consecutively? a.☐ yes, b. ☐ No.

If yes, location: home ☐/ workplace ☐

Details of exposure	Home		Workplace
Parameters relationship to self	Smoker 1	Smoker 2	Smoker 1
29a. No. of cigarettes/beedis smoked per day			
29b.Smoking in your presence < 5 min at a time (yes=1; no=0)	yes ☐ no ☐	yes ☐ no ☐	yes ☐ no ☐
29c. Exposure lasting > 5 min at a time (yes =1; no=0)	yes ☐ no ☐	yes ☐ no ☐	yes ☐ no ☐

relationship with the subject (at home) specify ……

30. If yes to Q 29, total no of smokers exposed to in a day ☐ ☐

31. Whether your's is a consanguineous marriage a.☐ yes, b.☐ no

ANTHROPOMETRIC INFORMATION (1st, 2nd and 3rd trimester)

1. Name:

2. Subject Id: | | | | |

3. Hospital Code: | | | | | | | |

4. ANC Code: | | | | | | |

Measurements	TRIMESTER 1,2 or 3		
Date			
Gest Age			
Head cir (cm)			
Height (cm)			
Weight (kg)			
Waist cir (cm)			
Hip cir (cm)			
MUAC (cm)			
biceps skin fold (cm)			
triceps skin fold (cm)			
Subscapular skin fold (cm)			

4. **Blood Pressure reading during 1st, 2nd or 3rd trimester:**

	Trimester 1,2 or 3		
	BP1	BP2	Clinical BP1
Systolic BP			
Diastolic BP			

INVESTIGATION FORM (Trimester 1, 2 & 3 trimester)

1. Name:
2. Subject Id: ☐☐☐☐
3. Hospital Code: ☐☐☐☐☐☐☐
4. ANC Code: ☐☐☐☐ ☐☐
5. Date: ☐☐☐☐☐☐ d d m m y y

SCAN DETAILS in the 2nd trimester:

Gest age ☐☐•☐ LMP ☐☐☐☐☐☐

PARAMETERS Date:	☐☐☐☐☐☐
CRL (mm)	☐☐☐•☐
CRL Gest Age	☐☐•☐
Gest Sac	☐☐•☐
Gest Sac gest age	☐☐•☐
Yolk Sac	☐☐•☐
Yolk Sac gest age	☐☐•☐
BPD (mm)	☐☐☐•☐
BPD Gest Age	☐☐•☐

FL (mm)	□□□•□
FL Gest Age	□□•□
HC (mm)	□□□•□
HC Gest age	□□•□
AC (mm)	□□□•□
AC Gest Age	□□•□
FHR (bpm)	□□□•□
FHR Gest age	□□•□
EDD	□□□□□□ d d m m y y
AFI (cm)	□□•□
EFBW (mm)	□□□□
Impression wks	□□•□
Impression	

Routine blood investigation

INVESTIGATIONS	**1st TRI**
HIV 1=positive, 2= negative	
VDRL 1=positive, 2= non reactive	
HBsAG 1=positive, 2= negative	
Blood Group	
Glu random	
PPBS	
Glu fast	
Glu 60 min	
Glu 120 min	

Routine investigations in the 2nd or 3rd trimesters

Hemoglobin	
Glu random	
PPBS	
Glu fast	
Glu 60 min	
Glu 120 min	
Routine urine	
Urine Albumin 1=nil, 2=traces	
Others	

Blood group:
1=O postive, 2=A positive, 3=B positive, 4=AB positive, 5=O negative, 6=A negative. 7=B negative, 8=AB negative

SUPPLEMENT FORM (Trimesters 1, 2 & 3)

1. Name:

2. Subject Id: ☐☐☐☐

3. Hospital Code: ☐☐☐☐☐☐☐

4. ANC Code: ☐☐☐☐ ☐☐

5. Date: ☐☐☐☐☐☐

d d m m y y

6. Have you suffered from any of the following medical conditions during the last trimester? ☐ yes; ☐ no

ILLNESS	Yes/No	Frequency	Duration of illness(no of days/episode)
Loose motion/diarrhea/ dysentery)	a.☐ ☐	b.☐☐	c.☐☐
Vomiting	a.☐ ☐	b.☐☐	c.☐☐
Giddiness/ Tiredness	a.☐ ☐	b.☐☐	c.☐☐
Tiredness	a.☐ ☐	b.☐☐	c.☐☐
Fever	a.☐ ☐	b.☐☐	c.☐☐
Cough/cold	a.☐ ☐	b.☐☐	c.☐☐
Wheezing	a.☐ ☐	b.☐☐	c.☐☐
Gastritis	a.☐ ☐	b.☐☐	c.☐☐
Constipation	a.☐ ☐	b.☐☐	c.☐☐

Intermittent spotting	a.☐ ☐	b.☐☐	c.☐☐
UTI	a.☐ ☐	b.☐☐	c.☐☐
Abdominal pain	a.☐ ☐	b.☐☐	c.☐☐
Itching	a.☐ ☐	b.☐☐	c.☐☐
Others	a.☐ ☐	b.☐☐	c.☐☐

7. Do you consume any vitamins or mineral supplement atleast once a week?

a.☐Yes, b. ☐ No

	Brand name / Type	**Dosage**	**No. / Week**
Folic Acid			
Iron			
Calcium			
Vit A			
Vit C			
B complex			
Multi Vit			
Others			

8. Have you taken any supplements before conception a. ☐Yes, b. ☐ No

	Brand name / Type	**Dosage (mg.)**	**No. / week**
Folic Acid			
Iron			
Calcium			
Vit A			

Vit C			
B complex			
Multi Vit			
Others			

9. Currently are you on any medication prescribed by the physician?

a. ☐Yes, b. ☐ No

If yes specify…….

EATING HABITS DURING PREGNANCY

1. Name:

2. Subject Id: ☐☐☐☐

3. Hospital Code: ☐☐☐☐☐☐☐

4. ANC Code: ☐☐☐☐ ☐☐

5. Date: ☐☐☐☐☐☐
d d m m y y

6. LMP ☐☐☐☐☐☐
d d m m y y

7. Gest age by Scan ☐☐•☐ 8. Gest age by LMP ☐☐•☐

9. How would you describe your food habits?

a. ☐ Vegetarian=1, b.☐ Non-vegetarian=2

10. Which is the cereal that you most consume?

a. ☐ Rice, b. ☐ Ragi, c. ☐ Wheat, d. ☐ Jowar, e. ☐Maize

11. Which is the second cereal that you most consume?

a.☐ Rice, b.☐ Ragi, c.☐ Wheat, d.☐ Jowar, e.☐Maize

12. How many litres of these oils/fats does your family consume in a month?

Code	Type	Weekly (ml.)	Monthly (Kg.)
a	☐ Sunflower oil	a. ☐☐☐	b. ☐☐.☐☐
b	☐ Groundnut oil	a. ☐☐☐	b. ☐☐.☐☐
c	☐ Coconut oil	a. ☐☐☐	b. ☐☐.☐☐
d	☐ Palm oil	a. ☐☐☐	b. ☐☐.☐☐
e	☐ Mustard oil	a. ☐☐☐	b. ☐☐.☐☐
f	☐ Dalda / Vanaspati	a. ☐☐☐	b. ☐☐.☐☐
g	☐ Butter	a. ☐☐☐	b. ☐☐.☐☐
h	☐ Ghee	a. ☐☐☐	b. ☐☐.☐☐
i	☐ Olive oil	a. ☐☐☐	b. ☐☐.☐☐
j	☐ Corn oil	a. ☐☐☐	b. ☐☐.☐☐
k	☐ Rice bran oil	a. ☐☐☐	b. ☐☐.☐☐
l	☐ Others, specify	a. ☐☐☐	b. ☐☐.☐☐

13. How many coconuts do you use for cooking in a month? (No. per month)☐☐

14. Do you add any of the following as a thickening agent for cooking at least 2 times a week?

a. ☐Yes, b. ☐ No

14a. If yes to Q14, tick the options.

a. ☐Coconuts, b. ☐ Groundnuts, c. ☐ Roasted bengal gram dhal

15. Which is the most common method of cooking vegetables?

a. ☐ pressure cooker, b. ☐ frying, c. ☐ with lid open, d. ☐ with lid closed,

e. ☐ prolonged cooking

16. Do you discard excess water after cooking vegetables? a. ☐Yes, b. ☐ No

17. How many meals do you consume in a day?

a. ☐ 1 meal, b. ☐ 1, 2 meals, c. ☐ 3 meals, d. ☐ >3 meals

18. Do you routinely remove fat/skin from meat before cooking?

a. ☐Yes, b. ☐ No

19. What type of milk do you regularly consume?

a. ☐ whole milk, b. ☐ skimmed milk, c. ☐ toned milk, d. ☐skimmed milk powder

20. Have you taken your 1st tetanus dose as prescribed by your physician

a.☐Yes, b.☐ No

21. Have you taken your 2nd tetanus dose as prescribed by your physician

a.☐ Yes,b.☐No

22. Do you consume tinned/canned food? a. ☐Yes, b. ☐ No

22a.If yes to Q.22 how many tins/cans do you consume in a month?
Specify…….

23. Are you on any special diet? a. ☐Yes, b. ☐ No

23a.If yes to Q.23, what diets are currently following:

a.☐ Diabetics diet, b.☐Low fat diet, c.☐ high fiber diet, d.☐Low salt diet,

e.☐Weight reducing diet, f.☐Others, specify----------

24. Since how many years are you on this special diet? Specify……..

Physical activity questionnaire

PHYSICAL ACTIVITY QUESTIONNAIRE							Study Code ID
Name			Study Code				
Occupation-			Age-	Height (cms)-			Weight(Kgs)-
1a. On an average, how many hours per day do you spend at work?							
1b. How many days in a week do you work?							
1c. Of the hours you spend at work, how many hours do you spend in							
Standing	Sitting	Walking	On activities more strenuous than walking				
2. On an average, how many hours do you sleep in a day?							
3. Apart from work, how do you spend your time. Fill in the table below							

TYPE OF ACTIVITY		**Daily**	**Weekly**			**Monthly**	
(Over the last three months)	Average Duration (mins)		Once	2 -4	4-6	once	2-3
Exercise							
Hobbies involving manual labour (for e.g. gardening)							
1.							
2.							
3.							
4.							
Household chores (for e.g. sweeping, cooking, washing etc.)							
1.							
2.							
3.							

4.							
5.							
6.							
Sedentary activities for e.g. Reading, watching TV, etc.							
1.							
2.							
3.							
4.							
Other Activities							
1. Eating							
2. Brushing & Bathing							
3. Dressing							
4. Socializing (talking)							
5. Travelling to and from work							

DELIVERY INFORMATION

1. Name_____

2. Sub Id: ☐☐☐☐

3. Hospital Code: ☐☐☐☐☐☐☐

4. ANC Code: ☐☐☐☐ ☐☐

5. Date of delivery: ☐☐☐☐☐☐

6. Time of delivery: ☐☐ ☐☐

7. Place of delivery: 1. ☐ St John's, 2. ☐ other medical facility

8. EDC: ☐☐☐☐☐☐

9. Gestational age at birth: by LMP ☐☐☐☐

9a. Gestation age at birth: by scan ☐☐☐☐

10. Gestational age: by 1st scan ☐☐☐☐

10a. Gestational age: by 3rd scan ☐☐☐☐

11. RISK FACTORS: a.☐ yes, b.☐ no.
(Information from the doctor's notes)

Risk Factors	YES	NO	If yes, specify
a. Antenatal	☐	☐	
b. Intranatal	☐	☐	
c. Postnatal	☐	☐	
d. Perinatal	☐	☐	

12. Mode of delivery:
(Information from the doctor's notes)

Mode of delivery	Yes	No
Vaginal	☐	☐
Spontaneous/ assisted	☐	☐
Vacuum	☐	☐
Forceps	☐	☐
Outlet/LSCS/AT/Kielland	☐	☐

13. Pregnancy outcome: 1.alive ☐ 2.dead ☐

14. Birth Details:
(Information from the doctor's notes)

BIRTH DETAILS	MEASURMENTS
Cord length (cm)	☐☐☐
Placenta (gm)	☐☐☐☐
Birth weight (gm)	☐☐☐☐
Gender	☐ Male ☐ Female
APGAR score	☐ 1min ☐ 5 min
Birth diagnosis 1=Normal, 2=preterm, 3-SGA, 4=Still birth, 5=IUG, 6=neonatal death	. ☐

15. Resuscitation: ☐Needed ☐ Nil:
if yes, specify……..

16. Vitamin K immunization : a.☐ yes, b.☐ no.

17. Was the baby admitted in NICU after birth: (yes=1/no=2)

a.☐ yes, b.☐ no.

No of times	Age (in days/month)	Duration of stay	Reason of admission

18. Blood pressure of the mother after delivery

Systolic Blood pressure: ☐☐☐ Diastolic Blood pressure: ☐☐☐

19. Pulse reading: ☐☐

20. Baby Blood Group: _____

21. Anthropometry of the neonate within 24 hours of birth

Date: | | | | | | |

Measurements	Reading 1	Reading 2	Reading 3
Baby weight (gm)			
Baby length (cm)			
MUAC (cm)			
Head circumference (cm)			
Chest circumference (cm)			
biceps skin fold (cm)			
triceps skin fold (cm)			
subscapular skin fold (cm)			
suprailiac skin fold (cm)			
Waist Circumference (cm)			
Hip Circumference (cm)			

22. Has the subject successfully completed the study

a. ☐ yes, b. ☐ no

22a. If no reason: _____

23. Date of completion / termination | | | | | | |

APPENDIX- F

Food frequency questionnaire

FOOD FREQUENCY QUESTIONNAIRE (URBAN)							
Name	Subject ID		Visit No			Interviewer	
Date							
FOODS LIST	***PORTION SIZE***	***AVERAGE CONSUMPTION***	***PER DAY***	***PER WEEK***	***PER MONTH***	***PERYEAR / NEVER***	**SEASONAL**
CEREALS							
Idlis	No						
Urud vada	No						
Plain dosa, uttappam etc.	No						
Masala Dosa	No						
Roti, pulkhas	No						
Chapathi, parathas etc.	No						
Poori, bhatura	No						
Uppuma, all types	Bowl						
Ragi ball	Ball						
Porridge	Bowl						
Corn flakes, cereal flakes etc.	Bowl						

Plain cooked polished rice	Bowl						
Plain cooked unpolished rice	Bowl						
Avalakki	Bowl						
Lime rice, veg pulao, puliyogre etc.	Bowl						
Bisibile bath, khichidi	Bowl						
Non-vegetarian fried rice, biriyani	Bowl						
Veg noodles, macroni, pasta, etc	Bowl						
Non-veg noodles, macroni, pasta etc.	Bowl						
Bread slices, toast, rolls, buns etc.	Slice/No						
Pizza, Burgers	No						
LENTILS/DHALS/GRAVIES							
Rasam	Bowl						
Sambar	Bowl						
Dhal	Bowl						
Channa, rajma, dry peas etc. curry	Bowl						
Vegetable kurma	Bowl						
Green leafy vegetable curry	Bowl						

Formatted: French (France)

Paneer gravy	Bowl						
CHUTNEYS							
Coconut chutney	Tbsp						
Groundnut chutney	Tbsp						
Tomato chutney	Tbsp						
Chutney powder	Tbsp						
SOUPS/SALADS/OTHERS							
Cream soup (veg or non-veg)	Bowl						
Clear soup	Bowl						
Fresh vegetable salad	Tbsp						
Raw carrots	No						
Vegetable raitha	Tbsp						
Pickle	Tsp						
Papad fried, sandige, vathal etc.	No						
Papad roasted	No						
NON VEGETARIAN / EGGS							
Lamb, beef, pork - fry	Tbsp						

Lamb, beef – cutlet	No						
Lamb, beef, pork - curry	Bowl						
Liver, brain, kidney etc.	Tbsp						
Chicken – curry	Bowl						
Chicken fry – roasted, grilled	No						
Fish – fry	No						
Fish – cutlet	No						
Fish - curry	Bowl						
Prawn, crab, shell fish etc.	Tbsp						
Dried fish, dried seafood	Tbsp						
Egg - boiled, poached	No						
Egg - fried, scrambled	No						
Egg gravy	Bowl						
Egg omelette	No						
Non-veg rolls	No						
Ham, salami, bacon etc.	Slices						
Sausages	No						

Non-veg kebab	No						
SNACKS							
Mixture, namkeen	Tbsp						
Nuts	Tbsp						
Murukku ,chakkli	No						
Chips, french fries	Bowl						
Masala vada Samosa, bajji , bonda	No No						
Chaat	Bowl						
Veg & non-veg puff	No						
Roasted or boiled corn	No						
Biscuits (salted)	No						
Biscuits (sweet, creamed, etc)	No						
DESSERTS / SWEETS							
Cakes or sweet pastries	No						
Puddings, souffle , custard	Bowl						
Payasam, kheer	Bowl						

Ice cream	Bowl						
Kesari bath	Tbsp						
Jamoon, jilebi, jangir etc.	No						
Mysore pak, ladoo, etc.	No						
Others, specify_____	No						
Indian milk sweet	No						
Chocolate pieces	Bar						
Candy	No						
BEVERAGES & MILK							
Plain milk	Glass						
Flavored Milk (bournvita, Horlicks)	Glass						
Curd, yoghurt	Bowl						
Buttermilk	Glass						
Tea	Glass						
Coffee	Glass						
Fresh fruit juice	Glass						
Soft drink, others etc.	Bottle						

Beer	Glass						
Wine	Glass						
Spirits (rum, whiskey etc.)	30 ml						
MISCELLANEOUS							
Butter/ cream	Tsp						
Ghee	Tsp						
Jam	Tsp						
Sugar	Tsp						
Cheese	Cube						
Ketchup, tomato sauce	Tbsp						
Added salt	Tsp						
FRUITS							
Banana	No						
Apple (S = 3 mo.)	No						
Orange (S = 4 mo.)	No						
Sweet lime (S = 4 mo.)	No						
Mango (S = 4 mo.)	No						

Guava	No						
Grapes	Bowl						
Pineapple	Slice						
Papaya	Slice						
Pomegranate (S = 3.5 mo.)	No						
Custard apple (S = 3 mo.)	No						
Sapota or Chikoo	No						
Watermelon (S = 3 mo.)	Bowl						
Jackfruit	No						
Fruit salad	Bowl						
Dried fruits	Tbsp						
VEGETABLES							
Palak, methi, other leafy vegetables	Tbsp						
Potato masala	Tbsp						
Carrot	Tbsp						
Beetroot, raddish, yam, etc.	Tbsp						
Cabbage	Tbsp						

Beans, cluster beans	Tbsp						
Ladiesfinger	Tbsp						
Cauliflower (S = 3 mo.)	Tbsp						
Chowchow, pumpkin, gourds, etc.	Tbsp						
Capsicum or green pepper	Tbsp						
Drumstick (S = 3 mo.)	Pieces						
Brinjal	Tbsp						
Mushrooms	Tbsp						
Fresh peas (S = 3 mo.)	Tbsp						

APPENDIX- G

24-Hour dietary recall

TWENTY FOUR HOUR FOOD RECALL - DATE ( )

TIME	MEALS	INTAKE	SERVINGS
	Morning		
	Breakfast		
	Mid meal		
	Lunch		
	Supper		
	Dinner		
	Bed time		

G = Glass, t = Teaspoon, T = Table spoon, B = Bowl, Ball to measure the ragi ball, Scale to measure the chapathi, paratha etc.

Standardised measures used for recording dietary intakes.