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FACULTY OF MEDICINE

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The effect of a micronutrient-rich food supplement on women's health and nutrient status

by

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Thesis for the degree of Doctor of Philosophy

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ABSTRACT

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THE EFFECT OF A MICRONUTRIENT-RICH FOOD SUPPLEMENT ON WOMEN'S HEALTH AND NUTRIENT STATUS

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Background: The prevalence of type two diabetes (T2D) is rapidly increasing in India and is predicted to affect 7% of the population by 2030. There is evidence that maternal nutrition before and during pregnancy affects foetal development and risk of T2D in the offspring. Green leafy vegetables (GLV), fruit and milk are micronutrient-rich foods that are acceptable to most of the Indian population. Intakes of these foods during pregnancy were positively associated with birth size in an observational study in rural India. The Mumbai Maternal Nutrition Project (MMNP) was a randomised controlled trial (RCT) that investigated the effect of a supplement containing these foods on offspring birth size and T2D risk factors in a slum population.
Objectives: 1) to conduct a systematic review of interventions assessing the effect of increasing fruit and vegetable intakes on micronutrient status of women of reproductive age; 2) to run a RCT to study the effect of consumption of the MMNP supplement on micronutrient status among low income, non-pregnant women aged 15-35y (MMNP Extension Study); 3) to study diet patterns among MMNP participants.
Methods: Published data on the effect of fruit and vegetable interventions on change in micronutrient status among women of reproductive age were identified by systematic review. The Extension Study was conducted in the Shivaji Nagar area of Mumbai in 2009. Women (n=222) were randomised to receive the MMNP intervention supplement or a control supplement daily for 12 weeks. Blood samples were collected at baseline, after 6 and 12 weeks of supplementation and analysed for retinol, β-carotene, folate, vitamin B12, vitamin C and ferritin concentrations. Diet, anthropometry, grip strength, blood pressure and self-reported health were assessed at baseline and after 12 weeks. A principal component analysis (PCA) of food frequency data was used to identify diet patterns among MMNP participants (n=4816).
Results: The systematic review identified 14 studies. Most had small numbers of participants and 13 were conducted in high income countries. Where assessed, vitamin C and β-carotene status improved in over 60% of the studies while findings were inconsistent for other nutrients. In the Extension Study, blood micronutrient data pre and post intervention were available for 170 women (77% of those randomised). The intervention supplement was associated with an increase in circulating β-carotene concentrations. There was no effect of the supplement on any of the other nutrients measured, or on the functional outcomes. Intakes of fruit, vegetables and milk were low in the MMNP and the Extension Study. Three diet patterns were identified. The first was characterised by frequent intakes of snacks and fruit, the second by non-vegetarian foods and the third by fish and coconut. Adherence to diet patterns was associated with age, education, occupation and standard of living.
Conclusions There is a lack of data on the impact of fruit and vegetable-based interventions on micronutrient status of young women in low income countries. The MMNP supplement may be an effective method to improve circulating concentrations of carotenoids in an Indian slum population. The findings from the PCA may be useful for informing diet guidelines, monitoring trends in diet behaviour and designing interventions to improve diet quality in this setting.

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Author's Contribution

The work in this thesis is largely based on two randomised controlled trials. 1) The Mumbai Maternal Nutrition Project (MMNP); 2) the MMNP Extension Study. Details of the contributions of myself and others are as follows:

1) The MMNP was conceived and designed by Caroline Fall and Ramesh Potdar with input from Barrie Margetts, Nick Brown, David Barker, HPS Sachdev and Alan Jackson. The project was managed by Siraj Sahariah and Meera Gandhi. Devi Shivashankaran, Purvi Chheda, Preeti Adekar, Harsha Chopra and I devised and developed the supplementation recipes and managed dietary data collection and nutrient status assessment. Data were collected by paediatricians and several project assistants. The MMNP was launched in January 2006 and I have worked on all nutritional aspects of the trial from September 2006 to date.

2) In 2008, I conceived and designed the Extension Study with input from Caroline Fall, Barrie Margetts, Ramesh Potdar, Dnyaneshwar Tarwande, Leena Joshi and the MMNP team. Harsha Chopra and I co-managed the study. Harsha Chopra, Bhavya Rhanadive, Vaishali Thakur and I collected data. Eleven health workers sensitised the community to the study, identified potentially eligible women and maintained contact with the women throughout the study. Three project assistants; Fouziya Sayeed, Manju Rajbhar and Gazala Sahik assisted with data collection, distributed supplements and recorded participant compliance with the protocol. Harsha and I processed the blood samples.

The Nair Hospital, Mumbai, MRC Human Nutrition Research Unit, Cambridge, Dr Dharap's Diagnostic Centre, Mumbai and the Diabetes Unit at the King Edward Memorial Hospital, Pune analysed blood samples. Patsy Coakley, Harshad Sane and Vanessa Cox managed the database, cleaned data and built analysis files. I conducted all statistical analysis with input from Clive Osmond, Sarah Crozier, Aravinda Guntupalli, Nicola Winder and Ella Marley-Zagar.

I conceived and designed the systematic review. The review was conducted by Elena Rayner, Barrie Margetts and myself.

DECLARATION OF AUTHORSHIP

I, Sarah Kehoe declare that the thesis entitled

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and the work presented in the thesis are both my own, and have been generated by me as the result of my own original research. I confirm that:

- this work was done wholly or mainly while in candidature for a research degree at this University;
- where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
- where I have consulted the published work of others, this is always clearly attributed;
- where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
- I have acknowledged all main sources of help;
- where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
- part of this work has been published as:

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Signed: ……..

Date:……

Acknowledgements

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Dr Ramesh Potdar, Dr Siraj Sahariah, Meera Ghandi and all the team at CSSC have been extremely helpful and supportive during my visits to India and I am very much inspired by the valuable work they have done and continue to do in running the Mumbai Maternal Nutrition Project.

My colleague Harsha Chopra has been a cheerful and conscientious research partner and a pleasure to work with. I appreciate all of the advice and support in planning and conducting the Extension Study from Leena Joshi and Dnyaneshwar Tarwande of Apnalaya. I thank the team that collected the data for the Extension study; Bhavya Rhanadive, Vaishali Thakur, Fouziya Sayeed, Manju Rajbhar, Gazala Sahik and all of the health workers at Apnalaya.

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This work would not have been possible without the time and interest of the women who took part in the MMNP and the Extension Study. I am extremely grateful to all of the participants and their families for their involvement.

Finally I thank Jamie for all the hot dinners and for being a hero! I thank my parents for the genes and environmental exposures that made me who I am. I am also very grateful for all of their support with my studies and for providing me with the opportunities to travel. Thanks also to Jon and Katherine, and all of my family and friends for their help and encouragement over the last four years.

Definitions and Abbreviations

BHT	Butylated hydroxytoluene
BMI	Body mass index
CBC	Complete blood count
CRP	C-reactive protein
CSSC	Centre for Study of Social Change
CVD	Cardiovascular disease
DASH	Dietary approaches to stop hypertension
DOHaD	Developmental Origins of Health and Disease
DNA	Deoxyribonucleic acid
EAR	Estimated average requirement
FAO	Food and Agriculture Organisation
FFQ	Food frequency questionnaire
FPIA	Fluorescence polarization immunoassay
GHQ	General health questionnaire
GLV	Green leafy vegetable
GNI	Gross national income
GRIN	Germplasm Resources Information Network
HDI	Human development index
HDL	High density lipoprotein
HEI	Healthy eating index
HIV	Human immuno deficiency virus
HPLC	High performance liquid chromatography
IFPRI	International Food Policy Research Institute
IGT	Impaired glucose tolerance
INCAP	Institute of Nutrition of Central America and Panama
IQR	Inter quartile range
ISX	Intestinal transcription factor
IUGR	Intra-uterine growth retardation
KMO	Kaiser-Meyer-Olkin

LBW	Low birthweight
LMIC	Low and middle income country
MDG	Millennium development goal
MMNP	Mumbai Maternal Nutrition Project
MPA	Metaphosphoric acid
MUAC	Mid-upper arm circumference
NCD	Non-communicable disease
NFHS	National Family Health Survey
NGO	Non-government organisation
PCA	Principal component analysis
PMNS	Pune Maternal Nutrition Study
PRISMA	Preferred reporting items for systematic reviews and meta-analyses
RAE	Retinol activity equivalent
RDA	Recommended daily allowance
SAH	S-adenosyl-L-homocysteine
SD	Standard deviation
SGA	Small for gestational age
T2D	Type 2 diabetes
Hcy	Homocysteine
UN	United Nations
UNDP	United Nations Development Programme
UNIMMAP	United Nations Multiple Micronutrient Preparation
USDA	US Department of Agriculture
VMNIS	Vitamin and Mineral Nutrition Information System
WHO	World Health Organisation

1. Introduction

This chapter introduces the setting of the Mumbai Maternal Nutrition Project (MMNP) and the MMNP Extension Study both of which were randomised controlled trials of a food-based intervention among women of reproductive age. The trials were conducted in slum areas of the Indian city of Mumbai (section 1.1.1). A background to the population health issues in India is given in section 1.1.2. The increasing prevalence of chronic disease alongside the persistent problems of communicable disease and undernutrition occurring in many low and middle income countries (LMIC) has been termed the 'double burden of disease'. This phenomenon is discussed in sections 1.1.3 and 1.1.4.

The concept of sub-optimal early development is introduced in section 1.2 and evidence for the association between early development and the risk of later chronic disease is summarised in section 1.3. Findings from studies investigating the relationship between maternal nutrient intake and offspring health are described in section 1.4. Data from national, state and local level surveys describing the nutritional status and dietary intakes of young Indian women are presented and discussed in section 1.5.

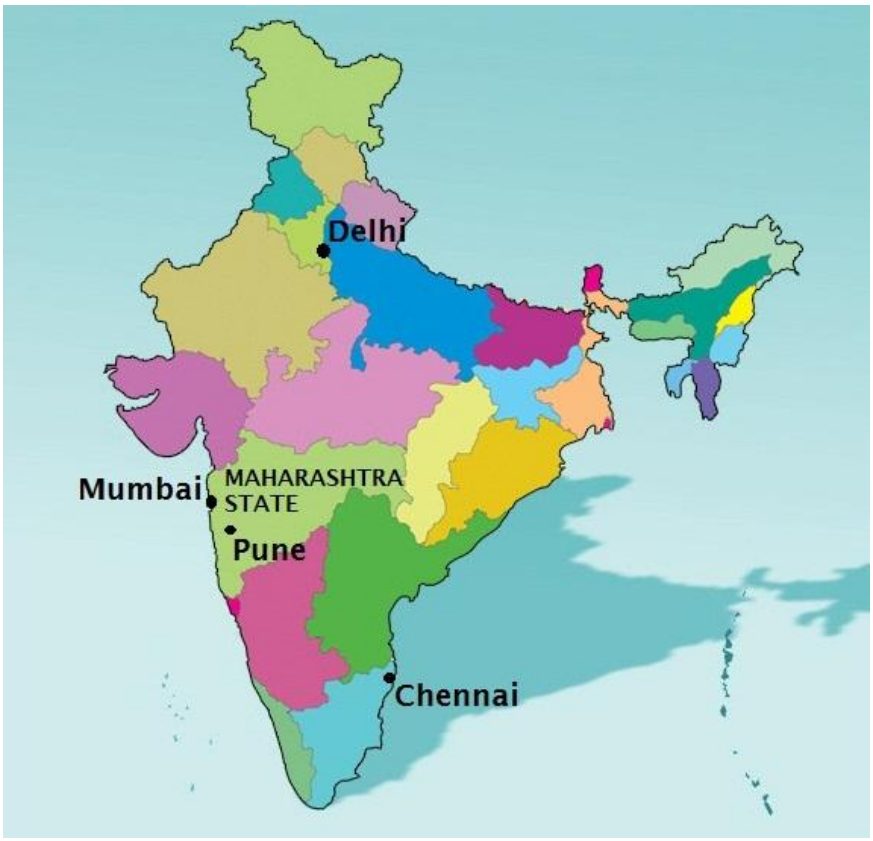
The MMNP was designed to investigate the effects of pre-conceptional maternal supplementation with a food-based intervention on birth size, mortality and developmental outcomes in the offspring (section 1.6). The objectives of the work contained in this thesis are given in section 1.7.

1.1 Study setting and population health in India

1.1.1 Introduction to the study setting; Mumbai slums

Situated on the west coast of the state of Maharashtra (**Figure 1.1**), Mumbai (Bombay) is the state capital and is thought to be India's most populous city with 12.5 million inhabitants according to Indian census data (1). In 2011, the combined population of the urban agglomeration of Mumbai and the nearby cities of Thane and Navi Mumbai was estimated at 19.7 million and is projected to rise to 26.6 million by 2025 (2).

Figure 1.1 Map of India showing the location of Mumbai, capital of the state of Maharashtra



Until the 16th century Mumbai consisted of seven islands and its geography was such that it was an ideal natural harbour. The city was named after the Goddess Mumba Devi, She was worshipped by Koli fishermen who were the original inhabitants of the islands before they were colonised by the Portuguese in the 16th century. In 1661 it became a British colony and was leased to the East India Company and developed into a major port and ship-building yard. It is now the financial hub of India and a cosmopolitan city with people from all over India and the world making it their home. The local language is Marathi.

The inner city area is approximately 603km^2. The average living space in Mumbai has been calculated as 4.5m^2 per person (3). The average space among slum dwellers is likely to be much smaller than this. It has been estimated that approximately 60 % of Mumbai's inhabitants live in slums (3). Slums have been defined as having the following physical and legal characteristics: inadequate access to safe water and sanitation; poor structural quality of housing; overcrowding; insecure tenure (4). There is a large degree

of variation between slum areas in terms of these characteristics. Slum housing in Mumbai for example can range from makeshift 'kacha' huts constructed using wood and plastic sheeting (**Figure 1.2**) to more structurally sound 'pukka' dwellings with concrete floors and walls, and a reliable power supply (**Figure 1.3**). The literacy rate among slum dwellers in Mumbai is 69% and it has been claimed that Mumbai's slums are the most literate in India (5).

1.1.2 Population demographics and health in India.

In 2010 the population of India was 1.22 billion with 70% living in rural areas (20% in the UK). The population density was 373 per km^2 (255 per km^2 in the UK). Between 2005–2010, the average life expectancy at birth of males was 62.8 years (77.4 in the UK) and 65.7 for females (81.7 in the UK) and of those who reached the age of 15 years, the proportion who survived until the age of 60 years was 779/1000 (921/1000 in the UK) (2).

In 2011 the gross national income (GNI) per capita in India was $1,410 ($37,780 in the UK). Based on this, India is classified by the World Bank as a 'lower middle income country' though its GNI is towards the lower end of the range for this category (GNI $1,026–4,035). The proportion of the population living below the national poverty line ($1.25/day at purchasing power parity exchange rates) was 29.8% in 2010 (6).

The Human Development Report published by the United Nations Development Programme (UNDP) calculates a Human Development Index (HDI) score (7). The HDI is derived using three indicators: a long healthy life; access to knowledge; standard of living. In 2011 India had a HDI score of 0.547 (0.863 in the UK) and was ranked 134 out of 187 countries (UK, 28 out of 187). India is a large, populous and diverse country and there are many disparities in terms of these three indicators. A small number of individuals at the higher end of the distribution for one or more indicators may influence the score disproportionately. When inequalities were accounted for, the score for India was 0.392, 28% lower than the overall index score. Almost half of this reduction was explained by mean duration of education. Inequalities in life expectancy and income accounted for 33% and 18% of the reduction respectively.

Figure 1.2 Kacha dwellings in a Mumbai slum (Shivaji Nagar)

Figure 1.3 Pukka dwellings in a Mumbai slum (Bandra East)

1.1.2.1 Gender inequalities in India

The UNDP Gender Inequality Index assessed the disparities in access to education, longevity and income between men and women. In 2011, India was ranked 129 out of 146 countries (UK was 34/146). Just over a quarter of women received education to secondary level or above compared with half of males (70% for both sexes in the UK). A third of women participated in the labour market compared with 80% of males (55.3% and 69.5% respectively in the UK) (7).

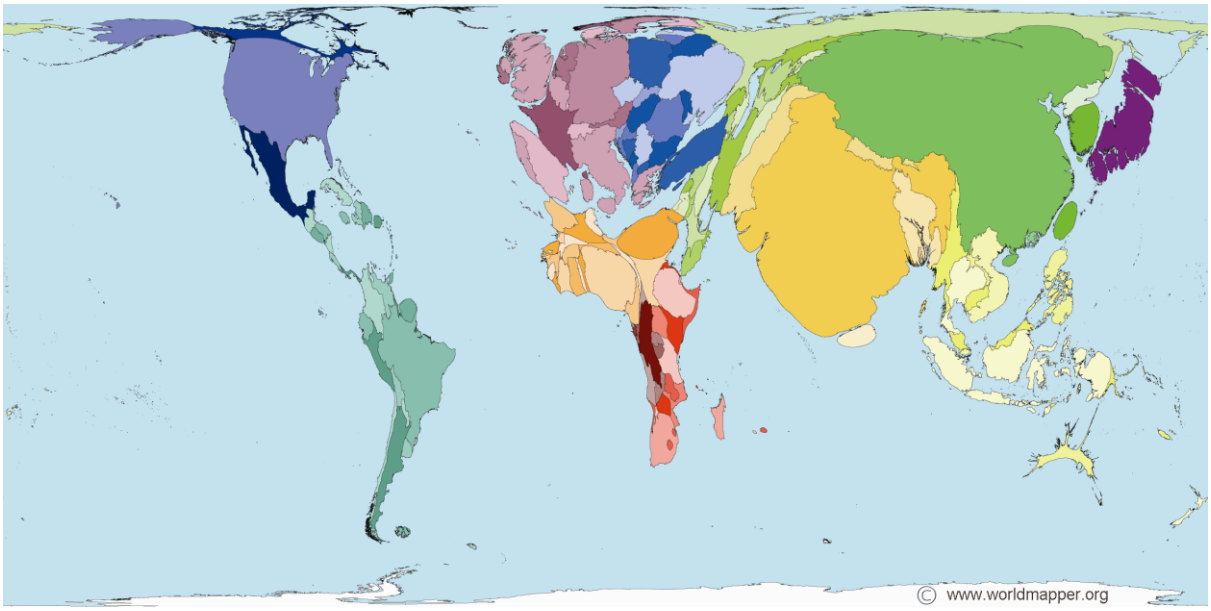
In 2010 the sex ratio in India was 108 males to 100 females (105:100 in the UK) (2). This may be an indication of the selective aborting of female foetuses (8).

In the same year, the median age of child-bearing in India was 25.3 years (29.2 in the UK) (2). This was the lowest of all United Nations (UN) countries with populations over 100,000. In 2011 the maternal mortality ratio was 230/100,000 live births (12/100,000 in the UK). The proportion of births attended by a health professional in 2005-6 in India overall was 47% and in urban areas was 62% (9). Women were more likely to have poor nutritional status as indicated by anaemia prevalence and measurements of vitamin A status than men (for prevalence data see section 1.5.1).

1.1.2.2 Prevalence of non-communicable diseases globally and in India

According to the World Health Organisation (WHO), of the 57 million deaths that occurred globally during 2008, 36 million (63%) were due to non-communicable diseases (NCD): cardiovascular disease (CVD); cancer; type 2 diabetes (T2D) and chronic respiratory disease (10). Almost 29 million (80%) of these deaths occurred in LMICs. Outside Africa, there were more deaths from NCDs than the total due to communicable, maternal and peri-natal conditions combined. The age-standardised death rate from non-communicable disease was 685/100,000 in India (401/100,000 in the UK). It can be seen from the cartoon map in **Figure 1.4**, that a disproportionately large proportion of the global deaths due to NCDs occurred in India in 2002.

Figure 1.4 Cartoon representation of the global burden of NCD deaths in 2002

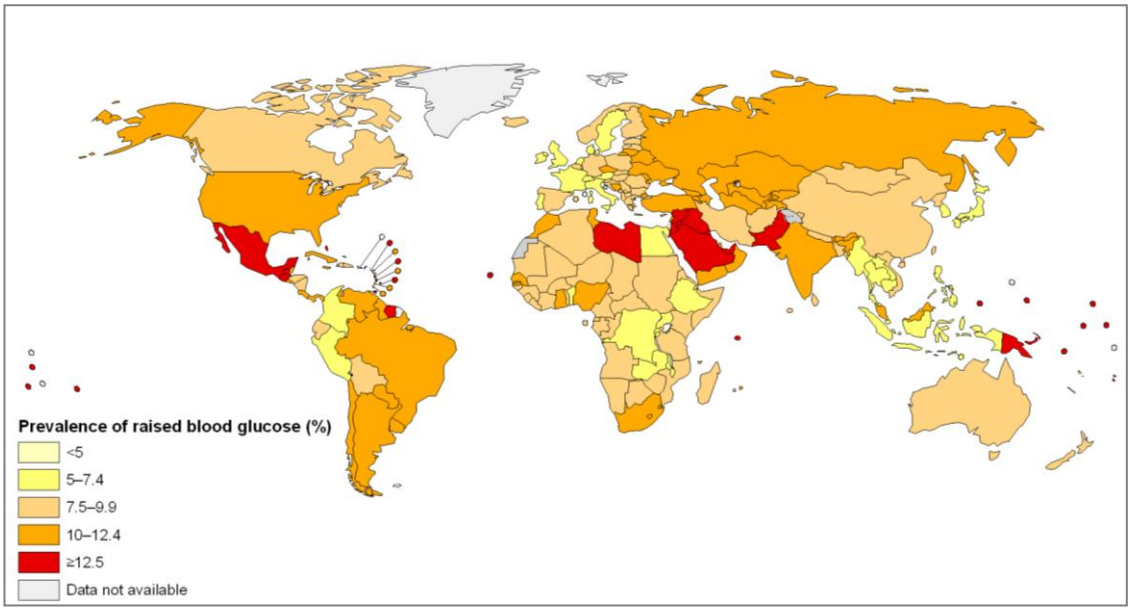


1.1.2.3 Type 2 Diabetes in India

T2D is a chronic condition, defined by levels of hyperglycaemia which cause microvascular damage and macrovascular complications. T2D develops when insufficient insulin is produced by the pancreatic β cells or when the insulin that is produced cannot be effectively used to control blood sugar concentrations. It is diagnosed clinically by fasting plasma glucose levels ≥7.0mmol/L or 2 hour plasma glucose of ≥11.1mmol/L (11). Its consequences can be reduced life expectancy and diminished quality of life due to increased risk of cardiovascular and peripheral vascular diseases, retinopathy and kidney disease (11).

India is facing a dramatic increase in the prevalence of T2D (12). In a health policy document, published in 2002, the Indian government stated that poor resources available for medical research meant that a systematically assembled health statistics database was not available. It also pointed out that methodologies were often not standardised meaning that information was collected in different ways from one state or district to another (13). Despite the lack of good quality data from India and many other countries it has been estimated that between 10–12.5% of the population of India aged over 25 have raised fasting blood glucose and/or are on medication for raised blood glucose (**Figure 1.5**) (10).

Figure 1.5 Age standardised prevalence of raised fasting blood glucose by country in 2008

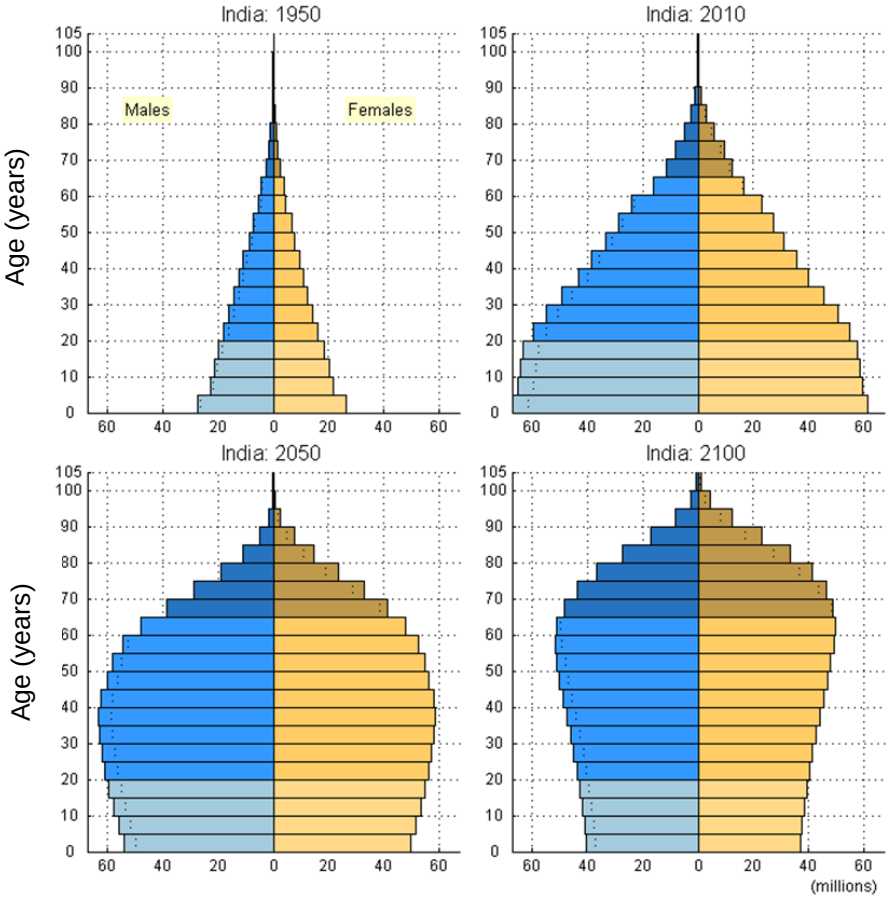


Source: WHO Global Health Observatory (14). Raised blood glucose is defined as ≥7.0mmol/L or being on medication for raised blood glucose ages ≥25years.

This proportion is currently not as high as some countries including Mexico, Saudi Arabia and Pakistan but the absolute numbers affected in India means that this is a significant public health problem. The prevalence estimate for 2012 was 63 million (15) and WHO predict that this figure will rise to 79 million (approximately 7% of the entire population) by 2030 (16).

If trends in wealth inequalities persist, it is likely that the poor will be disproportionately affected by this rise in T2D prevalence (17). An expanding and ageing population is likely to contribute to the rise in morbidity and mortality associated with T2D and other NCDs. It is estimated that the Indian population will rise to 1.56 billion by 2100 (2). In 2010, the median age in India was 25.1 years (39.8 years in the UK). The age distribution of the population is predicted to change dramatically between 2010 and 2100 (**Figure 1.6**) (2). This change in age distribution is likely to lead to an increase in the prevalence of NCDs in India.

Figure 1.6 Indian Population between 1950 and 2100 by age groups and sex (absolute numbers in millions)



Source: United Nations Department of Economic and Social Affairs (2).

The narrowing of the pyramid at the lower end of the age spectrum means that there will be fewer adults of working age to meet the cost of treatment and care for those affected. Furthermore, there is evidence that T2D tends to develop at a younger age among Asian populations than in white populations (18). This has implications for economic productivity and healthcare costs at the national and individual level. It has been reported that the urban poor who are diagnosed with T2D spend up to a third of their income on diabetes medication (19).

1.1.2.4 Trends in type 2 diabetes prevalence in India

Two cross-sectional studies conducted in Jaipur in 1995 and 2002 found that the percentage of adults diagnosed with T2D increased from 1.1% in 1995 to 7.5% in 2002 (17). Prevalence studies in Chennai (formerly Madras) on the east coast of India reported an increase from 13.9% to 18.6% of the population aged

20 years or over diagnosed with diabetes between the years 2000 and 2006 (20). People living in rural areas are often considered to be at lower risk of chronic disease, probably due to a combination of lower life expectancy, higher levels of physical activity and lower energy and fat intakes. Nevertheless, between 1989 and 2003 there was a three-fold increase in the prevalence of diabetes from 2.2% to 6.4% in a rural area 40 miles from Chennai (21).

1.1.3 Double burden of chronic and communicable diseases and the role of nutrition

One of the most critical health-related challenges facing many LMICs at present is the concurrent incidence and prevalence of communicable and non-communicable diseases. The phenomenon of 'epidemiologic transition', i.e. change from a disease pattern of infection pandemics to degenerative or chronic diseases, was described in the early 1970s (22) and has been supported by data from many industrialized countries over the last century (23). The two types of disease pattern require very different resources for treatment which means that important decisions must be taken about how best to allocate funds. In addition, public health messages and programmes must be carefully targeted towards the at-risk-groups. In many countries undergoing epidemiologic transition, including India, the disease double burden is associated with the paradoxical occurrence of over and under nutrition. Over-nutrition has been described as adherence to a 'Western' diet high in saturated fat, sugar, refined foods and low in fibre (24). Under-nutrition occurs when dietary intakes are not sufficient to meet macronutrient and/or micronutrient requirements (25). Data from the Indian National Family Health Survey (2005-6) show that there was geographical variation in the proportion of women who were both undernourished (Body Mass Index (BMI)<18.5kg/m^2) and overweight (BMI>25 kg/m^2) within India (**Figure 1.7**) (9). Over 40% of women living in the central states of India were chronically undernourished. This proportion was 35% in Maharashtra. Over 10% of women in Maharashtra were overweight or obese; this proportion was 20% or more in the southern states and in the Punjab. The dual burden of disease in India is associated with the large income differential between rich and poor. A study on

national level data from India found that over and under-nutrition were most likely to occur in states with the greatest wealth inequalities (26).

1.1.4 Undernutrition in India

1.1.4.1 Poverty and hunger

The first millennium development goal (MDG) was to 'eradicate extreme poverty and hunger' by 2015 (27). Globally, there has been a recent downturn in the progress towards this goal with approximately 1.4 billion people living in extreme poverty and this rate predicted to rise until the year 2015 and beyond (28). Improving the micronutrient status of individuals and populations is a vitally important aspect of improving health, well-being and productivity. Indeed, the UN Standing Committee on Nutrition has linked the right to good food and nutrition to achieving all eight MDGs (28). None of the targets or indicators associated with the MDGs are specifically related to micronutrient intake or status. This may be partly due to the numerous difficulties with assessing micronutrient status in low income countries including poor resources, lack of capacity, remoteness of location and lack of health infrastructure.

1.1.4.2 Prevalence of undernutrition in India

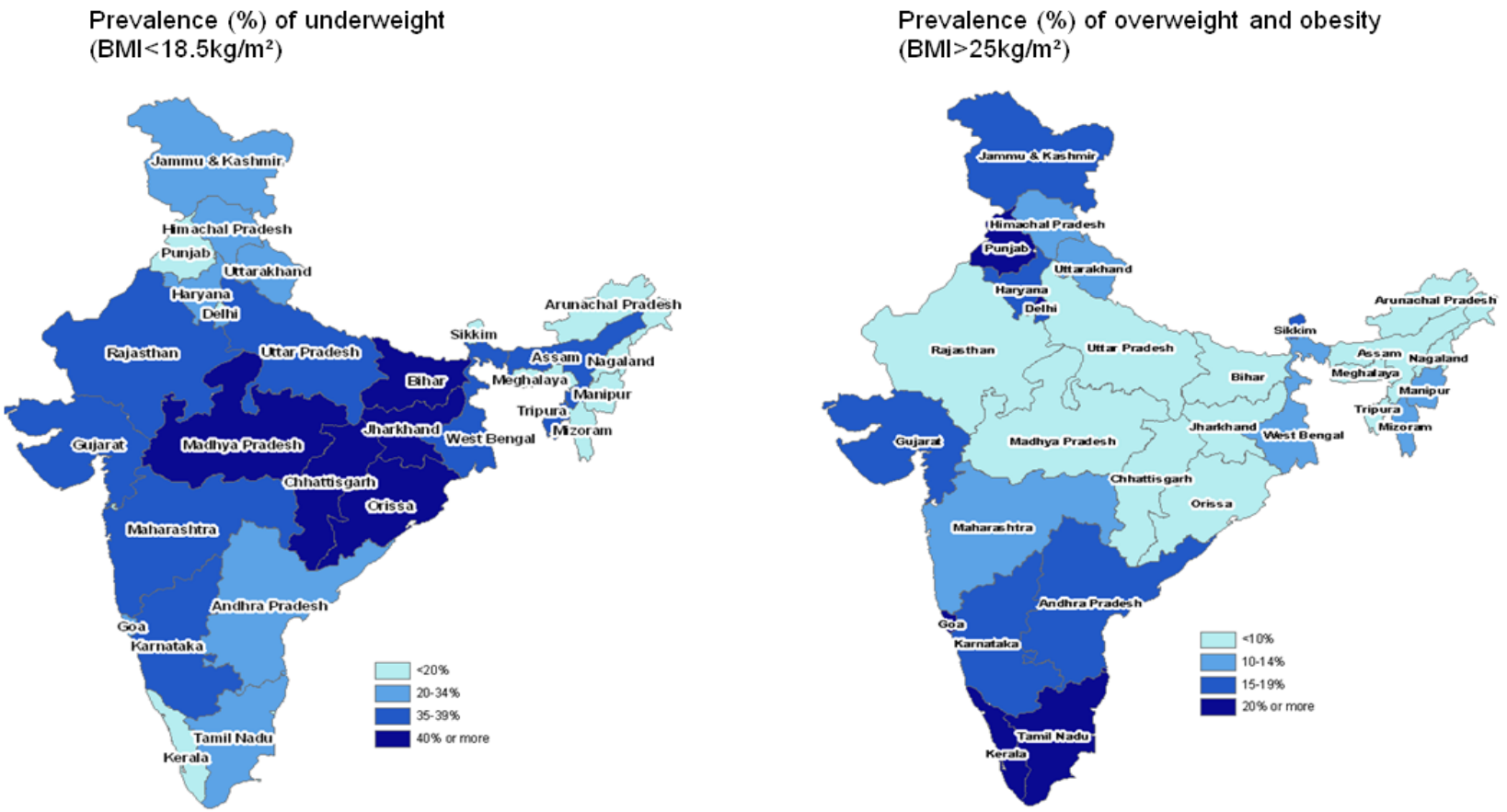
According to UN data over a fifth of the Indian population consume less than the recommended daily energy intake, based on sex and age, and nearly half of all pre-school children are underweight for age (**Table 1.1**). Indicators of undernutrition in India are described in sections 1.1.4.2.1-1.1.4.2.3.

Table 1.1 Indicators of progress with Millennium Development Goal 1 in the Indian population

.	Year			
MDG Indicator	1991	1996	2001	2005
Population undernourished[1] (%)	24.0	20.0	21.0	22.0
Population undernourished[1] (millions)	210	194	223	252
Children moderately or severely underweight[2] (%)	53.4*	ND	47.0**	47.8

[1]Energy intake <RDI [2]Weight for age <2SD below WHO mean reference value (29), *data from 2003, **data from 1999, ND no data.

Figure 1.7 Mapping of BMI of Indian women aged 15–49 by state (2005–6)



Source: National Family Health Survey 2005-6 (9)

1.1.4.2.1 Population undernourished

Data relating to population intakes are based on: 1) the amount of food available derived from food balance sheets (30); 2) data on inequalities in access to food (9); 3) the average minimum daily calorie requirement (29). Food balance calculations are based on yearly estimates of food production within the country along with data on imports and exports. The energy equivalent of all food available for human consumption is divided by the total population to derive average daily energy consumption. Inequalities in access are based on data from household surveys. The distributions of age, gender and body size from national census and anthropometric surveys respectively are also used. These calculations are crude but do provide some useful information for comparisons at the country and regional level. The estimates of people whose nutritional demands are not met are likely to be conservative as they are based on minimum energy requirements. These requirements have been established based on a sedentary level of activity and the lowest acceptable weight for height (29). Many people living in LMICs perform physically demanding tasks on a daily basis such as those for unskilled jobs, farming and transportation of water. Therefore, their demands may well exceed the average for the country and will almost certainly be greater than the minimum requirements. The calculations also assume that no food is wasted or thrown away so are likely to be an underestimate of actual intake (31;32).

1.1.4.2.2 Proportion of children underweight for age

The data on the proportion of children under five years who were moderately or severely underweight for age are from the NFHS (9). Caution must be applied when assessing changes over time in this parameter as the age range differs by time point. In 1993 the age range measured was 0-47 months and subsequently it was 0-35 months. It is likely that the distribution of underweight children varies by age making it difficult to ascertain trends over time. Despite the limitations of these data it appears from these crude indicators that there is a major public health problem of child undernutrition in India.

1.1.4.2.3 Proportion of children stunted for age

An indicator of chronic undernutrition among children is stunting, defined as a length or height-for-age of <-2 z-scores below the WHO reference population mean (33). The national figure for the proportion of children stunted in India in 2006 was 48%. The proportion of stunted children living in Mumbai slums is similar to the national figure at 47% (34). Of children who were born with a birthweight below 2.5kg in India, 47% were stunted compared with 36% of those born above this weight. Over half (54%) of children whose mothers have a BMI<18.5kg/m^2 are stunted at 5 years (9).

1.1.4.3 Inter-generational effects of undernutrition

The likelihood of a child achieving their genetic potential is highly dependent on the nutritional status of their mother before and during pregnancy and evidence indicates that it may also be dependent on the nutritional status of the child's grandmother (35). There is a common cycle in India and other LMICs whereby a woman who is born small, gives birth to a small baby who is then more likely to be stunted as a child and adult, and herself give birth to a small baby (36). Therefore, young women and those of reproductive age are a group that merit particular attention when designing interventions to reduce the prevalence of undernutrition.

1.2 Early development

There is considerable evidence that early life development has short and long term effects on health (section 1.3). This section describes the methods for assessing foetal growth and development. Birthweight is a widely used and reported method and is defined in section 1.2.1. The prevalence of low birthweight (LBW) in India is presented in section 1.2.2. While birthweight is a useful and relatively easy measure to collect, there are some important limitations which are discussed in section 1.2.3. A description of studies of neonatal anthropometry and an introduction to the concept of the 'thin-fat' phenotype are given in section 1.2.4. Finally the health and socio-economic consequences of sub-optimal early development are discussed in section 1.2.5.

1.2.1 Definition of low birthweight

Birthweight is defined as the first weight of the neonate or foetus obtained after birth (37). Ideally birthweight should be measured before there is any loss of weight post-natally. In practice this may not be achievable, particularly in remote locations or where delivery occurs outside of a medical facility. It is common in research studies to record a birthweight up to 72 hours after delivery. LBW is defined as less than 2.5kg regardless of length of gestation (38).

1.2.2 Prevalence of low birthweight in India

Several observational studies have shown that Indian babies are on average smaller than European babies (39-42). According to a report by the UN, 28% of babies born in South-central Asia were LBW and India accounted for 40% of all LBW babies in the developing world with 8 million babies being born LBW per year in 1999-2000 (37). Not all LBW babies are born at term; when age at gestation was taken into account the prevalence of LBW among babies born at term in India was approximately 20% (43).

These figures are estimations based on data from national surveys. There are difficulties obtaining data on birthweight in India due to the large number of home deliveries and those not attended by health professionals. The NFHS interviewers asked mothers either to recall the birthweight of their infant or to provide documentation. Data relating to the years 2005-6 showed that 34% of women surveyed were able to report a birthweight. Of these, 20% reported a birthweight <2.5kg. If no information on birthweight was available, a subjective assessment of size at birth was asked for as follows: ‘‘when the child was born, was he/she: large, average, small or very small?’’. Virtually all (99%) of women were able to give a subjective assessment of size at birth. Based on women’s subjective assessments, 21% of babies were described as very small and considered LBW which is consistent with the weight data. It was reported that there was good agreement between the two methods but no statistical analyses were conducted to test this. It is conceivable that women who recalled their child’s birth weight or had kept documentation may have been different to those who could not recall e.g. better educated or of higher socio-economic status. These women may have tended to give birth to larger

babies thus leading to a different birthweight distribution between the two sets of data. This should be considered when interpreting these findings.

1.2.3 Limitations of the use of birthweight data in developmental origins research

Birthweight is dependent on the growth of the foetus and gestational age. For this reason birthweight is frequently adjusted for gestational age in statistical analysis. Small for gestational age (SGA) is usually defined as a weight below the population 10th centile at a specific week of gestation (44).

Much research in humans has used birthweight as a proxy for assessment of intra-uterine growth retardation (IUGR) because it is a relatively easy measure to make and has been well recorded historically in several populations (45). It has been a very useful tool for studying the associations between early development and later disease risk. However, birthweight is a rather crude measure of whether the foetus has attained its genetic growth potential (46). In order to better understand NCD risk, it may be more useful to know about the body composition of the baby.

1.2.4 Neonatal anthropometry; comparison between Indian and European data.

An illustration of the limitations of using birthweight as a proxy for IUGR, is a comparison of anthropometric data between Indian babies born in India and British babies born in the UK (47). While the Indian babies were born smaller and had less muscle and viscera, indicated by smaller mid-upper arm circumference (MUAC) and chest circumference, there was relative 'sparing' of the truncal fat indicated by sub-scapular skinfold measurements. This implied that there was something different about these babies' early development which was not captured by measuring birthweight.

A study comparing foetal growth data from France with that from rural India found that the Indian foetuses were smaller at 18, 30 and 36 weeks gestation. There was a significant difference in abdominal circumference between the two groups while the difference in head circumference and femur length was less

pronounced (42). These findings support the concept of 'brain-sparing' at the cost of optimal development of abdominal organs and are consistent with studies comparing neonates from the UK with those from India (40).

A comparison of neonatal size from several developed and developing countries found that South Asian babies were smaller than African, East Asian and European babies in terms of birthweight and abdominal circumference. However, the Indian babies were relatively fatter as measured by subscapular skinfolds (48).

1.2.5 Consequences of sub-optimal foetal growth

A meta-analysis of birthweight and neonatal mortality data from India, Pakistan, Nepal and Brazil was recently carried out. It was calculated that babies born at term and with a birthweight of 1.5kg-1.99kg and 2kg-2.49kg were respectively 8 and 3 times more likely to die in the neonatal period than those weighing >2.5kg (49). In addition to this there is evidence from a systematic review of studies in India that LBW is associated with a greater risk of neonatal and infant death (50). Those infants that survive are more likely to be stunted as children and adults and give birth to LBW babies themselves, thus creating a cycle of sub-optimal development (36). An analysis of five prospective cohort studies and a systematic review of data in LMICs concluded that size at birth is positively associated with formation of human capital and highlights the 'vicious cycle' of intergenerational effects of LBW (51).

1.3 Evidence for developmental origins of type 2 diabetes

This section presents the evidence base for the Developmental Origins of Health and Disease (DOHaD) hypothesis (52). The idea was first based on data from the mapping of infant mortality and incidence of heart disease in the 20th century (53). This led to several epidemiological studies described in section 1.3.1. It is thought that a particularly damaging scenario for health is to be exposed to sub-optimal early development and then go on to live in a discordant environment with abundant calories and opportunities to be sedentary. This phenomenon termed 'Mismatch' is described in section 1.3.2.

Sections 1.3.3 and 1.3.4 present evidence to support the developmental origins theory from siege studies and animal research respectively. The concept of developmental plasticity is defined in section 1.3.5 and theoretical explanations of associations between early development and chronic disease in adulthood are given in sections 1.3.6 and 1.3.7.

1.3.1 Epidemiological evidence for developmental origins of chronic disease

It has been suggested that the rapid increase in incidence of CVD at the beginning of the 20th century in high income countries and more recently in LMICs is a strong indicator that the aetiology of CVD is multi-factorial and not solely genetic as it is impossible for the gene frequency within the population to have changed so dramatically in such a short time (45). An interesting observation is that while many NCDs are commonly thought of as 'diseases of the affluent', in general those living in more deprived areas are at greater risk of developing these conditions than those living in more wealthy areas both within and between countries (54;55).

A geographical study carried out by Barker et al (53) divided England and Wales into 212 regions and mapped infant mortality in the 1920s and CVD mortality in the 1970s. This showed that regions with high rates of infant mortality in the early 1920s also had high rates of CVD mortality between 1968-1978. It was suggested that this association reflected a relationship between poor growth in early life and subsequent susceptibility to CVD.

Further to this work, epidemiological studies in the UK and India have shown associations between early development and both mortality and risk of chronic disease including CVD and T2D (56-59).

Foetal growth is believed to be dependent on insulin (60), therefore an association between early growth, glucose and insulin metabolism, and later diabetes is conceivable. There are relatively few studies in India and other developing countries that have investigated the association between IUGR and later risk of diabetes. A systematic review by Whincup et al (61) reported a negative association between birthweight and risk of T2D in Europe and the

USA. Two of the 30 studies reviewed were conducted in India. The association between T2D and birthweight was less conclusive in the Indian studies than in Western populations. In a cohort study in urban and rural areas in and around Vellore, South India, a negative association was observed between BMI and length at birth, and glucose tolerance in adulthood when adjusted for current BMI (62). The authors concluded that being thin at birth followed by accelerated gain in BMI during adolescence was associated with Impaired Glucose Tolerance (IGT) and T2D in this population. A negative association between birthweight and glucose tolerance and blood pressure was observed in a cohort of Pakistani slum dwellers and supports these findings (63).

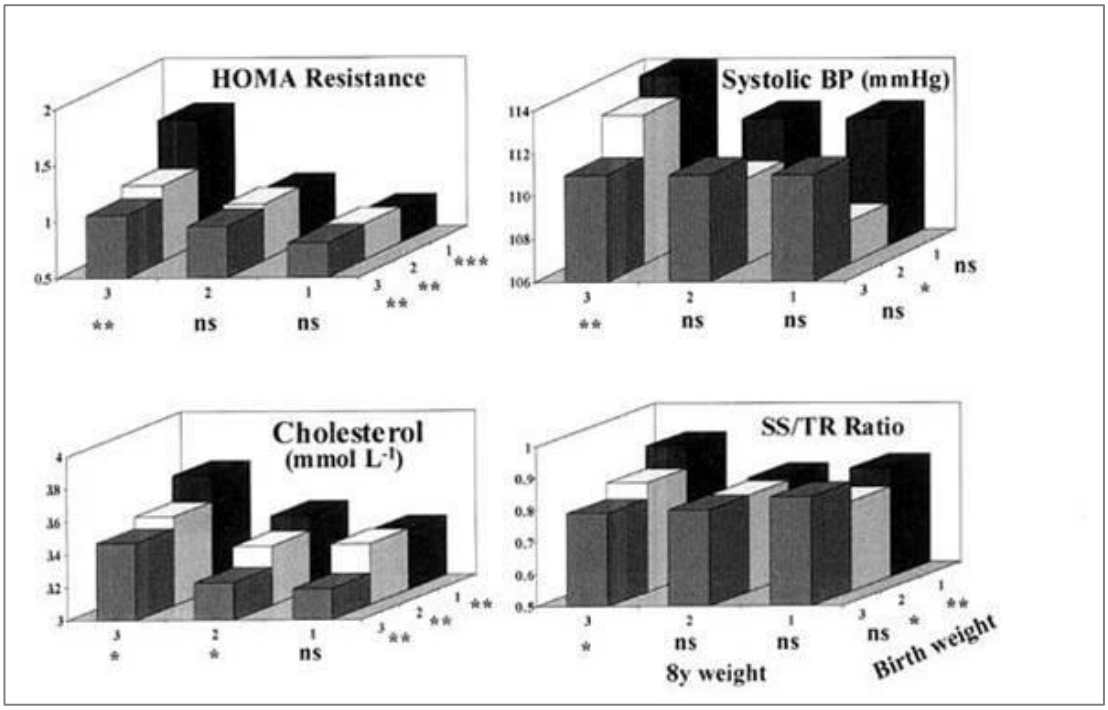
A retrospective study in Mysore, South India traced adults who had been born at Holdsworth Memorial Hospital. Oral glucose tolerance tests were administered to 487 men and women aged 35–68 years. IGT, insulin resistance and 120-minute glucose concentrations were all inversely related to birthweight when adjusted for current BMI (64). These associations were independent of socio-economic status.

1.3.2 Mismatch between early and later life environments, and chronic disease

While sub-optimal foetal development is associated with chronic disease, the combination of this early insult with a high energy diet and a sedentary lifestyle is likely to be far more detrimental than exposure to one of these factors alone. It is thought the nutritionally deprived developing foetus is 'programmed' to live in a nutritionally deprived environment (section 1.3.6) and a 'mismatch' between this phenotype and the environment is responsible for increased risk of chronic disease.

In Pune, birthweight was negatively associated with plasma insulin concentrations at both 4 years (65) and 8 years of age (66) when adjusted for current weight (**Figure 1.8**). The latter study found that children who were born smallest and were the largest at 8 years were most likely to be insulin resistant as measured by homeostatic model assessment. These children also had higher systolic blood pressure and cholesterol concentrations.

Figure 1.8 Association between birthweight, weight at 8 years and risk factors for metabolic disease among children from Pune



Source: Bavdekar et al (66)

In a Delhi-based cohort, birthweight was measured and participants were followed up every two years until adolescence (67). In young adulthood, glucose tolerance and plasma insulin concentrations were measured in 1492 men and women. Slightly more than half of the cohort were underweight according to international growth standards at age 2 years, whereas at age 26-32 years, just over half of the cohort were overweight with a BMI>25kg/m^2. Approximately 4% of the young adults were diagnosed with T2D and over 10% had IGT. There was no statistically significant association between birthweight and IGT in the Delhi cohort unless the model was adjusted for current BMI. Indeed the highest prevalence of IGT and T2D was observed in those participants with a low BMI in infancy and a high BMI, relative to their previous size, in adulthood. This was similar to the finding in Pune and implied that being born small was not necessarily deleterious to health; rather it was the combination of being born small and then becoming larger in later life that carried the greatest risk. It is important to note that relative to 'Western' populations these risks are present at lower BMI values. As the result of an expert consultation, the WHO recognises that health risks for Asians are present at lower BMI values than for other populations and have proposed

"public health action points" at lower values of BMI compared with the conventional thresholds (68).

1.3.3 Famine studies.

Famine studies allow observation of the impact of a developmental exposure which could not be replicated in an experimental setting for obvious ethical reasons. They allow 'direct' investigation of the effect on long term health of nutritional deprivation of the foetus without requiring birthweight as a proxy for early life exposure.

1.3.3.1 Dutch famine study

During the Second World War while the Netherlands was occupied by the German army there was an embargo on rail transport to cities in the west of the country. This was followed by an unusually early and harsh winter so canals that were commonly used for transporting food were impassable during the period from November 1944 until May 1945. This led to adult food rations in the cities of the area, including Amsterdam being reduced to <1000kcal per person per day. There was a dramatic drop in fertility during the famine (69) and women who were pregnant at the start of the famine lost weight during their pregnancy (70). A 300g reduction in mean birthweight was seen in babies born to women who were exposed to the famine in their third trimester of pregnancy (70;71). Exposure to the Dutch famine *in utero* was associated with IGT at both 50 and 58 years of age. An intravenous glucose tolerance test was performed in 94 normoglycemic participants (defined as 120-min glucose concentrations <7.8mmol/L), 54 of whom had been exposed to the famine *in utero*. Exposed participants, in particular during the first and second trimester, had lower glucose tolerance following the glucose load compared with those who were unexposed (72).

1.3.3.2 Leningrad siege study

The Leningrad siege took place from 1941-44 with variable food supply over the 872 day period. At times the rations provided a calorie intake as low as 300kcal/day and average birthweights fell by 17%. Stanner et al assessed

glucose tolerance and blood pressure in individuals who had been exposed to the effects of the siege *in utero* and infancy or solely in infancy depending on time of birth (73). Data were also collected from individuals who had been born at the same time but outside the siege limits. When comparing glucose tolerance and other CVD risk factors between these three groups, there were no differences between the two exposure groups in any outcomes, with the exception of a relationship between obesity and blood pressure among those exposed *in utero*.

The findings of the two famine studies described here seem to be conflicting, which could be due to the differing nature of the famines. The Dutch famine was considerably shorter and those affected are likely to have been well nourished before and after the famine. In contrast the inhabitants of the Leningrad area are likely to have been chronically undernourished before and after the siege leading to a less well defined exposure. In both studies the actual dietary intake of the mothers is not known and it is likely that there was variability within and between women. The extent of environmental mismatch among the Dutch group may have been greater due to the higher level of affluence and access to energy dense foods in this country compared with Russia. This may explain the lack of effect of siege exposure on CVD risk factors in Leningrad.

1.3.4 Animal studies

Animal research allows manipulation of maternal nutritional exposures and sampling of tissue at any stage of exposure or outcome development. It has helped elucidate the physiological mechanisms by which early development affects long-term health. Several models have been used to investigate these mechanisms including mouse, rat and sheep. Rodents' organs are generally less well developed at term than humans and therefore the timings of events related to development are different to humans. Therefore nutritional constraints on rodents during pregnancy do not tend to affect birthweight. This supports the idea that birthweight is a crude measure of IUGR (74).

One model of foetal deprivation is 'global nutrient restriction' whereby maternal calorie intake is reduced to 50% or as low as 30% of an *ad libitum*

diet. Offspring of mothers exposed to global nutrient restriction were born with endocrine and metabolic abnormalities including reduced pancreatic β cell mass. Animals that were exposed to a 50% maternal diet and then weaned onto a control diet regained body and pancreatic weight but still had reduced β cell mass in adulthood. Those animals exposed to a 30% diet remained smaller than control animals even if weaned on a control diet. In addition to a reduced β cell mass, these animals had higher fasting plasma insulin, exhibited more sedentary behaviour than control animals and were hyperphagic (75).

Amino acids are essential for foetal growth and production of enzymes, hormones and signalling molecules. In rat models of protein restriction, dams were fed diets of 5-8% protein, a similar proportion to that seen in some women of reproductive age in low-income countries (76). Pups born to these dams have reduced β cell and skeletal muscle mass. If they were suckled by low protein dams and then weaned to a control diet of 20% protein they still exhibited endocrine and metabolic abnormalities. As pups they demonstrated good glucose tolerance but developed IGT in later life (77).

The endocrine pancreas is formed when endodermal progenitor cells proliferate and differentiate to endocrine cell lines. Islet mass is then maintained by replication of already differentiated cells (78). It is thought that challenges in early life have an effect on the proliferation and differentiation of progenitor cells; this in turn affects the regenerative capacity of the pancreas. There is some evidence that excess glucocorticoids affect the foetal pancreas by reducing β cell mass. Glucocorticoids are thought to influence expression of genes with a role in generating the endocrine pancreas (78). Alterations to mitochondrial function have also been implicated as part of the developmental pathway to diabetes. It has been shown that feeding maternal rats high fat diets leads to disturbed glucose homeostasis in the offspring, but the mechanisms leading to altered mitochondrial structure and function are not yet clear (79).

1.3.5 Developmental plasticity

Developmental plasticity has been defined as ‘‘the ability of a single genotype to produce more than one alternative form of structure, physiological state or

behaviour in response to environmental conditions'' (80). Recently there has been much research attention focused on elucidating epigenetic mechanisms of developmental plasticity. Some of the most commonly studied epigenetic structural changes are methylation of CpG islands and modifications of histones. Patterns of deoxyribonucleic acid (DNA) methylation are set during embryogenesis or during early postnatal life. Methylation of the epigenome can inhibit transcription, for example by blocking the binding of transcription factors (74). These epigenetic modifications have been associated with methyl donors such as folates in the diet. The agouti mouse has been used to demonstrate how controlled doses of folic acid can affect coat colour and susceptibility to metabolic disease. These changes have been shown to be due to increased methylation of CpG islands involved in the expression of the 'agouti gene' (81).

1.3.6 Theoretical explanations for associations between early development and chronic disease in adulthood

The DOHaD hypothesis expressed the idea that an insult to early development had an irreversible effect on the tissues that could lead to an increased risk of chronic disease (52). The "Thrifty Genotype" hypothesis was based on the idea that a genotype had evolved as a result of exposure to famine and feast cycles which allowed storage of calories when supply was abundant in preparation for times when food was scarce (82). However, evidence suggests that famine/ feast cycles have only occurred since agricultural production of food began rather than in the times of the hunter-gatherer (83). It is therefore unlikely that a 'thrifty gene' would have had sufficient time to evolve. A recent review by Manolio and colleagues (84) highlighted that to date; genome-wide studies have explained only minimal increases in chronic disease risk and a small amount of the observed clustering of disease within families. The thrifty phenotype hypothesis (85) suggests that the environment in which one develops has an effect on the individual that may predispose them to later chronic disease. For example, if a foetus is poorly nourished *in utero*, it will be more likely to have a poorly developed pancreas which will predispose it to insulin resistance and diabetes in later life. Adaptations such as alterations to insulin secretion and sensitivity may also occur. One criticism of this hypothesis is that it does not account for the high rates of mortality among

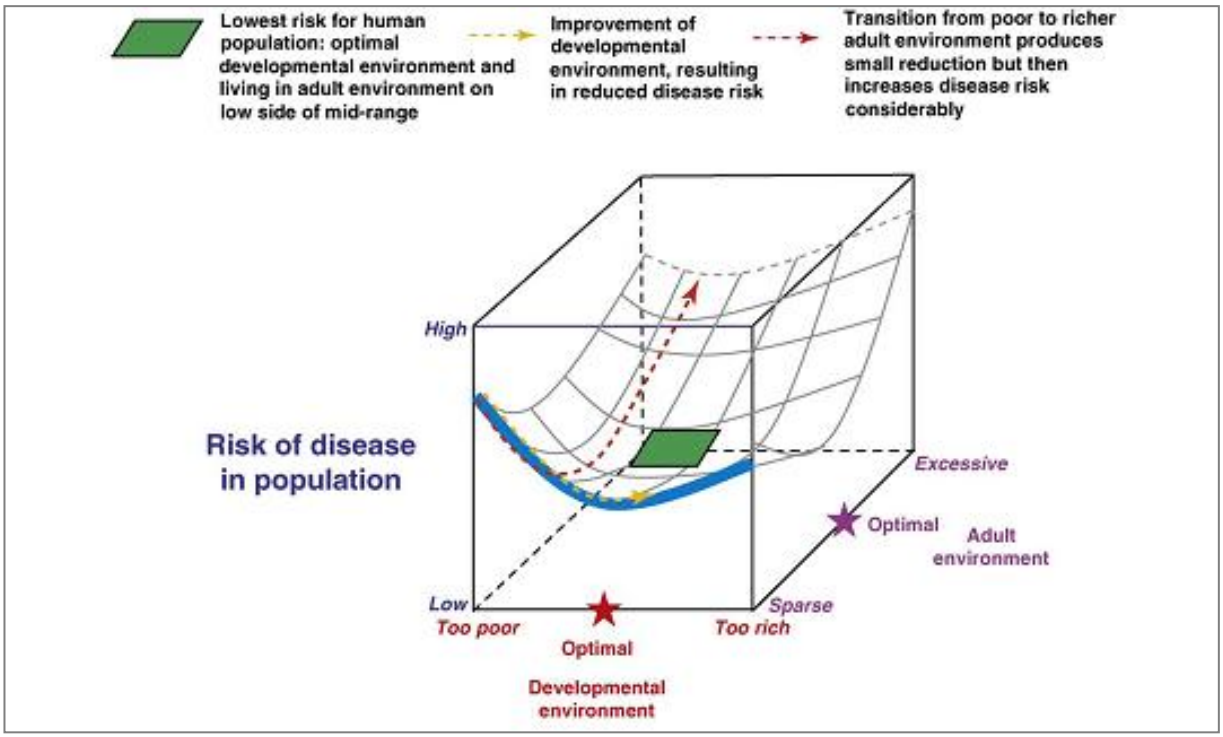
infants of LBW (86). It has been argued that this approach also assumes that extreme events rather than more subtle variations in development are important in terms of disease risk, leading to a focus on low (or high) birthweight as an important aspect of the causal pathway (83). McCance et al suggested that LBW offspring susceptible to insulin resistance and diabetes were more likely to survive than those without such a genotype, a hypothesis they called the "surviving small baby genotype" (86). Hattersley and Tooke put forward the 'foetal insulin hypothesis' which suggests that LBW and insulin resistance have common determinants and that these are principally genetic. However, it does suggest that development of the foetus is also affected by the early environment (87) and the authors point out that there is unlikely to be a single factor which can explain all of the variation in disease risk.

1.3.7 Global landscape of disease

A "life course approach" has been suggested as a means of better understanding the aetiology of T2D which incorporates genetics, epigenetics, childhood growth and lifestyle factors such as diet and activity (88). Based on data from studies in India (65;66), Yajnik proposed a compulsion of the growth-restricted foetus to conserve fat and thereby preserve brain growth at the expense of viscera and muscle tissue. He stated that this may result from a combination of genetic and nutritional factors. More recently the "Global Landscape of Disease" approach has been put forward (**Figure 1.9**), this emphasised the role of development and epigenetic factors in NCDs. It hypothesised that risk of disease is dependent on development, genetic makeup and lifestyle and in particular the relationship between the developmental and adult environments.

A possible scenario in low income countries is that an improvement in socio-economic conditions may reduce risk by enhancing the developmental environment. However, if this is accompanied by 'excess' in the adult environment, risk of disease increases dramatically, and more so than in those regions where the developmental environment is optimal. It has been suggested therefore, that interventions be targeted within the environment at the crucial window of developmental plasticity during early life (83).

Figure 1.9 Representation of the 'Global Landscape of Disease'



Source Godfrey et al (83)

1.4 The effect of maternal nutrition on short and long term offspring outcomes; evidence from human studies

The animal models described in section 1.3.4 have provided evidence that a reduced supply of maternal macronutrients affects the development of their offspring and increases chronic disease risk. While this evidence is invaluable in elucidating the underlying mechanisms of development, it is important that the findings are supported with evidence from human studies.

Section 1.4.1 defines the terms 'nutritional status', 'macronutrient', 'micronutrient' and 'dietary quality'. Section 1.4.2 describes studies that have assessed the effect of macronutrient intake on short-term outcomes such as child mortality and stunting. Section 1.4.3 presents evidence from trials showing the impact of such studies on longer term outcomes such as risk factors for T2D in adulthood and adolescence. Sections 1.4.4 and 1.4.5 report findings from studies that investigated the effect of increasing maternal micronutrient intake on short and long-term outcomes respectively.

1.4.1 Nutrition and diet quality

1.4.1.1 Macronutrients

Macronutrients have been defined as bulk components of foods and comprise carbohydrates, fat, protein and alcohol. Macronutrients are a source of energy and are required as substrates for physiological processes (89). Intakes are usually measured in grams.

1.4.1.2 Micronutrients

Minerals and vitamins are elements or compounds that are essential for health and metabolic functions. The daily requirements for micronutrients are expressed in milligrams or micrograms. Minerals are the elements other than carbon, hydrogen, nitrogen, and oxygen required by living organisms and include calcium, phosphorus, potassium and sodium. Minerals required in trace amounts include iron, cobalt, copper, zinc, molybdenum, iodine, and selenium (90). Vitamins are organic compounds with a variety of chemical structures (91). Micronutrients are required by the body for numerous functions including as co-enzymes (e.g. folate and vitamin B12), antioxidants (e.g. vitamin C and β-carotene), transportation of oxygen (e.g. iron), bone structure and function (e.g. calcium, phosphorous and vitamin D). With the exception of vitamin D, the vast majority of our micronutrient requirements must be met through the food and drink we consume (90).

1.4.1.3 Nutritional Status

Assessment of nutritional status requires information on rate of growth, body size and composition, biochemistry (e.g. blood micronutrient concentrations) and physiological measures such as blood pressure (76). In simple terms an individual's micronutrient status can be thought of as the balance between their intake of micronutrients and the requirements they have. These requirements tend to be greatest during growth, pregnancy and lactation (92). Several other factors can affect micronutrient status including infection, inflammation and parasitic diseases (93-95).

1.4.1.4 Diet quality

Often termed 'hidden hunger' micronutrient deficiencies represent a lack of quality in the diet (96). By definition a diet which is insufficient in quantity is likely to be insufficient in quality; therefore it is common for micronutrient deficiencies to occur among those who are chronically energy deficient. It is also possible to have a high quantity diet that is poor in quality. Such a diet is often characterised by high sugar, high fat foods, and is deficient in fruit, vegetables and other micronutrient-rich foods.

1.4.2 Maternal macronutrient intake and short-term offspring outcomes

The Institute of Nutrition of Central America and Panama (INCAP) trial was set up in rural Guatemala in 1969 to determine whether increasing protein intake in early life would improve children's mental development (97). Several other outcomes including child mortality and growth were also studied. Two pairs of villages were randomised to receive either an intervention drink (atole) or a control drink (fresco); in addition both villages received a health care intervention programme. The atole contained 11.5g of protein, 163kcal per 180ml cup and was rich in several micronutrients. The fresco contained no protein, 59kcal per 180ml cup and micronutrients were added to the drink such that the concentrations were similar to that of atole (97). The drinks were freely available to all villagers and consumption was recorded for all pregnant women and children less than 7 years of age. The design was such that one could not distinguish between supplementation during pregnancy and in childhood. The village-level infant mortality rate in the atole group was 60/1000 compared with 91/1000 in the fresco villages and 113/1000 in villages that did not receive any intervention (98). The authors point out the difficulty in attributing the effect to the nutrition or the health care intervention. There was no significant difference in mean birthweight of children born in the atole versus the fresco villages (99). However there was an effect on children's length at 3 years, with those from the atole villages growing 2.5cm longer on average (100).

It is important to take into account seasonality when designing an intervention study. A trial in the Gambia studied the effect on birth weight of maternal

intake of a protein-rich groundnut biscuit during the last 20 weeks of pregnancy. The control group received no intervention during pregnancy but were given the supplement post-natally, during lactation. The results demonstrated a mean increase in birthweight of 136g in the intervention group but there was considerable variability in the difference with the supplements having the greatest effect on children born following the 'hungry season' (101). Such differences may not be seen in settings where fluctuations in food supply are less common.

1.4.3 Maternal macronutrient intake and long-term offspring outcomes

In the longer term follow up studies of the Guatemalan INCAP trial, the offspring in the atole group were significantly longer than those in the fresco group at most ages. However the effect of treatment type was attenuated when adjusted for maternal height (102). In sub-group analyses an inverse relationship between consumption of the supplement and fasting plasma glucose was observed, especially among those born small (103). The intervention has been shown to improve economic productivity also. Among the male participants who had been exposed to atole before the age of 3 years there was a difference in average wages of 46% compared to the fresco group (104).

The study in the Gambia (101) also found no significant differences between the two groups in terms of BMI, body fat % or trunk fat % at 11-17 years despite the effect of consumption of the supplement during pregnancy on birthweight. The authors suggest that the lack of a difference could be due to the timing of measurement of body composition being during puberty which may have obscured any effects (105).

An explanation for the findings in the Gambian studies when compared with strong associations seen in animal experiments is that animal studies can involve interventions at extremes of macronutrient restriction as opposed to supplementation to the diet. In addition, the age of follow up in these studies may be an issue. It is possible that intervention effects may only be detectable in later adulthood. Finally, most trials intervene in a particular 'window' of

time, for example late pregnancy. It is likely that intervention before conception is necessary to achieve sustained positive changes in outcomes (106).

1.4.4 Maternal micronutrient intake and short-term offspring outcomes

Several randomised controlled trials have investigated the association between intake of antioxidant micronutrients including vitamins C and E and maternal outcomes such as pre-eclampsia and eclampsia with little consensus on causal relationships (107;108). A study in the UK collected food frequency data from women during pregnancy and found that maternal dietary intakes of β-carotene and vitamin C were positively associated with maternal and cord blood β-carotene and vitamin C concentrations at delivery (109).

A review by Kramer (110) in the late 1980s focused on randomised controlled trials that assessed the effect of supplementing women's diets during pregnancy on infant birthweight. The review concluded that there was little evidence to suggest that maternal intake of any one particular nutrient had an effect on development of the foetus in terms of birthweight or duration of gestation. A small case control study in China found that pre-conceptional B vitamin status was not associated with LBW or SGA status (111). It is perhaps unsurprising that this reductionist approach has yielded few positive results. It is more conceivable that the intake of a range of vitamins, minerals and other compounds in foods, and the interactions between them are required for optimal foetal development (112). An observational study in Nepal found that among Bhutanese refugees who received food rations, birthweight increased by 116g and LBW fell from 16% to 8% between 1996 and 1998. The authors concluded that the improvement was due to enhanced diet quality (113).

The majority of intervention trials that have investigated the association between micronutrient intake during pregnancy and offspring birthweight have supplemented women's diets with single or multiple micronutrient tablets. A meta-analysis of 12 randomised controlled trials from ten low income countries was recently conducted (114). Nine of the studies utilised the United Nations Multiple Micronutrient Preparation (UNIMMAP) which contains one dose

of the Recommended Daily Allowance (RDA) of 15 micronutrients (iron, zinc, copper, selenium, iodine, vitamin A, vitamin B1, vitamin B2, folic acid, niacin, vitamin B6, vitamin B12, vitamin C, vitamin D and vitamin E) the remaining three studies used a supplement with a similar composition and all contained at least 13 micronutrients In most of the studies the control group took iron (60mg) and folic acid (400µg) supplements as these were given to women routinely during pregnancy. The women typically started taking the supplements in the first trimester but in two of the trials supplementation started in the second or third trimesters. The outcomes investigated included birthweight, proportion of children born SGA, gestational duration and incidence of preterm deliveries. After adjustment for infant sex, maternal age, weight, parity and education, there was a small difference in the mean birthweight between control and intervention groups of 22.4g, the range was 4.9-75g. There was an interaction effect of maternal BMI in three of the studies and in the pooled analysis, birthweight was 39g higher in infants born to mothers with a BMI>20kg/m^2, compared with a negative effect in women with BMI<20kg/m^2, for whom mean birthweight was 6g lower than the control group. No effects on birth length were observed, nor on duration of gestation or pre-term delivery.

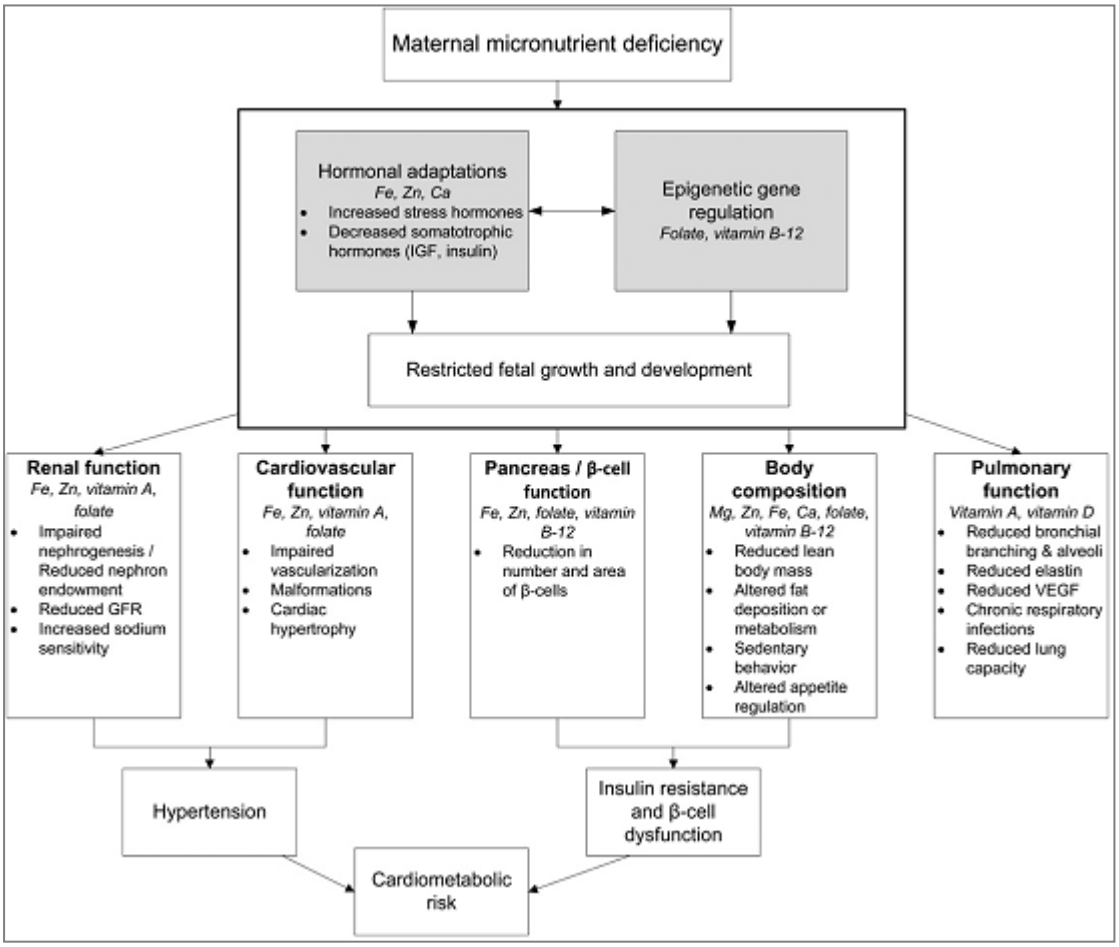
As pointed out in the review, an important question is whether the increase in birthweight translates to any developmental benefits for the children (115). A follow up study of one of the Nepali trials in this meta-analysis found that children of mothers who were supplemented with the UNIMMAP were approximately 200g heavier at 2.5y than those born to control mothers. Head, mid-upper arm, chest and hip circumferences as well as triceps skinfolds were larger in the intervention children. The differences were modest but statistically significant. There was no difference in mean height, waist circumference or waist-hip ratio. The authors pointed out that the iron content of the control supplements was 30mg higher than that of the UNIMMAP which may explain the differences. They also questioned whether the difference in body weight between the two groups was as a result of increased foetal supply of micronutrients leading to increased lean body mass, or due to greater adiposity as was suggested by the larger triceps skinfold measurements (116). It was found in Pune that low maternal vitamin B12 status as indicated by

plasma homocysteine concentrations was associated with LBW after controlling for offspring gender, maternal height, weight and gestational age (117).

1.4.5 Maternal micronutrient intake and long-term offspring outcomes

Few intervention trials have investigated the effect of maternal micronutrient intake on risk of chronic disease in the offspring (118). In a recent review of maternal micronutrient deficiencies and the effects on early development and risk of chronic disease, Christian and Stewart suggested some pathways by which maternal micronutrient status may affect the development of the foetus (119). They proposed that different nutrients play different roles in the pathways and that deficiency in any of these may result in suboptimal foetal development (**Figure 1.10**).

Figure 1.10 Proposed framework for pathways by which maternal micronutrient status affects the risk of chronic disease in offspring.



Grey boxes represent the hypothetical pathways (effects of micronutrients in italics on developmental outcomes have been studied). Source Christian and Stewart (119).

For example, iron, zinc, folate and vitamin B12 may be involved in the development of the pancreatic β cells. The former two nutrients may be required for hormonal adaptations and the latter two for regulation of epigenetic processes. A lack of any of these four nutrients may result in reduced quantity and size of β cells.

Offspring body composition is also thought to be influenced by maternal micronutrient status. It was proposed that several micronutrients are required to ensure that adequate lean tissue in the form of viscera and muscle is formed. An observational study in Bristol interviewed women about their diet at 32 weeks of pregnancy and found no substantial associations between intakes of any one micronutrient and offspring height at 7.5 years, there were weak associations with maternal magnesium, iron and vitamin C intakes but these were attenuated when adjusted for various confounders (120).

1.5 Nutritional status and dietary intakes of Indian women

Given the evidence that adequate maternal nutrition is required for optimal early development, it is important to understand the nutritional status and dietary intakes of women of reproductive age. Section 1.5.1 presents data suggesting that a large proportion of Indian women have sub-optimal nutritional status. Evidence that intakes of micronutrient-rich foods are low among Indian women, including those who live in slums, is described in section 1.5.2. The method of diet patterns analysis is introduced in section 1.5.3 and it is suggested that this approach may be a useful tool for better understanding these women's diets.

1.5.1 Nutritional status of Indian women

1.5.1.1 National level data

As described in section 1.1.3, data from the NFHS show that there is variability within India in the proportion of women who are both chronically undernourished and overweight (BMI<18.5kg/m^2 and BMI>25 kg/m^2 respectively) (9).

Data on specific micronutrient deficiencies (vitamin A and iron) are available in the Vitamin and Mineral Nutrition Information System (VMNIS) database collated by WHO. The most recent Indian data for vitamin A deficiency in young women are presented in **Table 1.2**. These data show that up to a third of women in India may be retinol deficient according to serum concentration measurements. The majority of studies, especially those with larger numbers of participants used data on clinical signs of deficiency and based on these data it appears that the prevalence of vitamin A deficiency did not decline in India between 1998 and 2003.

Table 1.2 Summary of information on Vitamin A status of women of reproductive age in India since 1999

Date of Study	Sample size	Xerophthalmia Prevalence (%)			Serum or plasma retinol concentration (µmol/L) Prevalence (%)		
		Current night blindness	Previous night blindness	Bitot's spots	<0.35	<0.70	<1.05
2003	151 (P)	15.9	-	-	-	-	-
2003	300 (P)	-	-	-	-	33.7	-
2003	220 (NP)	7.0	35.0	5.0	-	-	-
2002	736 (P)	2.9	-	-	3.5	27.3	65.0
2002	1506 (NP)	-	1.46	-	-	-	-
2001-2	129 (P)	-	-	-	-	17.1	-
2000	1130 (P)	3.21-15.86	-	-	-	-	-
2000	299 (NP)	-	18.3	-	-	-	-
1999-2000	*NS* (P)	5.2	-	-	-	-	-
1998-2001	5833 (P)	5.5	-	-	-	-	-
1998-2001	5786 (P)	6.1	-	-	-	-	-
1998-9	32393 (P&NP)	-	12.1	-	-	-	-
1998-9	300 (P)	-	-	-	-	4.0	14.7

Adapted from the WHO Vitamin and Mineral Nutrition Information System database (121). P, pregnant, NP, non-pregnant, NS, not stated

Data at the national and state level cannot always discriminate between urban and rural dwelling populations. Furthermore, it is clear that there are limitations to smaller studies in terms of generalisability, and the majority of studies in which more detailed measurement of biochemical indicators of nutritional status are collected are small due to the resources required. Serum

retinol concentrations have been described as the most acceptable measure of vitamin A status (122), however there are more data available on the prevalence of xerophthalmia. Estimates of night blindness among young Indian women range from 1.5–35%. Of the four studies that have measured serum retinol, two found that approximately one third of women were deficient using the WHO cut off (0.7 μmol/L).

According to NFHS data, the prevalence of anaemia among women nationwide in India was over 50% in 2005–2006 (**Table 1.3**). Of those affected, approximately 39% had mild anaemia (Hb, 10–12g/dL, 10–11g/dL in pregnancy); 15% had moderate anaemia (Hb 7–9.9g/dL; 2% had severe anaemia (Hb<7g/dL).

In Maharashtra the total prevalence of anaemia was slightly lower in non-pregnant women and higher in pregnant women when compared with the national prevalence. Interestingly, the prevalence among urban dwelling pregnant women is greater than among rural women. This does not reflect the national pattern and it is not clear why this would be so. There is a possibility that in Maharashtra there has been a more focused attempt to ensure that pregnant women receive and consume iron-folic acid tablets in rural areas than in the populous urban slums.

Table 1.3 Prevalence of anaemia; country level compared with Maharashtra state

	Prevalence of anaemia 2005-6 (%)					
	By setting		By number of years of education			
	Urban	Rural	Uneducated	<5y	5-9y	≥10y
All India						
Non-Pregnant	51.5	58.2	60.2	57.9	54.6	46.6
Pregnant women	54.6	59.0	63.0	58.5	56.2	47.4
Maharashtra						
Non-Pregnant	46.6	51.1	50.9	52.0	49.6	44.4
Pregnant women	60.1	56.4	61.2	-	61.8	47.9

Source: Arnold et al (34), - no data

In Mumbai the prevalence of anaemia was slightly higher among non-slum dwellers than slum dwellers (48% versus 46%). However, these data were not

broken down by severity of anaemia. The number of vegetarians in non-slum areas of Mumbai was over double the number in slum areas (13% versus 28%) (34). This may in part explain the differences in anaemia prevalence between these groups.

When NFHS data from 2005-6 (9) were compared with those from a previous national survey (1998-9) (123), among both pregnant and non-pregnant women there was a small increase in the prevalence of anaemia over time. A change to the way the respondents were selected was made between the two surveys which may go some way to explaining this. For the 1998-9 survey only married women were recruited to participate, whilst in the 2005-6 survey married and non-married women participated. It is possible that there was a greater prevalence of anaemia among non-married women. There have been calls for research aimed at assessing the micronutrient status of Indian women and the relationship between deficiencies and birth outcomes (124;125).

Overall, 22% of women in Mumbai were underweight and 27% were overweight. Among slum dwellers, the figures were 23% and 25% respectively.

1.5.2 Dietary intakes of Indian women

1.5.2.1 National Survey Data

In 2005-6, women in Mumbai slums were questioned about the frequency of intake of certain food groups: milk or curd; pulses or beans; leafy vegetables; fruit; eggs; fish; chicken. The possible responses were: daily; weekly; occasionally; never. Information on portion size was not obtained (9). **Table 1.4** shows that over two thirds of these women reportedly consumed leafy vegetables on a daily basis, however this could have been any amount and given that several Indian preparations contain very small amounts (<5g) such as coriander for seasoning, it is likely that fewer women consumed at least one portion of leafy vegetables; 100g as recommended in the Indian government guidelines (126). Approximately 40% of the women ate fruit less than once per week. More than 15% of women never consumed milk or curd (yoghurt) and 56% consumed these items less frequently than once a week.

These data are a useful overview and provide us with evidence that intakes of nutrient rich foods are infrequent in this population. However further detailed study of dietary behaviour is necessary to understand more about the diets of young women and how they relate to health outcomes of these women and their children.

Table 1.4 Frequency of consumption of food groups by women living in Indian census identified slums in Mumbai (n=1020)

	Daily	Weekly	Occasionally	Never	At least once a week
Milk or curd	33.3	10.6	40.9	15.2	43.9
Pulses or beans	79.2	16.4	4.1	0.4	95.6
Leafy vegetables	67.4	22.0	9.8	0.8	89.4
Fruits	27.7	31.7	38.8	1.8	59.4
Eggs	4.5	47.2	31.5	16.7	51.7
Fish	2.1	46.3	32.6	19.1	48.4
Chicken or meat	1.8	47.2	33.9	17.2	49.0

Source: NFHS 2005-6 (9)

1.5.2.2 Dietary Intake among Indian women; data from research studies

Studies investigating the diets of women of reproductive age living in India have tended to focus on tribal women and those living in rural areas (127-130). Detailed food frequency questionnaires (FFQ) were administered and nutrient composition tables were used to calculate micronutrient intakes. All of these studies found that the women were chronically energy deficient and the majority had intakes associated with deficiencies in at least one micronutrient.

Recently, quantitative data on dietary intake has been collected from women living in slums. This indicated that intakes of fruit and vegetables were very low (131;132). Anand et al (132) found that the mean number of servings per day of fruit and vegetables was 2.2 among women, with only 5.4% of women

consuming 5 servings per day. In the UK, national survey data suggest that a third of women aged 19-64 consume 5 or more portions per day (133).

It is important to consider that intake of nutrient rich foods may not always lead to improvements in nutritional status such as in cases of infection (93) or poor absorption due to anti-nutrients in the diet (134;135). An example pertinent to India is the effect of polyphenols in tea on the absorption of non-haem iron from vegetarian foods (136).

During the pilot phase of the MMNP (section 1.6), a 91-item FFQ was administered to women aged 16-40 years living in a slum area of Mumbai (n=1651). The data showed that a quarter of the women consumed fruit and green leafy vegetables (GLV) less than three times per week. Fewer than 50% consumed at least one portion of fruit per day and only 30% of women ate GLVs at least once per day. Apart from small quantities in tea, median consumption of milk and milk products was less than twice per week. There was some seasonal variation in intakes of fruit and GLVs with the lowest intakes occurring in June and July (the beginning of the wet season) (137).

1.5.3 Diet patterns approach

The data described in sections 1.5.2.1 and 1.5.2.2 use a 'single food approach' to understanding dietary behaviour. Foods are not eaten in isolation and it is conceivable that an understanding of the overall diet pattern of young Indian women may provide useful insights into developing methods for improving diet quality. This approach of determining which foods are commonly consumed together by different groups within a population has been widely used in Europe and the USA in relation to NCD risk factors (138).

There are a small number of studies investigating diet patterns among South Asians, these are discussed in detail in chapter 5, but to date no published studies investigating dietary patterns among women of reproductive age living in Indian slums have been identified.

1.6 Background to the Mumbai Maternal Nutrition Project

This section describes an observational study conducted in Pune which suggested that intakes of GLV, fruit and milk were positively associated with birth size (section 1.6.1). Based on these findings, a randomised controlled trial was designed to determine whether intakes of these foods before and during pregnancy were associated with improved birth outcomes. The rationale for the design of the MMNP is presented in section 1.6.2. A detailed description of the methods used is given in chapter 3.

1.6.1 Food intake and birth outcomes in India; Pune Maternal Nutrition Study

The study of the effect of single and multiple nutrient interventions has been informative in terms of understanding mechanisms by which maternal diet and offspring health are linked. However, it is probable that a combination of nutrients and other compounds present in foods are required for optimal foetal growth and development. The reductionist approach of attempting to attribute sub-optimal development to deficiencies in one or more micronutrients alone may not be sufficient to understand the association between maternal nutrition and offspring outcomes and indeed, may not be the most effective approach for intervention to improve such outcomes (110;124).

An example of an observational study investigating the associations between maternal food intakes and birth size was the 'Pune Maternal Nutrition Study' (PMNS) (139). The PMNS recruited low income pregnant Indian women who were living in rural villages surrounding the Indian city of Pune. The study was conducted between 1994 and 1996. Women were interviewed in the second and third trimesters of pregnancy about their dietary intake using a 111-item FFQ and anthropometric measurements were taken. Self report data on socio-economic status and physical activity were also collected. The babies were weighed within 72 hours of delivery and neonatal anthropometric measurements were made. It was observed that the frequency of mothers' intake of GLV at 28 weeks gestation was positively associated with all of the birth size measurements of their offspring after adjustment for pre-pregnancy

weight, weight gain during pregnancy, energy intake, physical activity and socio-economic status. The trend was strongest among the lightest mothers. Frequency of fruit intake at 28 weeks gestation was positively associated with birthweight, length and head circumference. This result remained significant when adjusted for the same variables as with GLV (with the exception of pregnancy weight gain) and the effect size was greatest among the lighter mothers. Milk intakes at 18 weeks gestation were associated with birthweight, birth length, MUAC and head circumference and these associations remained significant after adjustment. This study suggests that increasing intakes of these foods among women with poor quality diets may lead to improved health outcomes among their children. However the data were observational and it is possible that there were unmeasured confounding factors that would explain these findings.

1.6.2 Description of MMNP and design rationale

The MMNP was launched in January 2006. It was a randomised controlled trial designed to test the hypothesis that increasing maternal intake of micronutrient rich foods; GLV, fruit and milk, would enhance foetal growth, reduce infant mortality and improve the long-term development and risk of chronic disease in the offspring. The vast majority of intervention studies that have assessed the impact of a nutritional supplement during pregnancy on birth or other offspring outcomes have recruited women during pregnancy, usually towards the end of the first trimester. This means that the foetus is only exposed to the intervention for a maximum of two thirds of the pregnancy. In the MMNP, women were recruited before they became pregnant and were required to have consumed the supplement for at least three months before conception in order to be counted towards the target number of pregnancies (the project continued to supplement those who became pregnant within 3 months from the start of supplementation). It is thought that the pre-conceptional micronutrient status of the woman may have an impact on foetal development. There are several possible factors which may mediate this impact, including: adequacy of placental development; ability of the woman to store nutrients; signals that are sent to the developing embryo and foetus concerning the developmental environment (112). Three months was chosen as the minimum length of time of exposure to the intervention in the MMNP

because it is approximately similar to the lifespan of erythrocytes. It was hypothesised that an increase in dietary intake of nutrient-rich foods over this three month period should be sufficient to achieve changes in erythrocyte levels of nutrients such as folate. GLV, fruit and milk were chosen for the intervention based on the findings from the PMNS. In addition, they are all acceptable to the majority of the Indian population. These foods are rich in micronutrients and potentially other beneficial, compounds. For example, there is evidence of the beneficial health effects of phytonutrients such as flavonoids found in fruit and vegetables (140;141).

1.7 Objectives

This chapter provides evidence that maternal diet is fundamental to optimal early development (section 1.3, p16). The diets of young women should be of sufficient quantity and quality in order to fulfil the genetic potential of their children and grandchildren. Limited data suggest that the nutritional status of a significant proportion of Indian women living in low income settings is sub-optimal (section 1.5.1.1, p32). Furthermore, information on intakes of micronutrient-rich foods in the Mumbai slum community indicates that the dietary intakes of these women are of poor quality (section 1.5.2, p35). Little is known about the dietary patterns among low-income Indian women of reproductive age (section 1.5.3, p37). Such information may be useful in developing dietary guidelines for this population and in the design of interventions aimed at improving diet quality.

The main objectives of the work described in this thesis were:

1) To conduct a systematic review of intervention studies investigating the effect of fruit and vegetable interventions on micronutrient status among women of reproductive age.
2) To conduct a randomised controlled trial to assess the effect of consumption of a food based supplement containing GLV, fruit and milk over a 12 week period on the change in micronutrient status (as measured by blood micronutrient concentrations) and functional health-related outcomes among women living in Mumbai slums.

3) To identify diet patterns among MMNP participants using principal components analysis (PCA), and to examine associations between adherence to the identified diet patterns and anthropometric and socio-demographic variables.

2. Systematic review: The effect of fruit and vegetable interventions on micronutrient status among women of reproductive age

2.1 Introduction

It is estimated that 2 billion people globally are affected by single or multiple micronutrient deficiencies with women of reproductive age being particularly vulnerable (49;142). Over recent years there have been attempts to address this public health problem. Several programmes at global, national and local levels have provided women with single and multiple micronutrient tablets in order to prevent or treat deficiencies and associated morbidity and mortality, with inconsistent results for vitamin A, zinc and iron supplementation (143-145). In the case of iron there have been issues such as gastro-intestinal side effects and poor compliance with supplementation (146;147).

Fortification of frequently consumed foods, such as salt with iodine, is another strategy. In terms of reducing iodine deficiency, this approach has been relatively successful but for other nutrients, such as zinc, it has been more challenging (148). Both supplementation and fortification may have an important role in populations where requirements cannot be met from foods, such as in emergency situations, but both have been described as short term strategies. At the International Conference on Nutrition in Rome in 1992 it was declared that sustainable food-based strategies should be given priority in deficient populations and it was recommended that emphasis be placed on increasing availability of and enabling access to micronutrient-rich foods (149). The Indian National Institute of Nutrition has stated that it is essential for food-based dietary guidelines to be implemented in order to overcome the public health problems in India (150).

It may be difficult to achieve optimal intakes of nutrients using food-based approaches, particularly in remote areas with poor soil quality or those with high population densities. However, food based approaches are generally acceptable to the target population. An approach involving indigenous plant foods is likely to be sustainable and of economic and environmental benefit to

the community (151). With food based approaches there is little risk of exceeding safe upper limits of fat soluble nutrients such as vitamin A or of antagonistic interactions between nutrients e.g. iron disrupting zinc absorption (152–154). Such risks may be present when large doses of micronutrients are consumed from supplements and/or fortified foods (151).

There are multiple causes of micronutrient deficiencies impacting at the level of the population, community and individual (155). One important factor is that micronutrient deficient diets tend to be monotonous and mainly comprise staple cereal crops. They are usually low in fruit, vegetables and animal source foods (148). Fruit and vegetables are important sources of micronutrients and are acceptable to the vast majority of people, in contrast to several animal foods which are often relatively expensive (156) and not consumed by vegetarians or vegans. Plant foods are also likely to contain compounds that have not yet been identified as required for optimal human health and well-being and cannot therefore be added to synthetic micronutrient supplements (140;141).

To our knowledge a systematic review of studies assessing the effect of food-based interventions on the micronutrient status of women of reproductive age has not yet been undertaken. The objective of the current review is to determine whether dietary interventions that are entirely food-based and comprised of fruit and/or vegetables are effective in increasing circulating micronutrient concentrations among women of reproductive age.

2.2 Method

2.2.1 Study identification

The procedure of study identification and data extraction followed the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (157). We created search strategies individually tailored to the following electronic databases: MEDLINE (https://ovidsp.tx.ovid.com/); EMBASE (https://ovidsp.tx.ovid.com); AMED (https://ovidsp.tx.ovid.com); the World Health Organisation Global Health Library Databases: IMEMR; IMSEAR;

LILACS; WPIRM (http://www.who.int/ghl/directory/en/). We searched all database records to June 2012.

We used the terms 'fruit' OR 'vegetable' OR [all edible plant taxonomic names and synonyms available on the US Department of Agriculture (USDA) Germplasm Resources Information Network (GRIN) database (http://www.ars-grin.gov/cgi-bin/npgs/html/index)] 'AND' 'intervention study' [Mesh] OR 'intervention' OR 'trial' OR [York filter to identify randomised controlled trials]. For all databases we used the specific York filter to identify randomised controlled trials found at http://www.york.ac.uk/inst/crd/intertasc/rct.htm. For IMEMR, IMSEAR, LILACS, WPIRM, the York filter prescribed for MEDLINE was used. The search was limited to humans and studies published in peer-reviewed journals written in English were examined. Bibliographies of all papers identified were used to search for additional relevant papers.

2.2.2 Study selection

Studies that reported the results of an intervention involving fruit and/or vegetables, with the exception of fortified or bio-fortified foods, were included. The intervention could consist of supplying the participant with the food item(s), giving them the means to obtain the items (e.g. tokens) or giving advice, instruction, encouragement or education to increase consumption or achieve a certain intake. We included papers reporting on one or more of the following outcomes: anaemia, prevalence of micronutrient deficiencies or changes in blood micronutrient concentrations. We excluded studies in which the mean age was <16 or >40 years and where >50% of participants were male. In studies among men and women, where results were presented by sex, we extracted only data relating to females.

All article titles and abstracts identified in the database searches were examined for relevance by two authors: Elena Rayner and Sarah Kehoe. Where necessary the full article was obtained to assess eligibility. In cases where either reviewer was uncertain whether to include a paper, this was discussed and resolved by consensus.

2.2.3 Data analysis and presentation

Data were extracted by two reviewers: Barrie Margetts and Sarah Kehoe. The following data were extracted: country in which the study was conducted, description of the participants, sample size, description of the intervention, whether there was a control, duration of intervention, outcome measures, statistical methods and results including effect sizes and p values or confidence intervals where reported. Data were not aggregated due to differences in study design, outcomes and statistical methods between studies.

2.3 Results

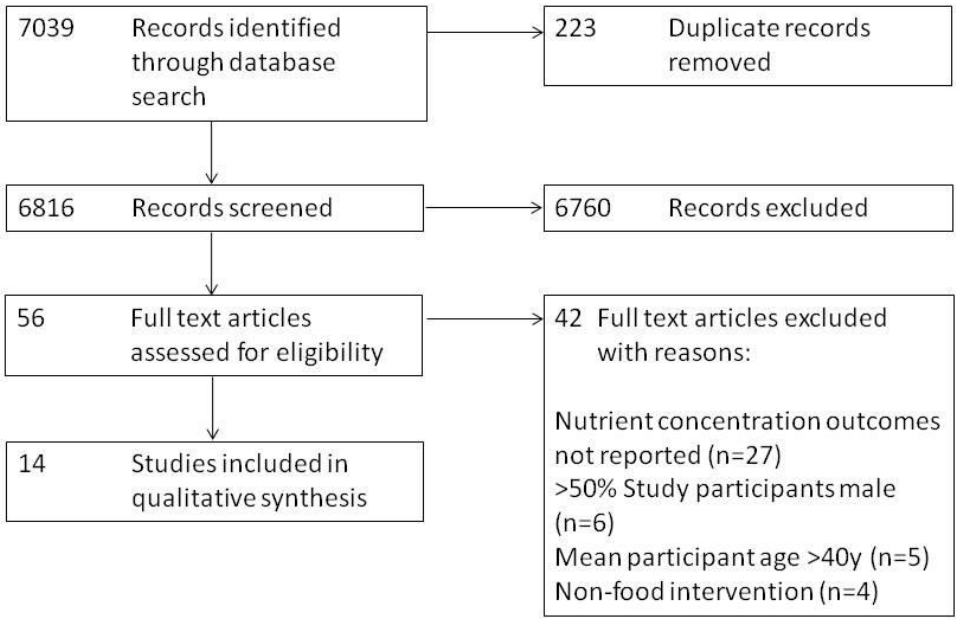
Of 6816 non-duplicate records obtained using our search strategy, 56 articles were identified as being potentially eligible at the screening stage. Full text versions of these 56 articles were assessed for eligibility and 14 studies were included in the review (**Figure 2.1**). Manual screening of the reference lists of included articles did not identify any eligible studies that had not been obtained in the original search. The 42 articles that were excluded either did not meet the inclusion criteria, or reported data from studies with >50% male participants, participants aged <16 or >40 years or in which the intervention did not meet inclusion criteria.

The majority of studies (n=12) were conducted in Europe or the USA with one study conducted in India and one in Japan (**Table 2.1**). The number of participants ranged from 6 to 97. The interventions ranged from single servings of a specific food to 600g of fruit and vegetables consumed per day. In eleven of the studies the participants were supplied with the intervention. In the remaining three, the participants were instructed or advised to consume a certain number of portions of fruit and vegetables. The intervention periods ranged from 1 week to 12 months. The median (IQR) period was 14.0 (8.5, 28.3) days. Ten studies investigated the effect of the intervention on carotenoids, nine on vitamin C, three on tocopherol, three on retinol, one on zinc and one on folate.

Assessment of the effect of the intervention differed between the studies. Six compared the treatment and control groups in terms of the change between

pre and post-intervention measurements (**Table 2.2**). Six studies compared the pre-intervention concentrations with post-intervention concentrations and did not have a control group (**Table 2.3**). Two studies compared the post-intervention nutrient concentrations of a treatment group with those of a control group (**Table 2.4**).

Figure 2.1 PRISMA flow diagram



PRISMA, Preferred Reporting Items for Systematic Reviews and Meta Analyses

2.3.1 Studies that compared the treatment and control groups in terms of the change between pre and post-intervention measurements

Of the six studies that compared changes in concentration between control and treatment groups and presented pre and post-intervention measures, two reported a statistically significant difference between groups in folate, β-carotene and lutein concentrations (**Table 2.2**). In the study by Brouwer et al (158), a diet containing folate-rich foods was associated with on average a 6.5nmol/L increase in plasma folate and a 59nmol/L increase in red cell folate in the treatment group compared with a small decrease in the control group. The article by Djuric et al (159) did not report any effect sizes but stated that there was a statistically significant difference between groups in terms of change in plasma α-carotene, β-carotene and vitamin C concentrations and no

difference for any of the other carotenoids measured. The Gill et al (2007) study (160), in which the intervention was 85g watercress per day for 8 weeks, did not report mean change in micronutrient concentrations but the difference between the mean post and pre-intervention plasma β-carotene concentrations was 100nmol/L in the treatment group and 10nmol/L in the control group. Plasma lutein concentrations almost doubled in the treatment group and decreased in the control group.

2.3.2 Studies that compared pre and post intervention measurements

The study by Maruyama et al (161), in which 480g of tomato juice was given daily, did not report the effect size and did not assess the difference in the change between groups statistically (**Table 2.3**). Instead they compared pre and post-intervention concentrations for the treatment and control groups separately. Plasma β-carotene concentrations were high at baseline compared with the Gill (2007) study and increased by approximately 1.2μmol/L on average in the treatment group and were unchanged in the control group. Plasma lycopene concentrations almost tripled in the treatment group and decreased in the control group. Gill et al conducted a study in 2004 (162) which assessed the effect of consuming cruciferous and leguminous sprouts for two weeks on carotenoid, retinol, vitamin C and tocopherol concentrations and found no effect of the intervention on any of the outcomes. The study by Møller et al (163) in which 600g of fruit and vegetables were consumed in addition to a controlled diet for 24 days reported a small increase in the mean vitamin C concentrations in the treatment group and a three-fold decrease in the control group. These changes were not tested for statistical significance.

Of the six studies that reported baseline and post-intervention measures but did not include control groups, four reported at least one statistically significant result. The remaining two studies did not report any statistical analysis.

The study by Agte et al (164) reported a 5% increase in vitamin C concentrations over a 3 week intervention of 100g cooked GLV per day. The two studies by Chopra et al reported approximately a two-fold increase in plasma β-carotene, lutein and lycopene over a seven day period during which

300g of carotenoid-rich fruit and vegetables were consumed in addition to a controlled diet (165). Neither study by Chopra et al presented any statistical analyses. Hoelzl et al did not report an average effect size but found that 300g of Brussels sprouts per day in addition to usual diet for six days was associated with an increase of approximately 13mg vitamin C (166). The studies by Sanchez-Moreno et al (167) and Tesoriere et al (168) reported increases in vitamin C of the same order when assessing the effect of daily consumption of 500ml of vegetable soup and 500g cactus pear respectively. In the Tesoriere study no change in retinol concentrations was seen.

2.3.3 Studies that compared post-intervention measurements of a treatment group with those of a control group

The studies by Brevik et al (169) and Martini et al (170) compared post-intervention measures between control and treatment groups (**Table 2.4**). The former study found an approximately 40% difference between the control and treatment groups in plasma α-carotene and β-carotene concentrations associated with a daily intake of 750g fruit and vegetables compared with 300g in the control group. The latter reported a two-fold difference in lycopene concentrations between groups and no difference in any other carotenoids.

2.3.4 Results by nutrient

Following on from the findings described in sections 2.3.1 – 2.3.3, I looked at the results of the studies by nutrient to determine whether there were differential effects of fruit and vegetable interventions on micronutrient status. Studies that reported results of statistical analysis (n=11) were considered. Of the six studies that reported the effect of interventions on β-carotene, 4 (67%) reported positive findings. The proportion in the case of vitamin C was 5 of 8 (62.5%). One of three studies (33.3%) reported a positive result for vitamin E. None of the three studies assessing the effect of interventions on retinol reported a positive finding nor did the one study assessing the effect on zinc. The one study with folate as an outcome reported a positive effect.

Table 2.1 Summary of the characteristics of the studies included in the systematic review

Ref	Author (Year)	Country	Participants	N	Treatment Intervention	Control Intervention	Duration	Outcome measure(s)
(164)	Agte (2006)	India	Students;100% female, aged 20-25y	12	100g cooked GLV/d +usual diet	No control group	3wk	Plasma Zn, vitamin C, β-carotene
(169)	Brevik (2004)	Norway	Medical students; 52% female, aged 19-34y	39	750g FV/d + controlled diet	300g FV/d + controlled diet	14d (CO)	Plasma carotenoids (α-carotene, β-carotene, lutein & lycopene)
(158)	Brouwer (1999)	Holland	63% female, aged 18-45y	67	Controlled diet based on habitual energy intake containing folate-rich foods ~395 µg of folate/d	Controlled diet with no folate-rich foods + placebo tablet	4wk	Plasma folate, red cell folate
(165)	Chopra (2000)	UK	100% female, aged 24-52y. Non-smokers	16	Controlled diet + 200g spinach &100 g mango puree	No control group	7d	Plasma β-carotene, lutein
(165)	Chopra (2000)	UK	100% female, aged 24-52y. Non-smokers	16	Controlled diet + 200g tomato puree &100 g watermelon	No control group	7d	Plasma lycopene
(159)	Djuric (2006)	USA	100% female, aged 21-50y	97	Counselling to achieve a goal of 9 servings FV/d	Usual diet	12mo	Plasma carotenoids (α-carotene, β-carotene, lutein, zeaxanthin, lycopene, β-cryptoxanthin, vitamin C, γ-tocopherol

N, Number in analysis. GLV, Green Leafy Vegetable. NS, non-statistically significant. FV, fruit and vegetables. CO, Crossover Design.

Table 2.1: Summary of the characteristics of the studies included in the systematic review (continued)

Ref	Author (Year)	Country	Participants	N	Treatment	Control	Duration	Outcome measure(s)
(160)	Gill (2007)	UK	50% female, aged 19-55y	60	85g watercress/d + usual diet	Usual diet	8wk (CO)	Plasma β-carotene, lutein, retinol, vitamin C, α –tocopherol
(162)	Gill (2004)	UK	50% female, aged 21-45y	18	113g cruciferous and leguminous sprouts + usual diet	Usual diet	2wk	Plasma carotenoids, retinol, vitamin C, tocopherol,
(166)	Hoelzl (2008)	Austria	50% female, mean age 33±7y	8	300g Brussels sprouts/d + usual diet	No control group	6d (CO)	Plasma vitamin C
(170)	Martini (1995)	USA	66% female, aged 20-34y	19	390g broccoli & 300g cauliflower + controlled diet	Controlled diet	9d	Plasma carotenoids (α-carotene, β-carotene, lutein, lycopene, β-cryptoxanthin)
(163)	Møller (2003)	Denmark	50% female aged 21-56y	31	Controlled diet + 600g FV/d	Controlled diet (no FV) + placebo	24d	Plasma vitamin C and β-carotene
(161)	Maruyama (2001)	Japan	100% female, aged21.3±0.8y	21	Usual diet + 480g tomato juice	Usual diet + 480g control drink	29d	Plasma carotenoids (α-carotene, β-carotene, lycopene, cryptoxanthin), α-tocopherol, vitamin C
(167)	Sanchez-Moreno (2006)	Spain	100% female aged22.0±0.5y	6	Usual diet + 500ml vegetable soup/d	No control group	14d	Plasma vitamin C
(168)	Tesoriere (2004)	Italy	56% female aged 33.3±11.3y	18	Usual diet with no fruit except 500g cactus pear pulp	No control group	14d	Plasma vitamin A vitamin C, vitamin E

N, Number in analysis. GLV, Green Leafy Vegetable. FV, fruit and vegetables. CO, Crossover Design

Table 2.2 Findings of studies with pre and post-intervention measures and comparison between control and treatment group

Author (Year)	Method of analysis	N in analysis	Nutrient	Group	Baseline	Post-intervention	Effect Size (δ)	P value
Brouwer (1999)	T-test used to compare δ between groups.	45	Plasma folate (nmol/L)	Control	13.2±3.4	12.7±2.9	-0.6±1.7	NS
				Treatment	13.8±3.0	20.4±3.5	6.5±3.0	<0.001*
			Red cell folate (nmol/L)	Control	347±79	345±79	-1.2±38.6	NS
				Treatment	338±81	400±114	59.3±55.5	<0.001*
Djuric (2006)	Linear effect of time on plasma micronutrient levels (i.e. slope) compared between treatment and control groups using mixed model repeated measures ANOVA.	97	Plasma α-carotene		NR	NR	NR	<0.001**
			Plasma β-carotene		NR	NR	NR	0.002**
			Plasma β-cryptoxanthin		NR	NR	NR	NS
			Plasma Lutein		NR	NR	NR	NS
			Plasma Zeaxanthin		NR	NR	NR	NS
			Plasma Lycopene		NR	NR	NR	NS
			Plasma Vitamin C		NR	NR	NR	0.006**
Gill (2007)	Wilcoxon signed-rank test to compare δ between groups.	60	Plasma β-carotene (nmol/L)	Control	320±210	330±200	NR	<0.001*
				Treatment	330±190	430±260	NR	
			Plasma Lutein (nmol/L)	Control	180±70	170±70	NR	<0.001*
				Treatment	180±70	350±180	NR	
			Plasma Retinol(μmol/L)	Control	1.89±0.43	1.79±0.37	NR	NS
				Treatment	1.89±0.39	1.84±0.36	NR	
			Plasma Vitamin C	Control	57.2±28.0	51.5±32.6	NR	NS
				Treatment	57.6±30.6	59.3±29.1	NR	
			Plasma α-tocopherol	Control	27.3±6.7	26.3±5.4	NR	NS
				Treatment	27.3±6.2	26.6±6.1	NR	

δ change in concentrations (post-intervention – baseline); † p value relates to difference between baseline and post-intervention values, *p value relates to difference between groups in terms of the effect size, **p value relates to difference in fitted slopes between groups. NR, not reported

Table 2.2: Findings of studies with pre and post-intervention measures and comparison between control and treatment group (continued)

Author (Year)	Method of analysis	N in analysis	Nutrient	Group	Baseline	Post-intervention	Effect Size (δ)	P value
Gill (2004)	Independent t test used to assess differences in Δ between groups	18	Plasma α-carotene (µmol/L)	Control	0.20±0.14	0.23±0.12	0.03±0.18	NS
				Treatment	0.20±0.14	0.17±0.12	-0.03±0.06	
			Plasma β-carotene (µmol/L)	Control	0.68±0.33	0.99±0.67	0.31±0.70	NS
				Treatment	0.66±0.48	0.57±0.40	-0.10±0.18	
			Plasma Lutein (µmol/L)	Control	0.32±0.16	0.39±0.17	0.08±0.16	NS
				Treatment	0.36±0.22	0.49±0.30	0.13±0.16	
			Plasma Retinol (µmol/L)	Control	2.27±0.44	2.36±0.39	0.09±0.75	NS
				Treatment	2.71±0.66	2.82±0.85	0.11±0.51	
			Plasma Vitamin C (µg/L)	Control	12.04±4.98	11.04±2.81	-1.00±3.85	NS
				Treatment	10.61±2.26	11.01±3.36	0.40±2.52	
			Plasma α–cryptoxanthin (µmol/L)	Control	0.08±0.02	0.10±0.03	0.02±0.002	NS
				Treatment	0.09±0.07	0.10±0.08	0.01±0.02	
			Plasma β-cryptoxanthin (µmol/L)	Control	0.25±0.19	0.31±0.17	0.05±0.23	NS
				Treatment	0.23±0.18	0.25±0.19	0.04±0.09	
			Plasma Lycopene(µmol/L)	Control	1.06±0.36	1.27±0.55	0.21±0.54	NS
				Treatment	0.98±0.53	0.95±0.66	-0.03±0.36	
Møller (2003)	No statistical analysis	31	Plasma Vitamin C (µM)	Control	67.3±17.3	21.1±11.8	NR	NR
				Treatment	74.8±19.5	78.5±13.6	NR	
			Plasma β-carotene (µg/100ml)	Control	19.7±14.5	10.4±8.5	NR	NR
				Treatment	18.6±9.5	30.9±15.7	NR	

δ change in concentrations (post-intervention – baseline); † p value relates to difference between baseline and post-intervention values, *p value relates to difference between groups in terms of the effect size, **p value relates to difference in fitted slopes between groups. NR, not reported

Table 2.2: Findings of studies with pre and post-intervention measures and comparison between control and treatment group (continued)

Author (Year)	Method of analysis	N in analysis	Nutrient	Group	Baseline	Post-intervention	Effect Size (δ)	P value
Maruyama (2001)	Wilcoxon signed rank sum test used to compare pre and post-intervention values.	20	Plasma α-carotene (µmol/L)	Control	0.205±0.112	0.189±0.086	NR	NS†
				Treatment	0.262±0.251	0.209±0.090	NR	NS†
			Plasma β-carotene (µmol/L)	Control	1.241±0.621	1.255±0.808	NR	NS†
				Treatment	1.456±0.927	2.686±0.857	NR	<0.01†
			Plasma Cryptoxanthin (µmol/L)	Control	0.243±0.117	0.465±0.272	NR	<0.01†
				Treatment	0.350±0.225	0.388±0.279	NR	NS†
			Plasma Lycopene (µmol/L)	Control	0.672±0.232	0.546±0.186	NR	<0.05†
				Treatment	0.647±0.245	1.842±0.216	NR	<0.01†
			Plasma α-tocopherol (µmol/L)	Control	22.23±2.22	22.37±2.34	NR	NS†
				Treatment	28.48±3.61	27.72±3.86	NR	NS†
			Plasma Vitamin C (µmol/L)	Control	75.88±16.42	70.17±10.75	NR	NS†
				Treatment	68.11±12.75	73.37±20.70	NR	NS†

δ change in concentrations (post-intervention – baseline); † p value relates to difference between baseline and post-intervention values, *p value relates to difference between groups in terms of the effect size, **p value relates to difference in fitted slopes between groups. NR, not reported

Table 2.3 Findings of studies with pre and post-intervention measures and no control group

Author (Year)	Method of analysis	N in analysis	Nutrient	Group	Baseline	Post-intervention	Effect Size (δ)	p value
Agte (2006)	Calculation of % increase between baseline and post-supplementation. Wilcoxon test to compare pre and post-intervention values.	12	Plasma β-carotene (mg/L)	Treatment	1.07±0.11	NR	+6%	NS†
			Plasma Vitamin C (mg/L)	Treatment	0.32±0.01	NR	+5%	0.002†
			Plasma zinc (mg/L)	Treatment	0.84±0.06	NR	+2.5%	NS†
Chopra (2000)	No results of statistical analysis reported.	16	Plasma β-carotene (nmol/L)	Treatment	412±340	738±340	NR	NR
			Plasma lutein (nmol/L)	Treatment	242±110	591±400	NR	NR
Chopra (2000)	No results of statistical analysis reported.		Plasma lycopene (nmol/L)	Treatment	319±330	852±340	NR	NR
Hoelzl (2008)	Two-factor ANOVA used to compare pre and post-intervention values.	8	Plasma Vitamin C (µmol/L)	Treatment	48±7	61±5	NR	<0.05†
Sanchez-Moreno (2006)	Repeated measures ANOVA used to compare pre and post-intervention values.	6	Plasma Vitamin C (µmol/L)	Treatment	50.2±2.3	61.2±1.5	22%	0.03†
Tesoriere (2004)	One way ANOVA used to compare pre and post-intervention values.	18	Plasma Vitamin A (µmol/L)	Treatment	2.00±0.38	2.19±0.35	NR	NS†
			Plasma Vitamin C (µmol/L)	Treatment	62.1±10.0	84.0±15.0	NR	<0.05†
			Plasma Vitamin E (µmol/L)	Treatment	18.3±1.4	20.8±2.0	NR	<0.05†

δ change in concentrations (post-intervention – baseline); † p value relates to difference between baseline and post-intervention values, *p value relates to difference between groups in terms of the effect size, **p value relates to difference in fitted slopes between groups. NR, not reported

Table 2.4 Findings of studies with a control and treatment group and no pre-intervention measures

Author (Year)	Method of analysis	N in analysis	Nutrient	Post Intervention measures		Effect Size	p value
				Control	Treatment		
Brevik (2004)	Parallel analysis, no adjustments (Mann Whitney U test)	40	Plasma α-carotene (nmol/L)	48±21	69±28	20 (42%)	0.013
			Plasma β-carotene (nmol/L)	436±244	627±233	190 (44%)	0.016
			Plasma β-cryptoxanthin (nmol/L)	205±80	187±83	-17 (-9%)	0.484
			Plasma Lutein (nmol/L)	254±79	311±113	57 (22%)	0.076
			Plasma Zeaxanthin (nmol/L)	27±9	32±15	5 (19%)	0.178
			Plasma Lycopene (nmol/L)	475±122	555±135	80 (17%)	0.057
Martini (1995)	Repeated measures ANOVA	19	Plasma α-carotene (µmol/L)	0.09±0.05	0.09±0.07	NR	NR
			Plasma β-carotene (µmol/L)	0.26±0.13	0.37±0.19	NR	NR
			Plasma Lutein (µmol/L)	0.26±0.09	0.53±0.16	100%	<0.001
			Plasma Lycopene (µmol/L)	0.32±0.12	0.29±0.13	NR	NR
			Plasma β-cryptoxanthin (µmol/L)	0.15±0.12	0.15±0.13	NR	NR

NR, not reported

2.4 Discussion

The present review was designed to determine whether dietary interventions comprising fruit and vegetables are effective in enhancing micronutrient status among women of reproductive age. We conducted a database search and identified fourteen studies describing such interventions in our target population. Of these, six compared the change in micronutrient concentrations pre and post-intervention between an intervention and control group. All but one of the studies with such a design reported a significant difference between groups for at least one micronutrient measured. Of the eight studies with less robust designs, two did not report any statistical analyses of the findings. The remaining six reported changes in nutrient concentrations associated with the intervention in the absence of a control group, or a difference between control and treatment groups in terms of post-intervention micronutrient concentrations. Most studies looked at the effects of fruit and vegetable interventions on carotenoids and vitamin C and, of those that did, about two thirds found a positive effect of the intervention on these nutrients. Fewer studies investigated the effect of the interventions on vitamin E, retinol and zinc and the results for these nutrients were mainly negative, apart from one positive result for vitamin E. It is important to evaluate these findings based on the fact that the analyses were conducted in different ways. For example the positive result for vitamin E was found in a study comparing pre and post intervention status with no control for comparison. Only one study assessed the effect on folate concentrations and found a positive result.

The majority of studies were conducted in high income countries. This is likely to be due to availability of resources and funds. The aim of interventions in such settings is usually a reduction in rates of chronic disease such as cancer or CVD. While the prevalence of these conditions is increasing in LMICs, there are also problems of undernutrition and communicable diseases associated with micronutrient deficiencies (section 1.1.4, p10). A systematic review of studies assessing the effect of multiple micronutrient supplementation on children's cognitive performance found that the effect size was larger in countries with a higher HDI ranking (171). The authors suggested that this may be due to higher pre-intervention nutrient concentrations in wealthier

countries and higher prevalence of protein-energy under-nutrition inhibiting absorption or utilisation of micronutrients in low income countries. In a review of maternal intervention studies in developing countries, multiple micronutrients were found to have a greater effect among women with higher BMI (115) which supports this suggestion. These findings highlight the need to design interventions that are tailored to the nutritional requirements of the at-risk-group.

2.4.1 Study quality

The studies in the present review were generally small and therefore some may have been affected by a lack of power to detect an effect. It is likely that the small numbers are due to the cost and logistics of blood micronutrient concentration analysis which usually involves specific processing of blood samples and storage below -70°C. There may also be difficulties obtaining blood in certain settings for cultural reasons, particularly among apparently healthy individuals.

Only six of the fourteen studies assessed the effect of the intervention by comparing the change in nutrient concentration over time with that in a control group. Changes in season or other environmental factors could affect nutrient concentrations and without a control group it is not scientifically sound to attribute any changes in concentrations to the intervention. Some of the intervention periods were short when considering the nutrient of interest. For example, the study by Brouwer et al (158) intervened for four weeks and assessed plasma and red cell folate. Given that the average turnover of red cells is 12 weeks, a greater effect may have been seen if the intervention period had been extended.

Another possible explanation in cases where there was no effect of the intervention is that the participants in the treatment groups consciously or otherwise reduced their fruit and vegetable intake so that the difference between the control and intervention groups was smaller than prescribed in the study protocol. This phenomenon was observed in the UK 'School Fruit Scheme' whereby children who received fruit at school as part of a nationwide programme were given less fruit at home by their parents or guardians. Thus

an intervention designed to increase children's intake of fruit by 1 portion per day effected only a 0.5 portion per day increase (172). Parents may have assumed that because children had eaten fruit at school they required less at home (173). This phenomenon represents an important challenge when designing food-based interventions and highlights the need for monitoring adherence to study protocols.

2.4.2 Limitations of the review

Due to the heterogeneity of interventions and outcome measurements within the studies, it was not possible to perform a meta-analysis which would have allowed an estimate of the effect size that could be achieved using fruit and vegetable interventions. This review was limited to intervention studies among women of reproductive age. It is likely that if the target population were more wide-ranging there would have been many more eligible studies with larger numbers of participants. Future research could consider a broader range of outcomes including more functional measurements of health and nutritional status such as dark adaptation, physical performance and psychological health and well-being.

2.5 Conclusion

The findings of this review suggest that increasing intakes of fruit and vegetables by as little as one serving per day can have beneficial effects on the micronutrient status of women of reproductive age. The evidence suggests that these interventions are most effective at increasing carotenoid and vitamin C concentrations. The limited studies with vitamin E, retinol and zinc as outcomes indicated that these nutrients were less responsive to such interventions. Our findings highlight the lack of data from LMICs where such interventions may be of particular benefit for the women themselves and the next generation. It is important that trials investigating the effect of food-based interventions in LMICs are made a research priority.

A substantial challenge when designing food-based interventions is ensuring that participants do not reduce their usual intakes of the intervention foods. It

is also very important that the prescribed changes to the diet are sustainable, accessible by, and acceptable to the community.

3. Methods

This chapter details the methodology of the Mumbai Maternal Nutrition Project (MMNP), and the MMNP Extension Study (Extension Study) conducted in 2009-2010. The MMNP was a large randomised controlled trial started in January 2006. It was designed to investigate the effect of consumption of a food-based supplement containing GLV, fruit and milk for at least three months prior to conception and throughout pregnancy on infant size, mortality and risk factors for chronic disease. The rationale for the MMNP is given in section 1.6 (p38). The Extension Study was a much smaller randomised controlled trial assessing the effect of the MMNP supplement on blood micronutrient concentrations of women of reproductive age. This was not assessed in the main MMNP trial to minimise blood collection which was thought likely to deter women from taking part. The Extension Study was conducted approximately 10km away from the MMNP study area and the participants were not registered in the MMNP at any time.

As explained in the Author's contribution section (page xv), the MMNP (known locally as 'Project Saras') was conceived and designed by Professor Caroline Fall and Dr Ramesh Potdar and colleagues. The project started with a two-year pilot study (2004-2005) in the Shetanchowki area of Mumbai, based in the Streehitakarini Health Centre. The main trial was launched in January 2006 in Bandra and was run from the Centre for the Study of Social Change (CSSC). I joined the MRC-Lifecourse Epidemiology Unit in September 2006. Since then I have visited Mumbai 2-3 times per year and have been working with the research team on the nutritional aspects of the MMNP: developing the supplements and organising lab tests for safety and micronutrient content; dietary data collection methods; liaising with collaborators on all nutritional aspects of the trial; analysis of dietary and nutritional status data. In 2008, I conceived and designed the Extension Study with input from my supervisors Professor Caroline Fall, Professor Barrie Margetts and the MMNP team. My nutritionist colleague in Mumbai, Harsha Chopra, was also keen to do a PhD and we decided to work together on the Extension Study. Her project involved assessing the effect of the MMNP supplement on erythrocyte fatty acid concentrations and markers of oxidative stress among the women. Harsha and I investigated several options for collaborators and study settings (section

3.2.2). We chose to run the study in Shivaji Nagar in conjunction with a non-government organisation (NGO) called Apnalaya. We employed a small team comprising a nutritionist Bhavya Rhanadive, a nurse Vaishali Thakur, and three project assistants: Fouziya Sayeed; Manju Rajbhar; Gazala Sahik. Harsha and I jointly supervised this team and an additional 11 Apnalaya health workers to run the study. We rented a room in Shivaji Nagar to be used for blood collection and processing. Harsha and I processed the blood samples collected in the Extension Study ourselves (section 3.2.9). The analyses of blood samples described in this thesis were conducted by laboratory staff at the Nair Hospital, Mumbai, MRC Human Nutrition Research Unit, Cambridge and the Diabetes Unit at the King Edward Memorial Hospital, Pune and Dr Dharap's Diagnostic Centre, Mumbai (section 3.2.10). All other data in the Extension Study were collected by us and our research team. Harshad Sane, Vanessa Cox and Patsy Coakley managed the database, cleaned the data and built analysis files. All statistical analysis in this thesis was conducted by me with input from Clive Osmond, Sarah Crozier, Aravinda Guntupalli, Nicola Winder and Ella Marley-Zagar.

3.1 MMNP methodology

3.1.1 Participants and setting

Participants in the MMNP were married women of reproductive age living in the slum areas of Bandra, Khar and Andheri in Mumbai. Women were recruited by trained health workers who were familiar with the communities living in the study area. The eligibility criteria were as follows:

1) Willing to join the project for at least one year
2) Married
3) Aged 15-35 years
4) Not pregnant but positively intending to have children
5) Planning to deliver in Mumbai

Women who met these criteria were asked to give their written consent to take part in the project.

3.1.2 Study design

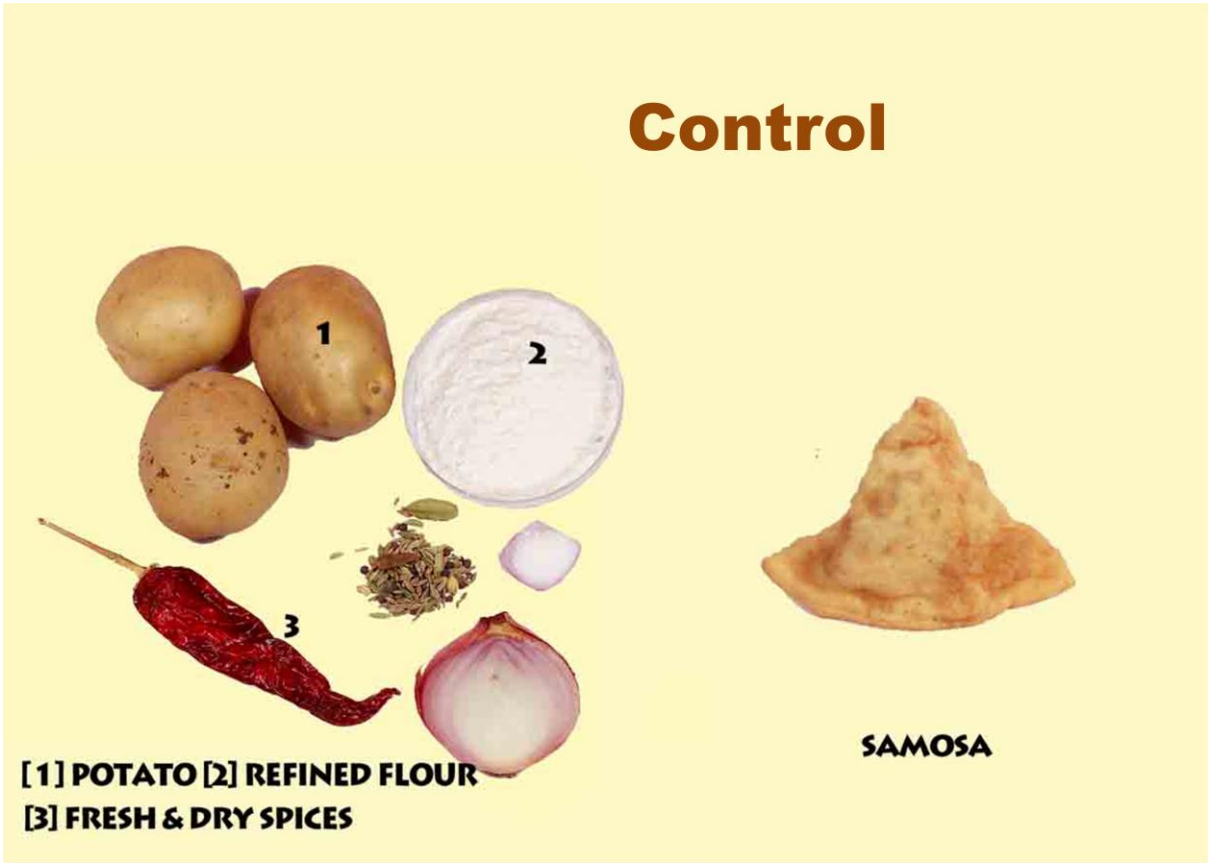
The MMNP was a randomised controlled trial. Women who fulfilled the eligibility criteria and gave consent to participate were randomised to receive the intervention or control supplement. Randomisation was carried out remotely from the study setting in Southampton and was stratified by age and BMI in order to balance the groups with respect to these variables. Blinding of the intervention was not feasible in this study as the intervention and control snacks obviously contained different foods. In order to provide some degree of allocation concealment, each arm of the intervention was divided into two groups to give a total of four sub-groups which were identified using colour coding (red and blue for the intervention group, green and yellow for the control group). The women were given an ID card after randomisation indicating which group they had been assigned to and the supplements were distributed in colour coded bags (**Figure 3.1**). The project staff who collected data and interacted with the women were not aware of the snack composition.

Figure 3.1 MMNP ID cards and supplement bags indicating treatment group allocation

3.1.3 Supplement

The supplement was a cooked snack such as a 'samosa' or 'patty' made from locally available food ingredients (**Figure 3.2**). They were prepared and cooked at the CSSC project kitchen (**Figure 3.3**). Intervention supplements contained approximately 25g fresh GLV (e.g. spinach, colocasia, coriander, and fenugreek), 10g dried fruit (e.g. figs, dates, raisins) and 12g whole milk powder. The control supplements contained foods of lower micronutrient content such as potato, sago or tapioca. Both types of supplement were prepared with binding ingredients such as wheat or corn flour, and spices were added to enhance palatability. They were fried in sunflower oil.

Figure 3.2 Examples of MMNP Supplement and Ingredients



There were some changes made to the ingredients and recipes used to produce the supplements over the course of the trial. A detailed description of the supplement development is given in section 3.1.4.

Several varieties of the intervention and control supplements were developed in order to provide variety. There were approximately 6-8 different recipes in each of the four groups giving a total of 24-32 different recipes in use at any point in the trial. On any given day all women in a particular group would receive the same supplement. There was no pattern to the days on which the different supplements were distributed so that it was not possible for the women to predict when a specific supplement would be available.

The average weight of the supplements was approximately 65g (intervention) and 36g (control). The women were advised to consume the supplement in addition to their habitual diet and they were therefore made available to the women at a time least likely to interfere with their usual intake; between 3:00pm and 6:00pm, Monday to Saturday.

Figure 3.3 MMNP kitchen

Left, raw supplements being shaped before frying. Right, supplements being shallow fried

3.1.4 Supplement development

Supplement development was carried out by Devi Shivashankaran, Purvi Chheda, Harsha Chopra, Preeti Adekar and myself (174). This section describes the stages of development of the intervention supplement. Nutrient content, acceptability, safety, cost, availability of ingredients, man-power and cooking facilities were considered when developing the supplements. There were four chronological stages of development: pilot study; main trial 1; main trial 2; main trial 3.

3.1.4.1 Target nutrient content

The starting point for setting the target nutrient content of the supplements was data collected during the PMNS on women's habitual food intake using a 111-item FFQ (139). Average intakes of 'marker' nutrients: β-carotene, riboflavin, folate and vitamin C were estimated. The amounts of nutrient that would increase daily intakes of the 'marker' nutrients above the 75th centile of intakes of the women in the PMNS were then calculated (**Table 3.1**). Because it was anticipated that on average MMNP participants would attend on alternate days (i.e. 3 days per week) rather than 6 days per week, the target nutrient content was set at double the amount that would move daily intake to the 75th centile of the PMNS. This amount was found to be approximately equal to one third of the UK estimated average requirement (EAR) for riboflavin and folate (175).

Table 3.1 Nutrient intake in the PMNS and target nutrient content of the MMNP supplement

	B-carotene (µg RE)	Riboflavin (mg)	Folate (µg)	Vitamin C (mg)
Median daily intake during MMNP pilot study	600	0.65	126	21
75th centile of intake in PMNS	654	0.82	164	23
Target nutrient content of supplement	108	0.34	76	4
UK Estimated Average Requirement	500	1.2	250	25
Safe Upper Limit (176)	1166	-	-	-

3.1.4.2 Assessment of nutrient content of supplements

At each stage of the trial, the supplements were tested for micronutrient content. Homogenised and frozen samples of the supplements were flown to the UK on dry ice and analysed at a commercial laboratory (Eclipse Scientific Group, Cambridge). The methods for each assay are described in sections 3.1.4.2.1–3.1.4.2.6.

3.1.4.2.1 B-carotene

B-carotene analysis was conducted away from natural light using amber glassware. The sample was saponified with ethanolic potassium hydroxide and the carotene was extracted into hexane. The hexane was evaporated off to dryness and the carotene dissolved in mobile phase and quantified by high performance liquid chromatography (HPLC) with UV detection, against a calibration standard of known concentration.

3.1.4.2.2 Riboflavin

Riboflavin was extracted by digestion with 0.1M hydrochloric acid followed by enzyme digestion with claradiastase. The filtered solution was analysed by reversed phase HPLC using fluorescence detection, against calibration standard solutions of known concentration.

3.1.4.2.3 Folate

Folates were extracted from the samples using 0.1M potassium phosphate buffer and heated for 15 minutes at 100°C. The filtrate was diluted to a suitable level and treated with deconjugase enzyme. L-Ascorbic acid was also added to prevent oxidation. This complex was incubated at 37°C for 4 hours. Using an autodiluter, sample extracts were diluted to values within the calibrated range. Folic acid casei media was added to the diluted samples which were then covered with aluminium foil and sterilized at 121°C in an autoclave. The assay was inoculated with *Lactobacillus rhamnosus*, and was incubated overnight at 37°C. The concentration of folate in the sample was measured spectrophotometrically.

3.1.4.2.4 Vitamin B12

Vitamin B12 was extracted from the samples using a buffer preheated to 50°C. Enzyme was added to the samples and they were left to stand for 30 minutes prior to being homogenised. Samples were then placed in the autoclave. After cooling, samples were made up to known volumes. Where necessary, dilutions were performed and finally the samples were filtered. Samples and known standard concentrations were then presented to the Biacore® Q (G.E. Healthcare, Amersham) on a microtitre plate. The samples were mixed with a constant amount of Vitamin B12-specific antibody or detecting molecule. This mixture was then run over a gold plated chip whose surface had been immobilised with a Vitamin B12 derivative on the chip. The amount of antibody binding to the chip was inversely proportional to the amount of Vitamin B12 in the sample. Binding of the antibody to the chip was measured by a change in the refractive index of the chip surface. The limit of quantification was 0.0020µg/g.

3.1.4.2.5 Vitamin C

Ascorbic acid was extracted from the sample using metaphosphoric acid (MPA) and EDTA. The ascorbic acid was then enzymatically oxidised to dehydro-ascorbic acid which was condensed with O-phenylene diamine to the fluorescent quinoxaline derivative. The latter was separated from interfering compounds by reverse phase HPLC with fluorimetric detection.

3.1.4.2.6 Minerals

To assess mineral content, samples were dried and ashed at 550°C for 16 hours, then dissolved in 5M hydrochloric acid and scandium internal standard/ caesium chloride solution was added. After filtration and dilution to known volumes with water, the concentration of each mineral was determined by 'Liberty series II' Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES).

3.1.4.2.7 Energy

The energy content of the supplements was calculated using the values for raw ingredients from Indian food composition tables (177).

3.1.4.3 Formulation

Initially, consideration was given to supplying the foods in the form of a piece of raw fruit, a cooked portion of GLV and a milk drink. This was not, however, feasible in the context of the trial, for a number of reasons: firstly, the daily purchase of all the fresh ingredients was too costly in terms of staff time; secondly, it was not possible to deliver these foods in a palatable state, or to maintain microbiological safety, during their distribution throughout the large slum area; thirdly, it would have been difficult to record the women's intake, in a simple way, with the foods in this form; finally, it was clearly impossible to make the intervention and control supplements appear similar using this approach. Therefore the three ingredients were combined and made into cooked snacks, similar to 'street foods' such as samosas, widely available in Mumbai.

During the pilot study phase, it was not possible to purchase and prepare sufficient quantities of fresh ingredients for the supplements due to man-power constraints. Initial formulations therefore contained dehydrated, powdered GLVs, fruit and milk. A Mumbai-based commercial company supplied vegetable and fruit powders prepared using a novel technique of room-temperature drying. These powders had superior aroma and flavour compared with heat-dried powders and nutrient retention was maximised. The use of powders allowed the inclusion of greater quantities of the GLV, fruit and milk in the limited volume available. They were combined with other 'binding' or 'covering' ingredients such as chickpea flour or semolina and seasoned with local spices.

3.1.4.4 Product development

The recipes for the supplements were initially developed by the project nutritionists, experimenting on a small scale at home in their kitchens.

Preparation of the supplements was then scaled up with the installation of a large project kitchen, staffed by 19 men and women and equipped with a range of basic kitchen facilities (including a large gas stove, oven, chilled storeroom and stainless steel preparation surfaces). Development of new recipes (to avoid monotony for the women), and the introduction of more palatable formulations, was an ongoing process.

3.1.4.5 Choice of green leafy vegetable

The choice of GLV to be added to the supplements was initially based on the availability of the dehydrated powders and the opinions of project staff as to acceptability. In early 2007, the dehydrated powders of ten different GLVs (spinach, coriander, colocasia, fenugreek, radish leaf, curry leaf, onion stalk, shepu, drumstick leaf, green amaranth and red amaranth) were analysed by a UKAS accredited laboratory (Eclipse Scientific Group, Cambridgeshire, UK) for micronutrient content. The powders were also analysed for polyphenol (Global Analytical Services, Heidelberg, Germany) and oxalate content (Lincoln University, Canterbury, New Zealand) (178). Polyphenols and oxalates are considered 'anti-nutrients' because they inhibit absorption of minerals, specifically iron and calcium respectively (179;180) . The dehydrated powders were crudely ranked according to nutrient and 'anti-nutrient' content; those with the lowest overall score being the most nutritious and containing the least anti-nutrient (appendix 1). The selection of GLVs used in the supplements was based on a combination of these data, acceptability to the women and seasonal availability.

3.1.4.6 Stages of development

As the study progressed, a series of major changes were made to the supplements (Table 3.2). These were largely to maintain nutrient content and achieve palatability. It was thought that the latter was likely to have an important impact on participant compliance. Firstly, the amount of GLV powder added to the supplements was reduced, to make the supplement taste less bitter (Main study 1). Next, 50% of the GLV powder was substituted with fresh GLVs (Main study 2). There were, however, other reasons for some of the changes; a problem with rat infestation on the premises of the commercial

dehydrated powder suppliers forced a complete change to the use of fresh rather than dried GLVs and dried fruit rather than dried fruit powder. The final formulation, used from June 2007 until the end of the trial, was named 'Main study 3'.

3.1.4.7 Assessment of the acceptability and safety of supplements

New supplement recipes were tested for palatability by project staff and small panels of local women before being distributed to the field. In addition to this anecdotal approach, acceptability was assessed more objectively using data on supplement consumption. The proportions of women attending the distribution centre and consuming the whole supplement (recorded as '1'), at least half but not the whole supplement (recorded as '0.5') or less than half (recorded as '0') were calculated and used as a proxy for the acceptability of each recipe. Microbiological testing of supplements was performed throughout the study; the supplements were tested for the presence of coliforms, salmonella, E-coli and aflatoxins at a commercial lab (Intertek Laboratory, Mumbai). All supplements were prepared and cooked fresh every day. There were some high levels of aflatoxins found in some of the supplements which were traced to the dried fruit. Based on this finding, we ensured that all dried fruit was frozen at -20°C until use. Tests on the supplements following this change in procedure showed that aflatoxin levels were within safe limits.

3.1.4.8 Production cost of the supplement

The costs of the ingredients, staff wages, cooking fuel and packaging were used to calculate the unit cost of the supplements. This was compared with the cost of the UNICEF multiple micronutrient tablet (181) The average unit production cost of snacks made using dehydrated leaves (main study 1) was 13 Indian rupees (approximately $0.33). This cost was reduced to 5 rupees ($0.13) for the snacks made with fresh GLVs. These prices were comparable to similar 'street' snack foods that the women consumed in this part of India. The unit cost was higher than that of the UNICEF multiple micronutrient tablet which is approximately $0.02 per daily dose (181).

Table 3.2 Mean nutrient composition and percentage contribution to nutrient requirements of the MMNP supplements

	Control	Intervention
Nutrient	**Mean (range) nutrient content per supplement**	
ß-Carotene (RE)	2 (0-3)	223 (21-595)
Riboflavin (mg)	0.01 (0.00-0.02)	0.12 (0.00-0.22)
Folate (µg)[a]	6.1 (2.7-12.1)	49.0 (5.2-93.0)
Vitamin C (mg)	0.00 (0.0-0.6)	4.1 (0.0-36.6)
Vitamin B12 (µg)	0.18 (0.00-0.60)	0.30 (0.00-0.74)
Calcium (mg)	25 (8-87)	169 (52-356)
Iron (mg)	0.90 (0.65-1.28)	3.65 (1.22-7.59)
Energy (kcal) [b]	90 (65-158)	164 (134-220)
Protein (g) [b]	2.4 (1.0-3.3)	6.7 (2.7-7.9)
	Mean % of EAR[c] per supplement	
ß-Carotene (RE)	<1	45
Riboflavin (mg)	1	10
Folate (µg)	2	20
Vitamin C (mg)	0	12
Vitamin B12 (µg)	14	24
Calcium (mg)	4	27
Iron (mg)	8	32

GLV: green leafy vegetable. [a] Values are total folate. [b] Macronutrient content calculated using Indian Food Tables (16). [c] UK Estimated Average Requirement during pregnancy (11).

3.1.5 MMNP procedure

At registration, demographic, anthropometric and dietary data were collected. Blood samples were not collected at this time as it was thought this would discourage women from participating. The women were then invited to consume the supplementary snack six days per week in addition to their normal diet. The supplements were provided at centres which were usually no more than 10 minutes walking distance from the women's homes. Health workers distributed the supplements and recorded consumption. Women who became pregnant before they had been consuming the supplement for 3

months were retained in the study but the pregnancy did not count towards the target of 1600 births. All women were followed up throughout pregnancy until delivery and there is currently ongoing follow up of the children. Aspects of the procedure are described in detail in sections 3.1.5.1-3.1.5.4.

3.1.5.1 Demographic data

Data on religion, education, occupation, husband's education, husband's occupation and household standard of living were collected by interviewer-administered questionnaire. The interviews were conducted by a trained project assistant in Hindi or Marathi depending upon the woman's first language. Registration data collection forms can be found in appendix 2.

Occupations were classified as: professional (e.g. doctor, lawyer, accountant, engineer); graduate/semi-professional (e.g. nurse, secretary, beautician); self employed (e.g. shop owner, rickshaw owner, estate agent); skilled worker (e.g. carpenter, tailor, electrician); semi-skilled worker (e.g. salesman, rickshaw driver, junior clerical staff); unskilled worker (e.g. daily wage labourer, household work assistant, loader).

The standard of living questionnaire was based on the NFHS data form from the survey in 1999-2000 (123). The questions were designed to obtain information about type of housing, toilet facilities, access to water and cooking fuel, possessions and land ownership. The multiple choice responses were coded numerically and summed to give a standard of living score.

3.1.5.2 Anthropometry

The following measurements were made while the woman was wearing light clothing and no footwear: height to the nearest 0.1cm using a portable stadiometer (Microtoise, UK); weight to the nearest 100g using electronic scales (Salter, UK); head, waist, hip and MUAC using anthropometric tape; triceps, biceps and subscapular skinfolds using Harpenden skinfold callipers (CMS instruments, UK). The protocols followed can be found in appendix 3. The measurements were made by trained project assistants. All measurers

took part in inter-observer variation studies and data were checked against an experienced measurer.

3.1.5.3 Dietary data collection

A 212-item FFQ (appendix 4) was administered in Hindi or Marathi by a nutritionist or trained project assistant at the time of registration and again at 6 months following registration in order to examine whether the women's food intake changed during the supplementation period. The reference period for the FFQ was the last seven days and the women were asked whether their usual intake was affected by factors such as illness or festivals. The FFQ was developed specifically for use in the MMNP following detailed focus group discussions with the women from the study area and consultation with local nutritionists to ensure all foods consumed by women in the community were included.

3.1.5.4 Compliance with supplementation

Compliance in the MMNP was assessed on a daily basis by a member of the research team who recorded whether the woman attended the centre and whether she ate all, half or less than half of the supplement.

3.1.6 Pregnancy

Project health workers recorded details of the women's self-reported menstrual cycle in order to identify possible pregnancies. Last menstrual period dates were recorded and women who missed two periods were referred for confirmation and dating of pregnancy by trans-abdominal ultrasound. At this time (between 7-16 weeks of pregnancy) a venous blood sample was drawn. Serum was analysed for retinol, folate and vitamin B12 concentrations (section 3.2.10, p85). Two further scans were conducted in the second and third trimesters to monitor foetal growth. Women received normal antenatal care throughout pregnancy from the obstetricians at the hospital chosen for their delivery and were supplied with routine iron and folic acid tablets (100mg iron, 500µg folic acid) as per Indian government guidelines.

3.1.7 Delivery and infant follow up

The women planned to give birth in a range of government and private hospitals, some returned to the village where they were themselves born, in order to be with their families. The health workers aimed to maintain close contact with the women prior to delivery but this was not always possible. The obstetric team aimed to measure all babies born in Mumbai within 72 hours of birth. The following anthropometric measurements were made: weight, length, head circumference, chest circumference, MUAC and triceps and subscapular skinfolds. Four follow up visits were scheduled for the first year after birth and then every year subsequently. At the follow up, anthropometric measurements were made and information on infant feeding and morbidity was collected from the mother. The Developmental Assessment Scale for Indian Infants was used to assess neuro-cognitive development. In cases of neonatal or infant mortality, age and cause of death was recorded.

3.2 Extension study methodology

The Extension Study was started in October 2009 and was run simultaneously with MMNP in a separate location. The Extension Study was run in collaboration with Apnalaya, a Non-Governmental Organisation that provides health care, legal advice and social support to communities in the Shivaji Nagar area of Mumbai for a nominal cost to the recipient. There was considerable overlap between the MMNP and Extension Study in terms of methodology and, where this is the case, referral to the appropriate section of the thesis will be made. The primary objective of the Extension Study was to determine whether the MMNP supplement effected a change in micronutrient concentrations in the women as well as indicators of functional health. The rationale for the micronutrients selected and the intervention period is given in section 3.2.1.

3.2.1 Rationale for the selection of micronutrients to be investigated

The decision as to which micronutrients to investigate in the Extension Study was based on several factors: 1) scientific importance i.e. whether there was evidence that there were deficiencies of the nutrient in the population; 2) available resources i.e. funding for the assays to be conducted and the

availability of a lab to perform the analysis; 3) whether the MMNP supplement contained the nutrient according to available composition data (Table 3.2). There was a limit to the number of assays that could be performed due to funding constraints. Initially we sought to have all of the samples analysed in India but this was not possible for all of the nutrients.

There is a lack of data on micronutrient status among young Indian women (124). The majority of evidence is based on small research studies, many of which have been conducted in rural parts of India. Therefore the baseline micronutrient concentrations of the urban slum-dwelling women recruited to the Extension Study were likely to be an important contribution to the literature.

Preformed and pro-vitamin A compounds (retinol and β-carotene) were selected due to the evidence of high prevalence of vitamin A deficiency among young Indian women of reproductive age (182). It was known that the intervention supplements contained at least 25% of the UK EAR for β-carotene. Folate, vitamin B12 and iron were also selected based on evidence of deficiency among women in India (183;184). Vitamin C was selected as there was evidence of low intakes of fruit and vegetables among Indian women (137;139;185). We also assessed ferritin concentrations due to the high prevalence of anaemia among Indian women.

The length of the intervention was decided upon based on physiological evidence and in order to inform the MMNP. Consideration was given to availability of resources, cost and participant burden. In practical terms, it was important to avoid times of the year when large numbers of city dwellers leave Mumbai for their native towns and villages. We believed it was crucial to achieve good compliance and follow up rates. It was decided that the length of the intervention period should be twelve weeks. This was for two reasons: 1) the average life span of an erythrocyte is approximately twelve weeks and it is therefore considered that this period is sufficient for red cell stores of nutrients such as folate and vitamin B12 to increase. We measured plasma concentrations and it was predicted that increases in stores would be reflected in increasing circulating concentrations; 2) it was hypothesised in the MMNP that the consumption of the intervention supplement for at least twelve weeks

prior to conception would effect an increase in birthweight. The Extension Study was designed to inform MMNP findings, so it was considered appropriate to supplement the women for this length of time.

3.2.2 Selection of collaborator and study area

Prior to the study we met with possible collaborators and visited potential study settings. It was important for us to work with an organisation that had an existing health worker infrastructure as was the case for MMNP. It was also desirable for the organisation to be sympathetic to the importance of the role of nutrition for health. It was crucial that the setting was located within a one hour journey time from CSSC where the MMNP kitchen and the -80°C freezer were located. We explored three options: the NGO 'Reality Cares' based in the slum area of Dharavi; Sneha Mumbai based in Sion; Apnalaya in Shivaji Nagar. We chose the latter as it had the most well established health worker infrastructure. It was felt that the Dharavi area did not have a suitable health worker infrastructure and there was an intervention study being conducted in Sion by Sneha which we did not want to interfere with.

3.2.3 Setting

The recruitment and data collection were carried out in conjunction with Apnalaya in the Shivaji Nagar area of Govandi, East Mumbai (approximately 10km from CSSC) (Figure 3.4**Figure 1.1**). Shivaji Nagar is very close to the city's main rubbish dump. The air quality and sanitation is very poor. Access to water is very limited for the majority of people who rely on supplies from tankers operated by private firms for all of their water requirements.

The study was carried out at three separate centres named Padma Nagar, Shanti Nagar and Rafiq Nagar. All of the centres were rooms leased by Apnalaya for their community activities including legal advice, vocational courses, a crèche, provision of meals to children (**Figure 3.5**). Most participants lived within 5 minutes walking distance from one of the three centres. **Figure 3.6** and **Figure 3.7** show some images taken in the study setting.

Figure 3.4 **Map of Mumbai showing location of MMNP and Extension Study areas**

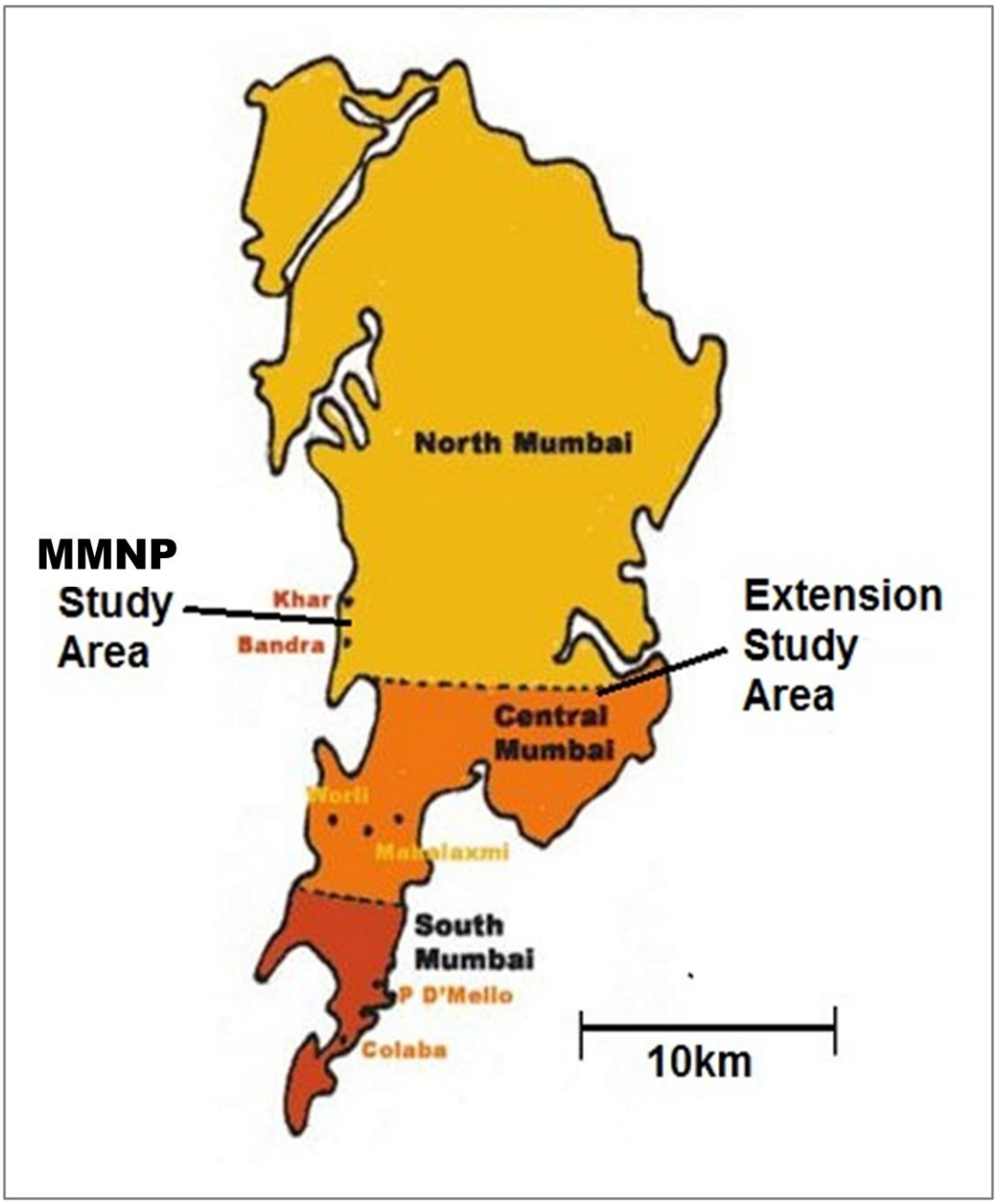


Figure 3.5 Community activities run by Apnalaya in Shivaji Nagar

Photograph A shows a member of Apnalaya staff (left) giving legal advice to a woman who is applying for a permit to collect items from the rubbish dump to be sold on.

Photograph B shows children being given a mid-day meal of rice and lentils at an Apnalaya community centre while their parents are working.

Photograph C shows some teenagers being taught how to apply henna (traditional Indian make up) during a vocational course being run and funded by Apnalaya.

Photograph D shows children having an afternoon sleep at a crèche run by Apnalaya for working parents.

Figure 3.6 Extension Study Setting

Photograph A shows the rubbish dump in Shivaji Nagar. Many of the members of the community collect and sell rubbish in order to make a living.

Photograph B shows a fish stall in the market in Shivaji Nagar

Photograph C shows green leafy vegetables being transported for sale in the market

Photograph D shows a queue for water. Water is transported to the Shivaji Nagar area by tanker and most people use this service for all their water requirements apart from drinking water which is usually purchased in bags

Figure 3.7 Daily life in the Extension study area

Photograph A shows a garment factory within the slum area of Shivaji Nagar.

Photograph B shows a self-employed man who runs his own small ironing business.

Photograph C shows a woman washing her clothes by the front door of her house.

Photograph D shows women preparing chapathis using a kerosene stove in one of the Extension Study centres.

3.2.4 Participants

Participants were women aged 15-35 years living in the Shivaji Nagar slum area of Mumbai. **Figure 3.8** shows some of the Extension Study participants consuming supplements in the study centres.

Figure 3.8 Extension Study participants at the supplement distribution centres

All women were non-pregnant and were not exclusively breast-feeding at the time of recruitment according to their self report. We chose to recruit non-pregnant, non lactating women in order to understand the effect of the supplement on the women themselves. It was thought that this would inform us about the effect of the supplement on the micronutrient status of women in the pre-conceptional period. This three month period was hypothesised to be important in terms of detecting an effect of the supplement on birthweight in the MMNP. There was a significant migration rate in some parts of the study

area so we asked women to consider taking part only if they had the intention of remaining in the study area for at least 3 months.

3.2.5 Recruitment

Local health workers informed women about the study during the months of August and September, 2009 and meetings were held to allow women to become acquainted with the research team and ask questions about the study. We chose to start the study at this time of year in order to avoid the summer and monsoon seasons and some of the festival periods when women were less likely to be available to take part.

3.2.6 Registration and Procedure

Registration took place over a two week period in November, 2009. Women were given an information sheet (appendix 5) and the study was explained to them on a one-to-one basis. If the woman agreed to participate, written informed consent was sought and recorded (appendix 6). At registration we collected data on age, religion, marital status, occupation, education and standard of living by questionnaire as in the MMNP (3.1.5.1, p73) (appendix 2). We asked about education and occupation of the woman's husband or father. Anthropometric measurements were also made at registration (appendix 3). Women were then randomly allocated to either one of two intervention or two control supplementation groups in the same way as for MMNP (section 3.1.2). **Figure 3.9** shows the study timeline.

We collected baseline data at visit 1 prior to starting supplementation. Baseline data included a blood sample, blood pressure measurement, FFQ (appendix 4), grip strength (appendix 7), (appendix 3) and general health questionnaire (GHQ) (appendix 8). **Figure 3.10** shows anthropometric and grip strength measurements being made. Women were invited to attend the centres six days per week throughout the supplementation period in order to consume the supplement. Attendance and consumption were recorded by project staff as for MMNP (section 3.1.5.4).

Figure 3.9 Extension study time line

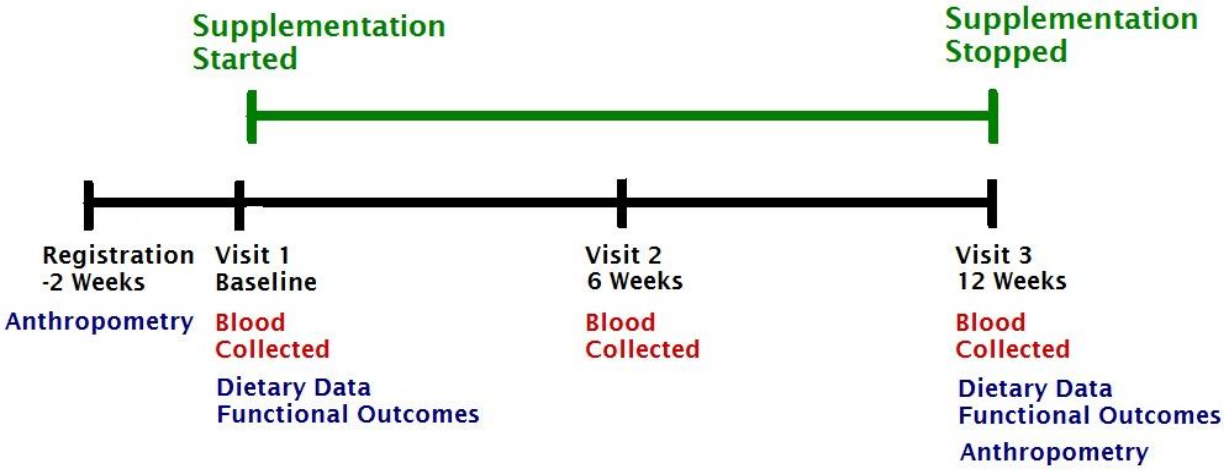


Figure 3.10 Anthropometric (left) and grip strength measurement (right) in the Extension Study

3.2.7 Sample size

The number of participants required to show an effect of the supplement on the 'nutrient status' outcomes was determined using published tables (186). A sample size of 82 per group was required to demonstrate a difference in the

proportion of women who were vitamin A deficient (defined as serum retinol <0.7μmol/L) of the order of 35% in the control group versus 10% in the experimental group at 90% power and at the p=0.05 significance level. This was based on data from the WHO VMNIS database whereby the prevalence of vitamin A deficiency ranged from 33-35% based on serum retinol concentrations in a study of 150 low income women in Calcutta (182). For each micronutrient measured, post hoc power calculations were conducted to determine the power achieved based on the number of women with blood measurement data at visit 1 and visit 3 and the effect size observed.

3.2.8 Supplementation

The participants were asked to consume the supplement 6 days per week (there was no distribution of the supplements on Sundays or public holidays) for a period of 12 weeks. The supplements were produced in the same batches as those sent out to the MMNP study area and supplementation methods were the same as in MMNP (section 3.1.3, p63). The mean micronutrient contents of the supplements provided to women in both the MMNP and Extension Study during the 12 week period are shown in Table 3.2.

3.2.9 Assessment of micronutrient concentrations

3.2.9.1 Blood collection and processing

A nurse, who had previous experience of blood collection in the MMNP study, took blood samples from the participants on three occasions:

1) 0 weeks supplementation (visit 1)
2) 6 weeks supplementation (visit 2)
3) 12 weeks supplementation (visit 3)

Approximately 12.5ml venous blood was collected by venipuncture using a needle and syringe while the participant was in a supine position. The blood was transferred from the syringe to vacutainer tubes for centrifugation as shown in **Table 3.3**. Tubes were centrifuged at 20°C for 10 minutes at 2000rpm using a refrigerated centrifuge (REMY, Pendleton, IN, USA). Depending on the assay, plasma or serum was pippetted into skirted 1.5ml vials and kept on dry ice until being transported to a -80°C freezer in Mumbai for storage until

analysis. For vitamin C analysis, 0.3ml plasma was added to 0.3ml of a 10% solution of MPA and stored at -80°C.

Table 3.3 Details of blood processing in the Extension Study

Vacutainer	Volume of blood (ml)	Assay	Vials
Lithium Heparin	2	Vitamin C	2 x 0.3ml plasma mixed with 0.3ml of MPA
Serum Separating Tube (kept in dark)	3.5	B carotene	2 x 0.6ml serum
Serum Separating Tube	4.5	Vitamin A	0.5ml serum
		Ferritin	0.5ml serum
EDTA	1.5	Folate, B12, homocysteine	0.5ml plasma
EDTA	1	Complete blood count	1ml whole blood (no centrifugation)

MPA, metaphosphoric acid. EDTA, Ethylenediaminetetraacetic acid

All samples were labelled with the participant ID number, assay, sample type and visit 1, 2 or 3. A complete blood count (CBC) was undertaken at a local commercial lab (Dr Dharap Diagnostic Centre, Mumbai) using an automated haematology analyser (ABX, Kyoto, Japan) a manual differential count was also carried out. Women were given the reports of the CBC within a week of blood testing. All results were shared with the doctor at Apnalaya. Women with haemoglobin concentrations of 10-12g/dL were advised to consult their doctor and consider taking iron supplements which they could obtain from Apnalaya. Treatment in the form of iron tablets and in some cases de-worming tablets was given by Apnalaya to women with haemoglobin counts of <10g/dl.

3.2.10 Laboratory methods

Serum was analysed for retinol and ferritin concentrations at the Nair Hospital, Mumbai. Folate, homocysteine, vitamin B12 and C-reactive protein (CRP) concentrations were measured at the King Edward Memorial Hospital, Pune. β-carotene assays and vitamin C and were conducted at the Medical Research

Council Human Nutrition Research Unit in Cambridge, UK. The methods used for each assay are described in sections 3.2.10.1 – 3.2.10.7

3.2.10.1 Serum retinol

A HPLC method was used. Two hundred microlitres of serum was added to 300µl acetonitrile and vortexed for 90 seconds then centrifuged at 3500rpm for 4 minutes. Supernatant was then separated and a 20µl aliquot injected. Serum standards were prepared by adding known amounts of retinol to pooled serum to give the concentration required for each of their calibration curves. The limit of detection was 30ng/ml and the limit of quantification 100ng/ml (187).

3.2.10.2 Serum β-carotene

Thawed subsamples of plasma were extracted with n-heptane in the presence of absolute ethanol, butylated hydroxytoluene (BHT) and α-tocopherol acetate (internal standard). The upper organic phase was evaporated nearly to dryness under vacuum, and was then re-dissolved in 250µl of the mobile phase. 50µl aliquots were then injected onto a 4µ Waters C18 column which was preceded by a 0.5µ reduced stainless steel filter frit, to remove any particles. The mobile phase was acetonitrile 44%, methanol 44%, dichloromethane 12%, by volume, with added BHT at 10mg/L. A Waters Millennium controlled HPLC system, with a photodiode array detector, was used. B-carotene was measured at 450nm. Peak area response factors were obtained from semi-pure, commercially available carotenoids. These were then corrected to 100% purity, by means of their HPLC patterns, and from their absolute optical densities and known extinction coefficients (188).

3.2.10.3 Plasma folate

Plasma folates were measured by microbiological assay using a chloramphenicol-resistant strain of *Lactobacillus. Casei* and using Victor-2 (PerkinElmer Life Science, Turku, Finland) (189;190).

3.2.10.4 Plasma Homocysteine

Total plasma homocysteine was measured by fluorescence polarization immunoassay (FPIA) using Abbott homocysteine kits on Abbott Axsym system (Abbott Laboratories, Abbott Park, IL 60064 USA). The Axsym homocysteine assay is based on the FPIA technology. Bound homocysteine (oxidized form) is reduced to free homocysteine that is enzymatically converted to S-adenosyl-L-homocysteine (SAH). SAH and labelled fluorecein tracer compete for the sites on the monoclonal antibody molecule. The intensity of the polarized fluorescent light was measured by the FPIA optical assembly (191).

3.2.10.5 Plasma vitamin B12

Plasma vitamin B12 was measured by microbiological assay using a colistin sulphate-resistant strain of L.Leichmanii (192;193).

3.2.10.6 Plasma C-reactive protein

Plasma CRP was measured in order to assess levels of acute phase reactants to interpret the results of serum retinol and ferritin analysis. CRP was assayed using a high-sensitivity ELISA kit (United Biotech, Mountain View, CA, USA); Victor-2 (PerkinElmer, Turku, Finland). The coefficient of variation for the assay was <11%. The cut off value for active inflammation was 5mg/L (194).

3.2.10.7 Plasma vitamin C

The assay was performed on a Roche Cobas Bio centrifugal analyser with fluorescence attachment. Ascorbic acid in the MPA stabilised plasma sample was converted to dehydroascorbic acid by ascorbate oxidase (Sigma, London). The resulting dehydroascorbate was coupled with o-phenylene diamine to give a fluorescent quinoxaline. The formation of this quinoxaline is linearly related to the amount of vitamin C in the sample, over the range 0-10µg/ml (0-5µM). The validity of the fluorimetric assay procedure used was by cross-correlation with HPLC-based assays, and by vitamin C spiking experiments (195).

3.2.10.8 Serum ferritin

A ferritin quantitative test kit based on solid phase enzyme linked immunosorbent assay was used. The assay system used one anti-ferritin antibody for solid phase (microtitre wells) immobilization and another mouse monoclonal anti-ferritin antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample was allowed to react simultaneously with the antibodies, resulting in the ferritin molecules being sandwiched between the solid phase and enzyme – linked antibodies. After 60 minutes incubation at room temperature, the wells were washed with water to remove unbound labelled antibodies. A solution of tetramethylbenzidine was added and incubated for 20 minutes, resulting in the development of a blue colour. The colour development was stopped with the addition of hydrochloric acid and the colour was changed to yellow and measured spectrophotometrically at 450 nm. The concentration of ferritin was directly proportional to the colour intensity of the test sample.

3.2.11 Functional outcomes

In addition to circulating concentrations, data on measures of functional health were collected. Initially it was hoped that it would be possible for us to measure dark adaptation. I looked into various options for acquiring a dark adaptometer that could be transported to India and used in our study setting. I made contact with Chris Hogg based at Moorfields Eye Hospital in London. He agreed to produce an adaptometer for us to use in India but unfortunately it was not ready in time to be used in the Extension Study.

3.2.11.1 Grip strength

A JAMAR Hydraulic Hand Dynamometer Model J00105 was used (Promedics, Blackburn, UK). Women were asked to sit comfortably in a standard chair with legs, vertical back support and fixed arms at a 90° angle to the back support. The same chair was used for every measurement. The forearms were rested on the arms of the chair with the wrist just over the end of the arm of the chair and thumb facing upwards (**Figure 3.10**, p84). The observer rested the base of the dynamometer on the palm of their hand as the woman held the dynamometer. The woman was encouraged to squeeze for as long and as

tightly as possible. Grip strength was recorded to the nearest 1kg. Three measurements were taken using each hand and the highest of the six measurements was used in statistical analysis.

3.2.11.2 Blood pressure

An automated blood pressure monitor was used to measure systolic and diastolic blood pressure at rest in a seated position (Omron HEM-711, Omron Healthcare, Hamburg). The cuff was applied to the left upper arm at the level of the heart. Three readings for systolic and diastolic blood pressure were recorded. The mean of the second and third measurements was used in statistical analysis.

3.2.11.3 Self-reported general health

An interviewer administered 12-item general health questionnaire (GHQ 12) was used to measure the woman's perception of her own health and well-being (196) (appendix 8). The questionnaire had previously been translated into Hindi and had been validated for use in a North Indian population (197). The questionnaire was translated into Marathi and back translated for use with participants who did not speak Hindi (~10%).

3.2.12 Assessment of diet and micronutrient supplement intake

The same 212-item FFQ as used in the MMNP was administered in Hindi or Marathi as appropriate by a nutritionist or trained nurse at registration and after 12 weeks of supplementation (visits 1 and 3 respectively). The form used for FFQ data collection can be found in appendix 4. Women were also asked if they were taking any synthetic micronutrient supplements in tablet or other form. Women who reported taking synthetic supplements were asked to bring the container to the centre so that the packaging could be examined and the micronutrients being taken recorded.

3.2.13 Ethical Approval

Ethical approval for the Extension Study was granted by the ethics committee of the JJ Hospital, Mumbai (appendix 9).

3.3 Data analysis

3.3.1 Handling of variables

Where data were normally distributed, means and standard deviations are presented, elsewhere the median and inter-quartile range is given. Where variables were not normally distributed the natural log was calculated. If this did not normalise the data, a Fisher-Yates transformation was made.

3.3.2 Calculation of outcome variables in the extension study

The change (δ) in blood nutrient concentration over the 12 week study period was calculated by subtracting the baseline (visit 1) value from the 12 week (visit 3) value. We calculated the proportion of women deficient in each of the micronutrients based on method specific or WHO published cut offs (92).

3.3.3 Statistical methods: MMNP and Extension Study

An intention to treat approach was taken in all statistical analysis. Women were included in the analysis if they had baseline and post-intervention data regardless of whether they had complied with the supplementation protocol. T tests were used to test for differences in means of continuous variables and chi-square tests were used to assess differences in proportions of categorical variables.

In the MMNP and Extension Study, logistic regression was used to calculate the relative risk of being deficient in early pregnancy and at visit 3 respectively for the control versus the intervention groups. The effect of allocation to the intervention group on change in nutrient concentration was assessed using univariate regression models. Although the women were randomised, in order to assess the effect of possible predictor variables on outcomes, we performed a multivariate regression adjusted for age, BMI, synthetic nutrient intake,

compliance status and visit 1 concentration with change in blood nutrient concentration as the dependent variable.

Retinol and ferritin are acute phase reactants, therefore high circulating levels can be a result of inflammation rather than an indication of replete stores. Women with CRP concentrations >5µg/ml were excluded from regression models relating to ferritin and retinol levels.

All statistical analyses were performed using the SPSS software package version 19 (SPSS Inc, Chicago, IL). Post hoc power calculations were carried out using G Power software version 3.1.3 (Universität Kiel, Germany).

4. Results

This chapter presents the analysis of data collected in the MMNP and Extension Study. The first section describes the participant flow in both studies (4.1). The second section (4.2) compares the MMNP and Extension Study participants in terms of socio-demographic and anthropometric characteristics. Dietary intakes of the women in both studies along with an assessment of change in diet over the course of the study period are presented in section 4.3. Section 4.4 presents data on the pregnancy micronutrient concentrations of MMNP participants. The effect of the intervention on micronutrient status among the women in the Extension Study and the results of post hoc power calculations are given in sections 4.5. Data on functional outcomes in the Extension Study are presented in section 4.6.

4.1 Participant flow in the MMNP and Extension Study

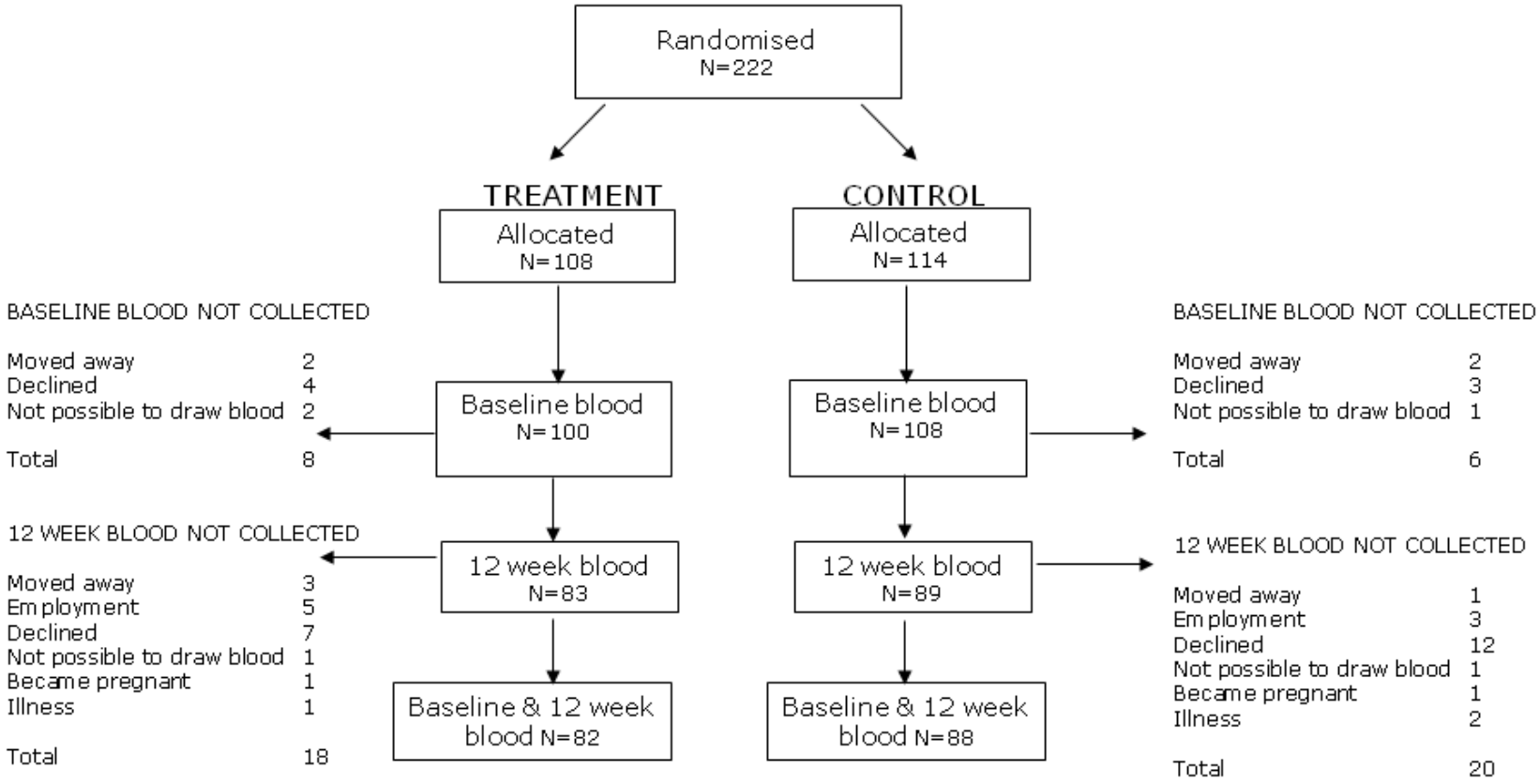
4.1.1 Participant flow in the MMNP

Of the 7096 women who attended MMNP registration meetings, 6513 (91.8%) were eligible to take part in the trial and gave consent. Of the 3308 women randomised to the control group, 1214 (37%) became pregnant, these figures were 3205 and 1134 (35%) respectively in the intervention group.

4.1.2 Participant flow in the Extension Study

Figure 4.1 shows the flow of participants through the Extension Study trial. Women who declined to have blood collected at visit 1 were excluded from the study and not supplemented. Of the 222 women randomised, 7 declined to have blood collected at visit 1, and 4 moved away before supplementation started. Three women attended the blood collection clinic but a sample of their blood could not be drawn, these women remained in the study and were supplemented. A total of 208 women with visit 1 blood measurements started receiving the intervention. Of these, blood was collected at 12 weeks from 172 women and blood samples were available at both time points for 170 women (77% of those randomised).

Figure 4.1Participant flow in the Extension Study



The data presented in section 4.3.1 (p100) relate to the 208 women who attended for visit 1 blood collection and were supplemented from the beginning of the trial. The data in section 4.3.2 (p102) and sections 4.5 and 4.6 (p137) relate to 170 women for whom visit 1 and visit 3 dietary and functional outcome data were available. Data relating to blood nutrient concentrations are presented for women whose blood was collected and whose samples were not haemolysed. On a small number of occasions (<4%) a smaller amount of blood was collected than was required for all of the proposed assays, in which case the complete blood count was prioritised.

4.2 Comparison of participant characteristics between the MMNP and the Extension Study

4.2.1 Demographic characteristics

Table 4.1 shows a comparison of participant characteristics from the MMNP and Extension Study. These data relate to all women who were eligible for the trials and randomised, n=6513 in the MMNP and n=222 in the Extension Study. The study groups were significantly different in all demographic characteristics. In terms of religion, just over two thirds of the women registered in the MMNP were Hindu, while over 80% of the women in the Extension Study were Muslim. Being married was an eligibility requirement for the MMNP but not for the Extension Study, half of the women in the Extension Study were married. The majority of women in both studies had been educated to secondary level (i.e. between 5-10 years). A fifth of the women in the MMNP reported that they were working compared with a third of women in the Extension Study. For most women in both studies the head of household was occupied in either skilled or unskilled work but over 10% in both studies were professionals (occupation categories are explained in section 3.1.5.1, p73). A greater proportion of women in the MMNP had husbands with skilled jobs than in the Extension Study. The first language of half of the women in the MMNP was Marathi, with the majority of the remainder being Hindi speakers. In the Extension Study over three quarters of women spoke Hindi as their first language which reflected the proportion of Muslim women in the study. Women in the MMNP were on average approximately 4 years older than women in the Extension Study.

Table 4.1 Comparison of MMNP and Extension Study participants at registration; demographic characteristics

		MMNP (n=6513)		Extension Study (n=222)		
		N	%	N	%	p*
Religion	Hindu	4561	70	34	15	<0.001
	Muslim	1671	26	181	82	
	Other	281	4	7	3	
Married	Yes	6513	100	112	50	n/a
	No	0	0	110	50	
Education (years)	0-5	809	12	46	21	<0.001
	5-10	4551	70	158	71	
	10+	1144	18	18	8	
	Not known	9	<1			
Occupation	Professional	180	3	12	5	<0.001
	Skilled	701	11	21	10	
	Unskilled	517	8	44	20	
	Not working	5115	78	145	65	
Husband/ Father's Occupation	Professional	1049	16	27	12	<0.001
	Skilled	4227	65	96	43	
	Unskilled	1084	17	78	35	
	Not working	97	2	15	7	
	Not known	56	<1	6	3	
First Language	Marathi	3344	51	29	13	<0.001
	Hindi	2457	38	176	79	
	Other	712	6	17	8	

	Mean	SD	Mean	SD	p*
Age (Years)	25.0	4.0	21.2	5.7	<0.001

*p relates to chi square test for categorical variables and t test for continuous variables.

4.2.2 Standard of living

Almost two thirds of women in the MMNP lived in houses with fewer than five residents while the same proportion of women in the Extension Study lived in houses with more than five persons (**Table 4.2**). Two thirds of women in the MMNP lived in joint family households compared with a fifth of women in the Extension Study. Approximately one fifth of houses in both study areas had more than one room and most households did not have a separate room as a kitchen. In the MMNP study area, the majority of drinking water was accessed from either private or public taps. In the Extension Study, over three quarters of women bought water in bags from shops that had a piped water source; however the research team observed that this supply was relatively insecure and there were regular shortages. The vast majority of women in both studies used pit toilets rather than flush, 5% of women in the Extension Study had no toilet facilities available. Approximately two thirds of women in the MMNP used liquid petroleum gas as cooking fuel, while in the Extension Study a similar proportion used kerosene. In both studies most families owned the house they lived in. Less than a tenth of MMNP families owned agricultural land, while a third of Extension Study families did. A relatively small proportion (4% and 11%) respectively of families in the MMNP and Extension Study owned livestock.

4.2.3 Anthropometry

Women registered in the MMNP had a greater BMI on average than women from the Extension Study and this was largely driven by women in the Extension Study being lighter (mean difference 3.9kg) than in the MMNP (**Table 4.3**). There was 1.7cm difference between groups in mean height with women in the MMNP being taller. There was no statistically significant difference in head circumference measurements between the two studies. For all other anthropometric measures, the Extension Study women had smaller circumference measurements and skinfolds. There was some indication that the women in the Extension Study had relatively large waist circumferences for their size indicating storage of fat centrally. There were differences in skinfold thicknesses between the studies.

Table 4.2 Comparison of MMNP and Extension Study; standard of living

		MMNP (n=6513)		Extension Study (n=222)	
		N	%	N	%
Persons in the house	1-5	3973	61	92	41
	>5	2540	39	130	59
Family type	Nuclear	2475	38	174	78
	Joint	4038	62	48	22
Number of rooms	1	5145	79	172	78
	>1	1368	21	50	22
Separate kitchen?	Yes	1043	16	10	5
	No	5470	84	212	95
Drinking water source	Piped water	3257	50	26	12
	Public tap	3248	49	10	4.5
	Tanker	8	<1	10	4.5
	Bought in bags	0	0	176	79
Household toilet	Flush	162	5	19	9
	Pit	6347	95	190	85.5
	No facility/Other	4	<1	13	5.5
Cooking fuel	LPG	4493	69	43	19
	Kerosene	1953	31	142	64
	Other	67	<1	37	17
Own the house	Yes	4879	75	153	69
	No	1634	25	69	31
Own agricultural	Yes	456	8	80	36
	No	6057	92	142	64
Own livestock	Yes	202	3	24	11
	No	6311	97	198	89

Table 4.3 Anthropometry of MMNP and Extension Study participants

	MMNP (n=6513)		Extension Study (n=222)		
	Mean	SD	Mean	SD	p*
Height (cm)	151.2	5.5	149.5	5.6	<0.001
Weight (kg)	*46.0*	*40.5-53.0*	42.2	8.8	<0.001
BMI (kg/m^2)	*20.0*	*17.9-22.9*	*18.6*	*16.3-20.7*	<0.001
Head circumference (cm)	52.6	1.5	52.6	1.6	0.679
Waist circumference (cm)	*69.1*	*63.4-77.0*	67.4	9.5	<0.001
Hip circumference (cm)	88.5	8.6	85.3	7.7	<0.001
MUAC (cm)	24.0	3.3	23.1	3.1	<0.001
Triceps skinfold (mm)	*13.8*	*10.1-19.4*	*10.7*	*8.3-14.5*	<0.001
Biceps skinfold (mm)	*6.2*	*4.4-8.6*	*5.4*	*4.2-7.2*	<0.001
Subscapular skinfold (mm)	*21.6*	*15.6-29.7*	*16.4*	*11.8-22.7*	<0.001

*p for difference between means, where variables were not normally distributed natural logs were used.
Values in italics are median and IQR.

4.2.4 Comparison of nutritional status indicators with national data

Table 4.4 shows that a third of women in MMNP and half of women in the Extension Study were chronically energy deficient (BMI<18.5kg/m^2). Both of these figures are substantially higher than those reported among slum and non-slum dwellers in the Mumbai NFHS sample (198). According to the NFHS a quarter of women in Mumbai slums were overweight or obese with 14% of MMNP participants and 5% of those in the Extension Study in this category. As there is an upward trend over time in the prevalence of overweight and obesity in India, it should be noted that the NFHS data were collected in 2005-2006, the MMNP data were collected in 2006-2011 and the Extension Study data in 2009.

There were greater proportions of women in the Extension Study with severe, moderate and mild anaemia than among the slum dwellers in the NFHS. There was little difference in anaemia prevalence between slum and non-slum dwellers in the NFHS sample.

Table 4.4 Nutritional status indicators; comparison with NFHS data

Indicator of Nutritional status		Percentage of women by survey			
		MMNP (n=6513)	Extension Study (n=222)	NFHS Mumbai slum*	NFHS Mumbai Non-slum*
Body Mass Index	<18.5kg/m^2	32	50	23	21
	18.5-25kg/m^2	54	45	52	48
	>25kg/m^2	14	5	25	31
Haemoglobin Concentration	<7.0g/dL	-	2	1	2
	7.0-9.9g/dL	-	15	12	10
	10.0-11.9g/dL	-	47	35	36
	≥12.0g/dL	-	36	54	52

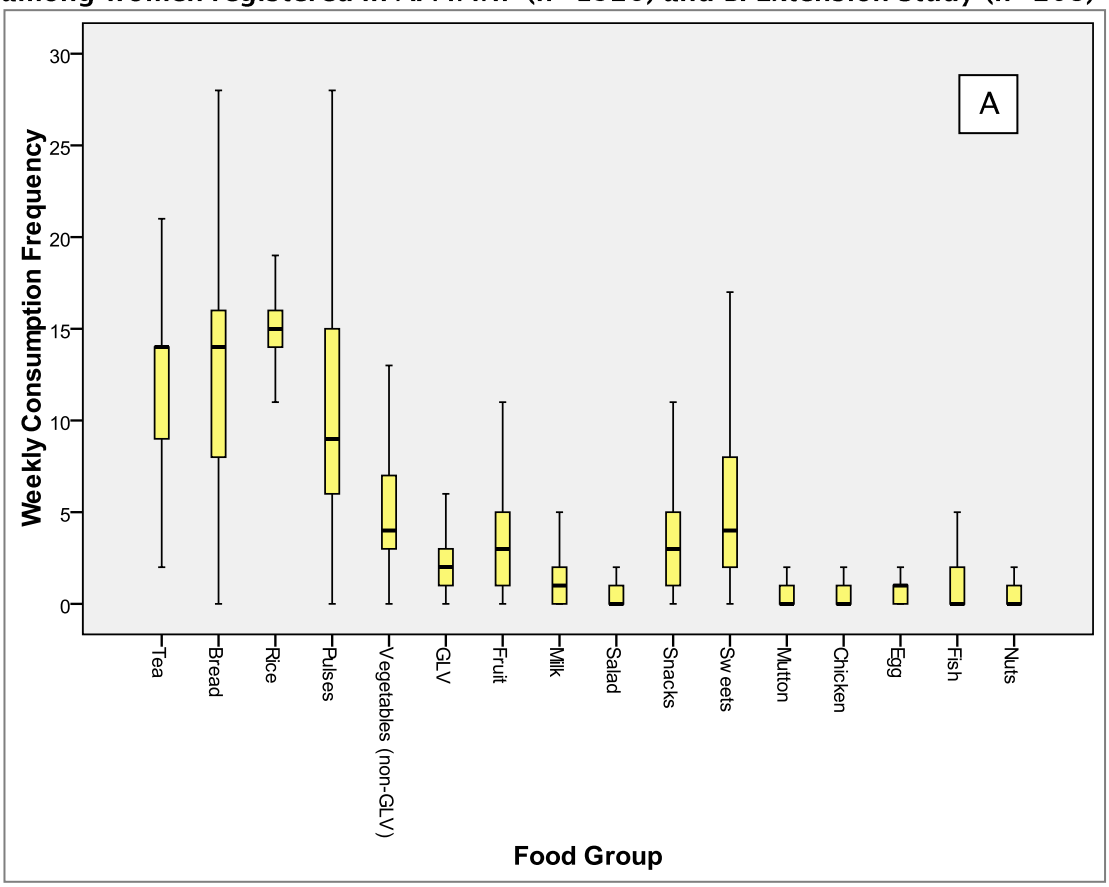
*****Number of participants was not reported in the NFHS analysis (34) – Non-pregnancy Hb concentrations not available.

4.3 Dietary intakes in the MMNP and Extension Study

4.3.1 Comparison of diets in the MMNP and Extension Study

The analyses of dietary data among MMNP participants relates to 1526 women for whom dietary data at two time points were available. Data were collected at registration and six months after supplementation had begun. Dietary data from the Extension Study were analysed for all women who attended for visit 1 blood collection and were supplemented from the beginning of the trial (n=208). The boxplots in **Figure 4.2**, A and B show the distributions of consumption frequency of sixteen food groups in the MMNP and Extension Study respectively. When the two studies are compared, for most food groups there was a broader distribution of intake frequency among women in the MMNP and median intakes were higher for the majority of foods, most notably bread pulses, vegetables and fruit. In the Extension study the distribution of frequency of mutton and egg consumption was slightly broader than in the MMNP and these foods were consumed more frequently on average.

Figure 4.2 Boxplots showing the baseline frequency of consumption of foods among women registered in A: MMNP (n=1526) and B: Extension Study (n=208)



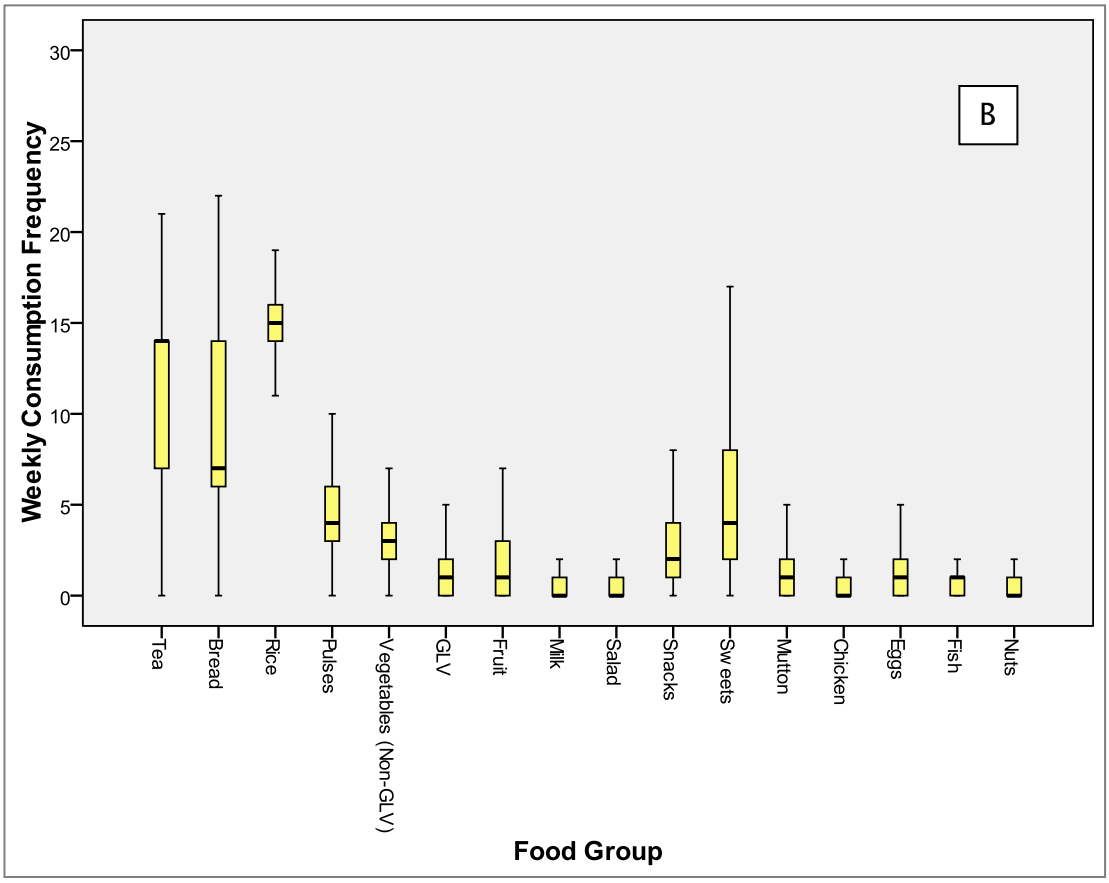


Table 4.5 shows that the majority of women drank tea daily. Cereal-based foods such as rice and bread were consumed daily by most women with a slightly smaller proportion of women in the Extension Study consuming bread on a daily basis. Only a quarter of MMNP participants consumed vegetables other than GLV daily and even fewer did so in the Extension Study. Half of the women in the MMNP and 57% in the Extension Study consumed GLV once per week or less. A fifth of women consumed fruit daily in the MMNP while only 7% in the Extension Study did so. A quarter of women in the MMNP and over a half of women in the Extension Study consumed fruit once per week or less. Over two thirds of women in both studies consumed milk and/or milk products, other than in tea, once per week or less. This proportion was greater in the Extension Study.

The majority of women in both studies consumed salad less than once per week. Foods of low micronutrient content such as snacks and sweets were eaten more than once per week by over half of the women in both studies and a third of women consumed sweet foods at least once per day. Non-vegetarian foods were consumed by women in both studies but intakes were low. Mutton was eaten more frequently in the Extension Study which is likely to be related to the larger proportion of Muslim women. Less than a fifth of women in both studies consumed chicken at least once per week. Fish and eggs were consumed at least once per week by approximately a quarter of the women in both studies. The majority of women consumed nuts once per week or less.

4.3.2 Changes in dietary intake over the supplementation period

Change in dietary intake over the supplementation period was assessed in both studies. In the MMNP a repeat FFQ was administered after 6 months of supplementation provided the woman had not become pregnant in that time period. Among women in the Extension Study, an FFQ was administered at visit 1 and again at visit 3. All Extension Study interviews were carried out within a two week period so there were no differences in terms of season of interview.

Table 4.5 Consumption of 16 food groups among women registered in the MMNP and Extension Study

Food Group	Percentage of women					
	MMNP (n=1526)			Extension Study (n=208)		
	≤1/wk	>1/wk	≥1/d	≤1/wk	>1/wk	≥1/d
Tea	9	1	90	7	4	89
Bread	4	4	92	10	18	72
Rice	1	1	98	1	2	97
Pulses	2	24	74	9	67	24
Vegetables (non-GLV)	11	63	26	21	67	12
GLV	50	47	3	57	42	1
Fruit	28	52	20	51	42	7
Milk	67	20	13	84	10	6
Salad	89	10	1	89	10	1
Snacks	29	54	17	43	42	15
Sweet Foods	21	44	35	24	42	34
Mutton	78	18	4	61	37	2
Chicken	87	13	0	92	8	0
Eggs	77	22	1	74	24	2
Fish	73	26	1	79	21	0
Nuts	85	13	2	79	18	3

4.3.2.1 Change in dietary intake; MMNP

Using data from the MMNP, we investigated the change in intake of fruit and GLV by season of baseline FFQ. **Table 4.6** and **Table 4.7** show the women's intakes of fruit and GLV respectively at registration and after six months of supplementation and the mean change (δ) in intake over this period. In both treatment groups there was an increase in fruit intake among women who registered in winter and a decrease in those who registered in the pre-monsoon and post-monsoon seasons. Among those who registered in the monsoon season, there was a decrease in consumption in the control group and a very small increase in the intervention group on average. For GLV,

among women registered in winter there was, on average, no change in consumption in the control group and a small decrease in the intervention group. All women registered in the pre-monsoon and post-monsoon seasons, decreased GLV intakes over the study period. Among those registered in the monsoon season the control group tended to decrease consumption whereas consumption in the intervention group increased. It should be noted that there was a large degree of variability around these mean changes as indicated by the large standard deviations.

There were no statistically significant differences in the change in consumption over time between the control and intervention groups for both fruit and GLV. Univariate linear regression analysis results also showed that there was no statistically significant effect of group allocation on change in fruit consumption between baseline and the 6-month FFQ (B=0.62; 95% CI -1.09, 2.33, where the control group was coded 0 and the intervention group coded 1). Similarly there was no effect of group allocation on change in GLV consumption (B=-0.12; 95% CI -1.15, 0.91).

4.3.2.2 Change in dietary intake; Extension study

The changes in fruit and GLV intake over the 12 week study period in the Extension Study are shown in **Table 4.8** and **Table 4.9**. There was an increase in fruit consumption of almost 7 servings per month in both groups and no difference between groups. For GLV, there was an increase in both groups. This increase was more than 3 times greater in the control than the intervention group although the difference in the change between groups did not reach statistical significance.

Table 4.6 Median monthly frequency of consumption of seasonal fruit at baseline and after 6 months in MMNP by season of baseline FFQ

Season	Control				Intervention				
	N	0 Month	6 Month	δ (6mo – 0mo)*	N	0 Month	6 Month	δ(6mo – 0mo)*	p**
Winter	215	4.0 (0.0,12.0)	4.0 (0.0,12.0)	1.1 (11.8)	184	4.0 (0.0,12.0)	8.0 (0.0,16.0)	1.5 (16.8)	0.333
Pre-monsoon	167	8.0 (4.0,16.0)	8.0 (4.0,12.0)	-1.2 (17.5)	150	8.0 (4.0,12.0)	8.0 (0.0,12.0)	-2.3 (12.8)	0.920
Monsoon	260	8.0 (4.0,16.0)	8.0 (4.0,12.0)	-1.3 (15.0)	236	4.0 (0.0,12.0)	8.0 (4.0,12.0)	0.1 (16.8)	0.257
Post-monsoon	170	8.0 (4.0,12.0)	8.0 (0.0,12.0)	-3.0 (26.1)	144	8.0 (1.0,16.0)	4.0 (4.0,12.0)	-1.6 (16.7)	0.267

*Mean (SD) **T test for difference in δ between treatment groups

Table 4.7 Median monthly frequency of consumption of GLV at baseline and after 6 months in MMNP by season of baseline FFQ

Season	Control				Intervention				
	N	0 Month	6 Month	δ (6mo – 0mo)*	N	0 Month	6 Month	δ(6mo – 0mo)*	p**
Winter	215	8.0 (4.0,12.0)	8.0 (4.0,12.0)	0.0 (11.6)	184	8.0 (4.0,12.0)	8.0 (4.0,12.0)	-1.2 (10.9)	0.291
Pre-monsoon	167	8.0 (4.0,12.0)	8.0 (4.0,12.0)	-1.1 (11.3)	150	8.0 (4.0,12.0)	8.0 (4.0,12.0)	-1.1 (8.0)	0.991
Monsoon	260	4.0 (0.0,12.0)	8.0 (4.0,12.0)	-0.8 (8.5)	236	4.0 (0.0,12.0)	8.0 (4.0,12.0)	1.6 (10.4)	0.365
Post-monsoon	170	4.0 (4.0,12.0)	8.0 (4.0,12.0)	-0.4 (8.4)	144	8.0 (4.0,15.0)	8.0 (4.0,12.0)	-0.7 (12.0)	0.757

*Mean (SD) **T test for difference in δ between treatment groups

Table 4.8 Median monthly frequency of consumption of fruit at visit 1 and visit 3 in the Extension Study

Control				Intervention				
N	Visit 1	Visit 3	δ(12wk–0wk)*	N	Visit 1	Visit 3	δ(12wk–0wk)*	p*
88	4.0 (0.0,8.0)	8.0 (0.0,21.0)	6.9 (22.6)	82	4.0 (2.0,12.0)	8.0 (0.0,22.0)	6.9 (23.7)	0.997

*Mean (SD) **T test for difference in δ between treatment groups

Table 4.9 Median monthly frequency of consumption of GLV at visit 1 and visit 3 in the Extension Study

Control				Intervention				
N	Visit 1	Visit 3	δ(12wk–0wk)*	N	Visit 1	Visit 3	δ(12wk–0wk)*	p*
88	4.0 (0.0,8.0)	8.0 (0.0,12.0)	3.2 (8.8)	82	4.0 (0.0,8.0)	4.0 (0.0,10.0)	1.0 (12.8)	0.144

*Mean (SD) **T test for difference in δ between treatment groups

4.4 Blood micronutrient concentrations among women during pregnancy in the MMNP

Blood was collected from women in the MMNP during early pregnancy by which point they had been consuming the supplements for at least 20 weeks. Women were invited to attend the clinic for blood collection at the same time as the first ultrasound scan was taken. The median (IQR) gestation was 10.1 (9.4, 12.0) weeks at the time of blood collection. Plasma concentrations of retinol, folate and vitamin B12 were measured and values were available for n=339, n=1304 and n=1303 respectively. The low number of retinol results was due to laboratory delays in processing samples and were the extent of the results available at the time of writing. **Table 4.10** shows the concentrations of nutrients by treatment group. There were no significant differences between groups in retinol or folate concentrations although there was a trend for the women in the intervention group to have lower concentrations of both nutrients. For vitamin B12, the difference between groups approached

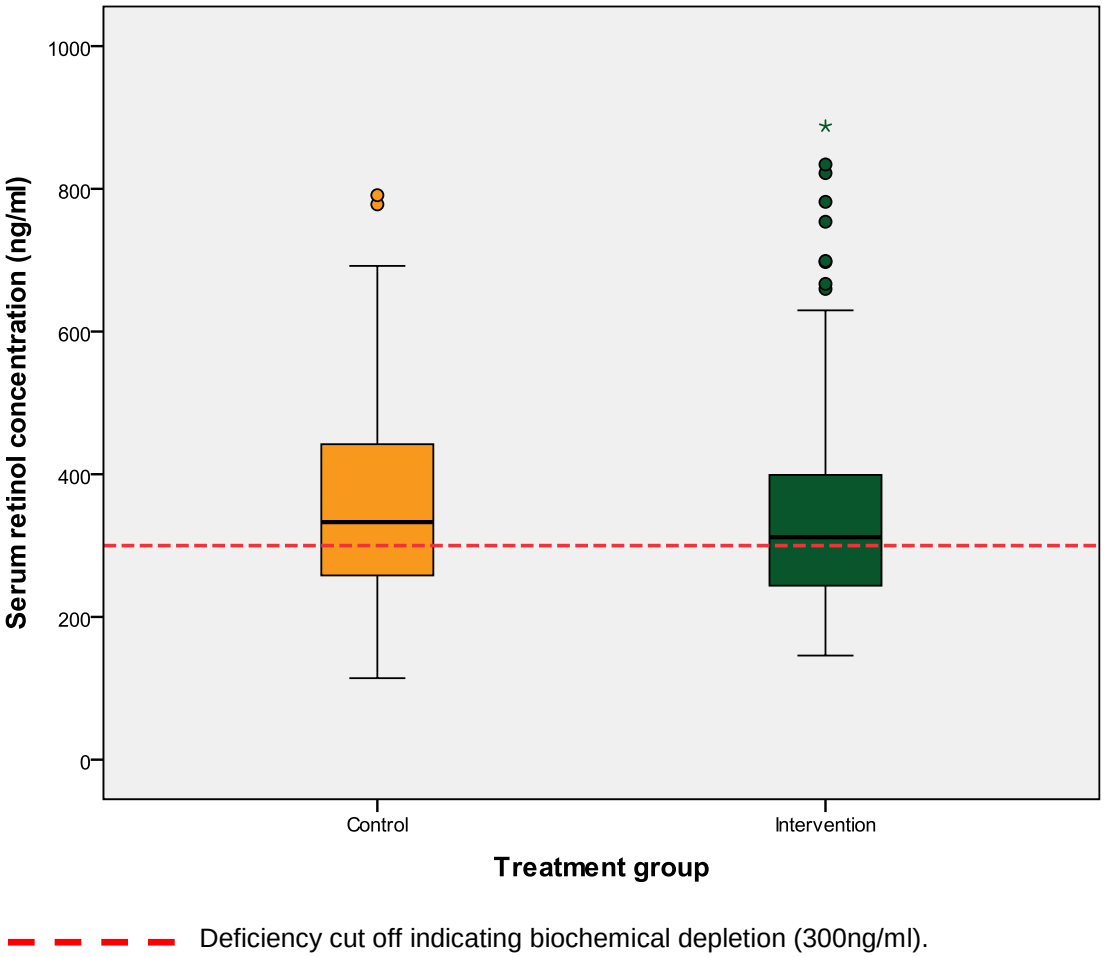
statistical significance with the intervention group on average having a lower status than the control group.

Table 4.10 Blood micronutrient concentrations in early pregnancy

	Control		Intervention		p*
Nutrient	n	Median (IQR)	n	Median (IQR)	
Retinol (ng/ml)	189	333 (258,442)	150	313 (244,410)	0.394
Folate (nmol/L)	688	31.5 (17.3,64.4)	616	28.7 (17.6,63.3)	0.954
Vitamin B12 (pmol/L)	687	233 (178,298)	616	217 (166,296)	0.061

*p value relates to t test for difference between means of variables transformed by taking the natural log

Figure 4.3 Distribution of serum retinol concentrations in early pregnancy



The distribution of serum retinol by treatment group is shown in **Figure 4.3.** A greater proportion of pregnant women were retinol deficient in the intervention group. The distributions of folate concentrations were similar between treatment groups (**Figure 4.4**). There were several extreme outliers in the distribution for vitamin B12 (**Figure 4.5**); therefore a second boxplot was produced omitting outliers to enable closer inspection of the distribution. It can be seen that more women in the intervention group were deficient (**Figure 4.6**).

Figure 4.4 Distribution of plasma folate concentrations in early pregnancy

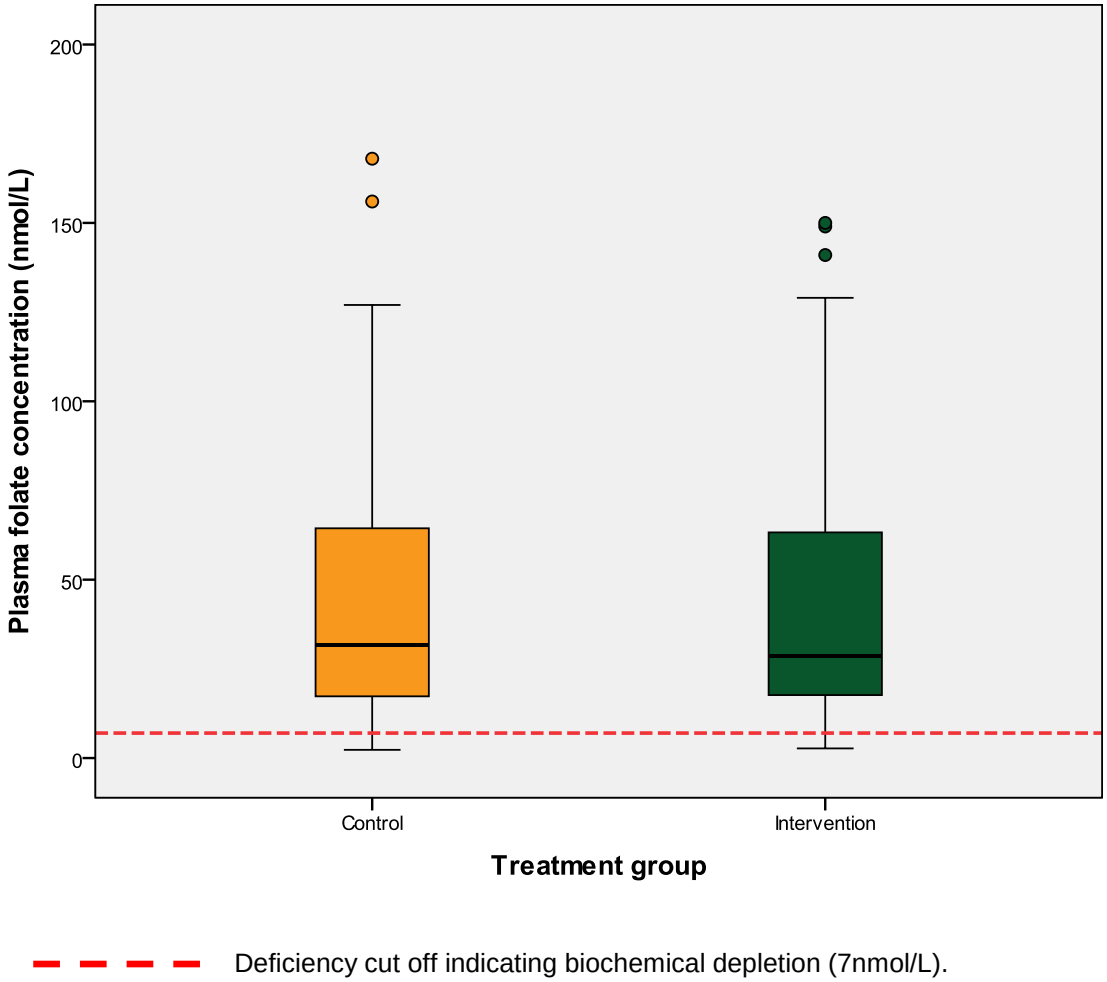


Figure 4.5 Distribution of plasma vitamin B12 concentrations in early pregnancy

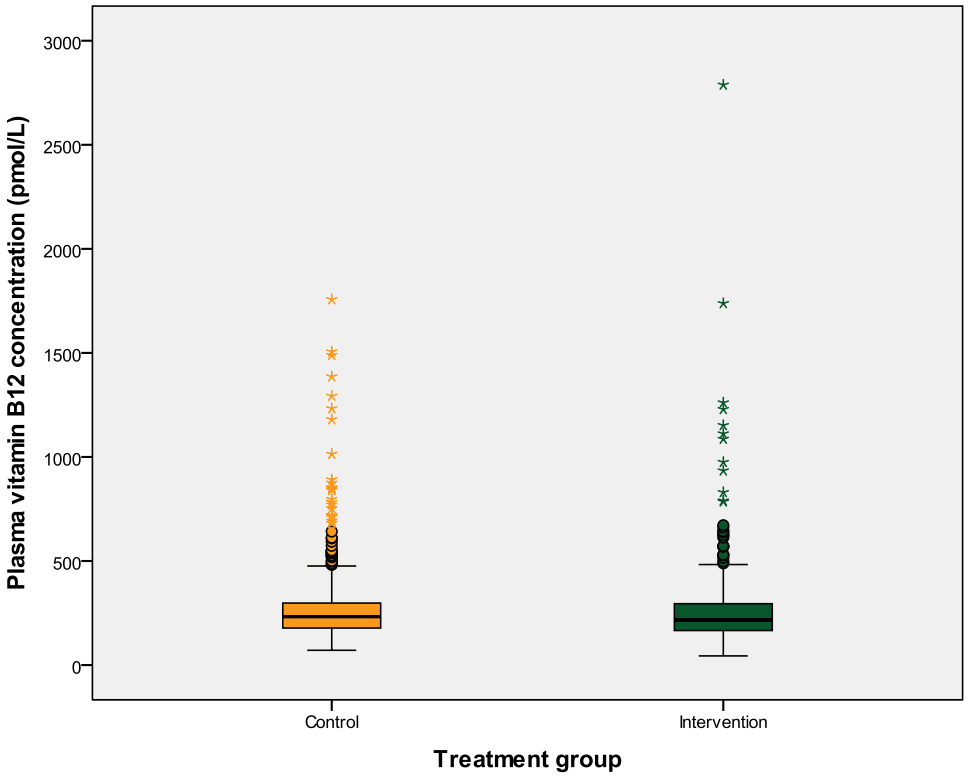
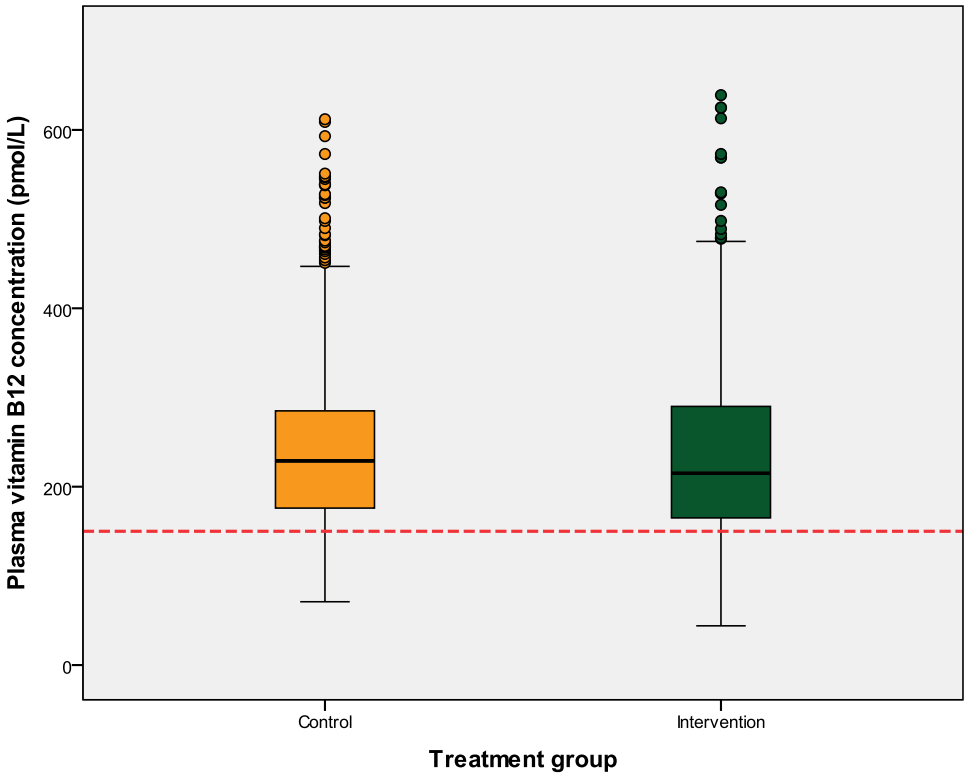


Figure 4.6 Distribution of plasma vitamin B12 concentrations in early pregnancy except outliers indicated by * in Figure 4.5.



Deficiency cut off indicating biochemical depletion (150pmol/L).

Table 4.11 shows the proportion of women who were retinol, folate and vitamin B12 deficient by treatment group allocation. For retinol and vitamin B12, there was a slightly higher proportion of women deficient in the intervention group but this finding did not reach statistical significance for any of the nutrients based on a chi-square test.

Table 4.11 Percentage of women with micronutrient deficiencies in early pregnancy

	Control	Intervention	
Nutrient	N (%)	N (%)	p*
Retinol	70 (37)	66 (44)	0.194
Folate	11 (2)	8 (1)	0.652
Vitamin B12	102 (15)	107 (17)	0.215

*p value relates to a Chi square test

4.5 Changes in nutrient status in the Extension Study

4.5.1 Comparison of characteristics between treatment groups

Table 4.12 shows that there were no statistically significant differences between the intervention and control groups for women included in the final data analysis in terms of demographics, anthropometry, baseline nutrient concentrations and compliance with the supplementation protocol. Ten women reported taking multiple micronutrient tablets at visit 1, half of these were in the control group (chi square, p=0.592). At visit 3, 30 women reported taking synthetic micronutrient tablets, 42% in the control group (chi square, p=0.174). Of these, four women were taking iron, eight were taking iron-folic acid and 18 were taking multiple micronutrients. There were 23 women with plasma CRP concentrations >5µg/ml at visit 1, of these 13 (56%) were in the control group (chi square, p=0.408). At visit 3, 28 women had CRP concentrations >5µg/ml, 14 (50%) were in the control group (chi square, p=0.602).

There were no statistically significant differences in terms of demographic or baseline measurements between women who remained in the study and those who were lost to follow up (data not shown).

Table 4.12 Comparison of participant characteristics of women included in the data analysis between treatment groups

	Control	Intervention	p*
Age (years)	21.1	21.1	0.999
Anthropometry			
Height (cm)	149.7	149.5	0.750
Weight (kg)	42.7	41.8	0.477
BMI (kg/m^2)	18.7	18.4	0.626
Head circumference (cm)	52.7	52.6	0.604
Waist circumference (cm)	68.1	66.9	0.373
Hip circumference (cm)	85.8	85.2	0.582
MUAC (cm)	23.3	23.1	0.670
Triceps skinfold (mm)	11.1	11.1	0.920
Biceps skinfold (mm)	5.8	5.7	0.773
Subscapular skinfold (mm)	16.8	16.7	0.872
Demographic			
Persons in household	6.01	6.01	0.998
Family Income (Rs)	4431	4347	0.802
Baseline blood nutrient concentrations			
Ferritin (ng/ml)	7.6	8.3	0.258
Haemoglobin (g/dL)	11.3	11.2	0.844
CRP (ng/ml)	830	737	0.595
Retinol (ng/ml)	435	414	0.360
Vitamin C (µmol/L)	14.7	15.9	0.385
Folate (nmol/L)	14.1	14.4	0.805
Homocysteine (µmol/L)	12.0	12.0	0.730
Vitamin B12 (pmol/L)	273	284	0.250
B-carotene (nmol/L)	398	414	0.428

*p value relates to t test for difference between means, where variables were not normally distributed, natural log values were used

4.5.2 Extension Study compliance with supplementation protocol

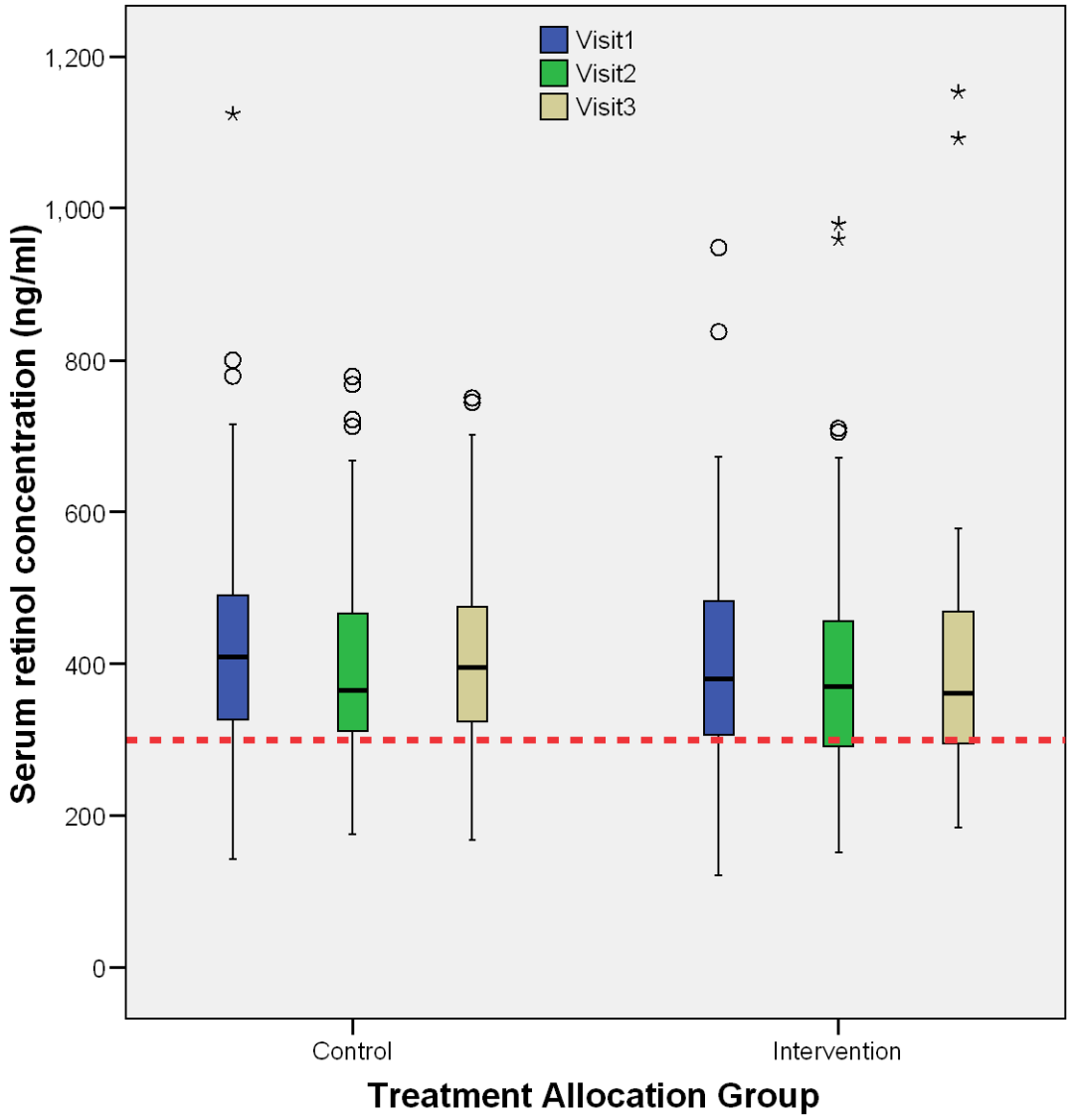
Compliance, defined as the proportion of women who consumed ≥3 snacks per week was 90% in the control and 85% in the intervention group. The median (IQR) weekly snack consumption of those in the final analysis was 5.0 (4.4, 5.5) supplements in the control group and 4.9 (3.8, 5.3) in the

intervention group. There was no significant difference between groups in terms of the percentage of 'compliers' and 'non-compliers' (chi square, p=0.304).

4.5.3 Distributions of indicators of micronutrient status during the Extension Study

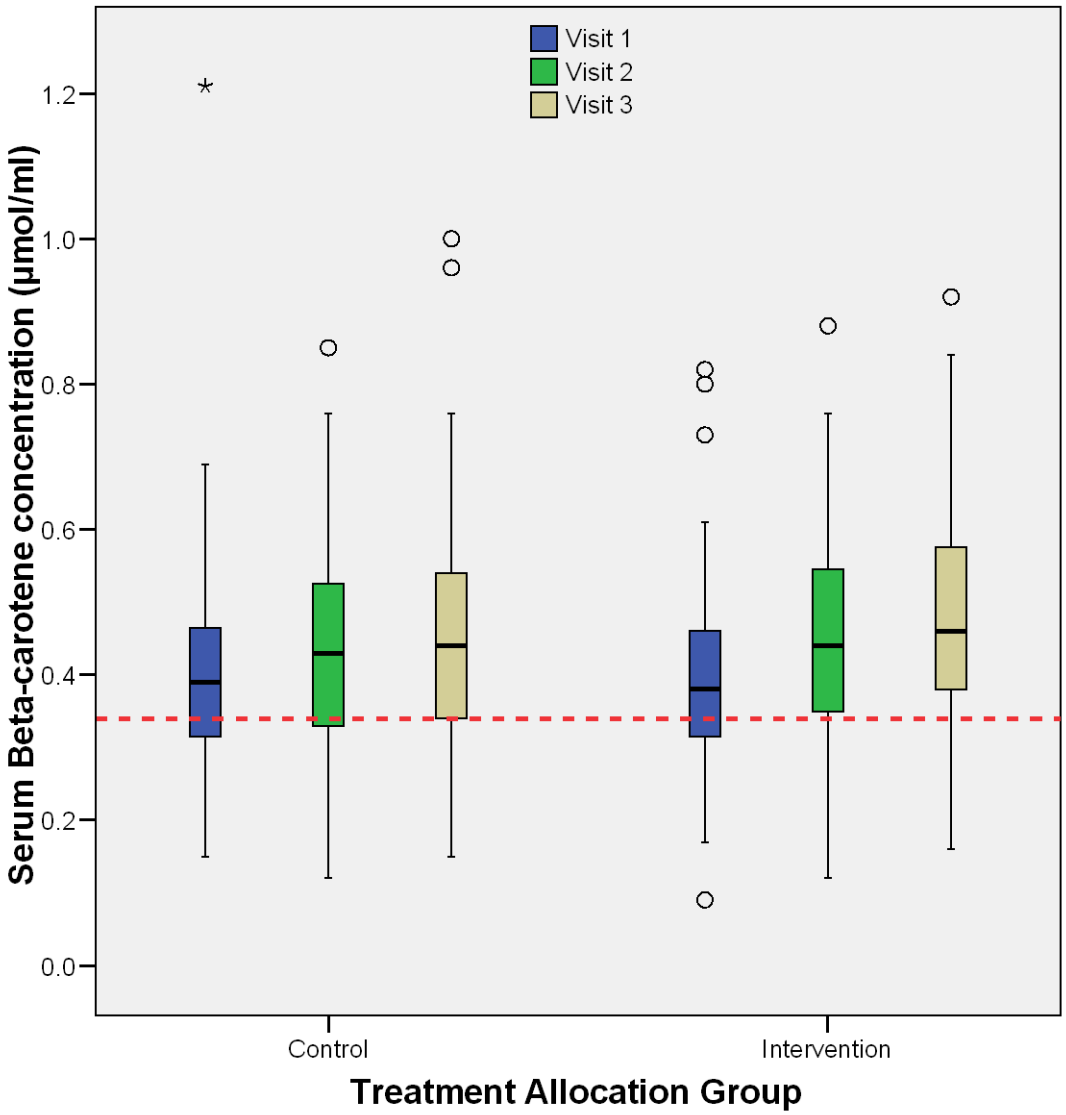
Figure 4.7-Figure 4.17 show the distributions of blood nutrient concentration indicators at visits 1, 2 and 3 split by treatment group for those women with measurements at all three time points. For some distributions (folate, homocysteine, vitamin B12, ferritin) there were extreme outliers. In these cases, a second boxplot was produced omitting those values that were >3xIQR above the 75th centile, in order to allow closer examination of the distribution of values. Deficiency cut offs are indicated on the boxplots and are based on WHO published deficiency values (92) with the exception of retinol for which the assay kit method value was used and β-carotene for which there is currently no published deficiency value. A reference line indicating the median plasma β-carotene concentration among women in the UK National Diet and Nutrition Survey, 2000 (133) is shown (**Figure 4.8**). The proportion of women who were deficient for each of the micronutrients studied is given in **Table 4.13.**

Figure 4.7 Distribution of plasma retinol concentrations at visits 1, 2 and 3 by treatment group (control, n=82, intervention, n=75)



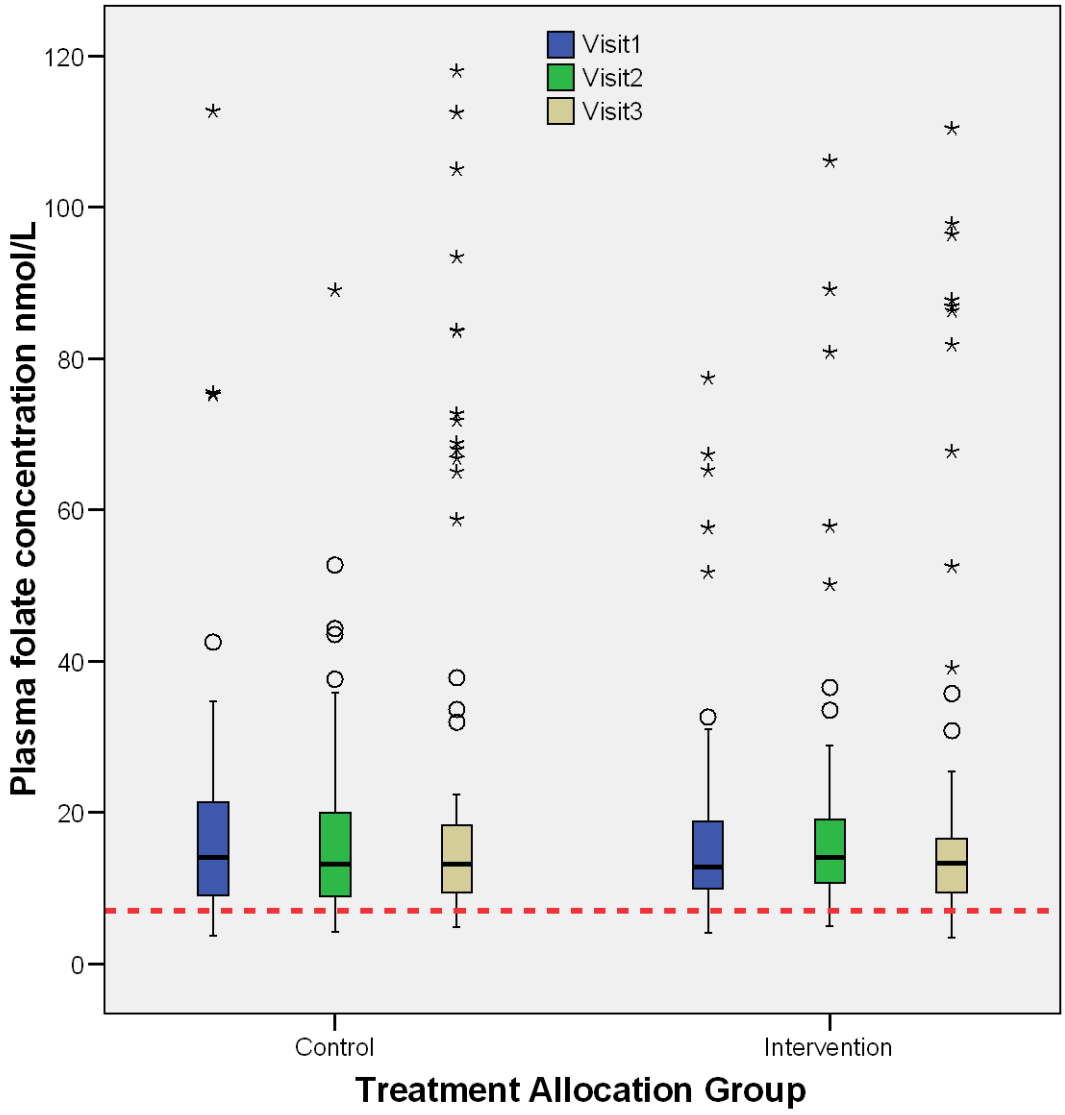
Deficiency cut off indicating retinol deficiency (300ng/ml).

Figure 4.8 Distribution of serum β-carotene concentrations at visits 1, 2 and 3 by treatment group (control, n=75, intervention, n=71)



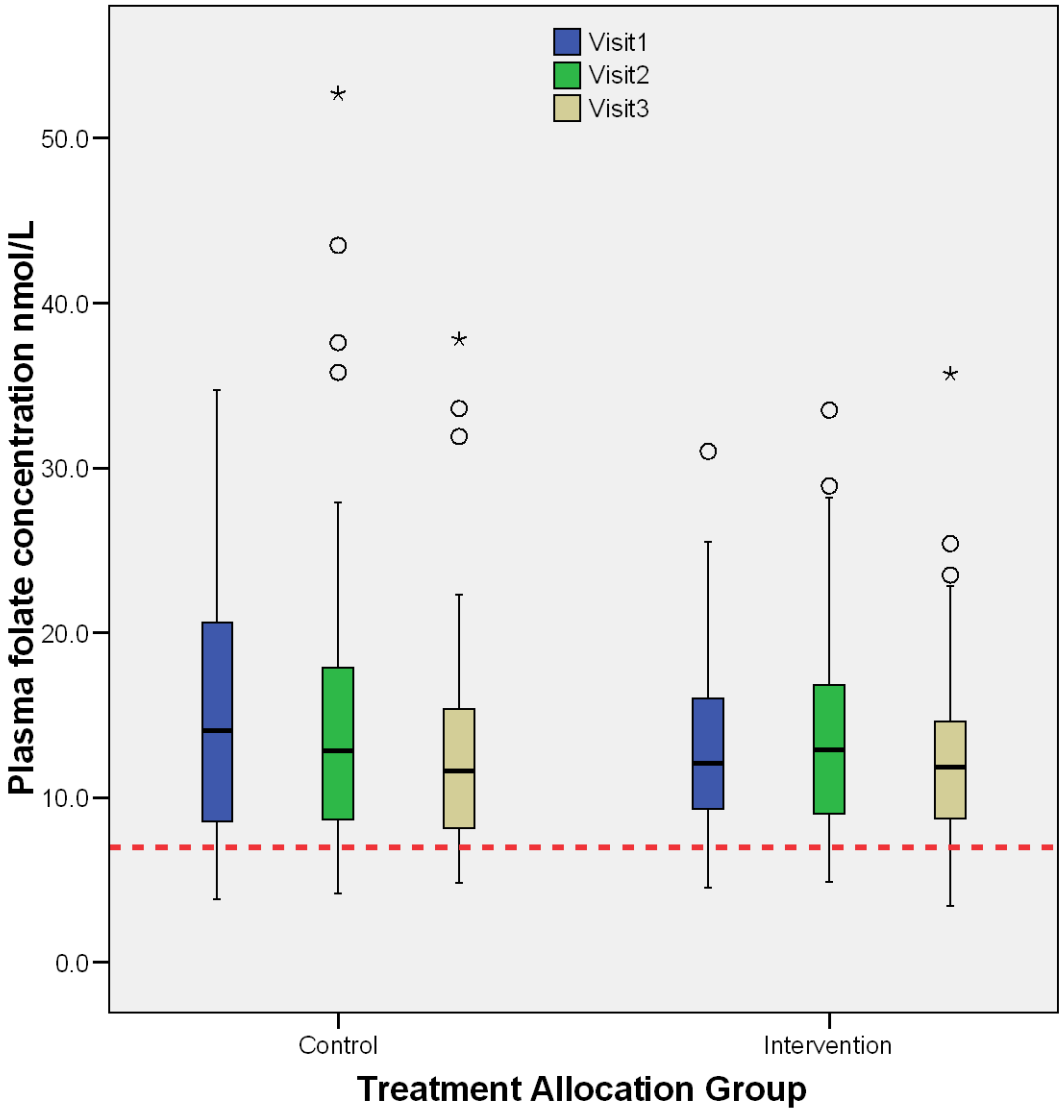
Median value for women in the UK National Diet and Nutrition Survey conducted in the year 2000.

Figure 4.9 Distribution of plasma folate concentrations at visits 1, 2 and 3 by treatment group (control, n=79, intervention, n=71)



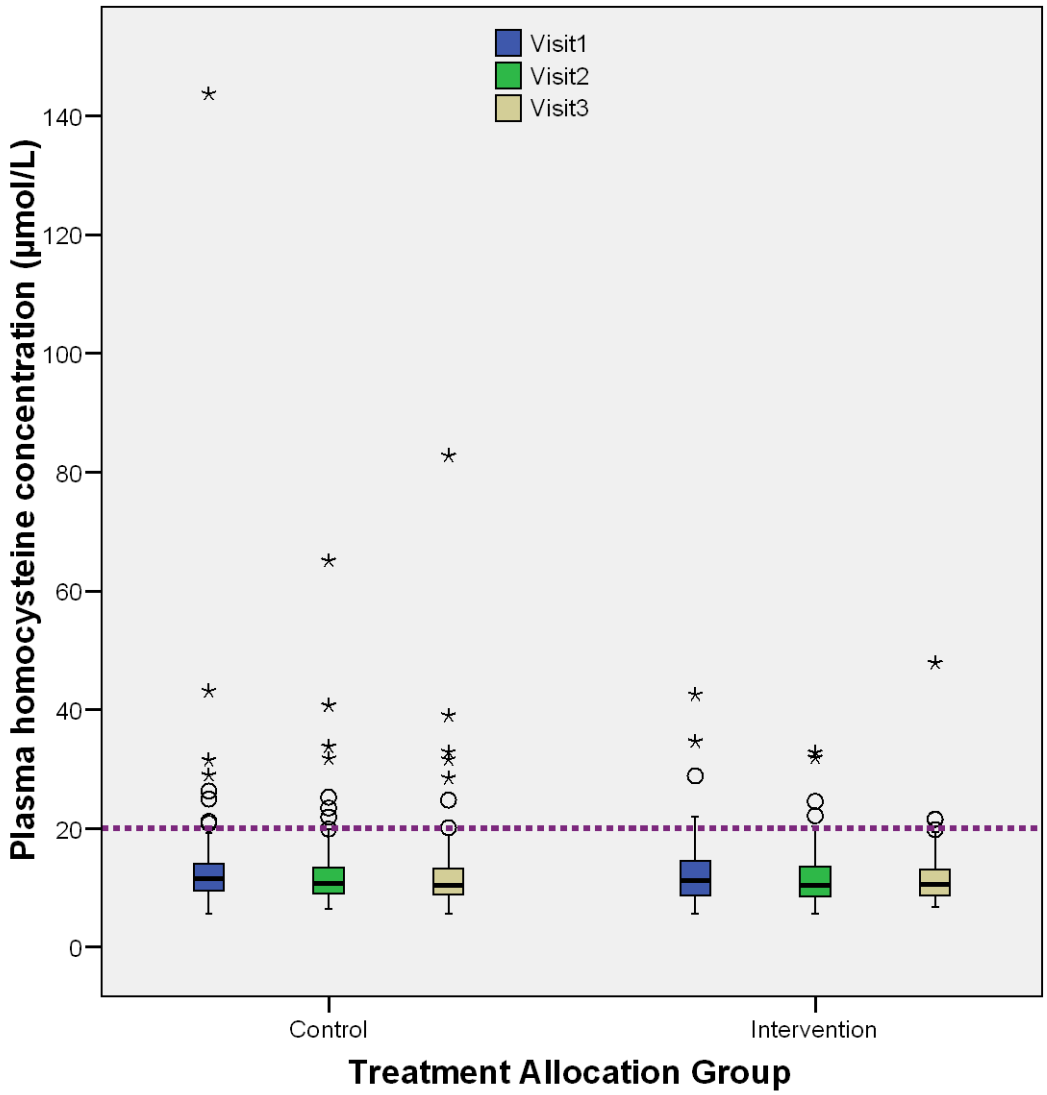
Deficiency cut off indicating biochemical depletion (7nmol/L).

Figure 4.10 Distribution of plasma folate concentrations at visits 1, 2 and 3 by treatment group excluding outliers (control, n=64, intervention, n=58)



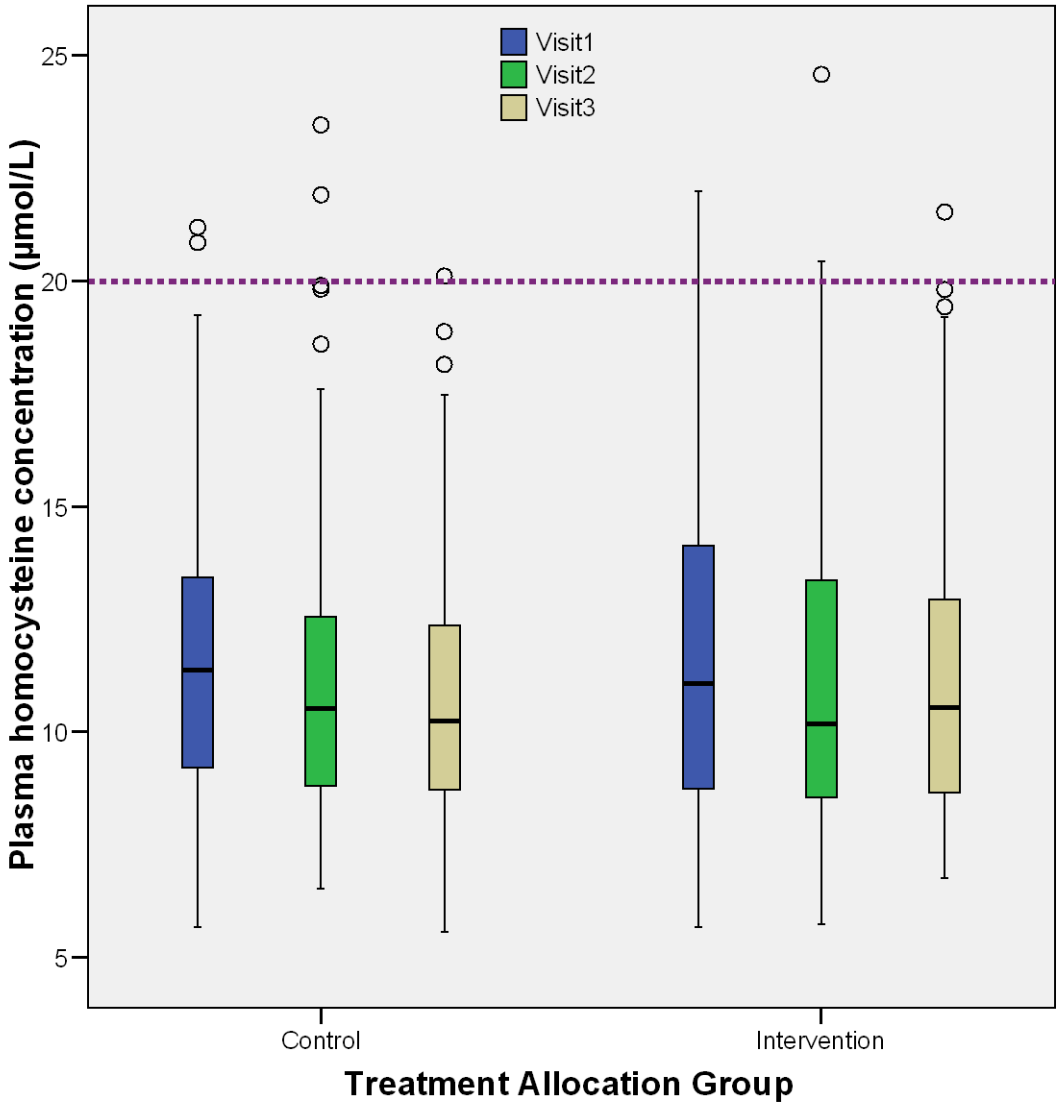
Deficiency cut off indicating biochemical depletion (7nmol/L).

Figure 4.11 Distribution of plasma homocysteine concentrations at visits 1, 2 and 3 by treatment group (control, n=79, intervention, n=70)



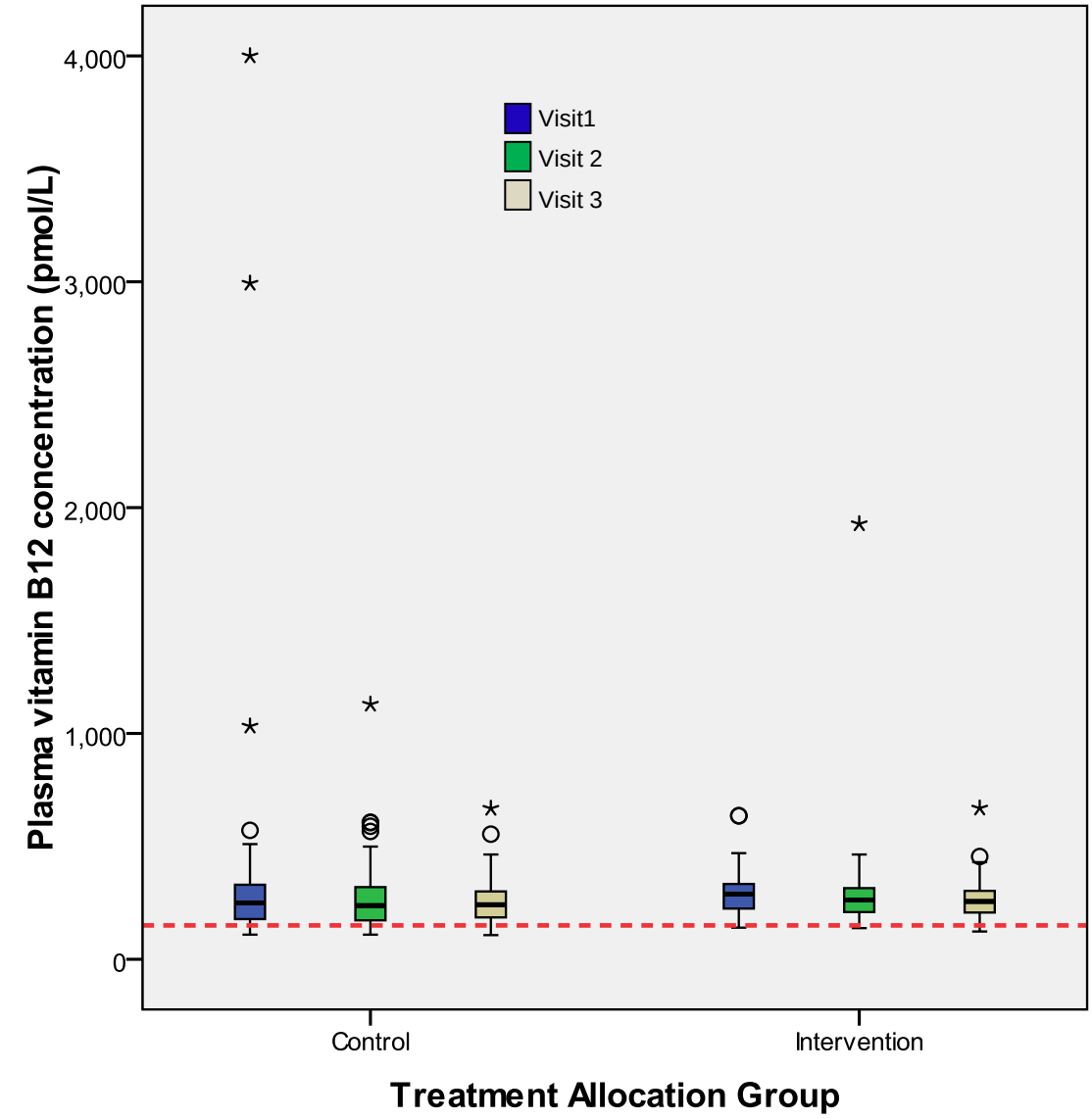
Values above this cut off indicate folate deficiency (20µmol/L).

Figure 4.12 Distribution of plasma homocysteine concentrations at visits 1, 2 and 3 by treatment group excluding outliers (control, n=72, intervention, n=67)



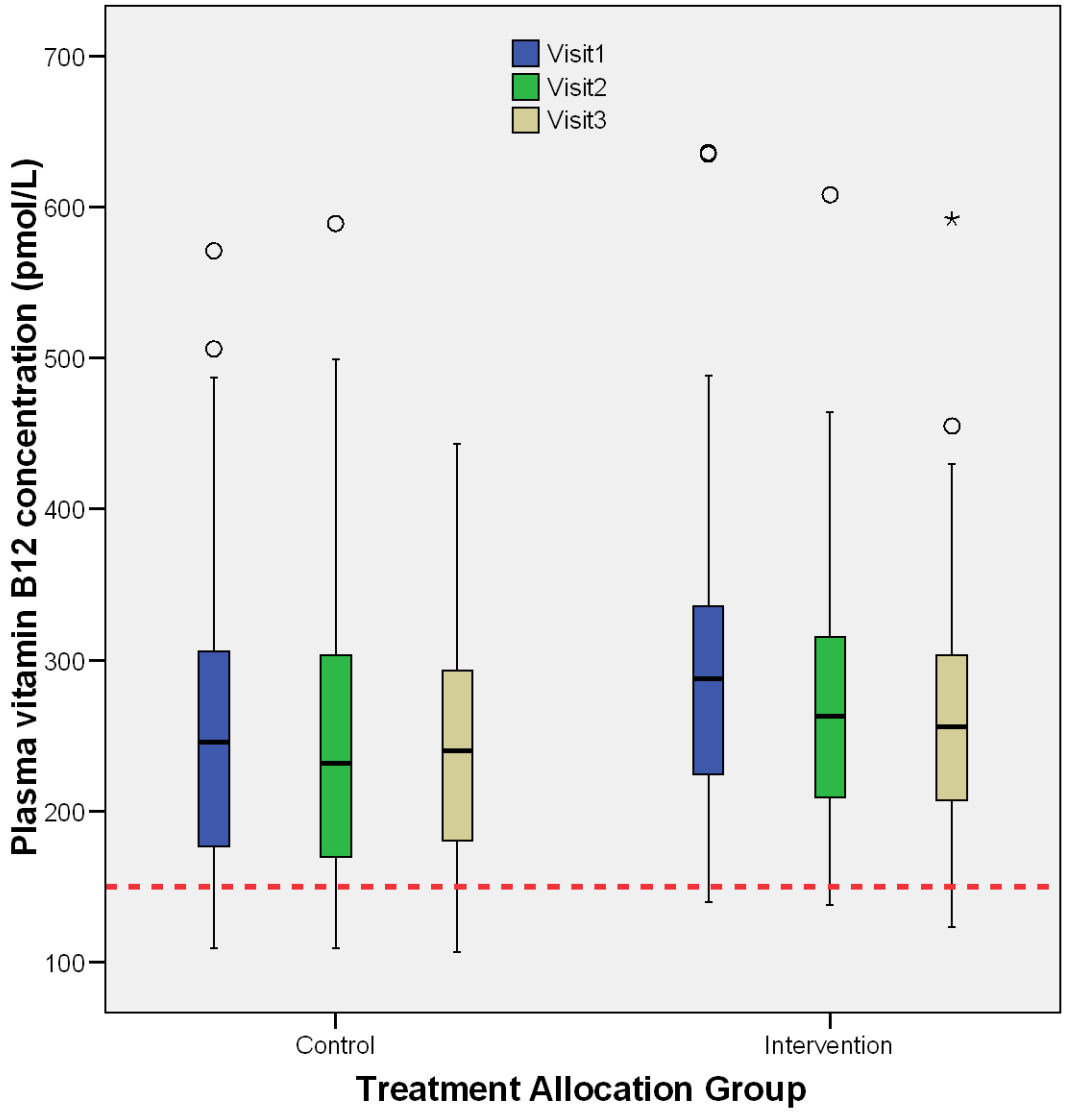
Values above this cut off indicate folate deficiency (20µmol/L).

Figure 4.13 Distribution of plasma vitamin B12 concentrations at visits 1, 2 and 3 by treatment group (control, n=79, intervention, n=72)



Deficiency cut off indicating biochemical depletion (150pmol/L).

Figure 4.14 Distribution of plasma vitamin B12 concentrations at visits 1, 2 and 3 by treatment group excluding outliers (control, n=75, intervention, n=71)



Deficiency cut off indicating biochemical depletion (150pmol/L).

Figure 4.15 Distribution of plasma vitamin C concentrations at visits 1, 2 and 3 by treatment group (control, n= 65, intervention, n=65)

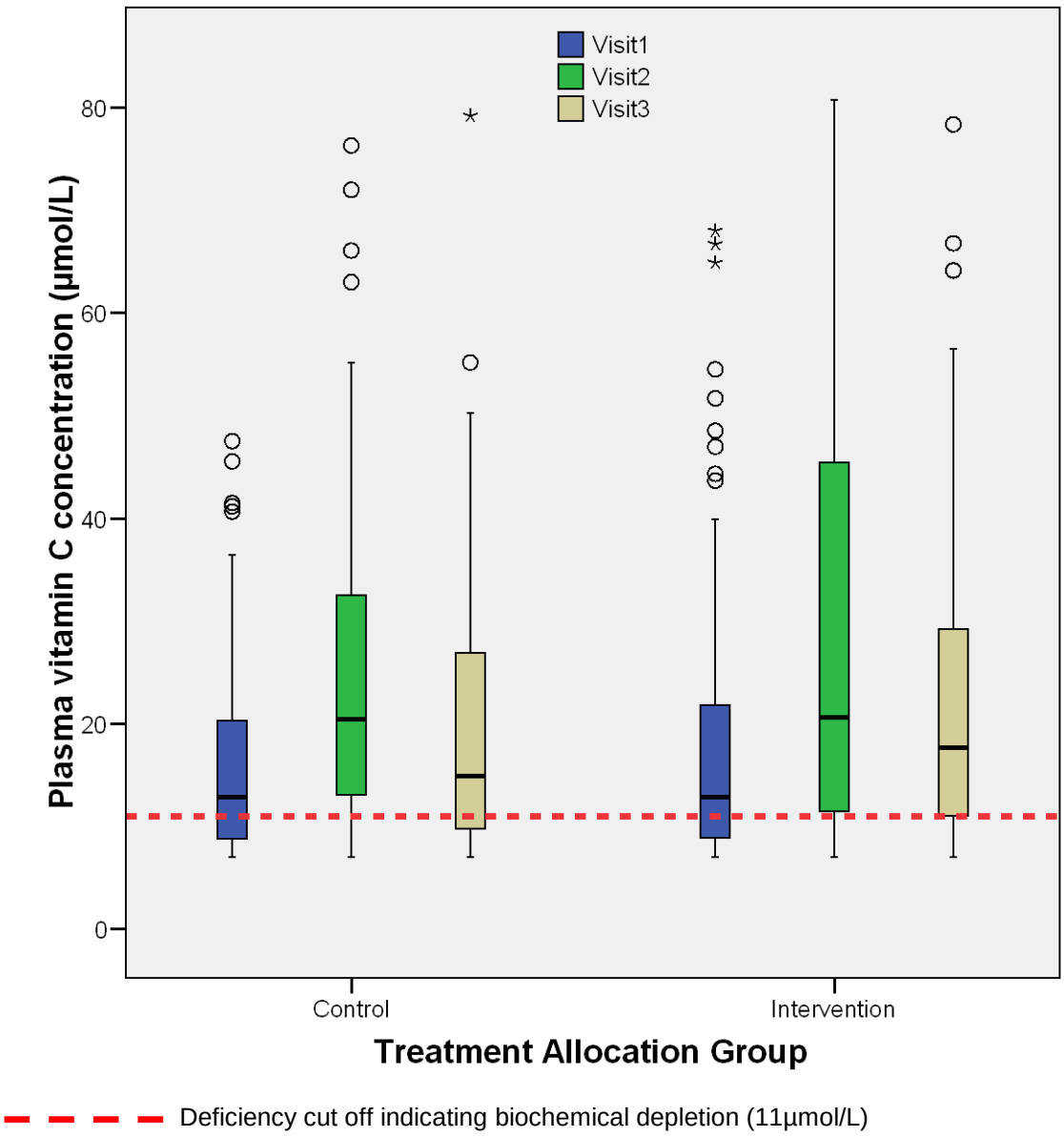
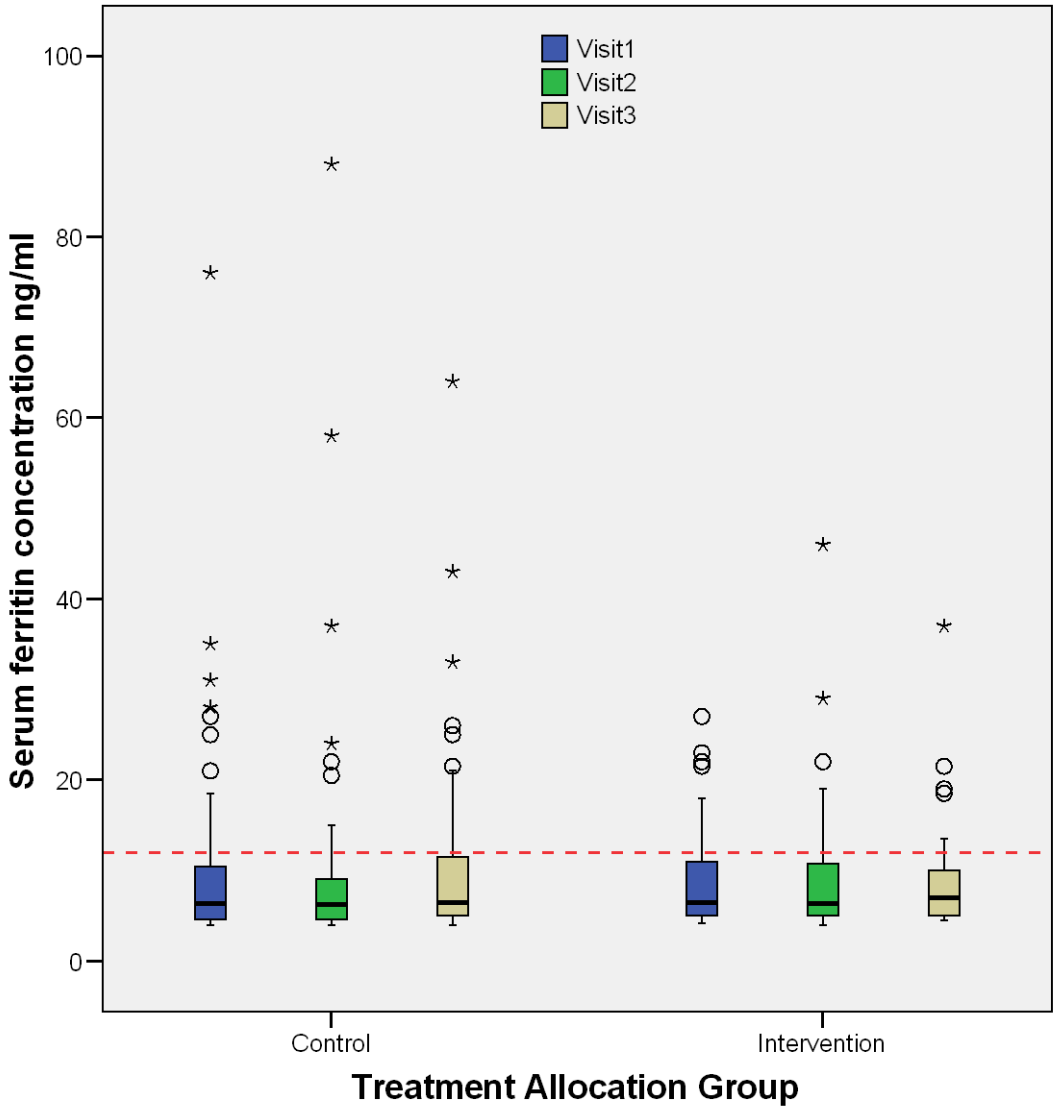
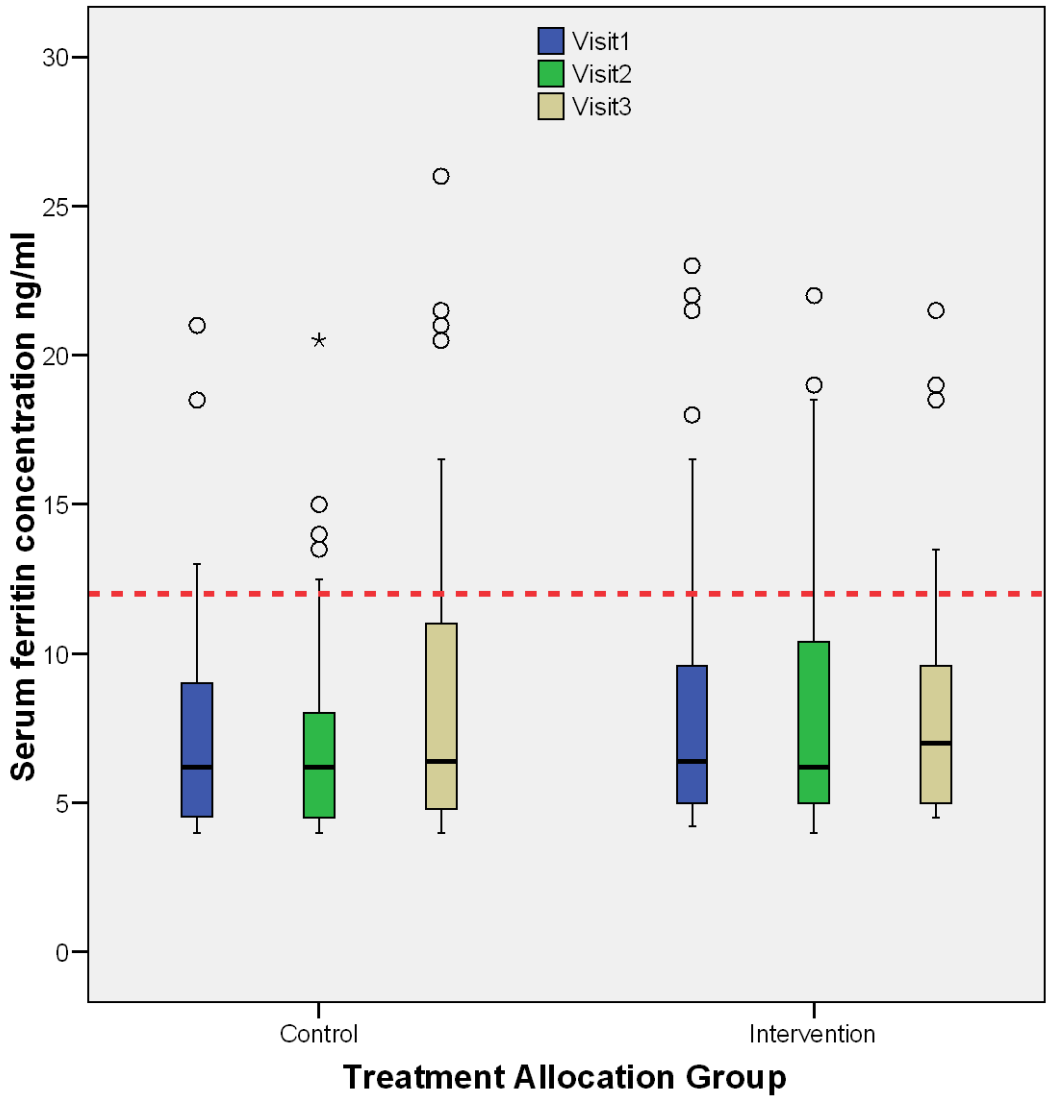


Figure 4.16 Distribution of serum ferritin concentrations at visits 1, 2 and 3 by treatment group (control, n=82, intervention, n=72)



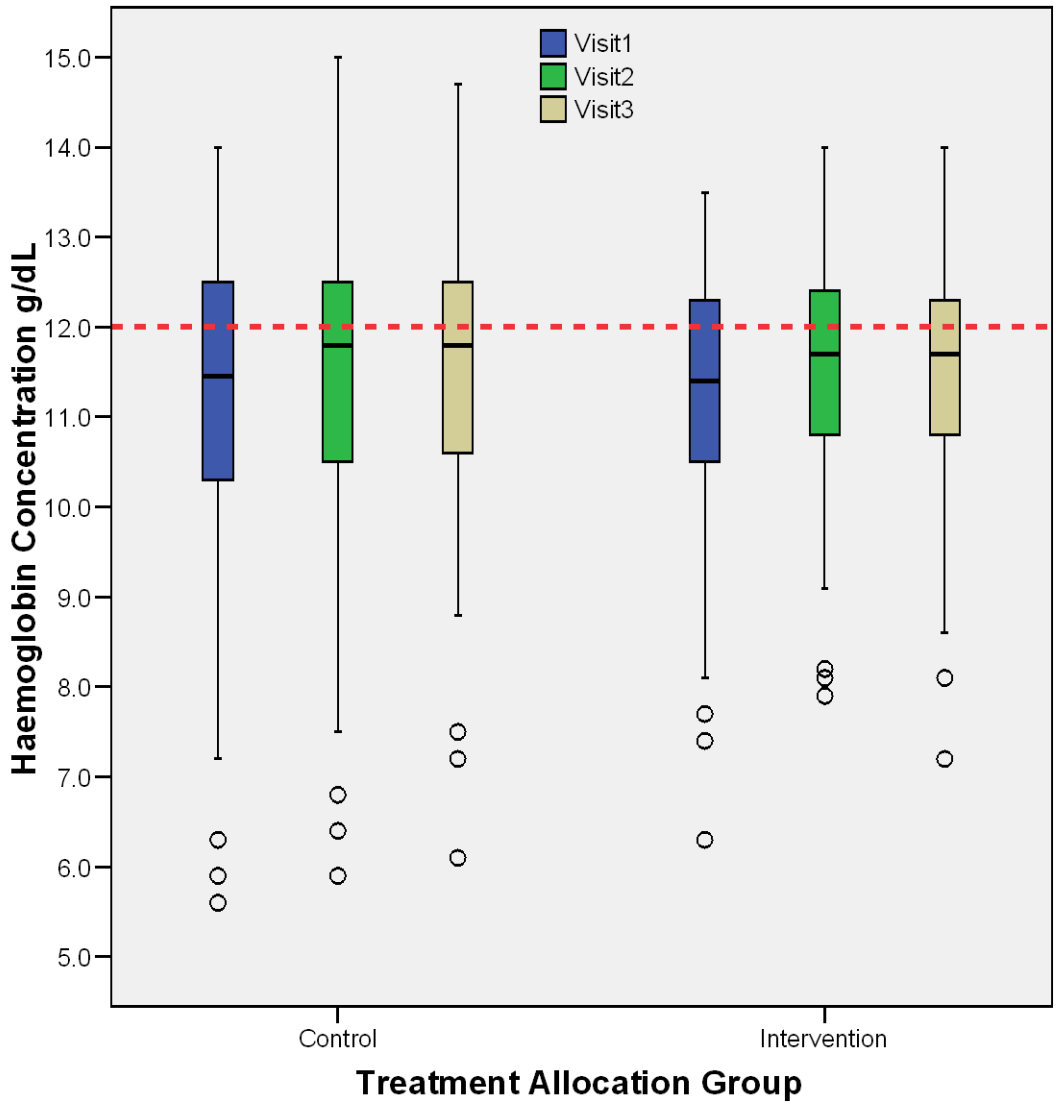
Deficiency cut off indicating biochemical depletion (12ng/ml).

Figure 4.17 Distribution of serum ferritin concentrations at visits 1, 2 and 3 by treatment group excluding outliers (control, n=76, intervention, n=70)



Deficiency cut off indicating biochemical depletion (12ng/ml).

Figure 4.18 Distribution of whole blood haemoglobin concentrations at visits 1, 2 and 3 by treatment group (control, n=86, intervention, n=78)



Deficiency cut off indicating iron deficiency anaemia (12g/dL).

4.5.4 Comparison between treatment groups in terms of changes in indicators of micronutrient status between visits 1 and 3

Table 4.13 shows the percentage of women whose blood measurements indicated that they were micronutrient deficient, by treatment group. The deficiency cut offs used were WHO published values (92). For retinol, percentages were also calculated using the cut off specified by the assay kit methodology. According to the WHO criterion, ≤5% of women were retinol deficient at both time points. However according to the kit method this proportion was higher and increased from one fifth to almost one third of women in the intervention group between visits 1 and 3. Women in the control group had a significantly lower risk of being retinol deficient at visit 3.

Table 4.13 Percentage of micronutrient-deficient women at baseline and after 12 weeks of supplementation; comparison between treatment groups

	Control			Intervention			RR (95% CI)	
	V1	V3	δ	V1	V3	δ		p
Retinol (<200ng/ml)*	1	3	2	5	5	0	0.68 (0.16,2.96)	0.610
Retinol (<300ng/ml)**	18	18	0	22	32	10	**0.56 (0.33,0.97)**	**0.035**
Folate (<6.8nmol/L)*	9	8	-1	8	11	3	0.72 (0.28,1.84)	0.487
Vitamin B12 (<150pmol/L)*	3	6	3	2	5	3	0.51 (0.47,5.91)	0.430
Vitamin C (<11µmol/L)*	40	36	-4	35	29	-6	1.29 (0.71,1.12)	0.308
Ferritin (<15ng/ml)*	88	82	-6	79	93	14	**0.89 (0.79,0.99)**	**0.044**
Haemoglobin (<12g/dL)*	61	57	-4	66	61	-5	0.93 (0.72,1.20)	0.406
Haemoglobin (<8g/dL)*	3	3	0	1	1	0	2.79 (0.30,26.34)	0.742

*Deficiency cut offs specified by WHO. **Deficiency cut off specified by kit method. V1, visit1. V3, visit 3, δ=Visit 3-Visit 1 values. RR, relative risk of deficiency at visit 3 (control=0 vs. intervention=1). CI, 95% Confidence Interval

Almost 10% of women were folate deficient at visit 1 and there was relatively little change between the time points. Few women were vitamin B12 deficient at visit 1, the proportion in both groups increased slightly but there was no

effect of group on risk of deficiency at visit 3. Over a third of women in both groups were vitamin C deficient and there was a small decrease in the proportions in both treatment groups. The majority of women were iron deficient at visit 1 based on serum ferritin concentrations. The proportion decreased in the control group and increased in the intervention group. Women in the control group had a significantly lower risk of being ferritin deficient at visit 3. There was evidence of mild anaemia (Hb<12g/dL) in approximately two thirds of the study group and the proportion who were anaemic decreased in both groups.

Further examination of the proportion of women who were retinol and ferritin deficient was conducted. **Table 4.14** shows the proportion of deficient women excluding those with CRP concentrations > 5µg/ml (n=28). As with the whole group analysis, the women in the control group had a significantly lower risk of being retinol deficient. The finding relating to ferritin was no longer significant but was in the same direction as the main group analysis. When women taking supplements (n=30) were excluded from the analysis, there was no statistically significant difference in the risk of being retinol or iron deficient at visit 3 between treatment groups (**Table 4.15**).

Table 4.14 Percentage of micronutrient-deficient women at baseline and after 12 weeks of supplementation excluding women with CRP >5µg/ml (n=28)

	Control			Intervention			RR (95% CI)	
	V1	V3	δ	V1	V3	δ		p
Retinol (<200ng/ml)*	1	3	2	1	3	2	0.95 (0.13,6.98)	0.962
Retinol (<300ng/ml)**	18	15	3	22	30	8	**0.41 (0.18,0.99)**	**0.045**
Ferritin (<15ng/ml)*	89	84	-5	83	94	11	0.34 (0.10,1.13)	0.078

*Deficiency cut offs specified by WHO. **Deficiency cut off specified by kit method. V1, visit1. V3, visit 3, δ=Visit 3- Visit 1 values. RR, relative risk of deficiency at visit 3 (control vs. intervention). CI, 95% Confidence Interval

Table 4.15 Percentage of micronutrient-deficient women at baseline and after 12 weeks of supplementation excluding women taking supplements (n=30)

	Control			Intervention			RR (95% CI)	
	V1	V3	δ	V1	V3	δ		p
Retinol (<200ng/ml)*	1	4	3	1	6	5	0.63 (0.14,2.94)	0.561
Retinol (<300ng/ml)**	19	20	1	22	30	8	0.57 (0.27,1.21)	0.147
Ferritin (<15ng/ml)*	87	84	-3	81	91	10	0.48 (0.17,1.35)	0.164

*Deficiency cut offs specified by WHO. **Deficiency cut off specified by kit method. V1, visit1. V3, visit 3, δ=Visit 3- Visit 1 values. RR, relative risk of deficiency at visit 3 (control vs. intervention). CI, 95% Confidence Interval

Table 4.16 shows the summary statistics broken down by group for micronutrient concentrations at visits 1 and 3 and the change (δ) between visit 3 and visit 1 measurements. Retinol concentrations decreased in both the control and intervention groups over the study period but there were no statistically significant differences between visit 1 and visit 3 concentrations in either group. There was no difference between groups in terms of the change in retinol concentrations over the study period. These results were similar when women with CRP>5µg/ml were excluded from the analyses. There was an increase in β-carotene concentrations in both treatment groups between the two time points and the difference between visit 1 and visit 3 values was significant in both groups. The increase was greater in the intervention group. The difference in the change between groups was 49nmol/L and was statistically significant.

There was a borderline statistically significant difference between visit 1 and visit 3 folate concentrations in the control group and no significant difference in the intervention group. Total plasma homocysteine concentrations decreased in both groups and the difference between visit 1 and visit 3 concentrations reached significance in the intervention group. There were no significant differences between groups for either folate or homocysteine in terms of the change over the study period. Median vitamin B12 values decreased in the intervention group, with little change in the control group.

Plasma vitamin C increased between visits 1 and 3 in both groups and the difference between visit 1 and visit 3 values was significant in the control group and approached significance in the intervention group. There was no difference between groups in the change in concentrations between time points.

When all women with plasma ferritin measurements at both time points were considered, there were no statistically significant differences between visit 1 and visit 3 values in either group but the difference between groups in the change over the study period approached significance. This was attenuated when women with plasma CRP concentrations >5µg/ml at visit 3 were excluded.

Table 4.16 Median (IQR) blood concentrations at visit 1 and visit 3, and change in concentrations, by treatment group

	Control					Intervention						
	N	Visit 1	Visit 3	p*	δ^c	N	Visit 1	Visit 3	p*	δ^i	p**	$\delta^i - \delta^c$ (CI)
Retinol (ng/ml)	83	409 (326,490)	395 (324,476)	0.278	-10 (-91,44)	79	378 (297,484)	358 (291,474)	0.387	-17 (-94,65)	0.829	4 (-34,43)
Retinol (ng/ml)†	65	429 (321,549)	394 (324,470)	0.229	-10 (-77,40)	62	375 (315,484)	372 (291,474)	0.875	-15 (-82,75)	0.464	-14 (-54,24)
β-carotene (nmol/L)	85	390 (305,470)	440 (340,540)	**0.009**	40 (-5,115)	80	385 (323,470)	470 (380,610)	**<0.001**	75 (23,158)	**0.020**	**49 (7,90)**
Folate (nmol/L)	80	14.1 (9.0,21.4)	13.1 (9.2,18.4)	0.085	-0.4(-5.5,4.0)	77	13.3 (9.9,19.6)	13.3 (9.2,17.5)	0.105	-0.6 (-3.9,2.4)	0.757	-1.3 (-10.0,7.3)
Hcy (µmol/L)	80	11.6 (9.5,13.9)	10.4 (8.8,13.4)	0.118	-0.5 (-2.5,0.5)	76	11.3 (8.7,14.6)	10.6 (8.6,13.4)	**0.022**	-0.3 (-2.8,1.0)	0.738	0.3 (-1.6, 2.3)
Vitamin B12 (pmol/L)	79	250 (178,351)	255 (187,304)	0.089	-10 (-52,19)	78	290 (228,340)	259 (208,326)	0.103	-17 (-50,24)	0.330	-34 (-103,35)
Vitamin C (µmol/L)	66	12.5 (8.8,20.3)	14.6 (9.6,27.0)	**0.033**	1.5 (-2.3,8.3)	70	12.7 (8.6,22.3)	17.0 (9.9,29.5)	0.276	0.6 (-3.7,8.0)	0.652	-1.2 (-6.2,3.9)
Ferritin (ng/ml)	83	6.4 (4.6,11.0)	6.4 (5.0,11.5)	0.238	0.0 (-1.0,2.5)	77	6.4 (5.0,11.8)	7.6 (5.0,10.7)	0.131	-0.4 (-2.5,1.0)	0.065	-1.5 (-3.1, 0.1)
Ferritin (ng/ml)†	69	6.2 (4.6,11.0)	6.4 (4.8,11.5)	0.387	0.0 (-1.0,2.1)	66	6.4 (5.0,12.0)	7.6 (5.0,11.0)	0.189	-0.3 (-2.6,1.1)	0.173	-1.3 (-0.6,3.1)
Hb (g/dL)	88	11.4 (10.3,12.5)	11.9 (10.6,12.6)	**0.027**	0.1 (-0.3,0.8)	82	11.4 (10.5,12.2)	11.7 (10.8,12.3)	0.095	0.1 (-0.3,0.5)	0.638	0.1 (-0.2,0.4)

δ^c, change in concentration in control group (visit 3 – visit 1), δ^i change in concentration in intervention group (visit 3 – visit 1). CI, 95% confidence interval. Hcy, homocysteine, Hb, haemoglobin.*p relates to the difference in mean values at visit 1 and visit 3. **p relates to independent t-test for difference in the mean δ^i and δ^c values. †Excluding women with CRP>5µg/ml at visit 3.

4.5.5 Univariate predictors of change in micronutrient concentrations between visits 1 and 3

This section presents the effect of group on change in circulating micronutrient concentrations. Data relating to homocysteine and haemoglobin are not presented in this section. **Table 4.17** shows the results of univariate analyses to estimate the effect of treatment group on change in micronutrient concentrations over the study period. Being in the intervention group was associated with an increase in plasma β-carotene over the study period. The effect size was approximately 10% of the median baseline β-carotene concentration. For all other nutrients, treatment group did not predict change in concentration, although for ferritin the result was close to being statistically significant. This was not in the expected direction with those in the intervention group experiencing a decrease in ferritin concentrations relative to the control group.

Table 4.17 Summary of univariate models with treatment group as the predictor variable and change in micronutrient concentrations as the outcome variable

Micronutrient	B	95% Confidence Interval		p
		Lower	Upper	
Retinol* (ng/ml)	5.76	-33.14	44.67	0.770
B-carotene (µmol/L)	0.049	0.010	0.090	**0.020**
Folate (nmol/L)	-1.39	-9.97	7.18	0.749
Vitamin B12 (pmol/L)	35.5	-32.78	103.76	0.306
Vitamin C (µmol/L)	-1.18	-6.17	3.81	0.641
Ferritin* (ng/ml)	-1.51	-3.10	0.09	0.064

*Women with CRP >5µg/ml were excluded from the analysis

4.5.6 Multivariate models to assess predictors of change in nutrient concentrations between visits 1 and 3

Table 4.18-Table 4.23 show the results of multivariate analyses with change in blood micronutrient concentrations between visits 1 and 3 as the outcome variables. For all nutrients, the concentrations at visit 1 were strong predictors of the change over the study with all associations being negative.

There was a borderline significant positive association between age and change in serum retinol concentrations, although the effect size was small (**Table 4.18**).

Table 4.18 Summary of multivariate model with change in serum retinol concentration as the outcome

	B	95% Confidence Interval		p
		Lower	Upper	
Group (0,1)*	-6.94	-41.99	28.11	0.696
Age (years)	3.32	-0.07	6.73	0.055
BMI (kg/m^2)	0.55	-4.82	5.93	0.838
Synthetic Nutrient Intake at visit 3**	37.80	-17.21	92.81	0.177
Compliance Status†	30.35	-61.45	122.16	0.515
Vitamin A at visit 1 (ng/ml)	-0.43	-0.55	-0.31	**<0.001**

*Treatment group; Control=0, Intervention=1, **Synthetic nutrient intake; No=0, Yes=1, †Compliance status; No=0, Yes=1

Table 4.19 shows that group allocation was a significant predictor of change in β-carotene concentrations when adjusted for potential confounders. The effect size (0.047μmol/L) remained similar to that observed in univariate analysis.

Table 4.19 Summary of multivariate model with change in serum β-carotene concentration as the outcome

	B	95% Confidence Interval		p
		Lower	Upper	
Group (0,1)*	0.047	0.007	0.087	**0.020**
Age (years)	0.003	0.000	0.007	0.083
BMI (kg/m^2)	-0.004	-0.010	0.002	0.170
Synthetic Nutrient Intake at visit 3**	-0.008	-0.069	0.053	0.795
Compliance Status†	-0.002	-0.027	0.023	0.874
B-carotene at visit 1 (natural log)	-0.321	-0.464	-0.179	**<0.001**

*Treatment group; Control=0, Intervention=1, **Synthetic nutrient intake; No=0, Yes=1, †Compliance status; No=0, Yes=1

Intake of synthetic nutrients was a significant predictor of change in folate concentrations (**Table 4.20**) but was not associated with changes in homocysteine levels (data not shown). Change in plasma vitamin B12

concentration was negatively associated with concentration at visit one and was not associated with any of the other variables (**Table 4.21**).

Table 4.20 Summary of multivariate model with change in plasma folate concentration as the outcome

	B	95% Confidence Interval		p
		Lower	Upper	
Group (0,1)*	-2.76	-11.15	5.64	0.517
Age (years)	0.18	-0.595	0.948	0.652
BMI (kg/m^2)	-0.73	-1.995	0.546	0.261
Synthetic Nutrient Intake at visit 3**	12.89	0.10	25.68	**0.048**
Compliance Status†	-3.74	-22.67	15.17	0.696
Folate at visit 1 (natural log)	-12.63	-19.49	-5.79	**<0.001**

*Treatment group; Control=0, Intervention=1, **Synthetic nutrient intake; No=0, Yes=1, †Compliance status; No=0, Yes=1

Table 4.21 Summary of multivariate model with change in plasma vitamin B12 concentration as the outcome

	B	95% Confidence Interval		p
		Lower	Upper	
Group (0,1)*	0.063	-4.194	58.952	0.089
Age (years)	0.042	-1.339	4.477	0.288
BMI (kg/m^2)	-0.033	-6.878	2.707	0.391
Synthetic Nutrient Intake at visit 3**	-0.026	-66.995	31.732	0.481
Compliance Status†	-0.008	-79.669	63.394	0.822
Vitamin B12 at visit 1 (natural log)	-0.899	-0.853	-0.725	**<0.001**

*Treatment group; Control=0, Intervention=1, **Synthetic nutrient intake; No=0, Yes=1, †Compliance status; No=0, Yes=1

The only statistically significant predictor of vitamin C status was visit 1 vitamin C concentration (**Table 4.22**). In the case of ferritin the effect of treatment group on change in serum concentrations was reduced when other predictor variables were added to the model (**Table 4.23**) compared with the effect size derived using the univariate model. There was a positive association between age and change in ferritin concentration which approached statistical significance.

Table 4.22 Summary of multivariate model with change in plasma vitamin C concentration as the outcome

	B	95% Confidence Interval		p
		Lower	Upper	
Group (0,1)*	-0.86	-5.69	3.96	0.724
Age (years)	-0.07	-0.51	0.38	0.776
BMI (kg/m^2)	0.11	-0.64	0.85	0.774
Synthetic Nutrient Intake at visit 3**	3.42	-4.07	10.90	0.368
Compliance Status†	-7.54	-17.72	2.64	0.145
Vitamin C at visit 1 (natural log)	-8.06	-11.88	-4.24	**<0.001**

*Treatment group; Control=0, Intervention=1, **Synthetic nutrient intake; No=0, Yes=1, †Compliance status; No=0, Yes=1

Table 4.23 Summary of multivariate model with change in serum ferritin concentration as the outcome

	B	95% Confidence Interval		p
		Lower	Upper	
Group (0,1)*	-1.24	-2.68	0.21	0.094
CRP at visit 1 (natural log)	0.05	-0.49	0.59	0.854
Age (years)	0.12	-0.02	0.26	0.082
BMI (kg/m^2)	-0.10	-0.34	0.13	0.382
Synthetic Nutrient Intake at visit 3**	1.62	-0.68	3.93	0.166
Compliance Status†	-1.21	-5.31	2.88	0.560
Ferritin concentration at visit 1(natural log)	-4.62	-5.89	-3.35	**<0.001**

*Treatment group; Control=0, Intervention=1, **Synthetic nutrient intake; No=0, Yes=1, †Compliance status; No=0, Yes=1

The scatterplots in **Figure 4.19–Figure 4.24** show the association between visit 1 nutrient concentrations and the change between baseline and visit 3 values. It can be seen that for all nutrients baseline concentrations are negatively associated with change over the study period such that those with the lowest concentrations tend to exhibit the greatest change over time.

Figure 4.19 Scatterplot of visit 1 serum retinol concetration by change in serum retinol concentration

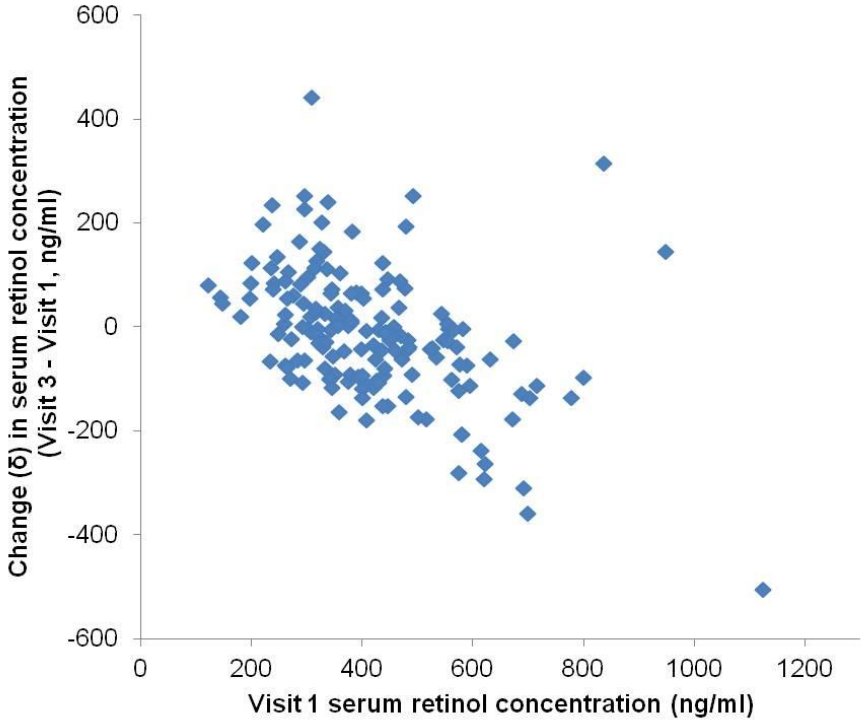


Figure 4.20 Scatterplot of visit 1 serum β-carotene concentration by change in serum β-carotene concentration

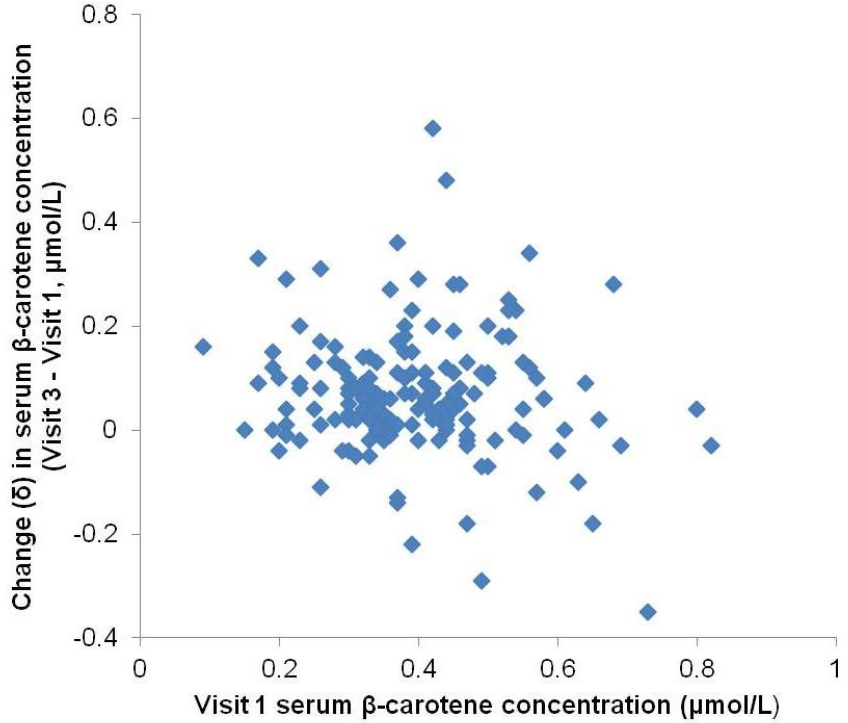


Figure 4.21 Scatterplot of visit 1 plasma folate concentration by change in plasma folate concentration

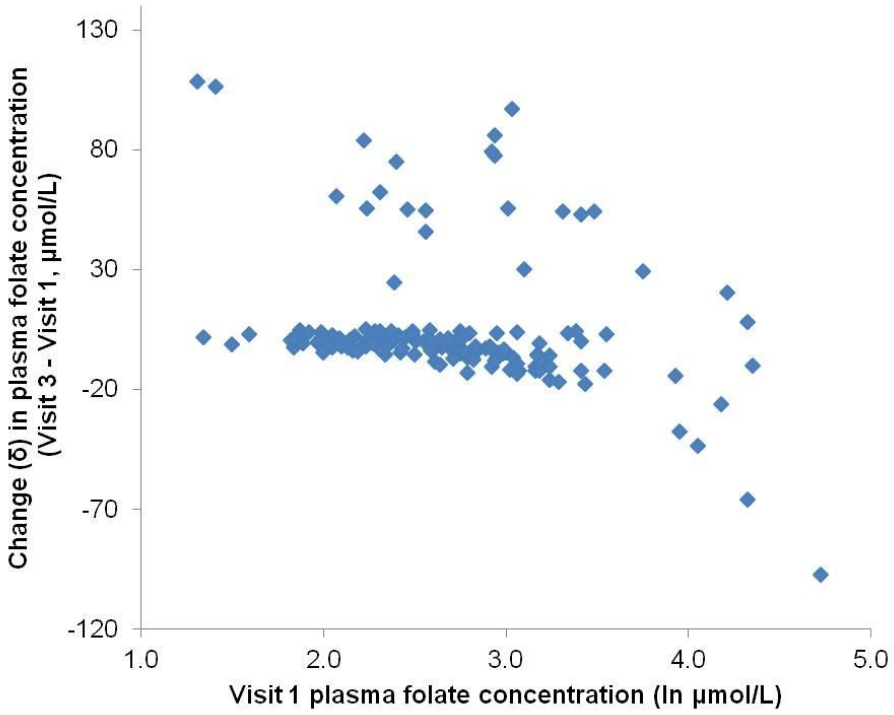


Figure 4.22 Scatterplot of visit 1 plasma vitamin B12 concentration by change in plasma vitamin B12 concentration

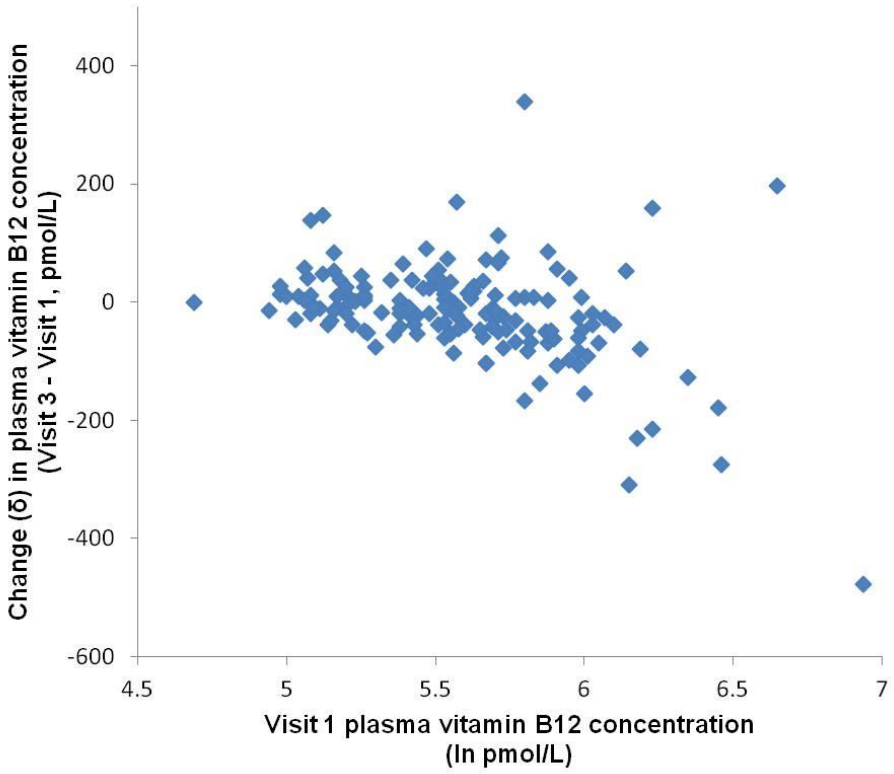


Figure 4.23 Scatterplot of visit 1 plasma vitamin C concentration by change in plasma vitamin C concentration

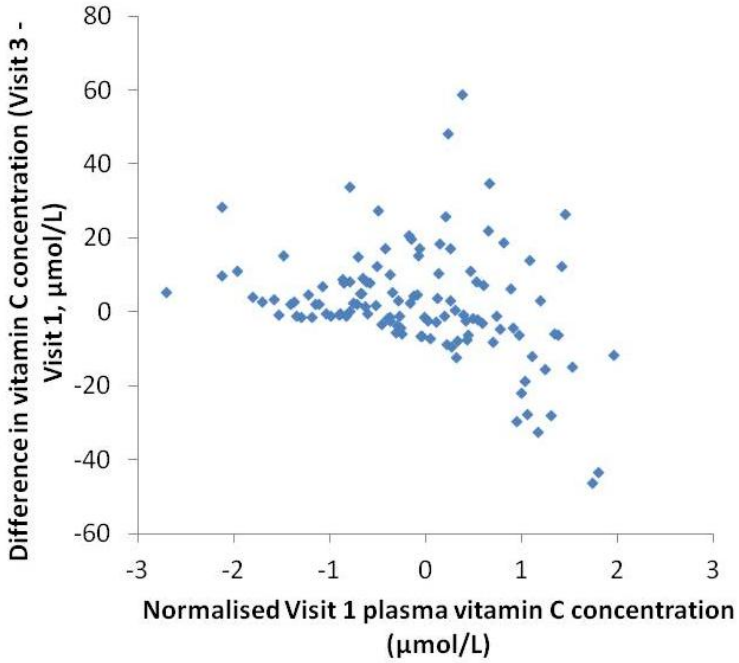
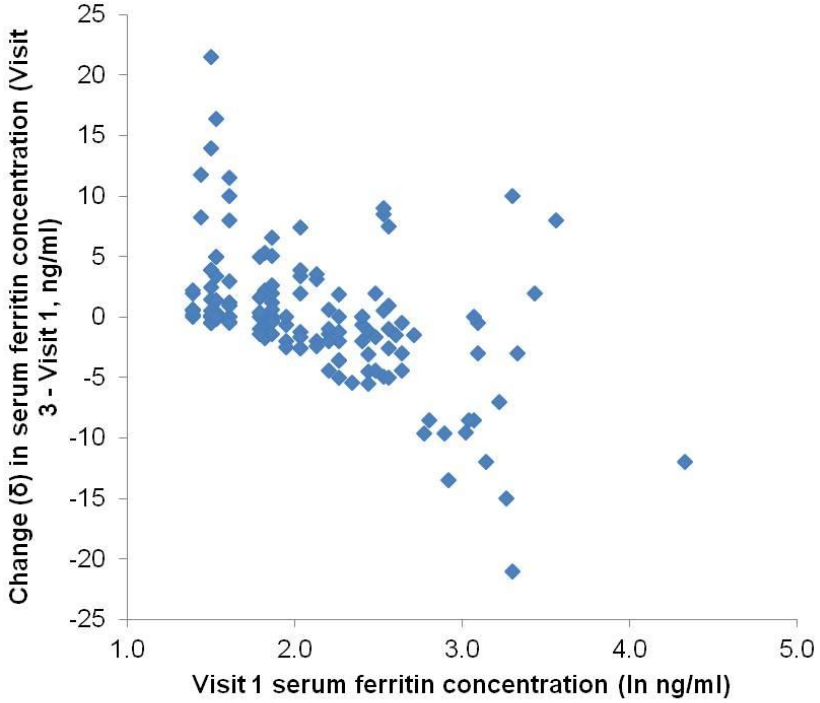


Figure 4.24 Scatterplot of visit 1 serum ferritin concentration by change in serum ferritin concentration



4.5.7 Sample Size

Post-hoc power calculations were conducted for each nutrient to determine the power achieved based on the sample size achieved and the effect size observed. The first set of calculations used the proportion of women who were deficient in each nutrient at visit 1 and at visit 3 (**Table 4.13**, p125) along with the sample size in each group. The power achieved was 64.7% for retinol (using the deficiency cut off specified by the assay kit method of <300ng/ml); 15.1% for folate; 8.0% for vitamin B12; 23.9% for vitamin C; 64.0% for ferritin; 24.0% for haemoglobin (<8g/dL).

The second set of calculations used the micronutrient concentration variables in continuous form. The power that was achieved given the sample size in the study and the difference between the control and intervention groups in the change in concentration between visit 1 and visit 3 was computed. Using this approach the power achieved was 7.6% for retinol; 76.4% for β-carotene; 9.3% for folate; 25.2% for vitamin B12; 13% for vitamin C; 58.8% for ferritin and 8.0% for haemoglobin.

4.6 Functional outcomes

In addition to investigating the change in blood micronutrient concentrations over the study period, changes in functional outcomes were monitored. Grip strength, blood pressure and self-reported health were studied at visits 1 and 3. As well as looking at the changes in these parameters over time, associations with anthropometric and dietary factors were examined.

4.6.1 Grip strength

Figure 4.25-**Figure 4.27** show that there was a positive relationship between grip strength at visit 1 and weight, height and BMI among all women in the study (n=208). The correlation coefficients were 0.39, p<0.001 for weight, 0.39, p<0.001 for height and 0.29, p<0.001 for BMI.

Figure 4.25: Scatterplot of weight by grip strength at visit 1

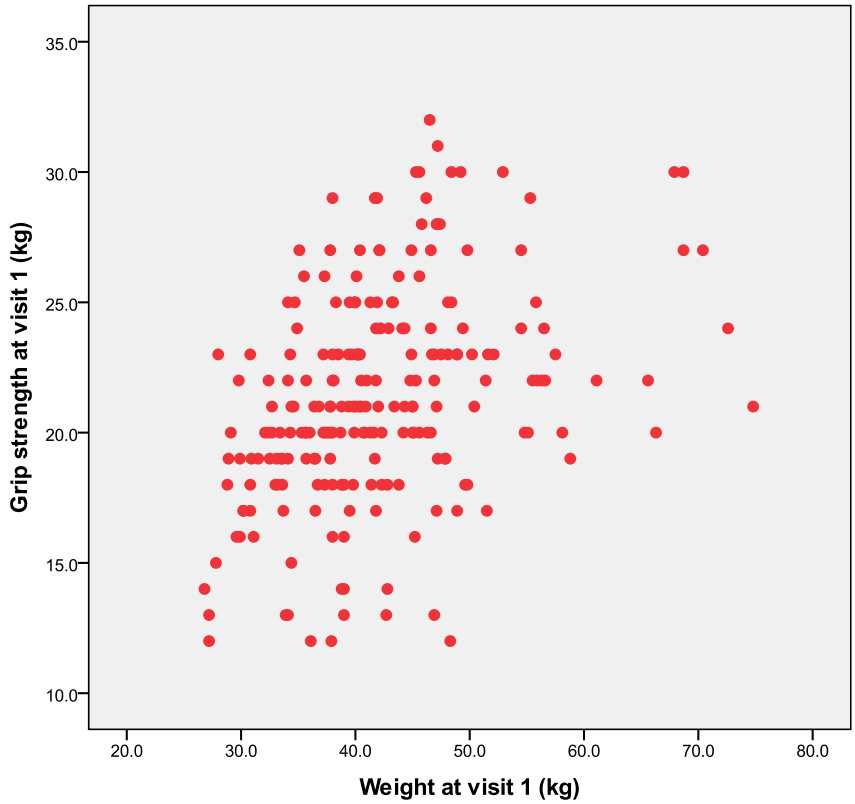


Figure 4.26 Scatterplot of height by grip strength at visit 1

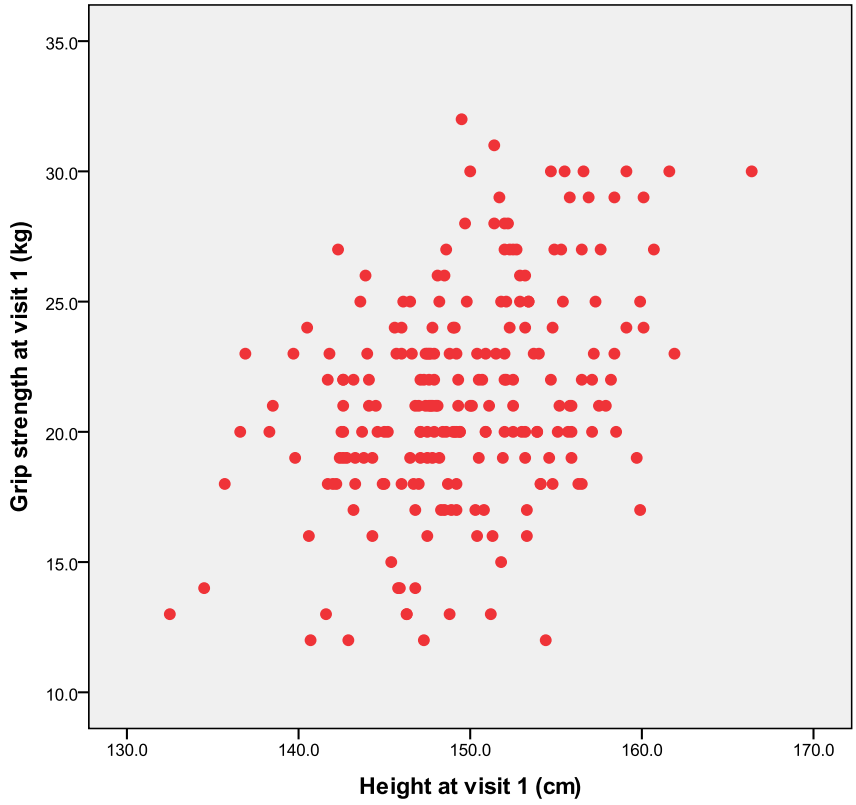


Figure 4.27 Scatterplot of body mass index by grip strength at visit 1

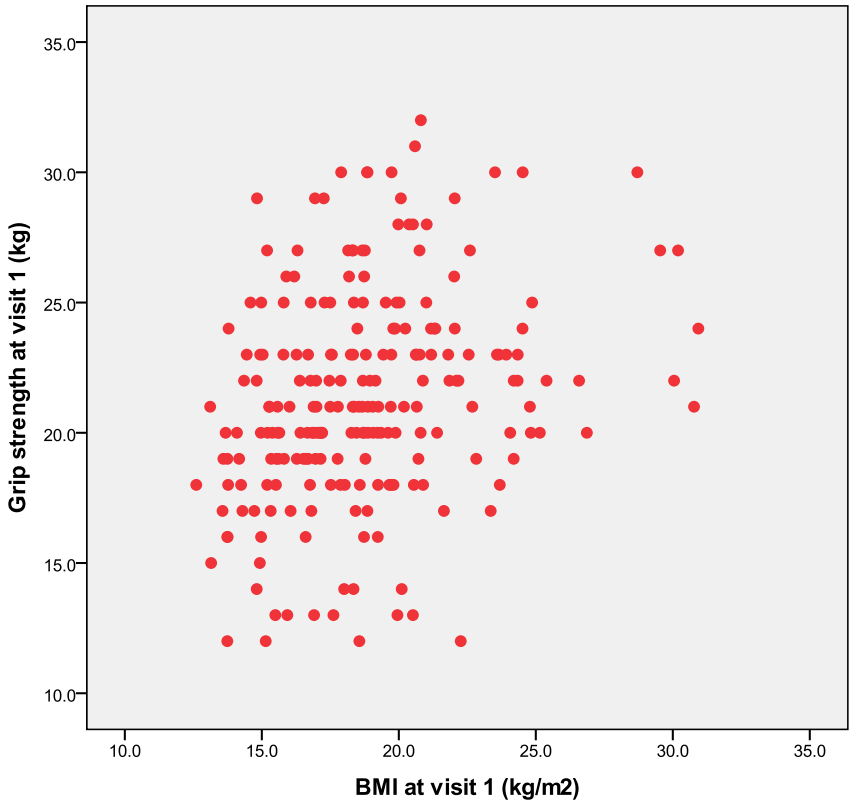


Table 4.24 shows the grip strength measurements at visits 1 and 3 by treatment group for all women with measurements at both time points (n=170), There was an increase in both groups over the study period. The mean difference was slightly higher in the intervention group but there was no statistically significant difference in the change over time between groups.

Table 4.24 Mean (SD) grip strength measurements (kg) by treatment group

Group	N	Visit 1	Visit 3	Visit 3 – Visit 1*
Control	88	21.7 (4.0)	23.2 (4.6)	1.4 (3.0)
Intervention	82	20.9 (4.3)	22.1 (4.3)	1.8 3.1)

*T test for difference between groups, p=0.449

Table 4.25 shows that for all participants, group allocation did not predict change in grip strength over the study period. However, the data show that among the women who were chronically energy deficient (BMI<18.5kg/m^2), there was a larger positive change among women in the intervention group

compared with the control group and this difference approached statistical significance.

Table 4.25 Summary of univariate model with treatment group as the predictor and change in grip strength as the outcome variable for all participants and by BMI category

	B	95% Confidence Interval		p
		Lower	Upper	
All participants	0.34	-0.55	1.24	0.449
BMI <18.5kg/m^2	0.92	-0.18	2.02	0.101
BMI 18.5-24.9kg/m^2	-0.37	-1.84	1.10	0.620
BMI>25.0 kg/m^2	0.75	-6.05	7.55	0.802

Treatment group coded as 0=control, 1 = intervention.

4.6.2 Grip strength and dietary intake

The boxplots in **Figure 4.28** – **Figure 4.31** represent the distribution of grip strength measurements by categories of intake frequency of the following micronutrient-rich food groups: GLV; fruit; milk and milk products; non-vegetarian foods.

Results of a one way ANOVA showed there was a borderline statistically significant difference in grip strength measurements at visit 1 between women who reported different consumption frequencies of GLV (p=0.055). There was no significant difference in grip strength between women reporting different frequencies of fruit consumption (p=0.262). Likewise there were no significant differences in grip strength by consumption of milk products or non-vegetarian foods p=0.157 and p=0.572 respectively.

Figure 4.28 Boxplot of grip strength at visit 1 by frequency of consumption of GLV

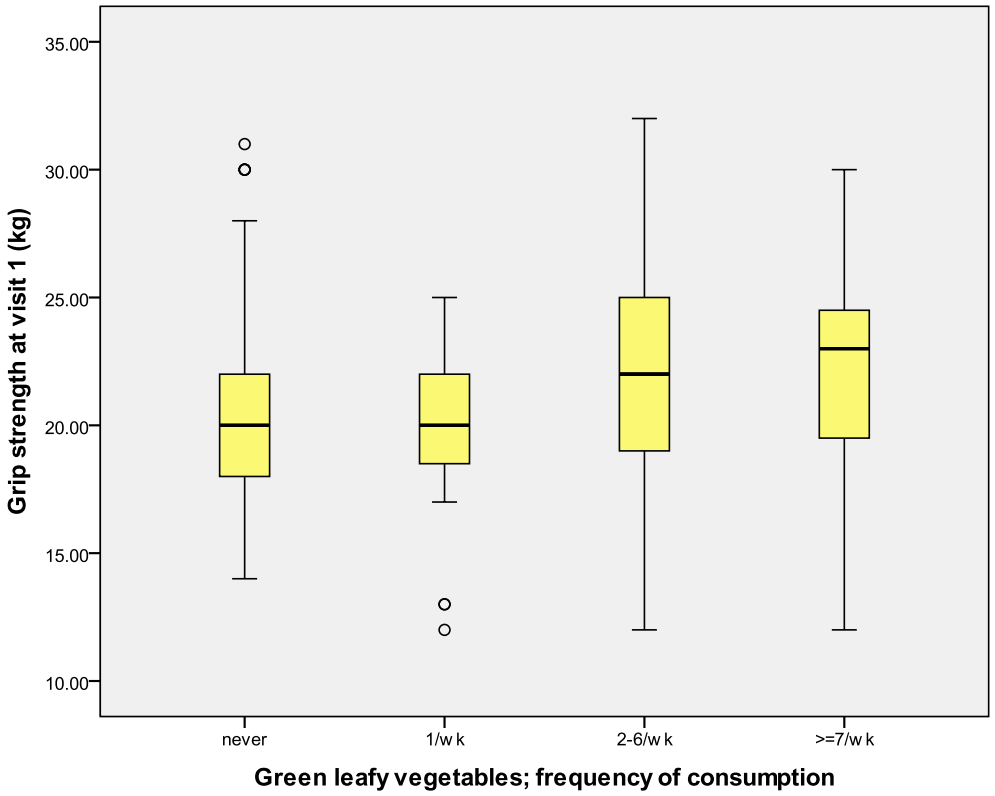


Figure 4.29 Boxplot of grip strength at visit 1 by frequency of consumption of fruit

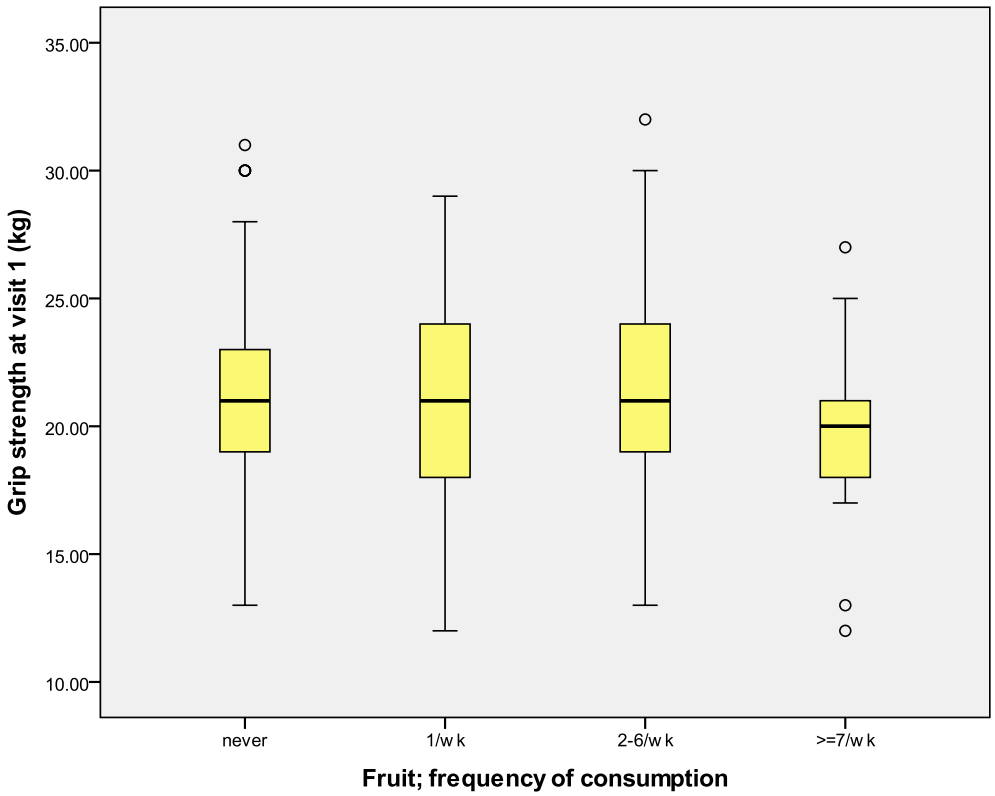


Figure 4.30 Boxplot of grip strength at visit 1 by frequency of consumption of milk and milk products

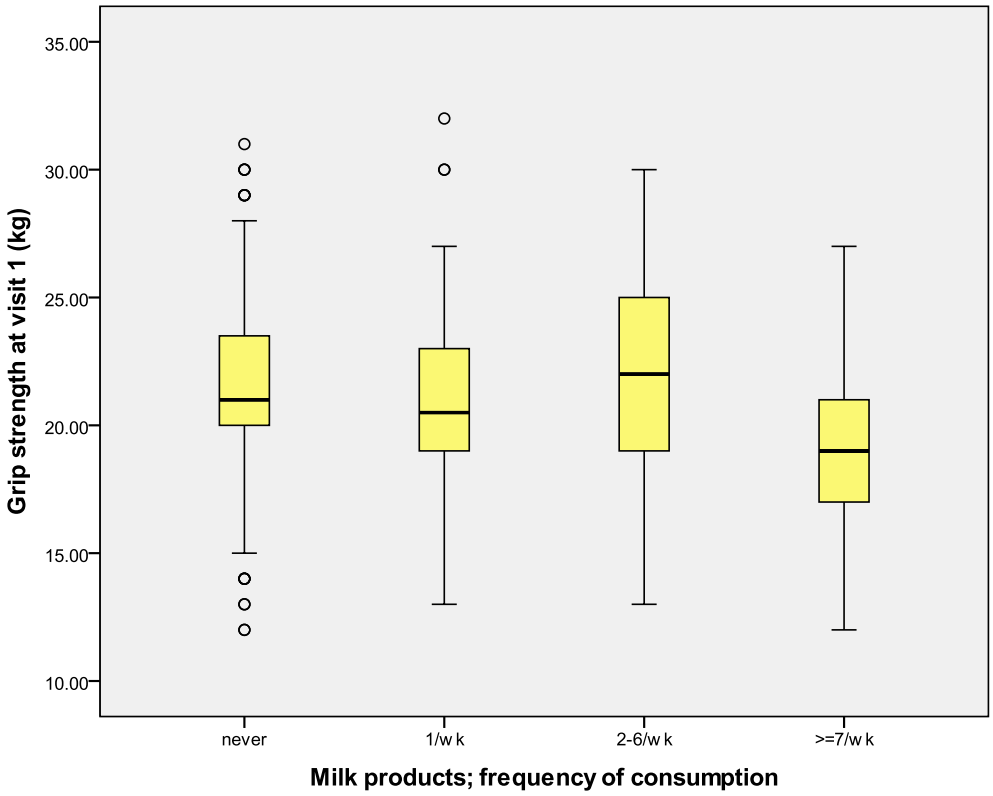
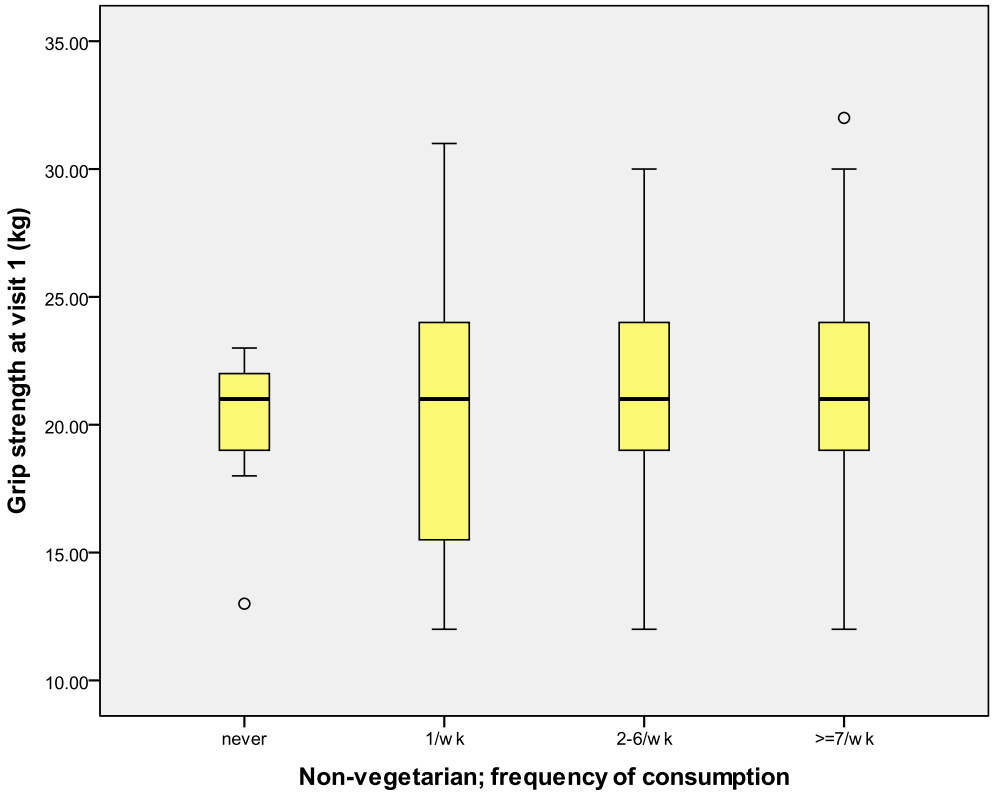


Figure 4.31 Boxplot of grip strength at visit 1 by frequency of consumption of non-vegetarian foods



4.6.3 Blood pressure

Systolic and diastolic blood pressure were monitored at visits 1 and 3. Data shown relate to 170 women for whom blood pressure measurements were available at both time points. **Table 4.26** shows that at baseline, mean systolic blood pressure was 105mmHg in both treatment groups. There was a small decrease in systolic pressure over the study period but there was no statistically significant difference between groups. Mean diastolic pressure was 67mmHg and this increased slightly in both groups, again with no significant difference between groups (**Table 4.27**).

Table 4.26 Mean (SD) systolic blood pressure measurements (mmHg)

Group	N	Visit 1	Visit 3	Visit 3 – Visit 1*
Control	88	105 (11)	104 (10)	-0.9 (10.5)
Intervention	82	105 (13)	105 (11)	-1.5 (13.1)

*T test for difference between groups, p=0.763

Table 4.27 Diastolic blood pressure measurements (mmHg), Mean (SD)

Group	N	Visit 1	Visit 3	Visit 3 – Visit 1*
Control	88	67 (8)	68 (9)	1.5 (9.9)
Intervention	82	67 (10)	68 (9)	0.1 (10.4)

*T test for difference between groups, p=0.352

The proportion of women with pre-hypertension as defined by a systolic blood pressure between 120 and 139mmHg decreased in both groups over the study period but there was no difference between groups. In the control group there was a small increase in the proportion of women who were pre-hypertensive and in the intervention group the proportion decreased. There were very few women categorized as hypertensive based on diastolic blood pressure at either time point. When the relative risk of having a diastolic blood pressure >80mmHg at visit 3 was calculated there was no significant effect of treatment group allocation (**Table 4.28**).

Table 4.28 Percentage of women with pre-hypertension and hypertension.

	Control (n=88)			Intervention (n=82)			
	V1	V3	δ	V1	V3	δ	RR* (CI)
SBP 120-139mmHg	12.5	6.2	-6.3	6.8	9.5	-2.7	1.04 (0.95,1.13)
DBP 80-89mmHg	5.3	7.3	5.0	9.3	1.2	-8.1	0.95 (0.87,1.04)*
DBP >90mmHg	10.9	3.1	2.2	0.9	4.8	3.9	

*The relative risk was calculated based on 2 categories of diastolic blood pressure; ≤90mmHg and >90mmHg. V1, visit 1. V3, visit 3. δ=Visit 3–Visit 1 values. RR relative risk of hypertension at visit 3 (control vs. intervention). CI, 95% confidence interval.

4.6.4 General health

Table 4.29 shows the median (IQR) GHQ scores at visit 1 and 3. There was virtually no change in average scores over the study period and there was no difference in the change between groups.

Table 4.29 General Health Questionnaire score, Median (IQR)

Group	Visit 1	Visit 3	Visit 3 – Visit 1*
Control	2.0 (1.0,4.0)	2.0 (1.0,4.0)	0.0 (-2.0,1.0)
Intervention	2.0 (1.0,4.0)	2.0 (1.0.3.5)	0.0 (-2.0,1.0)

*T test for difference between groups, p=0.882

When self-reported health was examined in terms of 'caseness' (defined as a score of >3 on the GHQ12), almost a third of women were defined as such. There was a reduction in the proportion of women scoring >3 from approximately 30% to a quarter in both groups but no significant difference between groups (**Table 4.30**).

Table 4.30 Proportion of women defined as displaying 'caseness' at baseline and after 12 weeks of supplementation; comparison between treatment groups.

	Control			Intervention			Control
	V1	V3	δ	V1	V3	δ	RR (CI)
Caseness* (%)	31	25	-6	29	25	-4	0.99 (0.84,1.18)

*Caseness is defined as scoring >3 on the GHQ12. V1, visit 1. V3, visit 3. δ=Visit 3 – Visit 1 values. RR relative risk of hypertension at visit 3 (control vs. intervention). CI, 95% confidence interval.

5. Discussion

A summary of the results of the MMNP and Extension Study is given in section 5.1. Preliminary results from analysis of the MMNP birth outcomes data are described in section 5.2. The way in which differences between the MMNP and Extension Study participants may affect the interpretation of the results is described in section 5.3. The results of the Extension Study are then discussed in detail in the context of other research in the literature (section 5.4). Strengths and limitations of the MMNP and Extension Study and implications for future research are presented in section 5.5.

5.1 Summary of findings from the MMNP and Extension Study

The MMNP was a randomised controlled trial set up to determine the effect of micronutrient rich foods consumed before and during pregnancy on offspring birthweight. As an extension to the MMNP, we conducted a second community-based randomised controlled trial to investigate the effect of a daily food-based supplement on micronutrient status and functional health-related outcomes among non-pregnant, slum-dwelling women of reproductive age living in Mumbai. The intervention supplement contained GLV, fruit and milk and the control supplement contained foods of lower micronutrient content such as tapioca and potato.

Data from the MMNP and the Extension Study were analysed to determine whether there were any changes in diet associated with supplementation (section 4.3, p100). Retinol, folate and vitamin B12 concentrations were measured during early pregnancy among the women registered in the MMNP and the difference between the intervention and control groups was assessed (section 5.1.2). The effect of treatment group allocation on blood micronutrient concentrations and functional outcomes in the Extension Study is described in section 5.1.3.

5.1.1 Changes in dietary behaviour during the MMNP and Extension Study

It was found that intakes of fruit and vegetables changed over the study period in both the MMNP and Extension Study (section 4.3.2). The changes in intake were as expected based on availability of seasonal fruit and vegetables. In the MMNP there was no difference in the change of fruit or GLV intake between treatment groups. In the Extension Study both groups reported increased consumption of GLV at the end of the study period compared with baseline measurements. This increase was greater in the control group but did not reach statistical significance.

5.1.2 Differences in pregnancy blood micronutrient concentrations between women in the intervention and control arms of the MMNP

Plasma retinol, folate and vitamin B12 concentrations were measured in early pregnancy (between 7-16 weeks gestation) among the MMNP participants. There were no statistically significant differences between the intervention and control groups in concentrations of any of these three micronutrients (section 4.4, p106). There was a borderline significant trend for the women in the control group to have higher vitamin B12 concentrations with a difference of 16pmol/L in the median concentrations between groups. These results support the findings from the Extension Study in that there does not appear to be an effect of consumption of the supplement on circulating levels of these micronutrients. A limitation of this analysis is that we did not have pre-supplementation nutrient concentration data so it is not possible to know whether there were differences at baseline between the groups.

5.1.3 Effect of the supplement on blood micronutrient concentrations and functional outcomes in the Extension Study

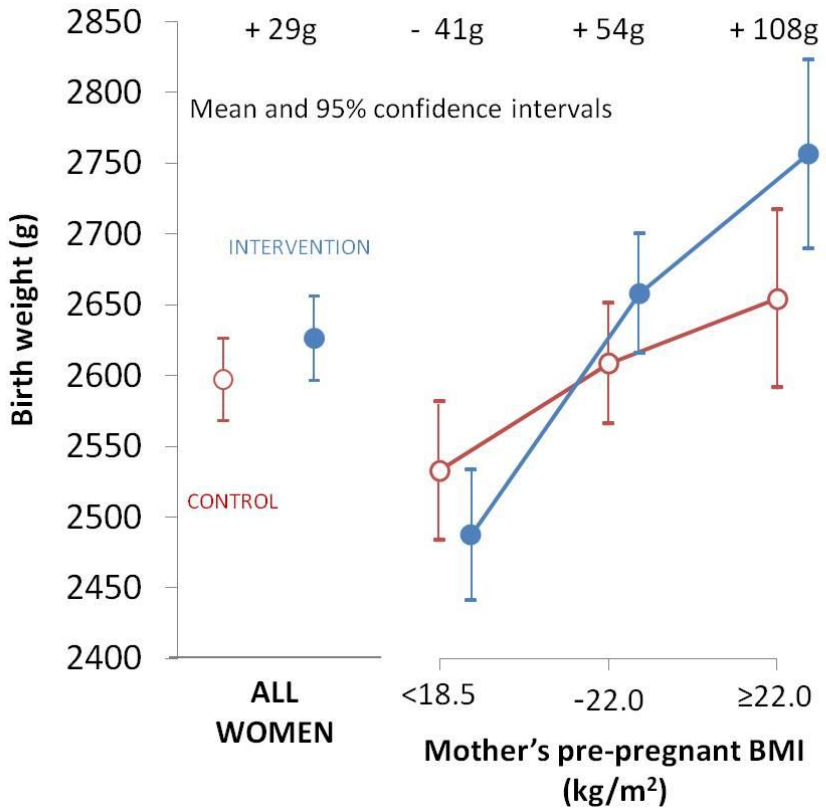
We recruited and randomised 222 women to receive the intervention or control supplement. Of these, 208 women had blood collected at visit 1 and began supplementation. Visit 3 blood data were available for 170 of these women. Compliance with the supplement was good and did not differ between groups. It was found that consumption of the intervention supplement for 12 weeks was associated with an increase in β-carotene concentrations relative to the

control group. The mean difference in the change between groups was 49nmol/L which was a difference of just over 10% of baseline β-carotene values (section 4.5.4). There was no significant effect of the treatment allocation on change in concentrations of any of the other micronutrients studied or on any of the functional outcomes assessed: grip strength, blood pressure or self-reported general health (section 4.5.7). There was a borderline statistically significant relationship between visit 1 grip strength measurements and frequency of GLV consumption.

5.2 Preliminary finding from the MMNP; birth outcomes

Intention to treat analyses of preliminary data from the MMNP indicate that randomisation to the intervention group was associated with increased offspring birthweight. Babies born to mothers in the intervention group weighed 29g more than those born to control mothers (**Figure 5.1**). This effect size is relatively small but may be important in public health terms. Furthermore, there was a significant interaction between treatment group and maternal pre-pregnancy BMI ($p<0.0001$), such that babies born to mothers with a BMI >22kg/m^2 were over 100g heavier in the intervention group compared with controls. Among the thinnest mothers (BMI<18.5kg/m^2), the intervention did not appear to confer a benefit compared with the control supplement. Babies born to intervention group mothers in this BMI category were on average 41g lighter than those born to control mothers. These findings suggest that the 'active' elements of the intervention supplement are most readily utilised and passed on to the foetus by women who are relatively well-nourished in terms of energy intake. It is possible that thinner women, who are chronically energy deficient lack the capacity to absorb, transport and store these active elements and thus are unable to pass on the nutrients required for foetal growth via the placenta. It is also possible that any active elements in the intervention supplement that were successfully absorbed and transported by the mother were utilised to meet her own requirements rather than those of the foetus. It is known that in the Extension Study compliance was slightly lower in the intervention group. Data on compliance in the MMNP are also available and an as-treated analysis will be conducted.

Figure 5.1 Effect of MMNP supplementation on birthweight



5.3 Comparison between MMNP and Extension Study participants

One of the main reasons for undertaking the Extension Study was to inform the findings of the MMNP. No effects on micronutrient concentrations were observed in the Extension Study except for β-carotene and this effect was relatively small. However, an effect on birthweight in the MMNP has been observed based on preliminary data analysis. It is therefore important to ascertain whether there were differences between the two study populations that might have affected how the nutrients and other active elements in the supplement were absorbed, transported and metabolised.

It is possible that the beneficial effects of the supplement were dependent on the woman's nutritional status prior to and/or during the study. For example, women with a BMI in the normal range may have been more able to metabolise, transport and store the micronutrients and other active elements in the intervention supplements than those who were chronically undernourished.

5.3.1.1 Age

The women in the Extension Study were on average three years younger than the women in the MMNP. Unlike the MMNP, being married was not an inclusion criterion in the Extension Study and 50% of the women were not married. This explains the higher proportion of younger women in the Extension Study. In most communities in India it is considered socially unacceptable to become pregnant when not married. In the Extension Study, 29% of women were under 18 years and 50% were under 21 years compared with 3% and 15% in the MMNP.

The younger age of the women in the Extension Study may have led to an underestimation of the effect of the supplement. If micronutrients were required for growth in the younger women, concentrations may have been low in their circulating pools. If the circulating nutrients were an indication of the storage pool then it is possible that these women's stores were not being expanded because the nutrients were being utilised for growth (199-201).

5.3.1.2 Religion

Over eighty percent of women in the Extension Study were Muslim which is a very different proportion to the MMNP and to the general Indian population of which 20% are Muslim (202). Religion dictates to a considerable extent which foods are acceptable and there are various taboos about eating certain foods in pregnancy and when to fast. In the diet patterns analysis of MMNP data, Muslims adhered to a diet pattern comprising non-vegetarian foods such as mutton and meat biriyani (section 6.1.8). Muslims are rarely vegetarian and are therefore less likely to be deficient in micronutrients that are mainly found in animal foods such as retinol and vitamin B12. It was not possible to know how the baseline retinol concentrations of the women in MMNP compared with the women in the Extension Study. However based on the pregnancy serum retinol concentrations it appears that a larger proportion of women in the MMNP were retinol deficient. It is possible that hemodilution may have explained these differences. Although, in a study among healthy French women, it was found that circulating concentrations of retinol among pregnant women were similar to those of non pregnant women (203). This indicated that hemodilution does

not affect retinol status in pregnancy. It is not clear whether these findings would be similar in LMIC settings.

5.3.1.3 Education and occupation

The women in the MMNP and their husbands had been educated for longer and a greater proportion worked in skilled occupations than those in the Extension Study. This is likely to mean that incomes in the Extension Study were lower than the MMNP and it follows that women in the Extension Study may have been less able to afford fruit, GLV, milk and other micronutrient-rich foods.

A study in Southampton, UK found that the educational attainment of women was positively correlated with their score on a prudent diet pattern (204;205) and was the most important predictor even after adjustment for activity, smoking and socio-economic factors. Occupation may affect consumption due to income, availability of time for preparing and cooking food, access to food, and autonomy in decision making when it comes to food choice.

5.3.1.4 Diet

The diets of the women registered in the two studies were different in some ways that might have affected the results. The women in the MMNP on average consumed more fruit and pulses. This may reflect the socio-economic status of the women and the timing of the dietary data collection. Women in the Extension Study were generally of lower socio-economic status and their interviews were conducted up to three years after those of the women in the MMNP. Over this time period there was a sharp increase in the cost of food, particularly fruit, vegetables and pulses which is likely to have impacted on the intakes of these women (206).

5.3.1.5 Summary

It is recognised that the differences in participant characteristics and dietary intakes must be considered when interpreting the findings of the Extension Study and in using these findings to inform MMNP. It would have been desirable for the Extension Study participant characteristics to have more

closely resembled those in the MMNP. However, it was deemed necessary to conduct the Extension Study without affecting the running of the MMNP. The requirement to recruit a sample that were as similar to the women in the MMNP as possible, had to be balanced with the practicalities of carrying out the Extension study with the time and resources available.

5.3.2 Generalisability of findings from the MMNP and Extension Study

It is important to consider whether the findings from the MMNP and Extension study can be applied to other populations. As is the case with the majority of trials, the recruitment process in the MMNP and Extension Study was subject to selection bias and it is likely that women who agreed to take part in the trials and adhered to the protocol in the long-term were different from those who did not. This may affect the external validity of the study findings. Furthermore, women who did not take part either through their own choice or due to choices made for them by others may have benefited most from the intervention. Such bias is a limitation of many trials and intervention programmes. We employed a community-based approach in this study and involved local stakeholders in the design of the MMNP intervention. This may have helped to maximise participation rates and to ensure that the women who took part were representative of the target population.

5.3.2.1 How representative of the Indian urban slum population were the study participants?

Compared with data collected in a national survey the women in both the MMNP and Extension Study were shorter, lighter and thinner and there was evidence of a greater proportion of anaemic women in the Extension Study (section 4.2.4, p99). It was not possible to compare prevalence of anaemia in the MMNP with that in the national survey or the Extension Study as the women in the MMNP only had blood collected during pregnancy. These results may indicate that the women in our study had a less favourable nutritional status than the average female slum dweller in Mumbai.

5.3.3 Changes to dietary intake during the supplementation period

Changes in intakes of fruit and GLV during the supplementation period were assessed in both the MMNP and Extension Study. In the MMNP, women were recruited on an ongoing basis over several years so were grouped according to season of first FFQ interview. Season of interview was associated with changes in intakes of fruit and vegetables but these changes did not differ by treatment group (4.3.2.1, p103). This indicates that group allocation was not a significant predictor of changes in fruit and/or GLV intake.

In the Extension Study, median fruit and GLV intake increased over the 12 week period (section 4.3.2.2, p104). This was as expected given the timing of the study and that all women were recruited at the same time of year. The study started in the winter and finished in the pre-monsoon season. The control group tended to increase their intakes of GLV to a greater extent than the intervention group over this period. In India availability and cost of fruit and vegetables varies considerably by season so it is unsurprising that intakes changed over time in both studies.

It is possible that the change in GLV intake differed between groups in the Extension Study because women in the control group observed the intervention supplements and consciously or otherwise increased their GLV intake as a result. Therefore, the effect size we have shown in relation to β-carotene concentrations may be an underestimation of the change in concentrations had the diets of the women in the intervention and control groups been the same throughout the study. An alternate explanation is that the women in the intervention group increased their GLV intakes to a lesser extent than those in the control group because they had consumed GLV in their supplement and consciously or otherwise chose to have less than they might have done had they not consumed the supplement.

A similar phenomenon was observed in the UK school fruit scheme. Children who received fruit at school were given less fruit at home. Thus an intervention designed to increase children's intake of fruit by one portion per day effected only a 0.5 portion per day increase (172). This may have been because parents assumed that their children required less fruit at home because they had eaten

some at school (207). This form of dietary 'substitution' represents an important challenge when designing food-based interventions. In the MMNP and Extension Study it was made clear to women that they should continue to eat their habitual diet and the timings of supplementation were decided on in order to avoid meal times.

The finding that there was no effect of group on change in GLV intake in the MMNP in contrast to the Extension Study could be explained by the different time frames. In the MMNP it was six months between diet assessments, while in the Extension Study it was 12 weeks. Differences between studies in the number of women per centre may have been a factor. There were more women per centre in the Extension Study and therefore the women in the control group were more likely to have regularly seen the intervention supplements. This may have influenced them to consume more GLV. Another explanation could be the change from dried to fresh GLV contained in the supplements over the course of the MMNP trial period (section 3.1.3). The MMNP FFQ data were from the early stage of the trial when supplements contained GLV powder. In the latter part of the MMNP and throughout the Extension Study they contained fresh GLV. The supplements containing powder may not have had the same visual appeal to the women in the control group as those containing fresh GLV.

5.4 Comparison of findings from the Extension Study with published research

5.4.1 Micronutrient concentrations

5.4.1.1 Vitamin A

Consumption of the intervention supplements for 12 weeks was associated with an increase in β-carotene concentrations relative to the control group. The mean difference between groups in the change in concentrations over the study period was 49nmol/L which was just over 10% of baseline β-carotene values.

Evidence suggests that in humans the proportion of ingested carotenoids that are absorbed is lower than for retinol (208). However it is thought that

carotenoid absorption is not under regulatory control in humans, therefore ingested β-carotene tends to be reflected in blood and tissue levels (209). This property means that carotenoid concentrations are a good indicator of fruit and vegetable intake regardless of baseline β-carotene status. It has also been reported that fat enhances absorption of β-carotene (210;211).

In the UK, the effect of adding 85g/d of raw watercress to the usual diet for eight weeks was to increase β-carotene levels by a mean of 100nmol/L (160). Considering the quantity of GLV in the intervention in the present study (~25g) it would appear that the mean effect size (47nmol/L) is consistent with this finding. Indeed the effect of the MMNP supplement appears to be proportionately greater than the watercress. It is possible that this is due to the fat content of the MMNP supplement. In terms of functional effects, the watercress intervention in the UK study was associated with a significant reduction in both basal and oxidative purine DNA damage.

The change in β-carotene concentration in the present study is considerably smaller than in studies where an alteration to the entire diet pattern, such as increasing daily intake of fruit and vegetables from two to ten portions per day, has been implemented. Such interventions have achieved up to a 5-fold increase in β-carotene concentrations (165;212;213). It is questionable how sustainable such changes to diets would be in the Indian slum population given the affordability of fruit and vegetables. The time and fuel required to prepare such quantities of vegetables may also be a barrier.

Given the results relating to β-carotene, the lack of effect of the intervention supplement on retinol concentrations requires explanation. After absorption into the small intestine, all-trans β-carotene is cleaved to produce two molecules of all trans retinal by the enzyme β,β-carotene 15,15′-monooxygenase 1 (BCMO1). The all trans retinol is then either oxidised to retinoic acid or reduced to retinol (210). Several factors affect the expression of BCMO1 including fat and protein intakes. However, it has been suggested that the most important factor is vitamin A status (210). A diet-responsive negative feedback mechanism has been identified whereby in the case of vitamin A deficiency, activity of intestinal transcription factor (ISX) is suppressed, this leads to increased activity of scavenger receptor class B type 1

(SR-B1) and BCMO1. SR-B1 increases intestinal absorption of carotenoids and other fat soluble molecules and BCMO1 cleaves the β-carotene to produce retinal. As retinol and retinoic acid concentrations increase and vitamin A sufficiency is achieved, ISX is activated and expression of BCMO1 is reduced (214). It is thought that this negative feedback mechanism exists to prevent toxic levels of vitamin A building up within the tissues.

The majority of the women in the Extension Study were not retinol deficient which may explain to some extent why there was a lack of effect of the supplement on retinol concentrations at the group level. There tended to be an increase in concentrations among those women with the lowest concentrations at baseline which is consistent with the evidence from animal and cell line studies (210). A recent study in Bangladesh assessed the effect of consumption of bio-fortified orange flesh sweet potato on vitamin A pool size among vitamin A deficient women living in a low income area of Dhaka city (215). Women were randomised to one of four intervention groups: 'low carotenoid white flesh potato', 'boiled orange flesh sweet potato', 'Vitamin A as retinyl palmitate capsule' or 'fried orange flesh sweet potato'. The white flesh potato contained 0μg retinol activity equivalent (RAE) while the other three interventions contained 600μg RAE per serving and were distributed to the women six days per week for 10 weeks. The orange fleshed potato increased circulating β-carotene concentrations but there was no difference between groups in terms of change in vitamin A stores. This finding indicated that the conversion of β-carotene to vitamin A was limited in this population. The authors concluded that protein deficiencies among these women may have led to reduced synthesis of BCMO1 which in turn would reduce the conversion of β-carotene to retinol. Furthermore, the unhygienic conditions in which they lived may have caused subclinical enteropathy which can lead to chronic intestinal inflammation. The women in the Extension Study lived in relatively unhygienic conditions often without a separate room as a kitchen; this may partly explain why there was no effect on retinol status in the Extension Study.

In contrast to these findings, a previous study by the same authors found that among Bangladeshi men, vitamin A pool size was increased following an intervention of spinach containing 375μg RAE per serving (216). The authors pointed out that these men were given a controlled diet throughout the study

period sufficient in macronutrients and micronutrients (apart from retinol) and that all food was prepared in hygienic conditions. Another suggestion related to genotype. There are two polymorphisms of the BCMO1 gene associated with reduced expression of BCMO1; the women in the sweet potato study were descendents of Bihar immigrants who migrated after partition of India in 1947. The men were indigenous Bengalis so it is possible that the differences in ethnicity are associated with differences in BCMO1 expression. Many of the women in the MMNP and Extension Study are migrants from different parts of India, yet it is possible that they would share the polymorphism of the BCMO1 gene with the Bihari women.

A study in a small group of healthy young females in Pune, West India compared the short term effects of consumption of a GLV meal with a standard meal containing no GLV (164). No differences in plasma β-carotene or vitamin C concentrations between the two groups were observed 4 hours after the meal. However, after a 3 week intervention period constituting daily supplementation with 100g cooked GLV and 10g oil, there was a significant increase in plasma concentrations of both nutrients. The authors concluded that intake of 100g GLV per day plus 10g oil could be an effective strategy for improving micronutrient status in young Indian women.

The intervention supplements in the present study contained approximately 25g GLV per serving; it is possible that there is a 'threshold intake' above which a change in nutrient concentration would be observed. For example consumption of four snacks (100g GLV) per day may have led to detectable increases in micronutrient concentrations.

The finding that the intervention snacks had an effect on β-carotene concentrations may be of particular importance in LMICs. Based on intake data in industrialised countries, it has been suggested that an adequate vitamin A intake cannot be achieved by consuming either retinol or β-carotene alone even where animal products are widely available (91). Despite the rapid increase in animal food consumption in many parts of the world, most people in LMICs still have low intakes of retinol-rich foods (217). Pro-vitamin A carotenoids are therefore nutritionally important; it has been estimated that in developing countries approximately 80% of vitamin A is from carotenoids (91).

In addition those who are vegetarian are likely to have low retinol intakes particularly if they avoid or cannot afford dairy foods. A food-based supplement that increases β-carotene concentrations may therefore be a useful intervention in these groups.

5.4.1.2 Folate

The trend for folate was for the median concentration in the control group to decrease while in the intervention group it remained stable; however the difference between treatment groups in the change over time was not significant. Over 80% of the women had blood folate concentrations above the cut off for deficiency at baseline, (**Figure 4.10**, p116) therefore it is likely that the majority of the women in this population were not folate deficient. This could be due to government iron-folic acid supplementation programmes. According to the dietary data collected in the study at baseline, folate-rich foods such as pulses, GLV and nuts were consumed less than once per day by the majority of women. It is unlikely that the intervention period was too brief for a change in folate status to be seen as serum measurements are a good indication of recent intakes. Effects on both serum and red cell folate concentrations have been seen in as little as four weeks as a result of increasing the proportion of folate rich foods in the diet (218) so it is unlikely that the time period of the study is an explanation for the lack of effect.

5.4.1.3 Vitamin B12

Median plasma vitamin B12 concentrations decreased for all women over the study period but to a greater extent in the intervention group than in the control group (**Figure 4.14**, p120). A very small proportion of the women were vitamin B12 deficient which may explain the lack of an increase in concentrations. Intakes of animal source foods in this population are likely to be sufficient to prevent deficiency.

5.4.1.4 Vitamin C

In the case of vitamin C, the content in the snacks was low which was probably due to the cooking process. There was some experimentation with different methods of cooking and preparing the snacks so as to preserve vitamin C

content but the most acceptable method to the women was shallow frying and it was important to achieve adherence to the supplementation protocol. It is unusual in India to consume vegetables that are not well cooked and, along with low fruit intake, this may explain the large proportion of women in the Extension Study who were vitamin C deficient at visit 1 (approximately one third, **Figure 4.15**, p121). Vitamin C concentrations increased in both groups over the course of the study which is likely to be due to increased availability and reduced cost of seasonal fruit towards the end of the study. Interventions comprising large quantities of vegetables (~300g/d) achieved a significant increase in plasma vitamin C concentrations even when the vegetables were cooked (166;167) however these were large amounts and may not be achievable currently in the Extension Study setting largely due to cost but there may also be acceptability issues for the women.

5.4.1.5 Iron

Serum ferritin was below the deficiency cut off among approximately 80% of the women at baseline (**Figure 4.17**, p123). The snacks contained on average 6.2mg of iron per serving. However, it is possible that inhibition of iron uptake due to frequent consumption of phytate-containing cereal based foods or tea containing polyphenols, and polyphenols in the GLV in the snacks themselves, meant there was insufficient bio-available iron in the snacks to improve iron status. Vitamin C can counteract the effects of these compounds therefore it is possible that vitamin C deficiency and low intakes of fruit among the women in the Extension Study in part explained these findings. There was an increase in median haemoglobin concentration in both groups and this was statistically significant in the control group but there was no difference between groups in terms of the change over the study period.

5.4.2 Functional outcomes

5.4.2.1 Grip strength

There have been few studies assessing the association between grip strength and dietary intakes. Some have looked at the relationship with diet patterns or individual foods, whilst others have assessed the effect of a nutritional intervention on changes in grip strength. The majority of such studies have

been conducted in older people living in Western countries. Sarcopenia is associated with ageing and morbidity in older life (219) and therefore dietary interventions aimed at decreasing the rate of muscle fibre loss are of great interest. At the time of the Extension Study, most of the women would have been undergoing muscle gain. It is thought that the higher the peak muscle mass attained, the lower the effect of sarcopenia on muscle strength in later life.

To our knowledge the Extension Study is the first study to assess the relationship between diet and grip strength in young Asian women. No effect of consuming the intervention supplement was observed but there was a positive association between reported consumption of GLV at visit 1 and grip strength. This result is supported by a prospective observational study in adults aged over 65 years in Italy which found a positive association between baseline plasma carotenoid levels and grip strength at a 6 year follow up (220). The authors concluded that adults with low fruit and vegetable intakes as indicated by low carotenoid status were at higher risk of skeletal muscle strength decline. Cross-sectional diet and grip strength data were collected from adults aged 59-73 years enrolled in the Hertfordshire Cohort Study. These data showed that men and women who adhered to a prudent diet pattern, characterised by high consumption of fruit, vegetables, whole grains and fatty fish had higher grip strength measurements (221). When associations with individual foods were studied, intakes of vegetables and fatty fish were found to independently predict grip strength among women. Two mechanisms were proposed for this association. The first was that increased levels of antioxidants in fruit and vegetables attenuate muscle damage. In a recent review of muscle aging and the oxidative system pathway, it was suggested that antioxidants such as vitamin C and carotenoids protect against muscle damage by reducing the accumulation of reactive oxygen species and down-regulating the production of pro-inflammatory cytokines (222). The second proposed mechanism is related to long chain n-3 polyunsaturated fatty acids found in oily fish. There is evidence that these fatty acids may protect against muscle wasting by reducing the production of cytokines such as tumour necrosis factor-α and interleukin-1 (223).

A systematic review of dietary advice and nutritional supplements on change in grip strength among adults with disease-related malnutrition found that both advice alone and advice given with multiple micronutrient supplements was associated with improved grip strength (224). To date no studies assessing the effect of food-based interventions on grip strength are known of. It is conceivable that a longer term change in dietary intake may be required to observe changes in grip strength measurements.

5.4.2.2 Blood pressure

The Extension Study showed no effect of the intervention on change in systolic or diastolic blood pressure over the course of the study. The majority of studies that have assessed the effect of dietary interventions on blood pressure have been conducted in populations that are at risk of cardiovascular disease and/or hypertensive. For example, a study in the USA randomised almost 50,000 women aged 50-79, with a reported 32% of energy intake from fat, to either a 'dietary change' group which involved increasing fruit and vegetable intake to five portions per day, or a control group (225). After three years of follow up, the study achieved an average increase of 1.1 portions of fruit and vegetables per day. There was a small but statistically significant difference in the reduction in diastolic pressure between the two groups. The mean reduction was 2.6 (9.4) mm Hg in the intervention group and 2.3 (9.4) mm Hg in the control group. There was no effect on systolic blood pressure.

A cross-sectional study in Bengali women aged 35 years and over, 48% of whom were hypertensive, assessed the association between adherence to one of three diet patterns and blood pressure (226). The three patterns identified were 'vegetables, fruit and pulses', 'hydrogenated and saturated fat' and 'red meat and high-fat dairy'. None of the three diet patterns identified were associated with blood pressure. There was a significant association between adherence to the vegetables, fruit and pulses pattern and being diabetic. Because this study was not longitudinal, it is not possible to know whether the women changed their diets as a result of being diagnosed with diabetes. Therefore causality cannot be inferred.

Very few of the women in the Extension Study were hypertensive which may explain the lack of effect on blood pressure. It is possible that a longer term intervention would be required to study such an effect and that an effect might not be seen until later in life when there is a greater risk of developing hypertension.

The Dietary Approaches to Stop Hypertension (DASH) diet has been shown to be effective in reducing blood pressure. The early studies of this diet on blood pressure outcomes did not look for possible mechanisms by which the foods in the DASH diet might reduce blood pressure. A recent controlled feeding study among hypertensive men and women investigated some of the possible mechanisms for this effect (227). The results of this small study indicated that the DASH diet led to increased nitric oxide bioavailability which in turn reduced blood pressure, it was also suggested that antioxidants in the DASH diet reduced oxidative stress which in turn prevented or attenuated endothelial dysfunction.

5.4.2.3 Self-reported psychological health and well-being

Self-reported psychological health, as assessed by the GHQ12 improved over the course of the study in both groups but there was no effect of the intervention supplement. It is likely that the daily contact the women were having with other study participants and the research team led to improved psychological health and well-being.

A cross-sectional study among older adults in Australia found that fruit and vegetable consumption was positively associated with self reported health such that for each daily portion consumed there was a 10% increase in the odds of reporting health as 'good' (228).

Intakes of B vitamins have been associated with psychological health and well-being in cross-sectional studies. One proposed mechanism for the relationship between fruit and vegetables and self reported health was increased folate stores. A longitudinal study among young women in Southampton, UK assessed the association between red cell folate concentrations and wellbeing

using the GHQ12 at baseline and examination of participants' medical records two years later (229). The data showed that while GHQ12 score was associated with folate concentrations, low folate levels were not associated with symptoms at 2 year follow up. It was concluded that there was no causal effect of folate status on psychological well being among young women. Another longitudinal study prospectively measured dietary intakes of thiamine, riboflavin, niacin, folate, vitamin B6 and vitamin B12 during childhood and adulthood and assessed psychological distress at 53 years (230). There were no significant associations between intakes of any of the B vitamins in childhood and later distress.

5.5 Strengths and limitations of the Extension Study

Prior to the present study, there was a paucity of data on the effect of long term food-based interventions on the nutritional status of women in low-income settings. We used a randomised controlled design to test our hypothesis and stratified by age and BMI to ensure that we had a similar distribution of these variables in either of the treatment groups. This is widely considered to be a robust method as the randomisation reduces potential for confounding; therefore any effect seen can reasonably be attributed to the intervention. However, as with many food-based intervention studies it was not possible to blind the intervention. It is unlikely that this would influence the findings given that the outcomes were objectively assessed and the group allocations were not known to the laboratory staff who made the nutrient concentration measurements. A strength of the study is that all of the participants started and completed the intervention at the same time, thus creating an internal control for seasonal changes in food intakes.

The sample size calculation was based on serum retinol concentrations as this was the nutrient with available data in the Indian population. Using previously published data, we over-estimated the proportion of women who would be retinol deficient and the study may have been underpowered for other nutrients for which no status data in this population were available. Post-hoc power calculations indicated that the study was under-powered for certain nutrients based on the effect sizes observed (section 4.5.7, p137).

The generalisability of the study may be a limitation. We selected a mainly Muslim population which may not have been representative of the general population in Mumbai or Indian slums. There is recently published evidence to indicate that there are genetic polymorphisms that affect mechanisms of nutrient absorption and metabolism (section 5.4.1.1, p153). Therefore it may be important to have some knowledge of the ethnic background of a population when conducting nutritional intervention studies.

A considerable challenge in conducting the Extension Study was finding labs that were able to measure plasma micronutrient concentrations. We were unable to find a lab in India that could measure vitamin C and β-carotene concentrations and so samples for these analyses were transported to the UK. The quality control and calibration methods used at the KEM Hospital and HNR Cambridge were well documented and fit for purpose. We had some concerns about the methods adopted at the Nair hospital where serum retinol and ferritin were measured using kits. We had these kit methods reviewed by Stephen Young at HNR Cambridge who commented that while the methods were somewhat out-dated, they were methodologically sound.

We used published nutrient concentration cut off values to determine the proportion of women who were deficient (92). It should be noted that these cut off values are based on data from high income countries and may not be the most appropriate indicators of deficiency among Indian women.

The food frequency questionnaire used in this study has not been previously validated against another dietary assessment method. The FFQ was developed specifically for the MMNP by local nutritionists who conducted several focus group discussions with women living in Mumbai slums. It was extensive and allowed recording of 'other' foods that were not mentioned so it is unlikely that intakes were underestimated due to women consuming foods that were not on the FFQ.

5.5.1 Implications for future research

The Extension Study was a relatively small scale project with the aim of informing the findings of the MMNP. If I were to design future efficacy or effectiveness studies of food-based interventions and had sufficient resource and funding, I would carefully consider the following points:

1) Sample size; the power calculations indicated that for some of the nutrients the sample size may have been insufficient to detect an effect. The sample size calculation carried out *a priori* was based on data that may not have been applicable to the Mumbai slum setting. A larger sample size would provide more power to detect effects and would allow inclusion of more treatment arms.

2) Multiple treatment arms could be included to assess dose response relationships between supplement intake and micronutrient status.

3) Proportion of the population deficient; before conducting a large scale randomised trial of an intervention it would be important to ascertain the proportion of the target group whose nutritional status was sub-optimal. This could be done using published data but often such data are not available particularly among at-risk-groups in LMICs. Therefore it would be prudent to conduct a surveillance study prior to supplementation. This would ideally involve measuring nutritional status biomarkers such as circulating nutrients and BMI. If this were not possible data on dietary intake could be used to infer nutritional status.

4) Comparisons between settings; it would be of interest to determine whether the findings of the MMNP and Extension Study would be replicated in alternative settings such as in rural areas.

6. Diet patterns analysis

This chapter presents the diet patterns of women registered in the MMNP. The patterns were derived using PCA of the baseline FFQ data collected in the trial. A rationale for the study of diet patterns and a description of PCA and other methods of assessing diet patterns is given in section 6.1. The methods used in the present analysis are described in section 6.2. The diet patterns derived are presented in section 6.3 along with data showing the associations between the patterns, and socio-demographic factors and anthropometric measurements. The findings are discussed in section 6.4.

6.1 Study of diet patterns

Diet patterns have been defined as "foods as they are actually consumed in various characteristic combinations" (231). The traditional epidemiological approach to studying diet and health outcomes has been to investigate associations between single nutrients and/or foods and the outcome of interest (138;232). In reality, nutrients and foods are not normally consumed in isolation so determining which foods are commonly eaten together as part of a diet pattern is likely to be more representative of typical eating behaviour. Knowledge of diet patterns and their association with health outcomes can be used to devise or inform public health recommendations and the design of interventions to improve dietary intake.

In terms of understanding how diet and health are related, a diet patterns approach has advantages over the more traditional single nutrient or food approach. It can assess the effect of combinations of nutrients that are synergistic, e.g. vitamin C and iron. There is also likely to be co-linearity between nutrient intakes which may make it difficult to attribute health effects to any particular nutrient. Furthermore, if a large number of single food items or nutrients are studied in relation to an outcome, it is more likely that a statistically significant result will be found due to chance.

Diet patterns cannot be measured directly, therefore statistical or theoretical methods are used to identify patterns from dietary data collected from the population of interest. There are three main approaches to studying diet

patterns: 1) Factor analysis and PCA; 2) Cluster analysis; 3) Dietary indices. Factor analysis, PCA and cluster analysis are all multivariate, data-driven techniques. This means that the patterns are not defined *a priori;* rather they are derived empirically using calculations. This data-driven approach is not limited by current consensus on what constitutes a 'healthy' diet. Dietary indices are based on model diet patterns, usually national dietary guidelines or patterns of eating thought to be associated with health outcomes. Each of the three methods is described in detail in sections 6.1.1– 6.1.7.

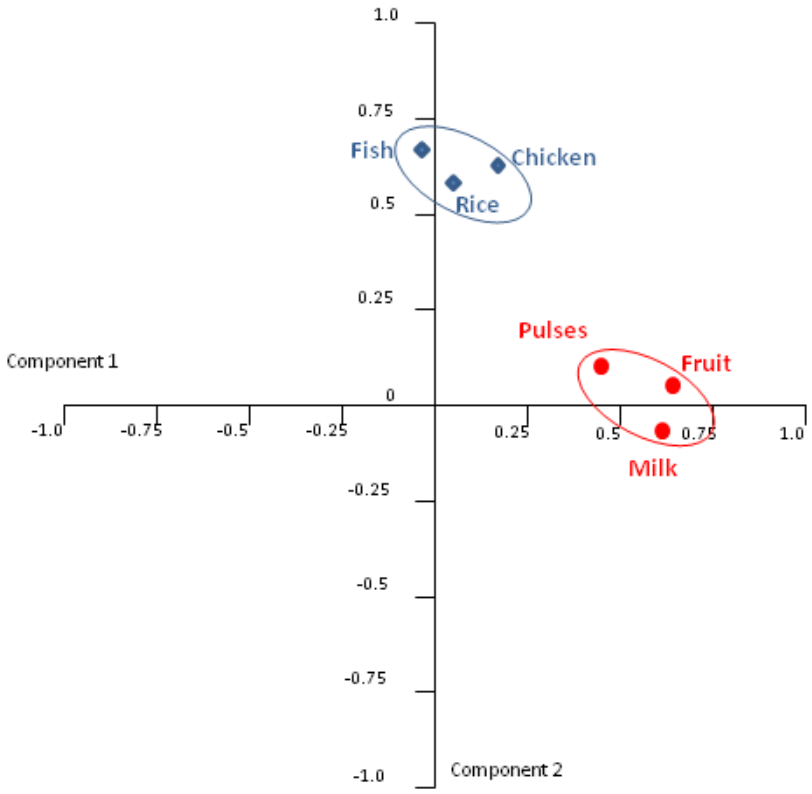
6.1.1 Factor analysis and principal component analysis

Within the diet patterns literature, the terms factor analysis and PCA are often used interchangeably and PCA is frequently described as a type of factor analysis. Statistical sources state that factor analysis and PCA are in fact distinct methods (233;234). They are similar in that they reduce a large number of input variables to a smaller number of independent variables that explain maximal variability within the study sample. However, the calculations used in either approach are different. The output of factor analysis is a mathematical model that estimates factors based on certain assumptions whereas PCA looks for linear components within the data and assesses the extent to which each variable contributes to each component (233).

It has been stated that PCA is conceptually simpler than factor analysis (234) and therefore has been more widely used in diet patterns analysis (138). It is often used as an exploratory technique for generating hypotheses. The technique derives a small number of linear 'components' from a larger number of input variables. These components are independent of each other and explain the maximum variation in the data. The input variables are usually 'intake frequency of foods' or 'weight of foods' consumed by the study population.

Figure 6.1 depicts a simplified version of two principal components plotted on the x and y axes. In most analyses there would be more than 6 food groups but for ease of explanation this example is used. There would also usually be more components. The third component would be plotted in the third dimension and so on.

Figure 6.1 Graphical representation of two components derived using a PCA of dietary data



If interpretable, components can be thought of as representing dietary patterns. Foods with coefficients of large magnitude on a given component are characteristic of the pattern represented by the component. In the case of the components presented in **Figure 6.1**, frequent consumption of fruit, pulses and milk would confer a high score on component 1. Similarly frequent consumption of chicken, fish and rice would be associated with a high score on component 2. The component scores are themselves continuous variables. Thus each individual case in a data set will have a score for each component. The higher the score the greater the adherence to that dietary pattern, the more negative the score the lower the adherence.

The component scores are calculated by multiplying the input variable (e.g. frequency of intake) by the component coefficient for that food. For example in figure 6.1 for Component 1, 'fruit' has a coefficient of 0.64, 'pulses' has a coefficient of 0.45 and 'milk' has a coefficient of 0.60 so a woman who

reported having fruit six times per week, pulses 5 times per week and milk 3 times per week would have a component 'score' calculated as follows:

(6x0.64)+ (5x0.45) + (3x0.60)

= 3.84 + 2.25 + 1.80

= 7.89

Component scores are usually standardised to have a mean of 0 and a standard deviation of 1.

There are several subjective decisions that must be made when conducting a PCA, these are listed below.
1) How the dietary data are collected; FFQ are likely to give a good representation of longer term diet patterns while 24 hour recalls may give a more accurate representation of foods eaten over a narrower time period. It is important to consider the impact of season and festivals or holidays on dietary behaviour when selecting a dietary data collection tool.

2) Whether and how the food data are collapsed into groups; a smaller number of input variables leads to a greater proportion of variance being explained by retained components (138). A study by McCann et al assessed the effect of the number of food groups included in a PCA on the patterns obtained and associations between patterns and risk of endometrial cancer. It was concluded that there is little difference in the patterns obtained using different numbers of food groups in the analysis but that a larger number of food groups may give a more precise estimate of disease risk (235).

3) Which input variables to use; possible input variables suggested by Schwerin (231) were frequency of intake, weight of intake or percent energy contribution to the overall diet. The majority of published studies have used frequency as the input variable. Frequency has been shown to discriminate between individuals to a greater extent than portion size or number of portions consumed (236).

4) Whether to use the mathematical techniques of orthogonal or oblique rotation in order to improve the interpretability of the patterns. Rotation of the components changes the axes upon which the coefficients are read and tends to polarise the coefficients so that they are closer to ±1 or 0. A review by Newby et al (138) found that most studies used orthogonal rotation, but in several studies there was no report of whether, or how components were rotated. There is little description of the utility of rotation and how rotation affects component solutions in the dietary patterns literature.

5) The number of output variables to retain; this is generally based on the eigenvalues being above a pre-defined value (usually >1), the break in the Scree plot and the interpretability of the component (237).

6) Naming patterns; patterns are often qualitatively named, for example 'healthy', 'traditional' etc. While these terms may be meaningful to the researchers they do not necessarily provide information about the foods that are characteristic of these patterns. Newby et al recommend naming patterns qualitatively based on the foods or nutrients that are associated with the pattern (138). However translation to different populations is dependent on availability of those foods in other settings.

Patterns derived using factor analyses were robust after varying the number of factors extracted, treatment of input variables and energy adjustment (238). It should be noted that the components derived in PCA do not necessarily define a complete eating pattern. A person with a high score on one component may have high or low scores on other components. Therefore a person's overall diet pattern is represented by their scores across all components rather than one single component (138).

6.1.2 Cluster analysis

Cluster analysis is also a data driven method that groups people by intake into mutually exclusive categories or clusters. Cluster analysis therefore examines whether there are distinct groups in the population with different diets. The intakes of individuals within the clusters are then examined and the clusters

are usually named according to the foods that are consumed most frequently or that contribute the highest percentage of total energy to the diet. As with factor analysis there are a number of subjective decisions which must be made by investigators when running cluster analysis. These are the same as for PCA with the exception of the decision as to whether to rotate the components. With cluster analysis, it is also necessary to define the number of clusters prior to analysis. In practice the analysis is usually run several times with different numbers of clusters and the solutions are examined for the best fit, yet there is no 'gold standard' for determining how many clusters to consider (239).

6.1.3 Comparison between PCA and cluster analysis

It has been suggested that the PCA procedure requires fewer subjective decisions and is more straightforward than cluster analysis (240). Another distinction between PCA and cluster analysis is that the former derives continuous scores which can be used as linear variables in analyses whereas the output variables in cluster analysis are categorical and therefore information on the extent of adherence to a particular pattern is lost. An advantage of cluster analysis is that the patterns derived are mutually exclusive and therefore individuals are grouped according to diet pattern. This provides information on prevalence of diet patterns whereas with PCA each individual is assigned scores for several different patterns so the 'prevalence' of a particular pattern cannot be observed (240).

Some studies using both the PCA and cluster analysis approaches in the same dataset have found very few differences in the derived patterns (204;241;242). Newby et al reported that there was no significant difference in the ability to predict blood lipid concentrations using either method (138). Crozier et al concluded that PCA was a more useful approach due to the derivation of a continuous pattern score for each individual. They also found that PCA was less sensitive to outliers in the data and to different combinations of foods as input variables (204).

6.1.4 Dietary indices

Dietary indices are theory-driven approaches to studying diet patterns and are often based on guidelines developed by national governments or international agencies such as WHO. Quantitative nutritional variables are summed to provide a measure of diet quality, variety or a combination of the two. Reviews by Waijers (243) and Arvaniti (244) of diet quality scores each found at least 20 distinct indices based on 4 original indices that were developed in the mid 1990s: Healthy Eating Index (HEI) and Diet Quality Index both of which were based on US dietary guidelines (245); Healthy Diet Indicator based on WHO guidelines (246); Mediterranean Diet Score (247). There is little difference between the US and WHO diet guidelines as they are largely based on the same body of evidence. As with empirical approaches, there are several subjective choices that investigators must make when developing indices:

1) Selection of input food or nutrient variables. The choice of foods to include in dietary indices are largely based on current knowledge, for example fish has tended to be included in more recent indices and grains have been split into refined and wholegrain in later indices. There are difficulties with handling items that may be beneficial in small quantities yet harmful in larger amounts e.g. red meat and full fat dairy products. Use of a nutrient-based score is reliant on availability of accurate food composition data.

2) Cut off values and scoring systems. Originally the approach taken was to assign a binary variable to each individual according to whether their intake met the guidelines or whether it was above or below the median value for the study sample. Other preferable yet more complex approaches are to assign scores for each food that are proportional to the percentage of the guideline met, this approach allows for U-shaped relationships between food intakes and health outcomes such as for red meat and dairy foods.

3) Possibly the most contentious decision is how to weight the scoring of input variables based on the relative harm or benefits attributable to the foods or nutrients. According to the Waijers review (243), this is often not well described by authors. The anomaly is that in order to assign weightings to food groups or nutrients it is necessary to know the effect of these individual

exposures on health outcomes. This tends to negate the 'raison d'être' of the diet pattern approach which is to understand the synergistic effects of several foods.

In order to maximise the usefulness of the diet index approach, it has been suggested that findings from data driven approaches can be used to feedback into the design of diet indices (138).

6.1.5 Food-based dietary guidelines in India

In a country as large and diverse as India it is challenging to produce one set of food based guidelines that will be applicable to and can be implemented by all. The dual burden of under-nutrition and chronic disease linked to overweight and obesity means that it is very important to send out a clear message that will serve the health requirements of the population. **Figure 6.2** shows the Indian food pyramid which is a representation of the food-based dietary guidelines published by the Indian National Institute for Nutrition (126). The aim is to prevent chronic energy malnutrition but also to tackle the growing problem of overweight and obesity in India (150).

The guidelines recommend adequate consumption of grains and pulses, plentiful consumption of fruit and vegetables, moderate consumption of oils, fats, salt and beverages, and low intakes of processed foods. The guidelines also recommend being physically active, (yet this is not quantified) and avoiding alcohol and smoking.

6.1.6 Evidence linking diet patterns to health outcomes

6.1.6.1 Empirical approach

The diet patterns approach was first suggested in 1969 at a White house conference on food, nutrition and health (248). One of the first analyses of dietary patterns by Schwerin and colleagues involved a PCA of data collected as part of state-level nutrition and health surveys in the USA in the late 1960s and early 1970s (231). The aim of the study was to examine associations between diet patterns and indicators of micronutrient deficiencies in the USA.

The authors found associations between the patterns and deficiencies. This included high dairy and low sugary food intakes being associated with low prevalence of deficiencies.

Figure 6.2 Indian Dietary Guidelines; Food Pyramid

The review by Newby et al identified studies on diet patterns derived using empirical methods between 1980 and 2004 (138). They found nearly 100 papers reporting such studies, two thirds of which assessed associations between diet and health outcomes. The health outcome focus of the studies had changed since Schwerin's preliminary work in this field with the majority of studies investigating the association between diet and chronic disease including CVD, T2D and cancer. In addition many studies looked at biomarkers

or risk factors for these chronic diseases. Adherence to 'healthy' or 'prudent' diets characterised by high intakes of fruit, vegetables, legumes, fish and low fat dairy foods combined with infrequent intakes of high sugar, high-fat, high salt and snack foods tended to be negatively associated with both prevalence of disease and risk factors (249-252). Unhealthy or 'Western' patterns characterised by high intakes of snacks, take away foods, sugar sweetened beverages and low fruit and vegetable intake were associated with increased risk of CVD and T2D (250;253;254).

6.1.6.2 Theoretical approach

Prospective observational cohort studies have shown that reported adherence to dietary guidelines is negatively associated with chronic disease incidence and mortality (255). A study by Willett and colleagues demonstrated the importance of how foods were represented in dietary guidelines (256). When they calculated a diet score using the HEI, based on the 1995 Dietary Guidelines for Americans (245), there was only a weak correlation between diet scores and risk of CVD and no correlation with cancer risk (255). It was noted that the HEI did not differentiate between meat and fish, potatoes were included as vegetables and type of fat was not taken into account. An Alternate Healthy Index which contained a 'fish and poultry:red meat' ratio; excluded potatoes from the 'vegetable' category; and included the ratio of polyunsaturated to saturated fats was proposed (257). Revised USA Dietary Guidelines were issued in 2005 (258) which more closely represented the patterns characterised by the Alternate Healthy Index. This index has been negatively associated with chronic disease risk factors in prospective studies (259;260). In a review of 39 studies, it was acknowledged that these modifications were helpful and that most indices were related to CVD risk and mortality, however the effect sizes were modest (243). This may be due to the arbitrary choices required in developing indices and the gaps in knowledge as to what constitutes a healthful diet.

6.1.7 Interventions aimed at altering diet patterns

Evidence suggests that interventions aimed at altering diet patterns may be more effective in terms of reducing risk of chronic disease than single nutrient

interventions (138). For example, a study of the DASH diet found that adherence to a low fat, high fruit and vegetable diet was associated with a reduction in blood pressure (261) and the DASH diet was more effective at lowering blood pressure than magnesium or potassium tablets (262). A potential disadvantage of diet pattern interventions is that they tend to require significant changes to behaviour which may be difficult to adhere to. Compared with a single nutrient intervention which could be given in tablet form, diet pattern approaches may be more challenging to implement. They require access to particular foods, skills and facilities for preparation and cooking as well as the motivation to adhere to the diet. Consideration should be given to these factors when designing interventions.

6.1.8 Socio-demographic correlates of diet pattern scores

As mentioned previously, many empirical studies in Europe and the USA have identified two diet patterns; 1)'Western' characterised by frequent consumption of processed foods including refined grains, processed meats, energy dense snacks such as crisps and biscuits, and infrequent consumption of fruit and vegetables; 2)'Prudent' characterised by frequent intake of whole grains, fruit, vegetables, fish and low-fat dairy produce (138). As well as these patterns being related to risk of chronic disease, it has also been demonstrated that they are associated with socio-demographic variables. Several studies have shown that sex is a predictor of diet with females being more likely to adhere to the prudent pattern (241;251;263;264). Age has been found to be positively associated with some healthy patterns (241;265;266) and negatively associated with others (263;267). The relationship is likely to be population specific. Socio-economic status and educational attainment are often positively associated with prudent pattern scores (205;268-270).

6.1.9 Study of diet patterns in India and South Asia

The vast majority of studies identified in reviews assessing associations between diet patterns and health outcomes have been conducted in the USA and Europe. Of 50 empirical studies reviewed by Newby et al (138), one data driven study was conducted in India, 19 in USA/Canada, 22 in Europe, 5 in Japan and 3 in other parts of the world. In a review of theory driven patterns

research (243), one of the diet indices was based on WHO dietary guidelines, the remainder used US guidelines or the Mediterranean diet as a reference. Of the 39 studies assessing the relationship between diet indices and health outcomes, 38 were conducted in the USA or Europe and one in China. A more recent review of the associations between diet patterns during pregnancy and maternal and infant health outcomes identified 7 studies; all were based in the USA or Europe (271). Despite the fact that diet patterns are likely to be population-specific, there is very little data from LMICs. However, there have been some recent data driven analysis studies carried out in India and South Asia as described below.

A study in urban-dwelling Pakistani men (n=355) and women (n=517) of low socio-economic status identified three diet patterns (268). Firstly, the 'prudent' pattern was characterised by high intakes of eggs, fish, raw vegetables, fruit juice, bananas and 'other' fruit. This pattern was negatively associated with plasma homocysteine concentrations and was positively associated with educational attainment. The second pattern was a 'high animal protein' pattern with high coefficients for meat, chicken and tea with milk and was positively associated with plasma homocysteine concentrations and waist hip ratio. Adherence to this pattern was positively correlated with education and income. The third pattern was a 'high plant protein' pattern which was characterised by high intakes of cooked vegetables and legumes and low intakes of meat. The high plant protein pattern was negatively associated with homocysteine concentrations.

A cross-sectional study in Bengali women living in the city of Kolkata aged 35 years and above (n=701) investigated associations between cardiovascular risk factors and diet patterns (226). Three patterns were identified. The 'vegetable, fruit and pulses' pattern was characterised by frequent intakes of GLV, sweets, fruit, pulses, nuts, poultry and eggs. Scores on this pattern were negatively associated with serum total cholesterol and non-high density lipoprotein (HDL) cholesterol concentrations and adherence was associated with younger age, higher educational attainment and greater income. The second pattern was named the 'hydrogenated and saturated fat and vegetable oil' pattern and was characterised by frequent intakes of butter, hydrogenated oil, ghee, vegetable oils, sweets, fish, high-fat dairy foods and refined cereals. This pattern was

positively associated with BMI, waist circumference and HDL cholesterol concentrations. It was also associated with younger age, higher educational attainment and greater income. The third pattern was named the 'red meat and high-fat dairy' pattern and was characterised by frequent intakes of red meat, high-fat dairy foods, whole grain cereals, high energy drinks and low intakes of fish, refined cereals and low fat dairy foods. Adherence to this pattern was not associated with any of the CVD risk factors measured and it was negatively associated with educational attainment and income.

A study of the diets of teenage girls in Pune (272) identified five patterns based on the most discriminating food group for each pattern: rice meal; snacks; wheat meal; pearl millet meal; sorghum meal. Girls with higher scores on the 'rice meal' and 'snack' patterns had lower micronutrient intakes and those with higher scores on the 'wheat meal' and 'sorghum meal' patterns had higher micronutrient intakes. Relationships between pattern scores and other health outcomes or demographic factors were not explored in this study.

Venkaiah et al (273) performed a factor analysis with diet data from 2864 men and 3525 women living in a rural area of the state of Orissa in the North East of India. Over half (55%) of the women were chronically under-nourished ($BMI<18.5kg/m^2$). They found similar patterns for men and women. The first pattern was characterised by high intake of milk and sugar which were described as 'income elastic' foods, the second by plant foods, the third by pulses, rice and legumes and labelled 'traditional', the fourth by micronutrient-rich foods, GLV and fruit, the fifth by fish and seafood and the sixth by nuts, seeds and meat. The intakes of the majority of the foods that characterised these patterns were very low, for example three quarters of women consumed less than 16g of fruit per day. The distributions of meat and fish intakes were highly positively skewed with a very small number of women consuming any of these foods. The main contributors to their diets were cereals, millet and vegetables other than GLV. It would have been interesting to have studied whether adherence to the patterns observed was associated with health outcomes and demographic or lifestyle variables in this population.

No reports of studies of theory driven assessment of dietary quality or variety conducted in India were found in the literature. The paucity of data

investigating patterns of diet among the Indian and South Asian population may be a barrier to designing intervention studies to improve health outcomes. In addition if more is known about diet patterns and socio-demographic correlates, it may be possible to target interventions at the population groups that are most at risk of ill-health. A greater understanding of diet patterns in India through the use of data-driven techniques may help to inform the design of dietary guidelines for the Indian population. The aims of this analysis were 1) to identify dietary patterns among women of reproductive age living in urban slums in Mumbai, 2) to investigate how different groupings of food items affected the patterns obtained 3) to study associations between diet patterns and anthropometric outcomes and 4) to investigate socio-demographic correlates of diet patterns.

6.2 Diet patterns analysis method

In order to study the dietary patterns of women in Mumbai, PCA methodology was selected. It was considered that having a continuous score would be more informative as opposed to a dichotomous outcome which would have been derived using Cluster Analysis. The *a priori* approach did not seem suitable given the lack of a diet index based on the Indian dietary guidelines (**Figure 6.2**, p173). Furthermore, this analysis was intended to be exploratory with the aim of informing food-based dietary guidelines.

Food frequency questionnaire data collected in the MMNP (n=4816) were used for the present analysis (see section 3.1.5.3, p74 for details of the FFQ). The 212 foods on the FFQ were condensed to a smaller number of food groups based on nutrient content and typical use. There is evidence that the method of grouping foods may affect the results of PCA (138), therefore we ran two sets of analyses, one with the foods divided into 16 groups and a second with 40 groups. The PCA input variables were the frequency of consumption of foods from each of the groups.

PCA was used to identify the women's diet patterns. As described previously, PCA produces new variables (components) that are independent linear combinations of the dietary variables accounting for maximum variance (237).

Another matter for debate within diet patterns research is whether to use rotation of components (138). The results of the present analysis are presented with and without orthogonal rotation using the Varimax rotation method.

The number of components to retain was based on the identification of the point of inflexion on the scree plot, the component eigenvalue being >1, and interpretability of the component as a plausible diet pattern for the study population. An arbitrary decision as to the definition of discriminatory foods was made. Food groups with factor loadings >|0.4| were considered to be discriminatory. The Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy was used to ensure that the sample size was sufficient relative to the number of input variables. KMO statistic values between 0.5-0.7 are 'mediocre', 0.7-0.8 are 'good' and above 0.8 are 'superb' (274).

Pattern scores were calculated for each woman based on the weekly frequency of consumption of items from the food group and the factor loading for that food group. Weekly frequencies were calculated by multiplying daily intakes by seven. These values were summed for all food groups to provide scores that represented the women's adherence to each of the patterns. Pattern scores were then standardised to give a mean (SD) of 0 (1). The first components for both the 16 group and 40 group PCAs were positively skewed and were normalised using a Fisher-Yates transformation for all further analyses. All analyses were performed using SPSS software package version 19 (SPSS Inc., Chicago, IL., USA).

6.3 Results of PCA analysis of MMNP data

This section presents results from the analyses with 16 and 40 food groups. Coefficients generated using rotated and non-rotated component solutions are shown. The non-rotated components were considered to be interpretable and three diet patterns were identified (section 6.3.1). Median weekly frequency of consumption of discriminatory foods by quarters of pattern score is presented for all patterns along with regression analysis assessing the effect of the patterns on anthropometric variables (section 6.3.2) and the association between demographic factors and pattern scores (section 6.3.3).

6.3.1 Diet patterns identified using PCA of 16 and 40 food groups

Figure 6.3 and **Figure 6.4** show scree plots for the 16 and 40 group analysis respectively.

Figure 6.3 Scree plot of 16 food group analysis

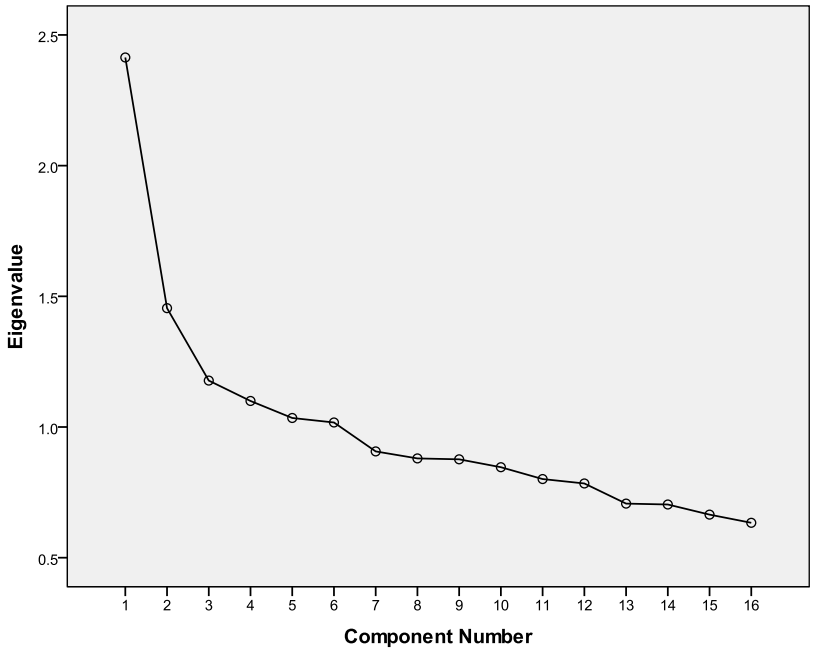
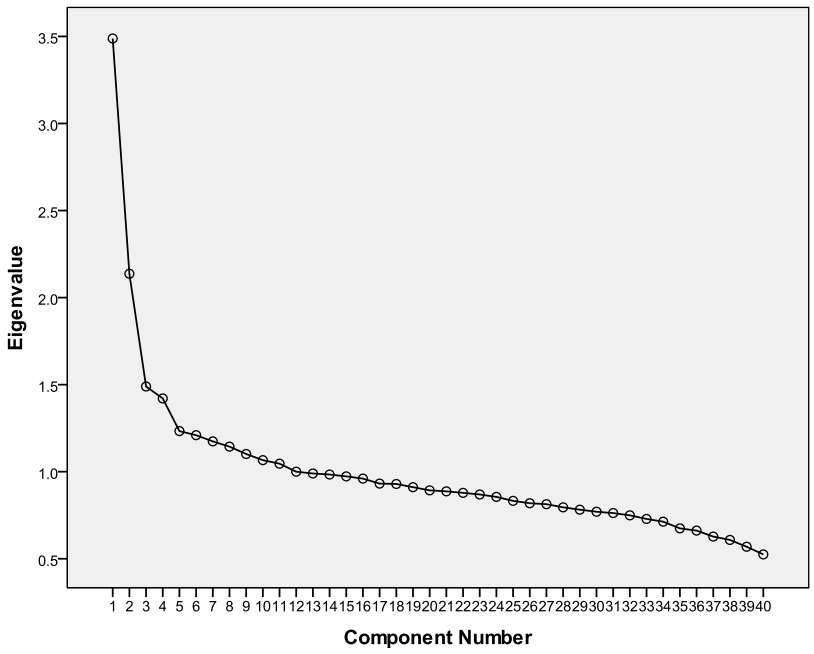


Figure 6.4 Scree plot of 40 food group analysis



Based on the criteria for defining components as meaningful patterns, three components were retained in both sets of analyses. **Table 6.1** and **Table 6.2** show the coefficients for each of the three retained components with and without orthogonal rotation. Foods were considered to be discriminatory if they had a coefficient of >|0.4|. Foods with coefficients of >0.4 were frequently consumed by women with a high score on that component whilst foods with coefficients of <-0.4 were infrequently consumed by women with a high score on that component. Foods with coefficients close to 0 are non-discriminatory for that component.

The KMO measure of sampling adequacy was 0.78 for the 16 group analysis and 0.73 for the 40 group analysis. These data indicated that the sample size was adequate for PCA in both instances.

It was decided that the interpretability of the patterns was not improved through the use of Varimax rotation, especially for the analysis using the 40 foods groups. Therefore further investigations were limited to the unrotated solutions.

For the first component, the coefficients were of greatest magnitude for GLV, vegetables, fruit, savoury snacks and sweet snacks in the 16 group analysis. The coefficients derived in the 40 group analysis were broadly similar to the 16 group analysis and indicated that mango and papaya were not drivers of the high 'fruit' coefficient and that desserts were more discriminatory than sweets, biscuits or sugar sweetened beverages in terms of sweet foods. The second component had large coefficients for mutton, chicken and eggs in the 16 group analysis and the coefficient for pulses and legumes approached the negative cut off for a discriminatory food of -0.4. In the 40 group analysis, coefficients for biriyani and mutton were large and positive and those for legume curry and coconut were negative.

Table 6.1 PCA coefficients from 16 foods in MMNP registration data (unrotated and rotated using Varimax rotation)

	Component (unrotated)			Component (Varimax rotated)		
Food	1	2	3	1	2	3
Bread	0.22	-0.26	0.40	0.10	-0.07	0.35
Rice	0.30	0.36	0.45	-0.09	0.58	0.27
Pulses & legumes	0.28	-0.36	0.54	-0.09	0.01	0.70
GLV	0.49	-0.11	0.13	0.31	0.15	0.39
Vegetables (except GLV)	0.59	-0.19	0.13	0.39	0.12	0.47
Salad	0.41	0.02	-0.24	0.46	0.10	0.02
Fruit	0.59	0.01	-0.36	0.67	0.13	0.03
Nuts	0.29	-0.32	-0.14	0.27	-0.16	0.30
Fish	0.32	0.25	0.21	0.09	0.41	0.16
Mutton	0.13	0.65	-0.11	0.10	0.56	-0.36
Chicken	0.20	0.43	-0.01	0.11	0.45	-0.13
Eggs	0.32	0.49	0.02	0.18	0.55	-0.10
Milk & yoghurt	0.33	-0.19	-0.46	0.56	-0.19	-0.06
Tea	0.11	0.12	0.40	-0.18	0.28	0.27
Savoury snacks	0.64	-0.12	-0.04	0.53	0.15	0.35
Sweet snacks & sugar sweetened beverages	0.50	0.04	-0.14	0.47	0.19	0.13

Coefficients ≥0.4 for pattern 1
Coefficients ≥0.4 for pattern 2
Coefficients ≥0.4 for pattern 3

Table 6.2 PCA coefficients from 40 foods in MMNP registration data (unrotated and rotated using Varimax rotation)

Food	Component (unrotated)			Component (Varimax rotated)		
	1	2	3	1	2	3
Chapathi	0.03	-0.19	-0.24	0.04	0.06	0.19
Rice bread	0.12	-0.32	0.16	-0.08	0.40	0.00
Bakery products	0.20	0.28	0.05	0.07	-0.04	0.09
Plain rice	0.03	-0.06	0.40	-0.06	0.06	0.05
Seasoned rice	0.29	0.09	0.12	-0.01	0.29	0.09
Biriyani	0.19	0.61	0.11	0.13	-0.19	-0.04
Pulses & rice dishes	0.25	0.10	-0.17	-0.01	0.03	0.26
Pulses curry	0.09	-0.29	0.02	-0.01	0.09	0.26
Legume curry	0.40	-0.41	0.15	0.04	0.52	0.30
GLV curry	0.43	-0.09	-0.14	0.19	0.10	0.51
Vegetable curry (no potato)	0.42	-0.08	-0.14	0.06	0.14	0.50
Vegetable curry (with potato)	0.42	-0.08	-0.32	0.15	-0.03	0.65
Bean & potato curry	0.29	-0.13	-0.14	0.02	0.11	0.46
Gourd curry	0.35	-0.14	-0.20	0.06	0.04	0.53
Salad	0.34	0.05	-0.04	0.35	0.14	0.10
Raita (yoghurt with salad)	0.16	0.12	-0.05	0.05	0.05	0.12
Citrus fruit	0.40	0.19	-0.13	0.60	-0.04	0.05
Mango & papaya	0.22	0.14	0.06	0.03	-0.05	0.07
Other fruit	0.50	0.08	-0.14	0.66	0.04	0.11
Fruit juice	0.32	-0.02	-0.08	0.38	0.02	0.12
Dried fruit	0.26	0.15	-0.00	0.46	-0.02	-0.14
Groundnut	0.16	-0.26	-0.08	0.06	0.33	0.03
Coconut	0.05	-0.47	0.46	-0.07	0.59	-0.21

cont.

Table 6.2 cont. PCA coefficients from 40 foods in MMNP registration data (unrotated and rotated using Varimax rotation)

Food	Component (unrotated)			Component (Varimax rotated)		
	1	2	3	1	2	3
Fish & seafood	0.25	-0.08	0.46	0.09	0.26	0.01
Fish curry	0.16	-0.03	0.44	0.01	0.13	0.01
Mutton	0.13	0.68	0.12	0.10	-0.30	-0.04
Offal	0.05	0.13	0.10	-0.01	0.04	-0.03
Chicken	0.16	0.22	0.27	0.04	-0.06	0.04
Eggs	0.26	0.27	0.27	0.11	-0.09	0.13
Milk & curd	0.28	0.00	-0.24	0.37	0.02	0.14
Tea & coffee with milk	0.07	0.07	0.17	-0.19	0.18	0.03
Fried snacks	0.53	0.03	0.03	0.44	0.26	0.10
Non-fried snacks	0.51	-0.08	0.00	0.42	0.30	0.12
GLV snacks	0.26	-0.29	0.04	0.04	0.38	0.20
Oily bread & savoury biscuits	0.23	0.16	0.02	0.07	-0.09	0.06
Papad	0.33	-0.21	0.13	0.16	0.38	0.10
Sweets	0.26	-0.02	0.09	0.19	0.28	-0.10
Desserts	0.52	0.00	0.02	0.35	0.25	0.13
Sweet biscuits	0.21	-0.20	0.08	0.08	0.35	0.05
Sugar sweetened beverages	0.34	0.18	0.08	0.30	0.04	0.06

Coefficients ≥0.4 for pattern 1
Coefficients ≥0.4 for pattern 2
Coefficients ≥0.4 for pattern 3

The two methods of grouping foods yielded differences in the third pattern. In the 16 group analysis, bread, rice, pulses and tea had highly positive coefficients while that for milk was negative. In the 40 group analysis, coefficients for rice, coconut, fish and seafood were all highly positive. Both of

these patterns were considered to be meaningful and so further analysis relates to the 16 group and 40 group PCAs.

In the 16 group analysis, components 1, 2 and 3 explained 15.1%, 9.1% and 7.4% of the variance respectively. The patterns were named 'Snack and Fruit' pattern, 'Non-vegetarian' pattern and 'Pulse and Rice' pattern. In the 40 group analysis, the first three components explained 8.7%, 5.3% and 3.7% respectively. These patterns were named 'Snack and Fruit', 'Non-vegetarian' and 'Fish and Coconut'.

Table 6.3 shows the median weekly intake of foods within the 16 group analysis for women in the lowest and highest quarters of pattern scores. The statistics for the Snack and Fruit pattern show that there is a 6-fold difference between these groups of women in their median frequency of intakes of fruit and savoury snacks. At least 75% of women in the lowest quarter of the Non-vegetarian pattern scores reported no intake of mutton, chicken or eggs whilst those in the highest quarter on average consumed mutton twice per week and chicken and eggs once. Median pulse and legume consumption was almost three times greater among women in the highest quarter of Pulse and Rice pattern scores.

Table 6.4 shows that in the 40 group analysis there was at least a three-fold difference in median weekly intakes of non-citrus fruit and fried snacks between women in the lowest and highest quarters of Snack and Fruit pattern score. Women with scores in the highest quarter for the Non-vegetarian pattern consumed mutton twice per week compared with 0 servings per week in the lowest quarter. Fish intake was on average 2 servings per week among women in the highest quarter of scores on the Fish and Coconut pattern and median coconut intake was at least 7 times higher on average than among those in the lowest quarter.

6.3.2 Diet patterns and anthropometry

In univariate analysis, BMI and all skinfold measurements were positively associated with Snack and Fruit and Non-vegetarian pattern scores and there

was a borderline significant negative association between triceps and biceps skinfolds, and the Pulse and Rice pattern scores (**Table 6.5**). There was a 0.5kg/m^2 difference in median BMI between women in the lowest and highest quarters of Snack and Fruit pattern scores Median triceps and subscapular skinfold thickness were respectively 1.2mm and 0.9mm greater in the highest quarter than the lowest quarter for Snack and Fruit pattern score. The trend across quarters of Non-vegetarian pattern score in terms of BMI and skinfold measurements was less clear. In the case of the Pulse and Rice pattern there was little difference in median BMI or skinfold measurements by pattern score (**Table 6.6**).

In the analysis based on 40 groups, the trends were broadly similar to the 16 group analysis (**Table 6.7**). The median difference in subscapular skinfold between the highest and lowest quarters of Non-vegetarian pattern scores was 1.2mm. There were no significant associations between the anthropometric measurements and the Fish and Coconut pattern scores (**Table 6.8**).

6.3.3 Diet patterns and socio-demographic variables

Data in **Table 6.9** show positive univariate associations between the 16 group Snack and Fruit pattern scores and age, educational duration, occupation, husband's duration of education, husband's occupation and standard of living scores. In the multivariate model, woman's occupation skill level and standard of living remained significant correlates of the Snack and Fruit pattern score (**Table 6.10**). Religion was not associated with this pattern.

In univariate models, age, educational duration, occupation skill level, husband's duration of education, husband's occupation skill level and standard of living score were all negatively associated with Non-vegetarian pattern scores. Religion was also associated with the Non-vegetarian pattern. The multivariate analysis indicated that Muslims, younger women and those whose husbands had low skilled jobs were more likely to adhere to the Non-vegetarian pattern.

The Pulse and Rice pattern was positively associated with age and standard of living score in the univariate analysis. It was negatively associated with the occupation skill level of the woman and with being of Hindu faith. In the multivariate model, age remained a significant positive correlate, occupation remained negative and being of Hindu religion was of borderline significance.

The univariate and multivariate models applied to the 40 group PCA scores showed that the same socio-demographic variables were associated with the Snack and Fruit pattern as for the 16 group PCA. There were some differences in the factors associated with the Non-vegetarian pattern. In the 40 group PCA, education and standard of living score were negatively associated with adherence to the Non-vegetarian pattern. Adherence to the Fish and Coconut pattern was associated with greater duration of education, higher standard of living and was not associated with religion (**Table 6.11** and **Table 6.12**).

Table 6.3 Weekly frequency of consumption of discriminating foods for each pattern by women in the MMNP whose pattern scores fall in the lowest and uppermost quarters of the distribution* (based on 16 food groups)

Snack & Fruit Pattern	Lowest quarter	Highest quarter
GLV	1.0 (0.0,1.0)	3.0 (1.0,4.0)
Non GLV vegetables	2.0 (1.0,3.0)	4.0 (6.0,9.0)
Salad	0.0 (0.0,0.0)	0.0 (0.0,1.0)
Fruit	1.0 (0.0,2.0)	6.0 (3.0,10.0)
Savoury snacks	1.0 (0.0,2.0)	6.0 (4.0,9.0)
Sweet snacks	2.0 (1.0,4.0)	7.0 (4.0,11.0)
Non-vegetarian Pattern		
Mutton	0.0 (0.0,0.0)	2.0 (0.0,4.0)
Chicken	0.0 (0.0,0.0)	1.0 (0.0,2.0)
Eggs	0.0 (0.0,0.0)	1.0 (1.0,3.0)
Pulse & Rice Pattern		
Bread	14.0 (7.0,15.0)	14.0 (9.0,17.0)
Rice	14.0 (8.0,15.0)	15.0 (14.0,17.0)
Pulses & legumes	6.0 (4.0,9.0)	15.0 (11.0,18.0)
Milk & curd	2.0 (0.0,7.0)	0.0 (0.0,1.0)
Tea	7.0 (3.0,14.0)	14.0 (14.0,15.5)

Table 6.4 Weekly frequency of consumption of discriminating foods for each pattern by women in the MMNP whose pattern scores fall in the lowest and uppermost quarters of the distribution* (based on 40 food groups)

Snack & Fruit Pattern	Lowest quarter	Highest quarter
Legume curry	1.0 (0.0,2.0)	3.0 (1.0,4.0)
GLV curry	1.0 (0.0,1.0)	2.0 (1.0,4.0)
Non GLV vegetables	0.0 (0.0,1.0)	1.0 (0.0,2.0)
Potato curry	1.0 (1.0,2.0)	3.0 (2.0,5.0)
Citrus fruit	0.0 (0.0,0.0)	0.0 (0.0,1.0)
Non-citrus fruit	1.0 (0.0,1.0)	3.0 (2.0,6.0)
Fried snacks	0.0 (0.0,1.0)	3.0 (1.0,4.0)
Non-fried snacks	0.0 (0.0,0.0)	1.0 (0.0,2.0)
Desserts	0.0 (0.0,1.0)	2.0 (1.0,3.0)
Non-vegetarian Pattern		
Biriyani	0.0 (0.0,0.0)	0.0 (0.0,1.0)
Legume curry	3.0 (2.0,4.0)	0.0 (0.0,2.0)
Coconut	7.0 (7.0,7.0)	0.0 (0.0,1.0)
Mutton	0.0 (0.0,0.0)	2.0 (1.0,4.0)
Fish & Coconut Pattern		
Plain rice	14.0 (7.0,14.0)	14.0 (14.0,14.0)
Coconut	0.0 (0.0,1.0)	7.0 (2.0,7.0)
Fish & seafood dishes	0.0 (0.0,0.0)	1.0 (0.0,2.0)
Fish curry	0.0 (0.0,0.0)	0.0 (0.0,1.0)

*Values are median (IQR).The Wilcoxon rank-sum test showed a trend across quarters for all foods ($p<0.001$)

Table 6.5 Univariate Analysis of anthropometric variables and diet pattern scores (based on 16 food groups)

	Snack & Fruit Pattern (SD)				Non-vegetarian Pattern (SD)				Pulse & Rice Pattern (SD)			
		95% CI				95% CI				95% CI		
	B	Lower	Upper	p	B	Lower	Upper	p	B	Lower	Upper	p
BMI (kg/m^2)	0.01	0.00	0.01	**0.004**	0.01	0.00	0.01	**<0.001**	-0.00	-0.01	0.00	0.118
Triceps SF (mm)	0.03	0.02	0.04	**<0.001**	0.03	0.02	0.04	**<0.001**	-0.01	-0.02	0.00	**0.049**
Biceps SF (mm)	0.02	0.01	0.03	**<0.001**	0.03	0.02	0.04	**<0.001**	-0.01	-0.02	0.00	0.067
SS SF (mm)	0.02	0.01	0.03	**<0.001**	0.02	0.01	0.03	**0.001**	-0.01	-0.02	0.00	0.138

SS, subscapular. SF, skinfold

Table 6.6 Anthropometric variables by quarters of diet pattern scores (based on 16 food groups)

	Snack & Fruit Pattern				Non-vegetarian Pattern				Pulse & Rice Pattern			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
BMI (kg/m^2)	19.8 (17.8,22.7)	20.0 (17.8,22.9)	20.1 (18.0,23.0)	20.3 (18.0,23.1)	20.1 (17.9,23.1)	19.8 (17.7,22.5)	20.1 (17.9,22.9)	20.3 (18.1,23.3)	20.1 (17.9,23.1)	20.1 (18.1,22.9)	20.1 (17.8,22.9)	19.9 (17.9,22.9)
Triceps SF (mm)	13.3 (9.5,18.3)	13.7 (9.9,19.4)	13.8 (10.1,19.1)	14.5 (10.3,20.4)	13.6 (9.9,19.4)	13.3 (9.6,18.4)	13.5 (10.0,19.2)	14.4 (10.3,20.3)	13.7 (10.1,19.9)	13.8 (10.1,19.2)	13.7 (10.0,19.1)	13.5 (9.7,19.4)
Biceps SF (mm)	6.1 (4.4,8.3)	6.1 (4.4,8.7)	6.2 (4.4,8.4)	6.4 (4.5,9.3)	6.2 (4.3,8.5)	6.1 (4.4,8.3)	6.2 (4.5,8.5)	6.4 (4.5,9.2)	6.2 (4.5,8.8)	6.2 (4.5,8.6)	6.2 (4.4,8.5)	6.2 (4.3,8.5)
SS SF (mm)	21.3 (15.3,28.5)	21.6 (15.5,29.5)	21.3 (15.4,29.2)	22.2 (15.9,31.0)	21.4 (15.5,29.4)	21.1 (15.2,28.5)	21.6 (15.6,29.1)	22.2 (15.6,30.6)	21.7 (15.6,29.7)	21.4 (15.9,28.8)	21.6 (15.1,29.6)	21.5 (15.3,29.8)

Table 6.7 Univariate Analysis of anthropometric variables and diet pattern scores (based on 40 food groups)

	Snack & Fruit Pattern (SD)				Non-vegetarian Pattern (SD)				Fish & Coconut Pattern (SD)			
		95% CI				95% CI				95% CI		
	B	Lower	Upper	p	B	Lower	Upper	p	B	Lower	Upper	p
BMI (kg/m^2)	0.01	0.00	0.01	**0.001**	0.02	0.01	0.02	**<0.001**	-0.00	-0.01	0.00	0.223
Triceps SF (mm)	0.04	0.03	0.05	**<0.001**	0.05	0.04	0.06	**<0.001**	0.00	-0.01	0.02	0.443
Biceps SF (mm)	0.03	0.01	0.04	**<0.001**	0.05	0.04	0.06	**<0.001**	0.01	-0.01	0.02	0.318
SS SF (mm)	0.02	0.01	0.03	**<0.001**	0.03	0.02	0.04	**<0.001**	0.01	-0.01	0.02	0.297

SS, subscapular. SF, skinfold

Table 6.8 Anthropometric variables by quarters of diet pattern scores (based on 40 food groups)

	Snack & Fruit Pattern				Non-vegetarian Pattern				Fish & Coconut Pattern			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
BMI (kg/m^2)	19.8 (17.8,22.8)	20.0 (17.8,22.9)	20.0 (18.0,23.0)	20.3 (18.0,23.1)	19.8 (17.9,22.8)	19.9 (17.8,22.7)	20.1 (17.9,22.9)	20.5 (18.1,23.6)	20.1 (17.9,23.0)	20.0 (17.9,22.9)	20.0 (18.0,22.9)	20.1 (17.8,22.9)
Triceps SF (mm)	13.3 (9.5,18.3)	13.7 (9.9,19.4)	13.8 (10.1,19.1)	14.5 (10.3,20.4)	13.4 (9.6,18.7)	13.4 (9.8,18.5)	13.5 (9.9,18.8)	14.6 (10.5,20.7)	13.6 (10.0,19.4)	13.5 (10.1,19.1)	13.8 (10.0,19.3)	14.1 (9.9,19.6)
Biceps SF (mm)	6.1 (4.4,8.3)	6.1 (4.4,8.7)	6.2 (4.4,8.4)	6.4 (4.5,9.3)	6.1 (4.3,8.3)	6.1 (4.4,8.3)	6.2 (4.4,8.6)	6.5 (4.6,9.4)	6.1 (4.4,8.7)	6.3 (4.5,8.5)	6.2 (4.5,8.5)	6.3 (4.4,8.7)
SS SF (mm)	21.3 (15.3,28.5)	21.6 (15.5,29.5)	21.3 (15.4,29.2)	22.2 (15.9,31.0)	21.2 (15.2,29.2)	21.3 (15.4,28.5)	21.6 (15.4,28.7)	22.4 (16.1,32.0)	21.4 (15.6,29.1)	21.4 (15.4,28.8)	21.8 (15.3,29.7)	21.7 (15.6,30.1)

Table 6.9 Univariate Analysis of demographic variables and diet pattern scores (based on 16 food groups)

	Snack & Fruit Pattern (SD)				Non-vegetarian Pattern (SD)				Pulse & Rice Pattern (SD)			
		95% CI				95% CI				95% CI		
	B	Lower	Upper	p	B	Lower	Upper	p	B	Lower	Upper	p
Age (years)	0.01	0.00	0.02	**0.004**	-0.02	-0.03	-0.02	**<0.001**	0.02	0.01	0.03	**<0.001**
Religion (Hindu=0, Muslim=1)	-0.02	-0.08	0.03	0.456	1.10	1.05	1.15	**<0.001**	-0.28	-0.34	-0.23	**<0.001**
Education*	0.18	0.14	0.23	**<0.001**	-0.14	-0.18	-0.10	**<0.001**	0.02	-0.02	0.06	0.408
Husband's Education*	0.20	0.15	0.24	**<0.001**	-0.31	-0.36	-0.27	**0.001**	0.01	-0.03	0.06	0.631
Occupation**	0.21	0.15	0.27	**<0.001**	-0.08	-0.14	0.03	**0.005**	-0.09	-0.16	-0.03	**0.003**
Husband's Occupation**	0.14	0.10	0.17	**<0.001**	-0.09	-0.13	-0.05	**<0.001**	-0.03	-0.07	0.00	0.092
SLI score	0.03	0.02	0.03	**<0.001**	-0.01	-0.02	-0.01	**<0.001**	0.01	0.01	0.02	**<0.001**

*Duration of formal education, 1=<5y, 2=5-10y, 3=>10y; **Occupation in 4 categories, 1=unskilled, 2=semi-skilled, 3=skilled, 4=professional

Table 6.10 Univariate Analysis of demographic variables and diet pattern scores (based on 40 food groups)

	Snack & Fruit Pattern (SD)				Non-vegetarian Pattern (SD)				Fish & Coconut Pattern (SD)			
		95% CI				95% CI				95% CI		
	B	Lower	Upper	p	B	Lower	Upper	p	B	Lower	Upper	p
Age (years)	0.01	0.00	0.01	**0.019**	-0.04	-0.04	-0.03	**<0.001**	0.01	-0.05	0.06	0.847
Religion (Hindu=0, Muslim=1)	-0.03	-0.09	0.02	0.248	1.47	1.42	1.51	**<0.001**	-0.28	-0.34	-0.23	**<0.001**
Education*	0.22	0.17	0.26	**<0.001**	-0.24	-0.29	-0.20	**<0.001**	0.17	0.12	0.21	**<0.001**
Husband's Education*	0.22	0.17	0.26	**<0.001**	-0.38	-0.42	-0.34	**<0.001**	0.02	-0.03	0.06	0.458
Occupation**	0.25	0.19	0.31	**<0.001**	-0.05	-0.11	0.01	0.095	0.01	-0.05	0.08	0.672
Husband's Occupation**	0.14	0.11	0.18	**<0.001**	-0.06	-0.10	-0.03	**0.001**	-0.02	-0.06	0.02	0.324
SLI score	0.03	0.03	0.03	**<0.001**	-0.02	-0.03	-0.02	**<0.001**	0.02	0.01	0.02	**<0.001**

*Duration of formal education, 1=<5y, 2=5-10y, 3=>10y; **Occupation in 4 categories, 1=unskilled, 2=semi-skilled, 3=skilled, 4=professional

Table 6.11 Multivariate Analysis of demographic variables and diet pattern scores (based on 16 food groups)

	Snack & Fruit Pattern (SD)				Non-vegetarian Pattern (SD)				Pulse & Rice Pattern (SD)			
		95% CI				95% CI				95% CI		
	B	Lower	Upper	p	B	Lower	Upper	p	B	Lower	Upper	p
Age (years)	-0.00	-0.02	0.01	0.897	-0.02	-0.03	-0.00	**0.027**	0.02	0.01	0.04	**0.002**
Religion (Hindu=0, Muslim=1)	-	-	-	-	1.08	0.95	1.21	**<0.001**	-0.15	-0.29	-0.00	**0.046**
Education*	0.05	-0.06	0.17	0.379	-0.09	-0.19	0.02	0.100	-	-	-	-
Husband's Education*	0.05	-0.07	0.17	0.430	-0.00	-0.11	0.11	0.985	-	-	-	-
Occupation**	0.10	0.03	0.18	**0.009**	0.03	-0.04	0.10	0.371	-0.13	-0.20	-0.05	**0.001**
Husband's Occupation**	0.06	-0.03	0.15	0.201	-0.13	-0.21	-0.05	**0.002**	-0.02	-0.11	0.07	0.681
SLI score	0.02	0.01	0.03	**0.005**	0.00	-0.01	0.01	0.799	0.00	-0.01	0.01	0.409

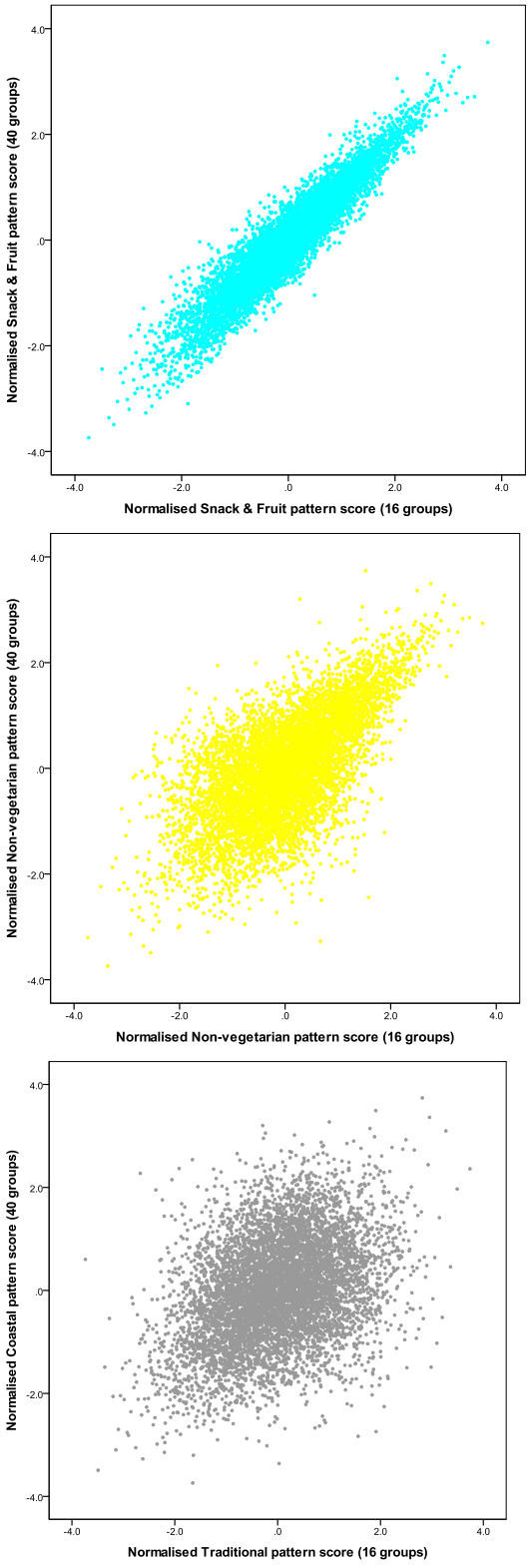
*Duration of formal education, 1=<5y, 2=5-10y, 3=>10y; **Occupation in 4 categories, 1=unskilled, 2=semi-skilled, 3=skilled, 4=professional; - not included in model

Table 6.12 Multivariate Analysis of demographic variables and diet pattern scores (based on 40 food groups)

	Snack & Fruit Pattern (SD)				Non-vegetarian Pattern (SD)				Fish & Coconut Pattern (SD)			
		95% CI				95% CI				95% CI		
	B	Lower	Upper	p	B	Lower	Upper	p	B	Lower	Upper	p
Age (years)	0.00	-0.01	0.02	0.854	-0.02	-0.02	-0.01	**<0.001**	-	-	-	-
Religion (Hindu=0, Muslim=1)	-	-	-	-	1.37	1.33	1.42	**<0.001**	0.05	-0.01	0.10	0.114
Education*	0.08	-0.03	0.20	0.151	-0.06	-0.10	-0.02	**0.004**	0.11	0.06	0.16	**<0.001**
Husband's Education*	0.09	-0.03	0.21	0.159	-0.02	-0.07	0.02	0.282	-	-	-	-
Occupation**	0.12	0.05	0.20	**0.001**	-	-	-	-	-	-	-	-
Husband's Occupation**	0.04	-0.05	0.13	0.379	0.01	-0.03	0.04	0.644	-	-	-	-
SLI score	0.02	0.01	0.03	**0.003**	-0.01	-0.01	-0.00	**0.004**	0.01	0.01	0.02	**<0.001**

*Duration of formal education, 1=<5y, 2=5-10y, 3=>10y; **Occupation in 4 categories, 1=unskilled, 2=semi-skilled, 3=skilled, 4=professional; - not included in model

Figure 6.5 Scatterplots of Principal Component scores using 16 groups vs. 40 groups



6.3.4 Analysis with 16 vs. 40 food groups

One of the aims of this study was to investigate how the number of input variables affects the result of PCA. The scatterplots in **Figure 6.5** show that there is a strong correlation between the first principal component derived using 16 foods and that derived using 40 foods, r=0.94. The correlation is weaker for the second principal component, r=0.65 and weaker still for the third component, r=0.37 (all correlation coefficients were significant at the p=0.01 level). Given that the patterns sequentially explain less of the variance in frequency of consumption, this is as expected and it explains why the multivariate regression statistics indicated that there were differences in terms of demographic factors that were associated with adherence to the Non-vegetarian, Pulse and Rice, and Fish and Coconut patterns. These results indicate that it is important to consider how the input variables are grouped. Using a larger number of input variables may enable greater precision in determining which particular foods contribute to a dietary pattern.

For example, non-citrus fruit and fried savoury snacks were particularly discriminatory within the Snack and Fruit pattern. It is important to ensure that when running the analysis with a large number of input variables the sample size is adequate.

6.4 Discussion

This analysis was designed to describe diet patterns among women of reproductive age living in a slum area of Mumbai. The pattern explaining most of the variance was named the Snack and Fruit pattern, and was largely characterised by foods that could be consumed with little preparation within the home. It may therefore represent a more modern or convenient mode of eating. This pattern was positively associated with all anthropometric measurements. The distribution of BMI is such that very few women were overweight or obese but it would be interesting to follow these women and determine whether a diet high in fried snacks leads to greater adiposity and other risk factors for chronic disease. The Snack and Fruit pattern was correlated with occupational skill level and standard of living score which fits with the suggestion that it is adhered to by a more affluent consumer. The cost of fruit, salad and GLV may be prohibitive for poorer women.

The second pattern was identified as Non-vegetarian as it was characterised by frequent consumption of mutton, chicken, eggs and biriyani. It was not surprising that this pattern was associated with being Muslim. It was also independently associated with younger age and lower skill level of husband's occupation. This may be a reflection of a departure from traditional vegetarianism among younger women of lower socio-economic status.

When 16 food groups were studied, the third component was characterised by frequent intakes of rice, pulses and legumes. This represents a traditional pattern of eating in South India. It is a diet that is usually comprised of home-cooked foods and would be less expensive than diets comprising non-vegetarian foods, fruit or dairy foods. This is supported by the positive association with age, being Hindu, and the negative association with occupational skill level of the woman. The third pattern identified when 40

food groups were input, was characterised by fish, rice and coconut. This represents a typical diet of people living in the Konkani coastal areas. This diet was associated with greater duration of education and higher standard of living.

A large cross sectional survey in rural parts of India studying intakes of single food groups as opposed to dietary patterns showed that low fruit and vegetable intake was associated with low socio-economic status (275). Maternal education is positively associated with adherence to a healthy diet pattern in Australia and the UK (269;276).

A recent study assessing the effect of rural to urban migration in India showed that urban dwelling adults consumed up to 80% more fruit than their rural counterparts (277). It is thought this is due to wider availability of produce and greater purchasing power among urban dwellers. It may also be due to a lack of indigenous fruit growing in rural areas due to deforestation. Urban dwellers also tended to have a higher energy and fat intake. This finding fits with the Snack and Fruit pattern that is described here.

The current analysis did not derive a typically healthy or prudent pattern, as has very often been found in other studies, albeit mainly in the USA and Europe. The Snack and Fruit and Non-vegetarian patterns found here are very similar to patterns identified among 9 year old children in a more middle class setting in Mysore, South India (278). It is important to consider how knowledge of this pattern which is comprised of some beneficial elements and some potentially harmful can be used to inform food-based guidelines and recommendations aimed at this population (279).

7. Public health relevance

Chapter 1 introduced the extent of the problem of NCDs in India and described evidence supporting the developmental origins of T2D. Improving the quality of young women's diets may lead to better developmental outcomes for their children and a reduced prevalence of NCDs in India and other LMICs. A recently published review on evidence based public health interventions for prevention of NCDs in India, highlighted the importance of good nutrition throughout the lifecourse (125). It stated that sub-optimal maternal health and prenatal nutrition should be addressed using a community-led approach. Policy measures to increase fruit and vegetable availability and to encourage consumption of locally available varieties were recommended.

The results of the systematic review described in Chapter 2, show that there has been very little research undertaken to assess whether increasing fruit and vegetable intakes can enhance the micronutrient status of women of reproductive age. In addition, the majority of such research has been done in high income countries where there is generally a lower prevalence of deficiency disorders. Section 7.1 describes approaches to reducing micronutrient deficiencies in LMICs and suggests that food-based approaches should be at least part of the solution.

It is clear from the analysis of dietary data among women registered in the MMNP and Extension Study that intakes of micronutrient-rich foods are low in this population. Barriers to consumption of a healthy diet at the community, country and global level are discussed in section 7.2.

7.1 Prevention of micronutrient deficiencies

Several approaches aimed at reducing the prevalence of nutrient deficiencies in LMICs have been implemented over the last few decades. Broadly speaking these approaches are: supplementation with pharmacological preparations of nutrients; fortification; dietary diversification.

7.1.1 Pharmacological supplementation

Pharmacological approaches involve intakes of one or more nutrients usually in tablet or capsule form or by injection. Such an approach has many attractive features; supplements are relatively inexpensive, easy to transport and store and have a long shelf life. Pharmacological micronutrient supplementation is often necessary to reverse clinical deficiency symptoms and in emergency situations such as famine, conflict and natural disasters (280). It may also be important for prevention of deficiency disorders among at-risk-groups such as infants, pregnant women and older people (281). For pregnant women, the current standard WHO recommendation is daily supplementation of 60mg iron and 400µg folic acid during pregnancy (282). Indian government policy is to supplement women in receipt of family planning and those who are pregnant or breastfeeding with 100mg elementary iron and 0.5mg folic acid per day (283). Several trials and systematic reviews have studied the effect of multiple micronutrient supplements among pregnant women on their own micronutrient status and on birth outcomes. The findings are discussed in sections 7.1.1.1 – 7.1.1.2 respectively. Section 7.1.1.3 describes pre-pregnancy interventions.

7.1.1.1 Micronutrient supplements and maternal micronutrient status

A review published in 2008 conducted by Bhutta et al (284) investigated the effect of a wide variety of interventions aimed at reducing undernutrition among mothers and children. These interventions ranged from home gardening to micronutrient supplementation programmes. The main conclusion relating to maternal micronutrient status was that iron-folic acid tablets were effective in increasing maternal haemoglobin at term, zinc showed little effect on maternal and birth outcomes and there was insufficient evidence to assess the effect of vitamin A supplements on maternal outcomes. A meta-analysis, published in 2006, found that iron plus folic acid supplementation increased maternal haemoglobin concentrations at term by 1.2g/dL on average compared with no treatment or a placebo (285). Another meta-analysis comparing the effect of multiple micronutrients with placebo or fewer than three micronutrients found that multiple micronutrients were associated with a 39% reduction in maternal anaemia (286).

There have been suggestions that multiple micronutrients may be more effective than iron-folic acid supplementation in improving maternal micronutrient status. This may be particularly true among women whose diets lack animal source foods (96;287) as retinol, riboflavin and vitamin B12 are required for haemoglobin synthesis. A pooled analysis of 13 studies conducted in LMICs assessed the effect of multiple micronutrient supplements among pregnant women on their haemoglobin levels and micronutrient status compared with iron-folic acid supplements (288). It was reported that multiple micronutrient tablets containing 30mg iron increased haemoglobin levels to the same extent as iron or iron-folic acid supplements containing 60mg iron. Overall, multiple micronutrient tablets had no effect on serum retinol or zinc concentrations, although there were conflicting results between some trials assessing changes in retinol (289;290) and zinc (291;292) status.

7.1.1.2 Micronutrient supplements and birthweight

In a 2009 Cochrane review of iron or iron-folic acid supplementation studies during pregnancy conducted in LMICs, there was no evidence to suggest an effect on birthweight (147). However a more recent meta-analysis detected a 20% reduction in LBW among mothers who were supplemented with iron (293). Compared with iron-folic acid tablets, daily multiple micronutrient supplements have been shown to be efficacious in terms of increasing birthweight in research settings. The Bhutta et al review concluded that multiple micronutrient supplements reduced the risk of LBW at term by 16% (284). Meta-analyses of data from low income countries showed that compared with iron-folic acid tablets, multiple micronutrient supplementation during pregnancy resulted in a 10% reduction in prevalence of LBW and a mean difference of 22.4g in birthweight between treatment groups but was not associated with reduced stillbirths or neonatal mortality (115;294). A recent update to a Cochrane review and meta-analysis of studies published up to February 2012 supported these findings (295). It showed that when compared to iron-folic acid tablets, multiple micronutrients were associated with a statistically significant reduction in LBW and SGA babies, risk ratio of 0.89 (95% CI 0.83-0.94) and 0.87 (95% CI 0.81-0.95). However, the authors concluded that there was insufficient evidence to support a universal change in micronutrient supplementation policy. Another recently published meta-

analysis of 15 trials with high compliance rates comparing iron folic-acid with multiple micronutrient tablets found a 44g mean difference in birthweight between groups (296). The authors of these meta-analyses also concluded that there was insufficient evidence to revise WHO policy (295;296).

The policy of folic acid supplementation during pregnancy has been questioned because the tablets are usually given to the women after the stage in pregnancy by which the neural tubes have closed (297). This is particularly pertinent in India and other countries where intakes of animal source foods are low, and B12 deficiency is likely to be common. In India maternal vitamin B12 deficiency in combination with high circulating folate concentrations is associated with insulin resistance and other risk factors for T2D (298;299). Among pregnant women who were vitamin B12 deficient and folate replete, vitamin B12 supplementation reduced total homocysteine concentration in late pregnancy which may be a mechanism for this effect (300).

A limitation of pharmacological supplementation is that compliance can be poor, particularly in a 'real world' scenario as opposed to a clinical trial (301). Iron supplementation has been reported to cause diarrhoea and other abdominal symptoms. A meta-analysis found that such side effects were more common among women who took iron when compared with a placebo or no treatment (147), although another study found these side effects did not affect compliance (302). The majority of research studies that have assessed the effect of pharmacological micronutrient interventions on maternal and infant outcomes have recruited women during pregnancy. Therefore the women may have started pregnancy with poor stores of nutrients. This may explain the relatively small effects of multiple micronutrient supplementation on birth outcomes described earlier in this section. However, a review which compared multiple micronutrients with iron-folic acid found that there was greater benefit among mothers who started supplementation after 20 weeks (296). The authors hypothesised that supplementation with micronutrients in early pregnancy may have adversely affected maternal metabolic regulation.

7.1.1.3 Pre-pregnancy multiple micronutrient interventions

As a result of an expert consultation in 2007, WHO recommended that weekly iron-folic acid tablets should be given to women of reproductive age in order to prevent anaemia before pregnancy and improve pregnancy outcomes (282). WHO stress that this approach should be implemented in settings with the necessary 'support, social marketing and interpersonal advocacy' and where 'necessary programme monitoring is feasible'. The consultation document recommends that strategies to combat iron deficiency and to improve iron and folate status should be integrated with better access to dietary sources of iron, de-worming and malaria prevention. As with all intervention programmes, it is essential that evaluation of supplementation programmes is properly carried out in order to determine the effectiveness of the intervention (303;304).

A study in anaemic Bangladeshi adolescent girls comparing long term once or twice weekly multiple micronutrient tablets with twice weekly iron-folic acid tablets over a 12 month period in a randomised trial found that all three treatments reduced iron deficiency as measured by haemoglobin and serum ferritin. However, the once weekly multiple micronutrient supplement led to less of an improvement in serum ferritin than the iron-folic acid tablets (267).

There is little evidence on the benefits of pharmacological micronutrient supplementation prior to pregnancy on birth outcomes. Such an approach may be of greater benefit than instigating supplementation programmes during pregnancy. A randomised controlled trial has recently been launched in Viet Nam to assess the effects of pre-pregnancy supplementation of either 2800µg folic acid, 60mg iron plus 2800µg folic acid or multiple micronutrient supplements containing 60mg iron plus 2800µg folic acid on birth outcomes and maternal and infant iron status. Five thousand women have been recruited from rural communes and will be followed up during pregnancy and post-partum (305).

7.1.2 Fortification

Fortification is a process whereby the nutrient content of a food is increased. This is done using pharmacological methods, for example in the case of flour fortified with iron at the milling stage (306). Alternatively, bio-fortification is achieved through plant breeding either by conventional selective breeding or by genetic modification. The World Food Programme recommendations for addressing micronutrient deficiencies rely on the implementation of food fortification (307). It is recognised that there are other effective strategies for addressing deficiencies, yet fortification is considered to be one of the most cost effective methods. Fortification of salt with iodine has been a successful strategy in reducing the prevalence of goitre and it has been recommended that iodisation of salt should be extended to universal coverage (284).

Staple foods are frequently used as fortification vehicles as they can usually be produced locally and are familiar to the population they are aimed at, thus enabling preparation and consumption of the foods in traditional ways. When designing and implementing fortification programmes, it is essential that the foods that are fortified are acceptable to the target population and reach those that are most in need, particularly in remote or rural areas.

An example of bio-fortification which has received much attention recently is genetically modified 'golden rice'. Golden rice is bred to contain high levels of β-carotene. In bio-availability studies it has been found to increase retinol stores in adults and children to the same extent as β-carotene in oil (308;309). As such it has the potential to provide vitamin A to millions of people who currently may not be able to meet their requirements through other dietary sources. However, it has been pointed out that many of the studies assessing efficacy in adults have been carried out in healthy volunteers in the USA and it is possible that the absorption and bio-conversion to retinol may be less efficient among populations in LMICs with multiple nutritional deficiencies and gastro-intestinal infections or parasites (310). While it is generally accepted that genetically modified foods are safe to eat, there is debate around consumer acceptability of genetically modified produce and potential environmental and ecological harm (311). The bio-availability study carried out in children has recently caused some controversy within the Chinese media

(312). There is also a concern that due to its bright yellow colour the rice will not be acceptable to many of the target populations who are accustomed to white rice (313).

A more general criticism of food fortification methods is that they tend to target one nutritional deficiency disorder and can obscure the overriding issue of a lack of available and affordable nutritious food (124). There are also concerns that the bio-fortification approach may lead to a loss of bio-diversity and over reliance on a small number of staple crops which may make populations vulnerable to famine if these species were to be affected by crop diseases or infestations (314).

7.1.3 Dietary diversification

There are three main aspects of dietary diversification and different populations may require improvements to one or more aspect in order to meet their nutritional requirements. First, overall food intake may need to be increased. Multiple micronutrient deficiencies frequently occur among those who are chronically undernourished. As well as increased quality of diet, sufficient substrate in the form of protein, carbohydrate and fat are required for optimal nutritional status. This is a particular challenge for the poorest and most marginalised communities largely due to lack of access and cost (281). Second, an increase in the variety of foods consumed. Third, increasing consumption of foods that enhance absorption of nutrients and controlling intakes of foods that inhibit absorption. For example, consumption of fats and oils with vitamin A-rich foods, and foods containing vitamin C with sources of non-haem iron should be encouraged (281). Consumption of foods and beverages containing phytates and polyphenols should be avoided when foods containing non-haem iron are consumed.

Broadly, there are two means of achieving an increase in quality to the diet. Which is more suitable depends on current diet and the requirements of the target population. One method is to make major changes to the long-term dietary pattern, such as increasing fruit and vegetable consumption from an average of two servings per day to nine servings per day. This would generally be accompanied by a reduction in other foods with the consequence of lower

consumption of calories, sugar, fat and salt. Typically, in research settings, these interventions have been aimed at groups diagnosed with NCDs or risk factors for NCDs. It could be argued that these people are likely to be motivated to change their behaviour if they have already experienced a health problem or adverse event. This approach tends to lead to the largest effects in terms of change in micronutrient concentrations and other health outcomes (170;218). The question is whether it is sustainable and indeed achievable in low income societies such as Mumbai slums. Adherence to such diets tends to be variable and may depend on the social support that is given to the recipient (315). Furthermore the majority of such interventions have been implemented in the USA and Europe where people have relatively greater purchasing power and a lower proportion of income is required for spending on food (316). In India such a modification to the diet of a young woman, who may not perceive long-term health as a priority and who is unlikely to have the resources or the autonomy to purchase such quantities of fruit and vegetables is probably unrealistic. A second approach is to supplement the habitual diet with a nutrient-rich food item. This approach, as adopted in the MMNP and Extension Study does not prescribe a change to the usual diet. If effective, it is likely to lead to a smaller shift in the distribution of outcome measurements than the former approach, although it may be more sustainable in terms of adherence and availability. Furthermore, in populations where micronutrient deficiencies are prevalent, a small shift in the distribution of micronutrient status may be important in public health terms (317).

An ideal scenario would perhaps be a combination of the two approaches. However programme planners are often required to design an intervention constrained by available resources that would be appropriate to the setting and acceptable to participants. It is perhaps more achievable in resource poor settings to implement a supplementation to habitual diet rather than a complete change to dietary behaviour. It is important that the supplement is appealing to the target group and is available to them outside of meal times so that it does not replace other foods.

There are several dietary diversification strategies that have been employed to achieve improvements in diet quality and quantity in LMIC settings. These include: increasing production and consumption of micronutrient-rich foods;

increasing consumption of enhancers of micronutrient absorption; changes to modes of preparing foods.

7.1.3.1 Increasing production and consumption of micronutrient rich foods

It has been suggested that the most efficient means of improving micronutrient status is through increasing consumption of animal source foods (151). Promotion of animal food consumption among women of reproductive age has resulted in reductions in anaemia prevalence and increased iron intakes (318). However, it is important to consider cultural and religious influences on diet. Plant based approaches are likely to be acceptable to most people (148). Furthermore a study in the UK has shown that diets containing more plant based foods, less meat and fewer high fat, high sugar foods are likely to be more sustainable and may help to mitigate climate change (319).

An approach to increasing access to fruit and vegetables is homestead gardening. There is evidence from observational studies that members of households with home gardens have higher intakes of fruit and vegetables (320) and a lower prevalence of night blindness (321). In Cuba, urban gardening, whereby waste land is used to grow fruit and vegetables has been employed to increase availability of fruit and vegetables in cities. This strategy was instigated out of necessity after the collapse of the Soviet Union led to reduced supplies of agricultural equipment and fertilisers to the country. A lack of fuel meant that food could not be transported easily. It has been estimated that fruit and vegetable availability per person in Havana has increased from 171g/day to 340g/day between 2001 and 2005 largely as a result of urban gardens (322). Other benefits include provision of employment, particularly to women (323), improvements to the environment and community development (324).

An evaluation of a homestead gardening programme in Bangladesh has shown that it has increased intakes of vitamin A rich foods (325). It has also provided increased income for the households, the majority of which has been spent on food. An evaluation of a community led gardening project in a rural village in South Africa found that compared with a control village, there was a significant increase in fruit and vegetable intakes and increased serum retinol concentrations among children under 5 years (326). In the review by Bhutta et

al (284), described in section 7.1.1 (p200), it was concluded that this approach was promising and culturally relevant but there was insufficient evidence to draw conclusions about its effectiveness (284). There are considerable difficulties with studying such approaches in a randomised controlled trial as there tends to be 'spill over' of benefits within and between communities (325).

7.1.3.2 Increasing consumption of enhancers of micronutrient absorption and changes to food preparation

There is mixed evidence from trials on the effect of consumption of citrus fruit on iron status among young women (135;327). Consumption of pro-vitamin A carotenoids with fats has been shown to enhance bioavailability (328). A review of interventions aimed at increasing micronutrient intakes by modifying food preparation techniques found that several methods were successful in increasing micronutrient content of foods and bioavailability of iron, zinc and calcium (329). Soaking or fermenting grains such as maize to reduce the phytate content (an inhibitor of iron, zinc and calcium absorption) (330) was one method. Another was to germinate flours which also reduced their phytate content. Germination of legumes is thought to reduce polyphenol and tannin content both of which inhibit iron absorption (331).

A point that has been raised in relation to vitamin A is that food-based approaches are often over-looked because indigenous plant foods grown in LMICs may not be recognised as rich sources of the nutrient due to composition data not being published in Western food tables (332). A report published by the International Food Policy Research Institute in 2001 highlighted the disproportionate focus on supplementation and fortification programs and policies to the detriment of food-based approaches (333). It proposed that this was due to the requirements of donors to demonstrate effectiveness rapidly. In addition the more complex nature of food-based research may discourage researchers from carrying out the studies that are required to assess such approaches. Furthermore, these studies are likely to require input from the agricultural, economic and education sectors which may be considerably more complex to co-ordinate than a randomised trial of a multiple micronutrient tablet. Blinding of interventions is more challenging when designing food-based studies and if randomised at the level of the

individual, it is possible that participants will alter their diets based on interventions given to other treatment groups. One way of addressing this is to cluster randomise at the village or some other suitable level, but this generally leads to a reduction in statistical power to detect an effect of the intervention.

Even with these challenges in mind, it has been suggest that the reductionist approach to resolving nutritional problems may not be adequate and dietary diversity approaches should always play a primary role in reducing micronutrient deficiencies with supplementation and fortification being used where absolutely necessary (124;281). The social, cultural and economic benefits of using food-based approaches are also of great importance. These include maintaining the tradition of collective meal times, preserving sustainable methods of cultivating indigenous crops and income generation for rural farmers.

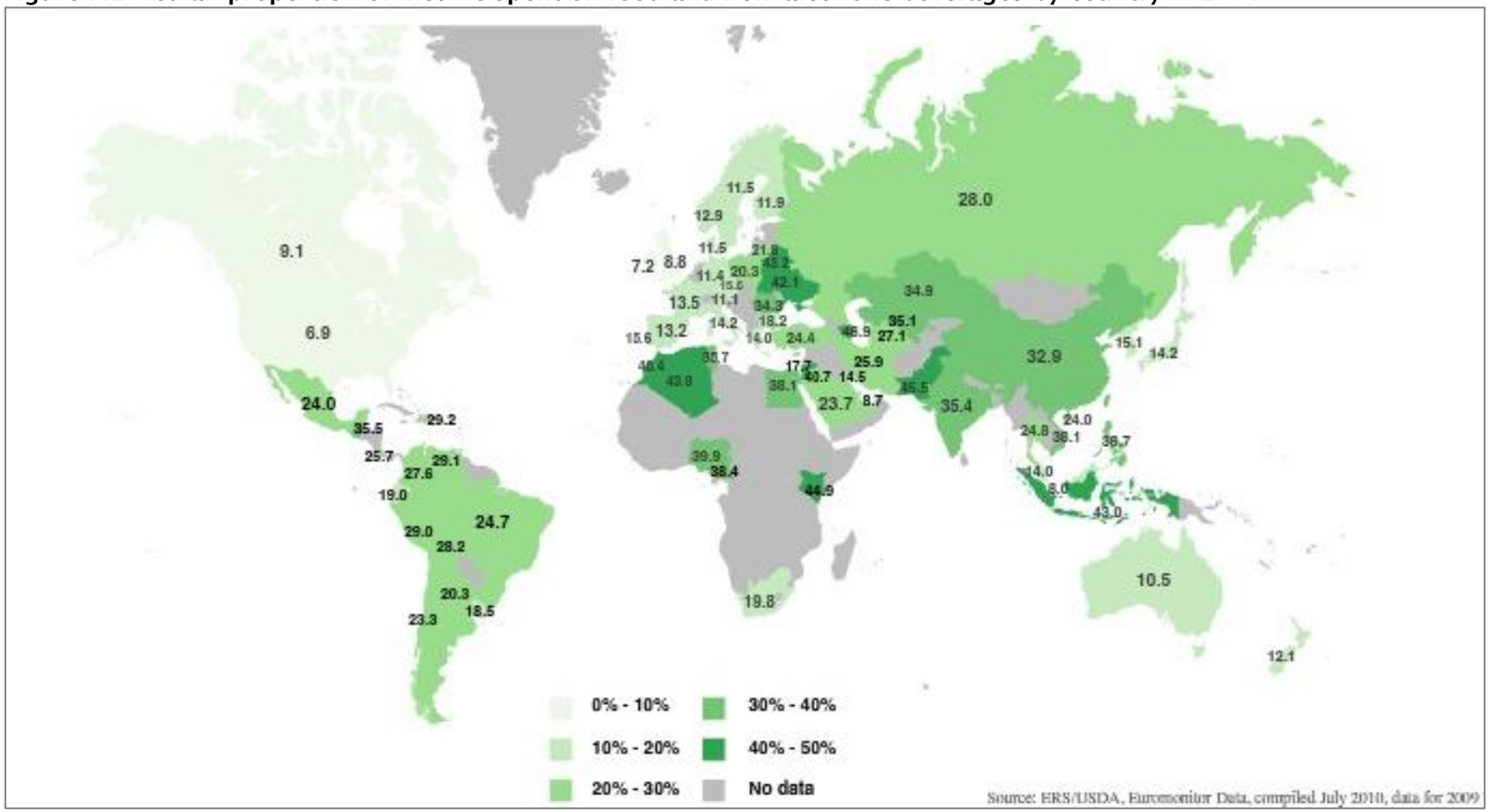
7.2 Factors affecting dietary intake

The work in this thesis (section 4.1) indicates that the dietary intakes of women living in Mumbai slums are of poor quality. This is likely to affect their health and may lead to sub-optimal developmental outcomes among their children. In India and many other countries in economic and nutrition transition the mis-match between early development and the food environment encountered later in life, particularly in cities, could vastly increase the burden of chronic disease. This section describes some of the barriers to consumption of a high quality diet for the majority of people living in LMICs.

7.2.1 Cost of food and food security

The proportion of income that is spent on food varies greatly between countries (**Figure 7.1**). In 2009 the average American spent under 7% of their income on food and non-alcoholic beverages while on average Indians spent over a third.

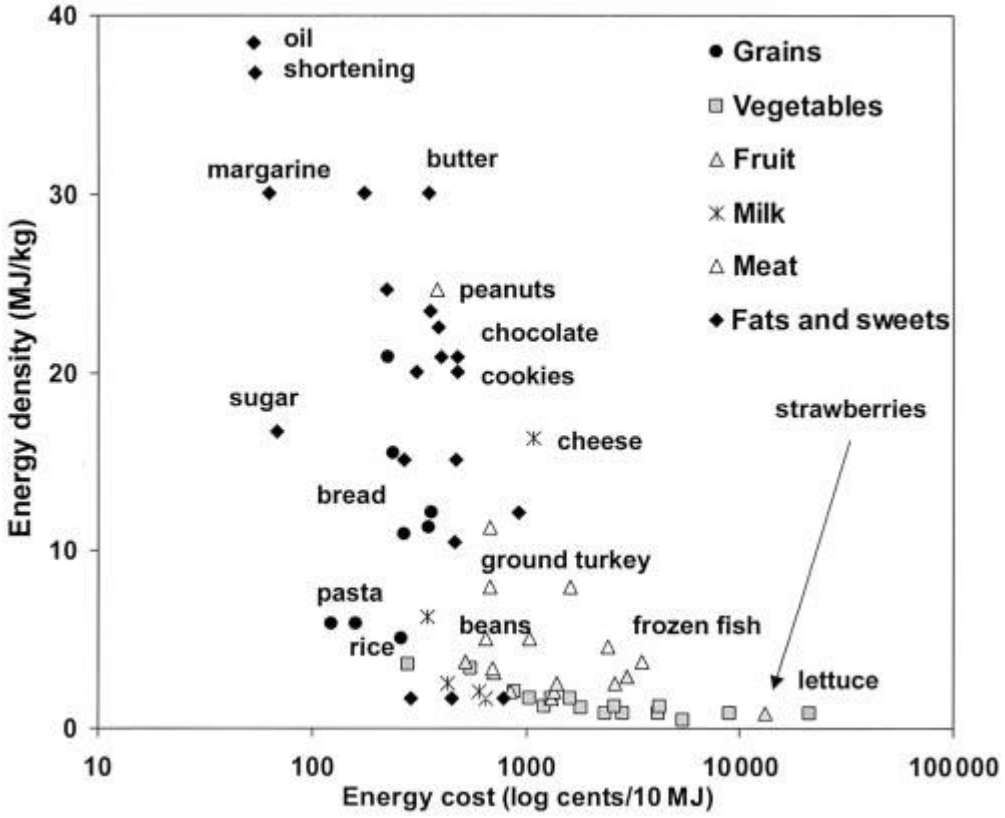
Figure 7.1 Median proportion of income spent on food and non-alcoholic beverages by country in 2009



Source: FRS/ USDA monitor data; compiled July 2011

Food choice among those of lower socio-economic status in particular is influenced by cost. A study in the USA found that when cost per calorie was calculated for sugar and strawberries, the latter was over 1000% higher (**Figure 7.2**) (334). In order to avoid hunger for themselves and their families, most people will seek to purchase calorie dense foods that will make them feel 'full' but which are generally low in micronutrients and fibre and high in refined carbohydrates, fat, salt and sugar.

Figure 7.2 Relation between the energy density of selected foods and energy costs*($/MJ)



*Food prices were obtained from Quality Food Centers supermarket, Seattle, winter 2003. (334).

The authors of a review on food insecurity and chronic disease in the USA highlighted the cyclic nature of food insecurity whereby in times when food supply is more abundant, people tend to consume more than they need in preparation for times when supply is scarce. Food insecurity was associated with a preference for energy dense diets, increased fat mass and reduced lean mass and was associated with risk factors for CVD including hypertension and T2D (335). It has been predicted that in LMICs such as India, food insecurity as

well as globalisation of food markets will contribute significantly to the increase in prevalence of chronic disease including T2D (336).

7.2.2 Migration

In India, as with many LMICs there has recently been rapid migration from rural to urban settings (337). This is an important driver of the nutrition transition and has been accompanied by a change in dietary intake (24;277). The Nutrition Transition has been described as a move towards consuming more processed, sugary, fatty, salty foods and lower consumption of fruit and vegetables and non-processed foods in combination with lower levels of activity. Data from the MMNP and Extension Study indicate that there is an identifiable dietary pattern characterised by frequent consumption of snacks, biscuits and sweets. The increased intake of fatty, salty and sugary snacks is of particular concern with respect to a potential mismatch of diet and physiology which may increase risk of chronic disease. However, this pattern was also characterised by more frequent intakes of fruit which does not fit with many descriptions of the transition diet (24). A study in India which investigated the changing dietary habits of those who migrated from rural to urban areas found that fruit consumption was more frequent among those who lived in urban areas (338). Fruit intake among women in the PMNS (section 1.6.1, p38) was lower than among women in the MMNP pilot study (137). It would seem that in India the transition diet is characterised by more frequent fruit consumption than in other settings. In any case fruit consumption is considerably lower than the recommended five portions per day even in urban areas and perhaps of greatest concern is the very low consumption in rural areas. One possible explanation for these low intakes is cost. It has been reported that exports of fruit from India have increased by 20% between 2010 and 2012 (339). This is likely to contribute to rising prices within India. Another possible explanation for such low intakes is that fruit does not tend to give a feeling of satiety; therefore if funds are limited it is likely that more substantial or 'filling' foods will be chosen.

7.2.3 Global food policy

Macro-policies and changes to the food supply chain may be required to reverse the problems of over consumption of unhealthy foods and under consumption of healthy foods (340). Between January 2006 and July 2008 global food prices rose by an average of 75%, causing an estimated 75 million additional people to become undernourished worldwide (341). These cost increases were not uniform for all foods. After adjusting for general inflation, the price of non-processed foods including fruit, vegetables, and fish has increased steeply over the past two decades whereas the price of processed and energy dense products such as sugar sweetened beverages, snacks, and take away food has fallen (342). Vegetable oils and sugar have remained relatively cheap on global markets, even as overall food prices have risen. Over five years between 2003-8 the global price increase for palm oil was only 4%, whereas wheat rose by 48%, bananas by 64%, and rice by 189% (343). Subsidisation of crops such as sugar and corn in the USA and Europe provides a cheap and abundant supply of ingredients for energy dense processed foods (344;345). These foods can be produced and sold with added value thus creating profit for the manufacturers. It has been reported that the majority of growth in sales of processed foods in the five years to 2011 occurred in LMICs (346). Such added value cannot so readily be applied to raw fruit or vegetables and generally these foods are not subsidised by governments so are more expensive for consumers (342).

Government taxation of high calorie foods and/or subsidisation of healthy foods are currently being piloted in some settings. A recent systematic review of research in this area found that most studies had been conducted in high income countries. The results suggested that fiscal measures could be beneficial to health but to date the evidence base is of poor quality (347).

Wealth inequalities have been increasing over recent decades in India and many LMICS (7). Government policy on taxation and spending on social welfare are mechanisms for reducing or increasing these inequalities. Providing state-funded education and good quality health care to all will generally mean that more resource at the household level is available for expenditure on food.

Compounding the problem of food price increases is price volatility. Financial speculation on commodity derivative markets has been identified as a contributory factor to both price increases and price volatility (341). This is due to food prices being driven by the performance of the markets rather than solely supply and demand (348). Governments, particularly those in high income countries could impose position limits on such trading by financial institutions. However, in October 2012, a European Commission proposal for more stringent limits to food speculation was not supported by members of the European parliament (349).

The World Development Report published in 2008 highlighted the need to increase agricultural productivity and diversity to meet health and development goals (350). The report called for support for agricultural research; improved access to education, credit, and technology for rural farmers; and investment in sustainable production systems. However, in recent years, biofuel production has led to a reduction in the area of land available for food cultivation. It has been claimed by Oxfam that 60% of all global land sold in the past 10 years has been to biofuel producers and 2/3 of these purchases have been made in LMICs (351). Production of biofuels is likely to be beneficial to large scale producers rather than rural farmers and may exacerbate the problem of undernutrition and poor diet quality among the poorest communities.

To date, global food policy has been largely negotiated between transnational food corporations, primarily responsible to their shareholders, and UN agencies including the FAO, the WHO and the World Trade Organisation. While there have been some improvements in transparency recently, the majority of these negotiations are not documented for public scrutiny and the parties are therefore not held to account (352).

Many of the factors affecting dietary intake described in section 7.2 are inter-related and only a multi-sectoral approach is likely to effectively combat the problem of health inequalities associated with a poor diet. Micronutrient deficiencies frequently coexist with infections such as malaria and parasitic worm infestations. Often without treating or preferably preventing these conditions, these deficiencies will persist. The MDG targets related to malaria

and HIV infection will go some way to tackling micronutrient deficiencies but it is important that a 'whole system' rather than a reductionist approach is taken. For example, preventing malaria through the use of bed nets is of course important but is unlikely to eradicate the disease completely. Individuals who are well nourished are likely to suffer less severe symptoms than those who have a poor micronutrient status. Nutrition interventions at the level of the individual are unlikely to be a solution. Improving the health and well-being of the world's poorest communities will require sustained inputs from governments and global governance organisations.

7.3 Concluding remarks

The evidence presented in the first chapter of this thesis shows that there is a growing problem of chronic disease in India including T2D. Poor quality maternal diets combined with offspring growing up in an environment of abundant energy dense food are thought to be major causative factors. The results of a systematic review of studies assessing the effects of fruit and vegetables on the micronutrient status of young women indicates that there has been little research into the potential of fruit and vegetable interventions for improving micronutrient status. There was a particular paucity of data from LMICs. The MMNP and the MMNP Extension Study were designed to assess the effect of a maternal food based supplement on birth outcomes and maternal micronutrient status respectively.

The findings from the Extension Study show that a daily food supplement containing GLV, fruit and milk can increase circulating β-carotene concentrations among slum dwelling women in Mumbai, however it did not have an effect on concentrations of other micronutrients or any of the measured functional outcomes.

The understanding of diet patterns among women in the MMNP (chapter 6) could be used to inform food based dietary guidelines for this population. Knowledge of the patterns could also be useful for designing targeted interventions to improve diet quality. In the context of increased availability of

inexpensive processed foods in India, it will be important to monitor changes in these women's diet patterns longitudinally.

It is clear that there is little data on the effectiveness and efficacy of food-based approaches to improving micronutrient status particularly in LMICs. It is generally thought that synthetic micronutrients are an important tool in preventing and treating micronutrient deficiencies in low resource settings. However, it may be important to consider a combination of food-based approaches with synthetic micronutrients and other interventions to reduce barriers to accessing a quality diet. Bio-fortification is another approach that could be successfully implemented to improve micronutrient intakes where specific deficiencies occur although caution towards reliance on a small number of potentially vulnerable crop species should be applied.

Recently there has been a focus on the 'first 1000 days' from pregnancy until the child's second birthday (353). While this period is of course extremely important, it is likely that a focus on the health, well-being and nutritional status of girls and young women before they become pregnant will be of benefit to the health of themselves and the next generation.

Interventions at the individual level are important but in order to achieve a reduction in the prevalence of NCDs such as T2D, government and global agencies must develop and implement policies aimed at reducing poverty and improving diet quality.

Appendices

Appendix 1

Total soluble oxalic acid and total polyphenol content of GLV samples tested January 2007.

GLV	Soluble oxalate mg/100g (% of total oxalate)*	Total Polyphenol mg/kg
Curry Leaves	ND	41000
Onion Stalk	ND	9040
Colocasia	1294.02 ± 21.36 (25.2%)	10300
Red amaranth	3558.03 ± 80.61 (43.9%)	16500
Green amaranth	4674.65 ± 92.8 (46.5%)	15900
Fenugreek	ND	15500
Coriander	ND	25900
Spinach	11899.83 ± 181.21 (94.6%)	8510
Drumstick	ND	17800
Shepu	ND	26200
Radish Leaves	ND	10900

*Oxalate results are presented on a dry matter basis as means ± standard error. ND = Not detected

Ranking of GLV dehydrated powders by nutrient and anti-nutrient content. Lowest scores reflect the **greatest** nutrient content and the **smallest** anti-nutrient content

Dehydrated GLV powder	Ca	Fe	Mg	Zn	B12	B2	Bcarot	RE	Vit C	FA	Oxalate	Poly-phenol	Total Score (minerals)*	Total Score (minerals and vitamins)
Curry leaves	2	10	6	7	4	11	10	11	7	10	-	11	**36**	**89**
Spinach	11	11	3	2	8	4	5	6	10	9	11	1	**39**	**81**
Shepu	8	4	7	6	6	9	8	9	3	4	-	10	**35**	**74**
Onion Stalk	7	6	9	10	1	8	7	8	6	7	-	2	**34**	**71**
Drumstick	1	7	5	9	7	5	9	10	2	8	-	8	**30**	**71**
Coriander	10	3	10	4	2	6	11	5	4	5	-	9	**36**	**69**
Colocasia	6	9	8	6	10	2	1	1	9	6	8	3	**40**	**69**
Green Amaranth	4	5	1	5	9	10	2	2	11	2	10	6	**31**	**67**
Fenugreek	9	2	11	8	7	1	6	7	1	4	-	5	**35**	**61**
Purple Amaranth	3	1	2	3	5	7	4	4	8	1	9	7	**25**	**54**
Radish Leaf	5	8	4	1	3	3	3	3	5	3	-	4	**22**	**42**

*Total Score (minerals) incorporates the rankings for Ca, Fe, Mg and Zn, NOT the vitamins because the anti-nutrients affect bio-availability of minerals

If the results are interpreted including all of the nutrients in the table, curry leaves and spinach have the lowest content of bio-available nutrients and radish leaves and purple amaranth have the highest.

Appendix 2

Checked by : _____ **Checked by : _____**

SARAS

NUTRIENT STATUS STUDY

REGISTRATION FORM

Name of Interviewer : _____

स्त्री क्रमांक Woman's Serial Number:		
केंद्र क्रमांक Centre No:		केंद्र का नाम Name of the Centre:
Woman's Name: स्त्री का नाम		
Woman's Surname: स्त्री का उपनाम		
Address: पता		

What is your Date Of Birth:
आपकी जन्मतारीख बताईये

Age of the subject if date of birth is not known:
अगर जन्मतारीख पता न हो तो, आपकी आयु

Height (cm)
ऊँचाई

Today's Date
आजकी तारीख

Weight (Kg)
वजन

Name of Health Worker : _____

1. क्या आपके घर पर टेलीफोन है ?

Do you have telephone at your residence: No=0, Yes=1 ☐

2. टेलीफोन नं.

Number: _____

3. धर्म

Religion ☐ (If Other, please specify) : _____

1=Hindu	2=Muslim	3=Christian	4=Sikh	5=Neo-Buddhist	6=Jain	7=Other
हिंदू	मुस्लीम	खिश्चन	सिक्ख	नवबौध्द	जैन	अन्य

4. क्या आप शादीशुदा है ?

Are you Married ? No=0, Yes=1 ☐

5. आपने कितनी पढाई की है ? ☐

What is your education level? _____

6. आपके पती का शिक्षण कितना है ? ☐

What is your Husband's education level? _____

"Education level key"

1	–	Post Graduate	द्वीपदवीधर
2	–	Graduate (B.A, B.Com, B.Sc, i.e. after XIIth Std)	पदवीधर
3	–	Higher Secondary (XIth and XIIth std)	११ वी / १२ वी
4	–	Secondary (Vth to Xth std)	५ वी से १० वी
5	–	Primary (Ist to IVth std)	प्राथमिक
6	–	Any Other	अन्य प्रशिक्षण
7	–	Illiterate	निरक्षर
8	–	Subject does not know	पता नहीं

7. किस माध्यम से पढाई की है ?

In which medium did you study ? ☐ (If other specify) _____

1. English 2. Marathi 3. Hindi 4. Urdu 5. Other

8. मातृभाषा

What is your mother tongue? ☐ (If other specify) _____

1. Marathi 2. Gujrathi 3. Punjabi 4. Bengali 5. Kanada 6. Tamil 7. Telugu
8. Malayalam 9. Konkani 10. Hindi 11. English 12. Other.

9. क्या आप कमाने के लिए कुछ काम करते है ?
Do you do any paid work? 1=Yes 0=No ☐

10. आपके परिवार की कुल मासिक आय कितनी है ? Rs. ☐☐☐☐☐☐
What is the total monthly income of the family?

11. आपका व्यवसाय क्या है ? ☐
What is your occupation?
Specify _____
स्पष्ट करें।

12. अगर आप शादीशुदा है, तो आपके पती का व्यवसाय क्या है ? ☐
If married, what is your Husband's occupation?
Specify _____
स्पष्ट करें।

13. अगर आप शादीशुदा नहीं है, तो आपके पिता का व्यवसाय क्या है ? ☐
If single, what is your father's occupation ?
Specify _____
स्पष्ट करें।

"Occupation level key"

1. - व्यावसायिक (डॉक्टर, वकील, इंजिनीअर, प्राध्यापक, शिक्षक, हिशेब तपासनीस, पुलीस वगैरे)
Professional (Doctor, Lawyer, Engineer, Professor, Teacher, Accountant, Police, etc.)

2. - पदवीधर (कारकून, कंडक्टर, परिचारीका, सेक्रेटरी, रिसेप्शनीस्ट, ब्युटिशीअन)
Graduate Semi-Professional (2nd Clerical, Conductor, Nurse, Secretary, Receptionist, Beautician)

3. - स्वयंरोजगार / स्वयं कोई व्यवसायिक (पान की दुकान, टेलर, खुद का दुकान, रिक्शा मालिक, टॅक्सी मालिक, तरकारी/मच्छी/ फुल बेचनेवाला, दलाल, घरमालिक, इस्टेट दलाल, अन्य)
Self Employed (Pan shop, Tailoring, shop owner, rickshaw owner, taxi owner, vegetable/ fish/flowers vendor, commission agent, house owner, estate agent, other)

4. - कुशल कारागिर (टर्नर, फिटर, सुतार, शिंपी, सुनार, वायरमन, मेकॅनिक अन्य)
Skilled worker (turner, fitter, carpenter, tailor, goldsmith, electrician, mechanic, etc.)

5. - अर्धकुशल कारागिर (सेल्समन, कनिष्ठ लिपिक, सिपाही, रिक्शा चालक, टॅक्सी चालक, प्लंबर, वॉर्ड-बॉय, आया)
Semi-skilled worker (salesman, lower clerical, peon, rickshaw driver, taxi driver, plumber, ward-boy, hospital, ayah)

6. - अकुशल कारागिर (रोजंदारी, घरकाम, दुकान मे नौकर, हमाल)
Unskilled worker (Daily wage labourer, Household work assistant, Loader, Shop assistant, Coolie)

7. - अन्य
Other

8. - पता नहीं
Subject does not know

9. - बेरोजगार
Not Working

Serial No : [. . . . .]

STANDARD OF LIVING INDEX

1. परिवार :
Family Type : [ ] (If other specify) _____
1=Nuclear विभक्त 2 = Joint एकत्र 3 = Other अन्य

2. परिवार में कितने व्यक्ती हैं ?
Number of persons : [ ]

3. आपके परिवार को पिने के लिए पानी कहाँ से मिलता हैं ?
What is the main source of drinking water for members of your household ? [ ]

1. Piped water	2. Hand pump	3. Well	4. Public tap/P. hand pump/P. well
5. River / Sream	6. Tanker	7. Other (Specify) _____	
१. नल	२. हात पंप	३. कुआँ	४. सार्वजनिक नल / हातपंप / कुआँ
५. नदीयाँ / झरा	६. टँकर	७. अन्य	

4. आपके परिवार के लिए किस प्रकार के शौचालय की व्यवस्था हैं ?
What kind of toilet facility does your household have ? [ ]

1. Own flush toilet	2. Shared flush toilet	3. Public flush toilet
4. Own pit toilet / Latrine	5. Shared pit toilet	6. Public pit toilet / Latrine
7. No facility / Bush / Field	8. Others (Specify) _____	
१. खुद का फ्लश का शौचालय	२. कुछ घरों का फ्लश का शौचालय	३. सार्वजनिक फ्लश का शौचालय
४. खुद का गड्ढे का शौचालय	५. कुछ घरों का गड्ढे का शौचालय	६. सार्वजनिक गड्ढे का शौचालय
७. सुविधा नहीं / खेत में	८. अन्य	

5. आपके घर में रोशनी का साधन क्या हैं ?
What is the main source of lighting for your household ? [ ] (If other specify) _____

1. Electricity	2. Kerosene	3. Oil	4. Gas	5. Other
१. बिजली	२. रॉकेल/केरोसीन	३. तेल	४. गॅस	५. अन्य

6. आपके घर में कितने कमरे हैं ?
How many rooms are there in your household ? [ ]

7. क्या आपके घर में अलग से रसोई घर हैं ?
Do you have a separate room that is used as a kitchen ? 1=Yes 0=No [ ]

8. खाना पकाने के लिए आप घर में क्या इस्तेमाल करते हैं ? [ ] (If other specify) _____
What type of fuel does your household mainly use for cooking ?

1. Electricity	2. Wood	3. Crop residues	4. Liquid Petroleum Gas
5. Biogas	6. Coal/Charcoal/Coke	7. Kerosene	8. Other
१. बिजली	२. लकडी	३. सुखे पेड-पौधे	४. एल.पी.जी.
५. बायोगॅस	६. कोयला	७. केरोसिन	८. अन्य

9. क्या आप इस घर या अन्य किसी घर के मालिक हैं?
Does this household own this house or any other house ? 1=Yes 0=No [ ]

10. घर का प्रकार (देखकर लिखिए) :
Type of house (Record Observation) :

Roof छत [ ] Walls दिवारे [ ] Floor जमिन [ ]

1. Pucca पक्का 2. Semi Pucca आधा पक्का 3. Kachha कच्चा

11. क्या आपकी खुद की जमीन हैं ?
Does this household own any agriculture land ? 1=Yes 0=No ☐
If Yes Specify acres _____

12. खुद की जमीन से कितनी जमीन उपजाऊ हैं ?
Out of this how much is irrigated land ?
Specify Acres _____

13. क्या आपका कोई पालतू जानवर जैसे गाय, बैल, बकरी आदी हैं ?
Does this household own any livestock ? 1=Yes 0=No ☐
If Yes Specify Number _____

14. निम्न दी गई वस्तुओं में से कौनसी वस्तुएँ आपकी खुद की हैं ?
Does the household own any of the following ?

1.	Mattress	चटाई	1=YES 0=NO	☐
2.	Pressure cooker	कूकर	1=YES 0=NO	☐
3.	Chair	खुर्सी	1=YES 0=NO	☐
4.	Cot / Bed	पलंग / कॉट	1=YES 0=NO	☐
5.	Table	टेबल	1=YES 0=NO	☐
6.	Clock / Watch	घडी	1=YES 0=NO	☐
7.	Electric fan	बिजली का पंखा	1=YES 0=NO	☐
8.	Bicycle	सायकल	1=YES 0=NO	☐
9.	Radio / Transistor	रेडिओ / ट्रान्सीस्टर	1=YES 0=NO	☐
10.	Television (B&W)	टी.व्ही.	1=YES 0=NO	☐
11.	Television (Colour)	कलर टी.व्ही.	1=YES 0=NO	☐
12.	Moped/Scooter/Motorcycle	स्कूटर/मोपेड/मोटर सायकल	1=YES 0=NO	☐
13.	Car / Jeep	गाडी / जीप	1=YES 0=NO	☐
14.	Water Pump	पाणी का पंप	1=YES 0=NO	☐
15.	Bullock cart	बैलगाडी	1=YES 0=NO	☐
16.	Thresher	मळणी यंत्र	1=YES 0=NO	☐
17.	Tractor	ट्रक्टर	1=YES 0=NO	☐
18.	Refrigarator	फ्रिज	1=YES 0=NO	☐
19.	Telephone	टेलिफोन	1=YES 0=NO	☐
20.	Sewing Machine	सिलाई मशिन	1=YES 0=NO	☐

प्रोजेक्ट क्लार्क का नाम
Project Clerk's Name_____

Appendix 3

Anthropometry protocols

1.1 Height

Height is measured using a stadiometer (CMS Instruments, London). The woman is asked to remove shoes and stand as tall and straight as possible with feet together, arms held loosely by the side and shoulders relaxed with her back, including the posterior surface of the head and heels applied to the wall. The head is positioned in the Frankfurt plane, such that an imaginary line joining the upper margin of the external auditory meatus and the lower border of the orbit of the eye is horizontal. The head plate of the stadiometer is pulled down and placed firmly on the top of the head in a horizontal position. The measurer aims to read the scale from as level a position as possible to minimise the errors due to parallax. The height is read once to the nearest 0.1cm.

1.3 Weight

Weight is measured using an electronic digital weighing scale. The base plate of the scale is placed on the most level and stable piece of ground possible. The monitor is switched on and checked for a 'zero' reading. The woman stands bare-feet on the base plate with no shoes and minimal clothing. One reading to the nearest 100g is taken.

1.4 Head circumference

The woman is asked to stand erect and look straight ahead. A measuring tape is passed firmly around the head such that it passes around the most posterior part on the back and just above the eyebrows anteriorly, and the maximum antero-posterior circumference is thus measured three times to the nearest 0.1cm.

1.5 Abdominal circumference

After ensuring that the abdominal wall is relaxed, the tape is placed horizontally around the abdomen at the level of the umbilicus, taking care not to pull the tape too tight as to indent the skin. Three readings to the nearest 0.1 cm are taken at the end of the expiration.

1.6 Hip circumference

The tape is placed firmly and horizontally at the widest part, usually between the greater trochanter and the lower buttock level with legs and feet together. A minimum of three readings are taken and the largest is recorded.

1.7 Mid upper arm circumference (MUAC)

The woman is asked to stand with her back to the measurer, arm being flexed at 90°. The tip of the acromion (the point of the shoulder) and the olecranon are palpated and a point halfway between them (measured with a tape) marked on the skin. This marks the vertical level at which the skinfold will be made. The woman is then asked to relax, with the arm hanging by the side. The tape is placed around the upper arm such that its upper border is at the level of the marking. The tape should be horizontal all round, should be firmly resting on the skin, but should not be pulled too tight. Three readings to the nearest of 0.1cm are taken.

1.9 Skinfold thickness

The woman should be as relaxed as possible while doing these measurements. Measurements are made with the 'Harpenden' John-Bull callipers (CMS Instruments, London).

1.9.1 Triceps skinfold

The tape is placed round the upper arm at the level of the mark done while measuring MUAC. With the tape in position, a horizontal line is drawn on the skin posteriorly at the level of the mark. Another vertical line is marked on this line at the most dorsal part of the upper arm. This level is determined by 'eyeballing' the mid-point or by a pen held vertically with one end on the olecranon process and the other end pointing towards the acromion. The point at which the fold is to be measured is now marked by a cross, formed by a horizontal line indicating the vertical level, and a vertical line marking the lateral level. The skin is picked up over the posterior surface of triceps muscle, above the cross, on a vertical line passing upward from the olecranon to the acromion. The callipers are applied below the fingers such that the marked cross is at the apex of the fold. The readings are taken at exactly 5 seconds after the application of the callipers jaws. Three readings to the nearest 0.1 mm are taken.

1.9.2 Subscapular skinfold

The inferior angle of the scapula is identified and the skin is marked with a cross immediately below the angle. The skinfold is picked up above the mark with the fold slightly inclined downward and laterally, in the natural cleavage of the skin. The calliper jaws are applied below the fingers, such that the marked cross is at the apex of the fold. Three readings are taken

Appendix 4

Serial No :

NUTRITION PROFORMA

FOOD FREQUENCY QUESTIONNAIRE
(Reference period: Last week)

D1 - Once a day, D2-Twice a day, D3- Thrice a day D4-Four time a day, D5 - Five time a day, D6 - Six times a day
W1 - Once a week, W2-Twice a week,
W3 - thrice a week, W4- four times a week, W5 - 5 times a week and W6- 6 times a week (Encircle for size and thickness in the appropriate box)

Item code	Food-items	पदार्थाचे नांव	Freq	Unit of measurement (don't enter data here)	Number of units consumed (enter only nos. in this column)	Size (circle)	Thickness
A	**BEVERAGES**	**पेये**					
1	TEA (WITHOUT MILK)	चहा (कोरा)		cup			
2	TEA (WITH QUARTER MILK)	चहा (पाव दुधाचा)		cup			
3	TEA (WITH HALF MILK)	चहा (अर्ध्या दुधाचा)		cup			
4	TEA (WITH FULL MILK)	चहा (पुर्ण दुधाचा)		cup			
5	COFFEE (WITH MILK)	कॉफी (पुर्ण दुधाची)		cup			
6	OTHERS 1, SPECIFY:	इतर 1 नमुद करा		cup			
7	OTHERS 2, SPECIFY:	इतर 2 नमुद करा		cup			
B	**BAKERY PRODUCTS**	**वेकरी पदार्थ**					
1	PAV	पाव		Number			
2	BREAD SLICE	ब्रेड		Number			
3	TOAST (RUSK)	टोस्ट		Number		S / M / B	
4	KHARI	खारी		Number		S / M / B	
5	BUTTER BISCUIT	बटर बिस्किटस		Number		S / M / B	
6	BISCUITS: SWEET (E.G GLUCOSE / PARLE-G/TIGER) (SPECIFY BRAND:)	बिस्किटस : गोड (उदा. ग्लुकोज पार्ले-जी/टाईगर)		Number			
7	BISCUITS: SALTED (E.G MONACO / 50-50 / KRACKJACK / CHASKAMASKA) (SPECIFY BRAND:)	बिस्किटस : खारे (उदा. मोनॅको /५०-५०/क्रेकजॅक/चस्का-मस्का) नमुद करा		Number			

13

Item code	Food-items	पदार्थाचे नांव	Freq	Unit of measurement (don't enter data here)	Number of units consumed (enter only nos. in this column)	Size (circle)	Thickness
8	BISCUITS:CREAM (SPECIFY BRAND:)	बिस्किटस : क्रिम नमुद करा		Number			
9	OTHERS 1, SPECIFY:	इतर 1 नमुद करा		Number			
10	OTHERS 2, SPECIFY:	इतर 2 नमुद करा		Number			
C	**CHAPATI/ROTI**	**चपाती / भाकरी**					
1	JOWAR FLOUR BHAKRI	ज्वारीच्या पीठाची भाकरी		Number		S / M / B	T1 / T2
2	CHAPATI (WHEAT FLOUR)	चपाती		Number		S / M / B	T1 / T2
3	PURI (WHEAT FLOUR / MAIDA / RICE)	पुरी (कणीक/ मैदा/ तांदुळाचे पीठ)		Number		S / M / B	T1 / T2
4	RICE FLOUR BHAKRI	तांदळाच्या पीठाची भाकरी		Number		S / M / B	T1 / T2
5	RICE FLOUR + JOWAR FLOUR BHAKRI	तांदुळ + ज्वारीच्या पीठाची भाकरी		Number		S / M / B	T1 / T2
6	RICE FLOUR + JOWAR FLOUR + BLACK GRAM DAL FLOUR BHAKRI	तांदुळ पीठ + ज्वारीचे पीठ + उडीद डाळ पीठाची भाकरी		Number		S / M / B	T1 / T2
7	OTHERS 1, SPECIFY:	इतर 1 नमुद करा		Number		S / M / B	T1 / T2
8	OTHERS 2, SPECIFY:	इतर 2 नमुद करा		Number		S / M / B	T1 / T2
D	**RICE**	**भात**					
1	RICE PLAIN	साधा भात		FlatLdl			
2	RICE SEASONED	फोडणीचा भात		FlatLdl			
3	MASALA RICE/PULAO	मसाले भात/ पुलाव		FlatLdl			
4	VEGETABLE BIRIYANI	व्हेज बिर्यानी		FlatLdl			
5	CHINESE FRIED RICE	चायनीज फ्राइड राईस		FlatLdl			

Item code	Food-items	पदार्थाचे नांव	Freq	Unit of measurement (don't enter data here)	Number of units consumed (enter only nos. in this column)	Size (circle)	Thickness
6	KHICHADI (RICE + ANY DAL)	खिचडी (भात + कुठलीही डाळ)		FlatLdl			
7	VEGETABLE NOODLES	व्हेज नुडल्स		FlatLdl			
8	DHIRIDE (BENGAL GRAM FLOUR + TOMATO)	धिरडे (बेसन + टॉमेटो)		Number		S / M / B	T1 / T2
9	AMBOLI	आंबोळी		Number		S / M / B	T1 / T2
10	OTHERS 1, SPECIFY:	इतर 1 नमुद करा					
11	OTHERS 2, SPECIFY:	इतर 2 नमुद करा					
E	**PULSE (DAL)**	**डाळ**					
1	(RED GRAM)-PLAIN	तुरीची डाळ		Cup Ldl			
2	RED GRAM + TOMATO + ONION	तुरीची डाळ + टोमॅटो + कांदा		Cup Ldl			
3	GREEN GRAM + ONION + TOMATO	मुगाची डाळ + टोमॅटो + कांदा		Cup Ldl			
4	GREEN GRAM	मुगाची डाळ		Cup Ldl			
5	LENTIL DAL + ONION + TOMATO	मसुर डाळ + टोमॅटो + कांदा		Cup Ldl			
6	BLACK GRAM + TOMATO + ONION	उडीद डाळ + टोमॅटो + कांदा		Cup Ldl			
7	DRUMSTICK CURRY + ONION + TOMATO	शेवग्याच्या शेंगाची आमटी + टोमॅटो + कांदा		Cup Ldl			
8	PITHALE (BENGAL GRAM FLOUR + ONION + TOMATO)	पिठले (बेसन पीठ + टोमॅटो + कांदा)		Cup Ldl			
9	PITHALE/PITHI (HORSE GRAM FLOUR TOMATO + ONION)	पीठले/ पिठी (कुळीथ पीठ + टोमॅटो + कांदा)		Cup Ldl			

Item code	Food-items	पदार्थाचे नांव	Freq	Unit of measurement (don't enter data here)	Number of units consumed (enter only nos. in this column)	Size (circle)	Thickness
10	WADE (RICE FLOUR + JOWAR FLOUR+ RED GRAM DAL FLOUR+ DAL FLOUR + BLACK GRAM DAL FLOUR)	वडे (तांदुळ पीठ + ज्वारीचे पीठ + तुर + चणा डाळ + उडीद डाळ)		Number		S / M / B	T1 / T2
11	OTHERS 1, SPECIFY:	इतर 1 नमुद करा					
12	OTHERS 2, SPECIFY:	इतर 2 नमुद करा					
F	**LEGUMES (USAL / CURRY)**	**कडधान्ये (आमटी / उसळ)**					
1	BENGAL GRAM WHOLE + TOMATO + ONION	हरभरा + टोमॅटो + कांदा		Cup Ldl			
2	MOTH BEAN + ONION+ TOMATO	मटकी + टोमॅटो + कांदा		Cup Ldl			
3	GREEN GRAM WHOLE + ONION + TOMATO	मुग + टोमॅटो + कांदा		Cup Ldl			
4	KIDNEY BEANS (ONION+TOMATO)	राजमा + टोमॅटो + कांदा		Cup Ldl			
5	COWPEA + ONION + TOMATO	चवळी + टोमॅटो + कांदा		Cup Ldl			
6	LENTIL WHOLE + TOMATO + ONION	मसूर + टोमॅटो + कांदा		Cup Ldl			
7	FIELD BEANS + TOMATO + ONION	वाल + टोमॅटो + कांदा		Cup Ldl			
8	PEAS GREEN / WHITE / BLACK	वाटाणा (सफेद / हिरवा / काळा)		Cup Ldl			
9	MIX USAL / DAL (ALL PULSES MIX)	मिक्स उसळ / (सर्व प्रकारच्या डाळी)		Cup Ldl			
10	OTHERS 1, SPECIFY:	इतर 1 नमुद करा		Cup Ldl			
11	OTHERS 2, SPECIFY:	इतर 2 नमुद करा		Cup Ldl			

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G	OTHER VEGETABLES	फळभाज्या					
1	POTATO-DRY	बटाटा सुकी		BSp			
2	POTATO- GRAVY (ONION + TOMATO + GARLIC)	बटाटा रस (कांदा + टोमॅटो + लसूण)		BSp			
3	POTATO + CAULIFLOWER	बटाटा + फ्लॉवर		BSp			
4	POTATO + BRINJAL+ TOMATO + ONION	बटाटा + वांगी + टोमॅटो + कांदा		BSp			
5	POTATO+ CABBAGE	बटाटा + कोबी		BSp			
6	POTATO+ LADIES FINGER	बटाटा + भेंडी		BSp			
7	POTATO +FIELD BEANS	बटाटा + वाल		BSp			
8	POTATO+ GREEN PEAS	बटाटा + मटर		BSp			
9	POTATO+ CLUSTER BEANS	बटाटा + गवार		BSp			
10	BRINJAL +ONION (DRY/ROAST)	वांगी + कांदा (सुकी / भाजलेली)		BSp			
11	BOTTLE GOURD +ANY DAL	दुधी भोपळा + कुठलीही डाळ		BSp			
12	CABBAGE +ANY DAL	कोबी + कुठलीही डाळ		BSp			
13	CAPSICUM (STUFFED WITH BENGAL GRAM FLOUR / SPRINKLED WITH BENGAL GRAM FLOUR)	भोपळी मीरची (बेसन भरुन / शीजवलेली)		BSp			
14	SNAKEGOURD+BENGALGRAMDAL /BEANS (FIELD BEANS)	पडवळ + चणा डाळ + वाल		BSp			
15	BITTERGOURD+ONION+TOMATO	कारले + कांदा + टोमॅटो		BSp			
16	YAM + ONION + TOMATO	सुरण + कांदा + टोमॅटो		BSp			

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17	RIDGE GOURD + ONION + TOMATO + BENGAL GRAM DAL	शिराळी / दोडका + कांदा + टोमॅटो + चणा डाळ		BSp			
18	YELLOW PUMPKIN +ONION	पिवळा भोपळा + कांदा		BSp			
19	TOMATO + ONION / TOMATO CURRY	कांदा + टोमॅटो / *टोमॅटो करी*		BSp			
20	COCONUT (DRY/FRESH)	खोबरे (सुके / ताजे)		Spoon		tbsp	
21	COCCINIA DRY	तोंडली सुकी		BSp			
22	COCCINIA + ANY DAL	तोंडली सुकी + कुठलीही डाळ		BSp			
23	OTHERS 1, SPECIFY:	इतर 1 नमुद करा		BSp			
24	OTHERS 2, SPECIFY:	इतर 2 नमुद करा		BSp			
H	**GREEN LEAFY VEGETABLES**	**हिरव्या पालेभाज्या**					
1	SPINACH - DRY	पालक सुकी		BSp			
2	SPINACH +POTATO	पालक + बटाटा		BSp			
3	SPINACH + COTTAGE CHEESE (PANEER)	पालक पनीर		BSp			
4	SPINACH + ANY DAL	पालक + कोणतीही डाळ		BSp			
5	FENUGREEK-DRY	मेथी सुकी		BSp			
6	FENUGREEK + POTATO	मेथी + बटाटा		BSp			
7	FENUGREEK + LENTIL (MOONG DAL)+ONION+TOMATO	मेथी + मुग डाळ + कांदा + टोमॅटो		BSp			
8	GREEN AMARANTH + ONION	चवळी + कांदा		BSp			

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9	RED AMARANTH (MATH)+ ONION + TOMATO	माठ + कांदा + टोमॅटो		BSp			
10	ONION STALK + BENGAL GRAM FLOUR	कांद्याची पाती + बेसन		BSp			
11	COLOCASIA (AALUU PAN) + BENGAL GRAM FLOUR	अळूची पाने + बेसन		Cup Ldl			
12	COLOCASIA WADI	अळूवडी		Number			
13	COLOCASIA + FIELD BEAN + GROUNDNUT/ANY DAL	अळू + वाल + शेंगदाणे+ कोणतीही डाळ		BSp			
14	RADISH LEAVES + ONION + GREEN GRAM DAL	मुळ्याची पाने + कांदा + मुग डाळ		BSp			
15	CORIANDER WADI	कोथिंबीर वडी		Number			
16	DRUMSTICK LEAVES + ONION + ANY DAL	शेवग्याची पाने + कांदा + कोणतीही डाळ		BSp			
17	DILL LEAVES + BENGAL GRAM FLOUR	शेपू + बेसन		BSp			
18	DILL LEAVES + ONION + ANY DAL	शेपू + कांदा + कोणतीही डाळ		BSp			
19	CORIANDER GARNISH	कोथिंबीरीची पाने		Spoon		tsp	
20	OTHERS 1, SPECIFY:	इतर 1 नमुद करा					
21	OTHERS 2, SPECIFY:	इतर 2 नमुद करा					
I	**CHUTNEY**	**चटणी**					
1	GARLIC + RED CHILLI	लसूण + अख्खी लाल मीरची		Spoon		tsp / tbsp	
2	GARLIC + RED CHILLI+ DRY COCONUT	लसूण + अख्खी लाल मीरची + सुके खोबरे		Spoon		tsp / tbsp	

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3	GROUNDNUT + GARLIC + CHILLI	शेंगदाणा + लसूण + मीरची		Spoon		tsp / tbsp	
4	COCONUT + GREEN CHILLI + GARLIC + GINGER	खोबरे + हिरवी मीरची + लसूण + आले		Spoon		tsp / tbsp	
5	CORIANDER + GREEN CHILLI + GINGER + GARLIC	कोथिंबीर + हिरवी मीरची + आले + लसूण		Spoon		tsp / tbsp	
6	PICKLE (LEMON/RAW MANGO ETC)	लोणचे (लिंबू, कैरी इ.)		Spoon		tsp / tbsp	
7	PAPAD	पापड		Number			
8	OTHERS 1, SPECIFY:	इतर 1 नमुद करा					
9	OTHERS 2, SPECIFY:	इतर 2 नमुद करा					
J	**FRUITS**	**फळे**					
1	APPLE	सफरचंद		Piece			
2	GUAVA	पेरु		Piece			
3	ORANGE	संत्रे		Piece			
4	SWEET LIME	मोसंबी		Piece			
5	BANANA	केळी		Number			
6	SAPOTA	चिकू		Piece			
7	RAW MANGO CUT	कैरी		Piece			
8	RIPE MANGO CUT	पिकलेला आंबा		Piece			
9	MANGO PULP	आमरस		Cup Ldl			
10	BERRIES (JAMUN, ZIZYPHUS, KARVANDE, CHERRIES)	बोरे (जांभूळ / चेरी / करवंदे / बोरे)		Number			

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11	SAFED JAMB	सफेद जाम		Number			
12	JACK FRUIT	फणस		Number			
13	PEAR	पेर (नासपती)		Piece			
14	GRAPES	द्राक्षे		Number			
15	PINEAPPLE	अननस		Slice			
16	WATERMELON	कलींगड		Slice			
17	POMEGRANATE	डाळींब		Piece			
18	PAPAYA	पपई		Slice			
19	CUSTARD APPLE	सीताफळ		Piece			
20	FRUIT SALAD	फ्रुट सॅलड		Bsp			
21	LEMON	लिंबू		Piece			
22	OTHERS 1, SPECIFY:	इतर 1 नमुद करा					
23	OTHERS 2, SPECIFY:	इतर 2 नमुद करा					
K	**MILK PRODUCTS**	**दुधाचे पदार्थ**					
1	MILK (BUFFALO)	म्हशीचे दुध		Glass			
2	MILK (COW)	गाईचे दुध		Glass			
3	CURDS	दही		Spoon		tsp / tbsp	
4	BUTTERMILK	ताक		Glass			
5	KADHI	कढी		Cup Ldl			

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6	LASSI	लस्सी		Glass			
7	ICE-CREAM/KULFI	आईस्क्रीम / कुल्फी		Cup/cone			
8	CHOCOLATE (SPECIFY BRAND:)	चॉकलेट नमुद करा		Piece			
9	OTHERS 1, SPECIFY:	इतर 1 नमुद करा					
10	OTHERS 2, SPECIFY:	इतर 2 नमुद करा					
L	**SALADS**	**कोशिंबीर**					
1	TOMATO +ONION	टोमॅटो + कांदा		Spoon		tsp / tbsp	
2	TOMATO + CUCUMBER	टोमॅटो + काकडी		Spoon		tsp / tbsp	
3	TOMATO + CUCUMBER + ONION	टोमॅटो + काकडी + कांदा		Spoon		tsp / tbsp	
4	CABBAGE + ONION + CARROT + TOMATO	कोबी + कांदा + गाजर +टोमॅटो		Spoon		tsp / tbsp	
5	CUCUMBER	काकडी		5 inch portion			
6	OTHERS 1, SPECIFY:	इतर 1 नमुद करा					
7	OTHERS 2, SPECIFY:	इतर 2 नमुद करा					
M	**RAITA**	**रायते**					
1	ONION RAITA	कांदा रायता		Spoon		tsp / tbsp	
2	CUCUMBER RAITA	काकडी रायता		Spoon		tsp / tbsp	
3	BOONDI RAITA	बूंदी रायता		Spoon		tsp / tbsp	
4	OTHERS 1, SPECIFY:	इतर 1 नमुद करा		Spoon		tsp / tbsp	
5	OTHERS 2, SPECIFY:	इतर 2 नमुद करा		Spoon		tsp / tbsp	

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N	**CARBONATED AND OTHER DRINKS**	**शितपेये आणि इतर पेये**					
1	FROZEN PEPSI COLA / ICE CANDY STICK	पेप्सीकोला / आईसकँडी		Number			
2	SHERBET	सरबत		Glass			
3	SUGARCANE JUICE	ऊसाचे सरबत		Glass			
4	PEPSI/FANTA/THUMBSUP /MIRANDA	पेप्सी / फँटा / थम्स अप / मिरींडा		Glass			
5	FROOTI/APPY/LITCHI DRINK	फ्रूटी / ॲप्पी / लीची		Glass			
6	ORANGE JUICE/LEMON JUICE/ SWEET LIME JUICE/ PINEAPPLE JUICE	संत्रारस / लिंबूपाणी / मोसंबीरस / अननसरस		Glass			
7	COCONUT WATER	नारळाचे पाणि		Glass			
8	OTHERS 1, SPECIFY:	इतर 1 नमुद करा					
9	OTHERS 2, SPECIFY:	इतर 2 नमुद करा					
O	**SPICY SNACKS**	**तिखट पदार्थ**					
1	SEMOLINA SPICY	तिखट सांजा		Dish			
2	UPPIT (RICE FLOUR / WHEAT FLOUR)	उपीट (तांदूळ रवा / गहू रवा)		Dish			
3	RICE FLAKES	पोहे		Dish			
4	BHAJIA (PAKODA)	भजी		Number			
5	BHAJIA PAV	भजी पाव		Number			
6	POTATO WADA	बटाटा वडा		Number			

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7	POTATO WADA + PAV	बटाटा वडा + पाव		Number			
8	SAMOSA	समोसा		Number			
9	SAMOSA + PAV	समोसा + पाव		Number			
10	BHELPURI	भेळपूरी		Dish			
11	SEVPURI	शेवपूरी		Number			
12	PANI PURI	पाणीपूरी		Number			
13	RAGDA PATTICE	रगडा पॅटिस		Number			
14	IDLI	इडली		Number			
15	MASALA DOSA	मसाला डोसा		Number			
16	MEDU WADA	मेंदू वडा		Number			
17	SANDWICH	सॅन्डविच		Number			
18	FARSAN /SEV/ CHIVDA	फरसाण / शेव / चिवडा		Dish			
19	OTHERS 1, SPECIFY:	इतर 1 नमुद करा					
20	OTHERS 2, SPECIFY:	इतर 2 नमुद करा					
P	**SWEET FOODS**	**गोड पदार्थ**					
1	SWEET SEMOLINA (SHEERA)	शिरा		Dish			
2	PURANPOLI	पुरणपोळी		Number			
3	SHRIKHAND	श्रीखंड		Spoon		tsp / tbsp	
4	GULABJAMUN	गुलाबजाम		Number			

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5	RICE/VERMICELLI / RAVA (PORRIDGE)	तांदळाची / शेवई / रवा खिर		Cup Ldl			
6	TIL LADDU /CHIKKI	तिळाचे लाडू / चीक्की		Number			
7	KARANJI / MODAK	करंजी / मोदक		Number			
8	JELEBI	जीलेबी		Number			
9	PEDHA/BURFI	पेढा / बर्फी		Number			
10	LADDU (BESAN / RAVA ETC)	लाडू (बेसन / रवा इत्यादी)		Number			
11	CARROT HALVA	गाजर हलवा		Spoon		tsp / tbsp	
12	BOTTLE GOURD HALVA	दुधी हलवा		Spoon		tsp / tbsp	
13	BASUNDI	बासुंदी		Cup Ldl			
14	OTHERS 1, SPECIFY:	इतर 1 नमुद करा					
15	OTHERS 2, SPECIFY:	इतर 2 नमुद करा					
Q	**NON - VEG**	**नॉन व्हेज**					
1	GOSHT KURMA	गोश्त कुर्मा		Cup Ldl			
2	KHEEMA (KOFTA / KABAB)	खीमा (कोफ्ता / कबाब)		Number			
3	KHEEMA SALAN (CURRY)	खीमा रस्सा		Cup Ldl			
4	LIVER SALAN (CURRY)	लीव्हर रस्सा		Cup Ldl			
5	CHICKEN SALAN (CURRY)	कोंबडी रस्सा		Cup Ldl			
6	BIRYANI (MUTTON / CHICKEN)	बिर्याणी (मटण / कोंबडी)		FlatLdl			

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7	KHICHADA (ALL DALS + WHEAT + RICE + GOAT MEAT + LEMON)	खीचडी (सर्व डाळी + गहू + तांदूळ + बकऱ्याचे मटण + लींबू)		FlatLdl			
8	GOSHT PULAV (RICE +GOAT MEAT +CURD + PUDINA)	गोश्त पुलाव (तांदूळ + दही + पुदीना + बकऱ्याचे मटण)		FlatLdl			
9	CHICKEN LOLLYPOP / FRY / CHICKEN PIECES	चीकन लॉलीपॉप / तळलेले / चीकन तुकडे		Number			
10	VAJDI	वजडी		Bsp			
11	STUFFED BITTER GOURD (KHEEMA)	खिमा भरलेले कारले		Number			
12	SHEEK KABAB (KHEEMA+ EGG +BESAN)	शीक कबाब (खीमा + अंडे + बेसन)		Number			
13	BHUNA / TANDORI GOSHT (ROASTED MEAT)	भाजलेलं मटण		Number			
14	BHUNA / TANDORI CHICKEN (ROASTED CHICKEN)	भाजलेली कोंबडी		Number			
15	DAL GOSHT (RED GRAM DAL + MEAT + BOTTLE GOURD)	डाळ गोश्त (तुर डाळ + मटण + दुधी भोपळा)		Cup Ldl			
16	CHICKEN CURRY (ONION + POTATO)	कोंबडीचं कालवण (कांदा + बटाटा)		Cup Ldl			
17	MUTTON CURRY (ONION + POTATO)	मटणाचं कालवण (कांदा + बटाटा)		Cup Ldl			
18	EGG CURRY (TOMATO + ONION)	अंड्याचं कालवण (टोमॅटो + कांदा)		Cup Ldl			
19	EGG BHURJI	अंड्याची भुरजी		Bsp			
20	EGG (BOILED/HALF FRY / OMELETTE)	अंडे (उकळलेले / हाफ फ्राय / पोळी)		Number			

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21	JAVLA FISH SUKAT (TOMATO + ONION)	जवळा माश्याचे सुके (टोमॅटो + कांदा)		BSp			
22	JAVLA FISH BHAJIA	जवळा माश्याची भजी		Number			
23	POMFRET FRY	पापलेट तळलेले		Number			
24	SURMAI FRY	सुरमई तळलेली		Number			
25	PRAWN FRY / DRY	कोळंबी तळलेली / *सुकी*		Number			
26	BOMBIL FRY	बोंबील तळलेले		Number			
27	HALWA FRY	हलवा तळलेला		Number			
28	BANGDA FRY	बांगडा तळलेला		Number			
29	CRAB CURRY	खेकड्याची आमटी		Cup/Ldl			
30	FISH CURRY (ANY FISH EXCEPT PRAWN) (ONION + TOMATO)	माशाची आमटी (कोळंबी व्यतिरिक्त कुठलीही) (कांदा +टोमॅटो)		Cup/Ldl			
31	BOMBAY DUCK + BRINJAL	बोंबील + वांगी		Cup/Ldl			
32	KARDI	कर्दी		BSp			
33	PRAWN BIRYANI	कोळंबी बिर्याणी		FlatLdl			
34	OTHERS 1, SPECIFY:	इतर 1 नमुद करा					
35	OTHERS 2, SPECIFY:	इतर 2 नमुद करा					
R	**MASALA PASTE**	**मसाले वाटण**					
1	GINGER GARLIC PASTE	लसूण आले वाटण		Spoon		tsp / tbsp	
2	TOMATO PUREE / PASTE	टोमॅटोचो रस / वाटण		Spoon		tsp / tbsp	

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3	ONION PASTE	कांद्याचे वाटण		Spoon			
S	**SWEET SPECIALS**	**खास गोड पदार्थ**					
1	MALIDA (WHEAT FLOUR + SEMOLINA+MILK+GHEE+SUGAR)	मलिदा (गव्हाचे पीठ + रवा + दुध + तुप + साखर)		Number			
2	DATES (KHAJUR / KHARIK / APRICOT / RAISIN / FIG)	खजुर /खारिक / मनुका / अंजीर		Number			
4	SHEER KURMA (SEVIYA KHEER)	शिरखुर्मा (शेवयांची खीर)		Cup Ldl			
5	FALOODA (MILK + SUGAR + KHOA + COCONUT DRY)	फालुदा (दुध + साखर + खवा + सुके खोबरे)		Glass			
6	MALPUA	मालपुआ		Number			
7	CHINA GRASS	चायना ग्रास		Cup Ldl			
8	SWEET ROTI (RAVA + GHEE +SUGAR)	गोड पोळी (रवा + तुप + साखर)		Number			
9	OTHERS 1, SPECIFY:	इतर 1 नमुद करा					
10	OTHERS 2, SPECIFY:	इतर 2 नमुद करा					

Appendix 5

Extension Study Information Sheet

SARAS

MRC-CSSC-SNEHA

PROJECT INFORMATION SHEET

The aim of our project is to improve the health of young women in Mumbai. Poor nutrition of young women is a common cause of ill health. In order to decide whether food supplementation can improve health, and which foods will be best, there is a need for a proper research study.

SARAS will study the effect of 4 nutritional supplements on the health of young women. Those who volunteer to participate will consume the supplement six days per week for 3 months. The 4 supplements contain different combination of calories, proteins, vitamins and minerals. They will be made as palatable and tasty as possible but their main purpose is for health rather than enjoyment or pleasure. Please note that these are only supplements – this means that your normal daily diets should continue as usual.

Which of the 4 supplements is best will be known to us at the end of the study analysis and only one or two experts will know the precise constituents of these supplements. However, they are all made from locally available foods and are completely vegetarian.

IF YOU VOLUNTEER TO PARTICIPATE

At first, your medical history will be recorded by a health worker or project clerk. A nutritionist will note down information about your daily food intake and activity/workload. A trained researcher will take measurements of your

weight, height and skin folds thickness. A qualified nurse will take a small sample of blood to test for nutrient levels before supplementation starts. The research team will also ask you to do some very simple tasks to get some information about your health. All of this will take about 1-2 hours of your time. All this information will be kept secure and confidential. You should therefore give all information accurately and truthfully without fear. When you join the project, you will be photographed for making your project identity card.

Two weeks after the above, your supplementation can start. This will take place at the designated community centre, six days a week (Monday to Saturday) between the hours of 3-6pm. The project clerk will give you the supplement and we will ask you to eat it on the spot. The project clerk will check your identity card and ensure that you get the correct supplement and will also record the quantity of supplement that you have eaten.

We ask that you come to the centre and eat the supplement every day for the full three months. This will best allow us to find out how the supplement improves your health.

We will be taking a very small sample of blood from you so that we can test the levels of nutrients in your blood. We will be looking at levels of nutrients such as iron, vitamin A and vitamin C. We will quickly provide you with a report about your blood count which will allow you to be treated for anaemia if necessary. We will take three blood samples from you in total. One at the start of the study, one more after 6 weeks and a final sample at the end of the study. The blood samples will be taken by a qualified experienced nurse.

When this project is completed and analysed, we shall organize a special meeting for all of you and inform you the results of the analysis and give further guidance publicly and definitely.

We, therefore request all women who participate in the study to co-operate fully and help us to promote the interests and health of all young women.

WHAT YOU NEED TO DO

- If you decide that you would like to participate in the project, please inform the health worker and she will advise you when you will be first required to attend for registration

- Attend the community centre on the assigned day when the nurse and other research team will be there to interview you and take measurements. We will provide refreshments for you on these days.

- Please make arrangements to come for supplementation on **all of the 6 days**. The supplement is for **YOU** alone – it is designed for women and should not be shared with others, even your children. You have been assigned to receive one of four types of supplement – you should not eat somebody else's supplement, nor give your supplement to other women. You will not be able to change the type of supplement – but should stick to the same type of supplement throughout the project.

HELPLINE TELEPHONE NO:

HEALTH WORKER TELEPHONE NO:

Appendix 6

Mumbai Maternal Nutrition Project – Extension Study

CONSENT FORM

- The aims and procedure of the study have been explained to me by the research team
- I am aware that I have the right to refuse/ withdraw my participation from the study at any time and that there will be no adverse consequences or penalty if I do so
- I understand that taking part in this study will not cause me any harm
- I understand that the research team are responsible for ensuring that the supplements are safe for me to consume
- I understand that the data collected during the study will be stored securely and will remain confidential. The result of my personal details will be kept confidential but pooled results will be used for dissemination of the study findings
- I will not gain financially for my participation in the project and will not claim any compensation for participation.
- I will allow blood samples to be taken and understand that these samples can be stored for future use
- With the considerations above in mind, I hereby give my voluntary consent to participate in this study

Signature_____

Print Name_____

Date_____

Appendix 7

GRIP STRENGTH DATA ENTRY SHEET

Date			

Grip strength (kg)
3 X both sides alternately
(record to nearest 1kg)

RIGHT		
LEFT		

Which hand do you <u>mostly</u> write with

Left ☐

Right ☐

Ambidextrous ☐

(N.B can <u>write</u> with both hands)

Observer _____

Appendix 8

General Health Questionnaire (GHQ12)

मानसिक स्वास्थ प्रश्नावली (जी.एच.क्यू. १२)

I'm going to ask about your health in the last two weeks
मै आपको आपके शारीरिक तकलीफों के बारे में पुछूँगी / पिछले दो हफ्तों में आपका स्वास्थ कैसा रहा है इसके बारे में मै जानना चाहती हूँ ।

Instruction:
निर्देश :-
Question number**1, 3, 4, 6, 7, 8, 12**: If the answer is: **No** =1 **Yes** = 0
प्रश्न क्रमांक १, ३, ४, ६, ७, ८, १२ का जवाब ना है तो १ और हाँ है तो ० लिखिए ।

Question number**2, 5, 9, 10, 11:** If the answer is: **No** =0 **Yes** = 1
प्रश्न क्रमांक २, ५, ९, १०, ११ का जवाब हाँ है तो १ और ना है तो ० लिखिए ।

OR या
Questions in the shaded rows: If the answer is: **No** =1 **Yes** = 0
रंग भरे (शेडेड) सवाल का जवाब ना है तो १ और हाँ है तो ० लिखिए ।

Questions in the non-shaded rows: If the answer is: **No** =0 **Yes** = 1
बिना रंग के सवाल का जवाब हाँ है तो १ और ना है तो ० लिखिए ।

In the last two weeks have you:
इन दो हफ्तों मे आप :-

GHQ1.	आप जो कुछ करते हैं उसमें आपका ध्यान लगता है ? **been able to concentrate on whatever you're doing ?**	
GHQ2.	क्या जादा चिंता के कारण आपकी नींद कम हुई है ? **lost much sleep over worry ?**	
GHQ3.	क्या आपको लगता है कि आप कोई भी उपयोगी / फायदेमंद काम कर सकते हैं ? **felt that you are playing a useful part in things ?**	
GHQ4.	क्या आप कोई भी फैसला ले सकते हैं ? **felt capable of making decisions about things ?**	
GHQ5.	क्या आप हमेशा किसी दबाव में रहते हैं ? **felt constantly under strain ?**	
GHQ6.	क्या आपको लगता है कि आप अपनी तकलीफों से बाहर आ सकते हैं ? **felt you could overcome your difficulties ?**	
GHQ7.	क्या आपको रोज का काम करने में खुशी मिलती हैं ? **been able to enjoy your normal day-to-day activities ?**	

GHQ8.	क्या आप अपनी मुश्किलों का सामना कर सकते हैं ? **been able to face up to your problems ?**	
GHQ9.	क्या आपको लगता है कि आप दु:खी और निराश हैं ? **been feeling unhappy and depressed ?**	
GHQ10.	क्या आपको लगता है कि आपका आत्मविश्वास कम हुआ है ? **been losing confidence in yourself ?**	
GHQ11.	क्या आप ऐसा सोचते है कि आपकी कोई किमत नहीं है ? **been thinking of yourself as a worthless person ?**	
GHQ12.	इन सब बातों को ध्यान में रखते हुए क्या आपको लगता है कि आप सुखी है ? **been feeling reasonably happy, all things considered ?**	
	कुल अंक **Total**	

Appendix 9

(Government of Maharashtra)

Institutional Ethics Committee

Department of Pharmacology,
Grant Medical College & Sir J.J. Group of Hospitals,
Byculla, MUMBAI 400 008
Ph: +91-22-2373 5555 Extn. 2322

No.IEC/Pharm/ 482 /09, Dt. 7/10/2009.

To,
Dr.R.D.Potdar,
Hon.General Secretary,
Centre for the Study of Social Change,
M.N.Roy Human Development Campus,
'C' Wing, Plot No.6, 'F' Block,
Opp.,Govt. Colony, Bldg.No.326,
Bandra (East), Mumbai-51.

Dear Dr.

The Institutional Ethics Committee of Grant Medical College and Sir J.J. Group of Hospital ,Mumbai, reviewed and discussed your application to conduct the Project entitled ""Extension of MMNP - "Proposed project entitled: "The effect of a micronutrient rich food supplement on Indian women's health and nutrient status".

The documents submitted by you, were reviewed by the IEC.

The following members of the Institutional Ethics Committee were present at the meeting held on 06/10/2009 at Department of Pharmacology, Grant Medical College and Sir J.J.Gr.of Hospitals,Mumbai.

Name of Members	Qualification	Affiliation/Designation within EC
1) Dr.Danial Joseph	M.D. Pharmacology	Professor of Pharmacology, Mahatma Gandhi Medical College, Navi Mumbai. Chairman, IEC.

2) Dr.A.D.Rathod	M.D. Paediatric D.M. Cardiology.	Professor & HOD Paediatric, Grant Medical College, Mumbai. Member, IEC.
3) Dr.M.B.Tayade	M.S. Gen.Surgery	Professor & HOD Surgery, Grant Medical College and Sir J.J.Group of Hospitals, Mumbai. Member, IEC.
4) Smt F.M.Merchant	Social Worker	Member, IEC.
5) Mr.Eknath Thakur	Lay Person.	Member, IEC.
6) Dr.S.B.Patel,	M.D.Pharmacology. D.C.H. Paedicatics.	Professor & Head of the Deptt. of Pharmacology, Grant Medical College, Mumbai. Member Secretary, IEC.

The following members of the Institutional Ethics Committee were absent at this meeting.

1)Shri U.R.Patil	Hon'ble Rtd.Judge	Member, IEC.
2) Dr.P.B.Sawant	M.D. (P.S.M.)	Associate Professor of PSM, Grant Medical College, Mumbai. Member, IEC.

We approve the study to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study, any SAE occurring in the course of the study, any changes in the protocol. Patient should not be put to any financial hardship and subject information/informed consent to be taken and ask to provide a copy of the final report.

The Institutional Ethics Committee functions based on GCP and ICMR Guidelines.

Yours Sincerely,

(Dr.S.B.Patel),
Member Secretary
Institutional Ethics Committee &
Prof. and Head Department of Pharmacology,
Grant Medical College, Mumbai.

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