The long-term effects of antenatal multiple micronutrient supplementation in Nepal

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Declaration

I, Delanjathan Devakumar, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signed…….. Date……...

Thesis abstract

The PhD thesis investigates the long-term effects of antenatal multiple micronutrient (MMN) supplementation. A growing body of evidence suggests that changes in early-life environment can have lasting effects on health and disease. To investigate this, we followed up children from a double-blind randomised controlled trial of MMN in pregnancy. The trial found that the intervention group were a mean 77 g heavier at birth and 204 g at 2.5 years, with a 2.5 mmHg lower mean blood pressure. The project described in the thesis set out to investigate the role of antenatal MMN intervention in the programming of future health and disease risk factors in mid-childhood. It sought to determine whether the differences in anthropometry previously present were sustained into mid-childhood and if this was due to an increase in lean mass or fat mass. It also looked at whether antenatal MMN supplementation resulted in an improvement in lung function in the children. Finally, two secondary analyses were conducted to investigate the association between socioeconomic status and growth, and air pollution and asthma.

We measured anthropometry, body composition using bioelectrical impedance (with population specific isotope calibration), blood pressure, kidney dimensions by ultrasound and lung function. Data were also collected on potential confounders: socieconomic status, food security and personal air pollution exposure estimates.

We assessed 841 children (422 controls, 419 intervention) at a mean age 8.5 years. Other than maternal education and residence, children lost to follow-up were no different. The unadjusted differences (intervention minus control), were 0.05 z-scores (95% CI -0.09, 0.19) for weight-for-age, 0.02 z-scores (95% CI -0.10, 0.15) for height, -0.08 z scores (95% CI -0.19, 0.04) for forced expiratory volume in the first second, and -0.05 (95% CI -0.17, 0.06) for forced vital capacity. There was no difference in blood pressure, body circumferences, lean mass, skinfold thicknesses or kidney measurements. The adjusted differences were similar for all outcomes. When considered together, just over half the children had low weight-for-age, and approximately one-third had stunting and low body mass index. Only 1.4% of the children were overweight and mean fat mass proportion was 14.5%. When lung function was expressed in relation to predicted values for Caucasian children, FEV_1 was 14% lower and FVC 12% lower. Our measures of socioeconomic status produced a multidimensional poverty index score of 0.155 and approximately one in ten households were considered food insecure in the previous year. The air pollution data showed a mean 24 hour time-weighted average of 168 $\mu g/m^3$. We found an overall low prevalence of asthma, with air pollution associated with cough at night in boys only (odds ratio 1.15 per 10 $\mu g/m^3$ increase in air pollution; 95% CI 1.05, 1.26). Socioeconomic status was associated with the growth of

children when families owned more expensive assets and appeared to have the greatest effect on skeletal growth in early life.

Differences in phenotype, body composition and lung function between children born to mothers who received antenatal MMN supplements and children whose mothers received iron and folate were not apparent at 8.5 years. While generally poor, households were comparable to those in more affluent regions of the country and were relatively food secure. The air pollution data showed that the children were exposed to levels much higher than national and international recommendations.

Dedication

To my family, upon whose shoulders I have always stood

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Acknowledgements

I would like to thank the many people who have been involved in this project and have helped me with my PhD. I am extremely grateful to all the participants who graciously gave up their time, and to the Wellcome Trust for funding me to conduct this work.

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I would particularly like to thank the members of the MIRA "micronutrient team" in Janakpur, headed tirelessly by Shiva Shankar Chaube. The team consisted of Chandra Maya Thapa, Durna Thapa, Gagan Chaube, Ram Narayan Mahato, Rupesh Yadav, Sonali Jha and Sushil Yadav. I'd also like to thank the many other people who helped: Bishnu Bandari and Raghbendra Sah for their involvement in the isotope calibration, Mahendra Paswan who helped with the air pollution measurements, Manju Baruwal for conducting the public engagement project, Maharudrakumar Thaker who performed the ultrasound measurements, Surya Narayan Yadav to whom we referred the children, Kariram Yadav and Bed Bikram Karki for providing security and general help, and finally Bimala Paudel for looking after us all. In Kathmandu, I would like to thank Bhim Shrestha for his coordination and leadership and Bidur Thapa for organising the finances.

Within UCL and the other UK universities, I would like to thank Naomi Saville for all her support during my time in Nepal; medical students Sebastian Roberts and Suzanne Bartington who worked so hard during their electives; James Beard for answering my many database and computer-related queries; Carlos Grijalva-Eternod and Jane Williams for their advice on running the anthropometry and isotope calibration; Professor Janet Stocks and Jane Kirkby for all their advice and support with the spirometry; Sean Semple and Om Kurmi for their help with the air pollution measurements; and Andrea Rehman and Rhian Daniel for statistical advice.

Finally, I must thank my family for all their love and support throughout my PhD and long before; especially my wife who coped so well in my absence.

Abbreviations

BAZ = BMI for age z score

BIA = Bioelectrical impedance

BMI = Body mass index

CH = Conditional height

CI = Confidence intervals

COPD = chronic obstructive pulmonary disease

CRW = Conditional relative weight

DAG = directed acyclic graph

DALY = Disability adjusted life years

DHS = Demographic and Health Survey

DNA = Deoxyribonucleic acid

FM = Fat mass

GDP = Gross domestic product

HAZ = Height for age z score

HDI = Human development index

ISAAC = International study of asthma and allergies in children

HFIAS = Household Food Insecurity Access Scale

IUGR = Intra-uterine growth restriction

LBW = Low birthweight

LM = Lean mass

LMIC = low- and middle-income countries

LGA = Large for gestational age

MCAR = Missing completely at random

MIRA = Mother and Infant Research Activities

MNAR = Missing not at random

MPI = Multi-dimensional poverty index

MUAC = Mid-upper arm circumference

NGO = Non-governmental organisation

OR = Odds ratio

PCA = Principal components analysis

R^2 = coefficient of determination

RDA = recommended daily allowance

SEM = structural equation model

SES = Socioeconomic status

SGA = Small for gestational age

TWA = time-weighted average

UNICEF = United Nations children's fund

UNIMMAP = United Nations International Multiple Micronutrient Preparation

USAID = United States Agency for International Development

WAZ = Weight for age z score

WFP = World Food Programme

WHO = World Health Organisation

1 Introduction

"If we knew what it was we were doing, it would not be called research, would it?"

Albert Einstein

The first chapter describes the rationale for conducting the study, the research question I set out to answer and the aims and objectives. It gives an outline of the thesis and summarizes the data collected within the study. I then describe my contribution to the work and the training I undertook within the PhD.

1.1 Scope of thesis

Micronutrient deficiency is common throughout the world, with pregnant women at particular risk due to their higher metabolic demands.[1] Impaired antenatal nutrition can affect fetal development and growth in the short term and risk of chronic disease in the longer term. The importance of micronutrients in this process is becoming increasingly apparent, especially in resource-poor settings where women may enter pregnancy with multiple micronutrient deficiencies.[2-4] In addition to its immediate effects on health, monitoring growth in early childhood is an important outcome because of its association with adult health and human capital. [5]

The study focuses on the role of multiple micronutrients (MMN) and summarises the second follow-up of a randomised controlled study (RCT) in Dhanusha district, Nepal, in which pregnant women received either daily multiple micronutrient supplements[6] or a control supplement of iron and folic acid.[7,8] The trial found that babies born in the MMN group were a little heavier at birth, with no difference in gestation or other anthropometric outcomes. It was one of several trials of the supplement,[9-16] meta-analyses of which have shown an increase in birthweight of 22 to 54 g, a reduction in low birthweight and small-for-gestational (SGA) age, but no other changes in anthropometry, gestation or mortality.[17-21] Follow-up of the children at 2.5 years found that the difference in weight was maintained, their body circumferences were a little larger and systolic blood pressure was 2.5 mmHg lower.

It set out to investigate the role of antenatal MMN intervention in the programming of future health and disease risk factors in mid-childhood, and performs the important function of following up the children born in a RCT to look at the long-term effects of the intervention. Figure 1 shows a summary of the previous research findings and the current project.

We collected anthropometry and body composition data to create a detailed picture of the child's phenotype. In addition, kidney measurements and lung function were collected to see whether there were differences in organ size and function. This has implications for both our understanding of growth and lung development and its potential epigenetic influences, and for the design of nutrition recommendations and programmes for pregnant women in low-resource settings.[22] In addition, saliva samples were collected for epigenetic analysis, but this is not covered in the thesis.

In designing the study I thought it imperative to look at the anthropometry of the children as this followed directly from the previous work. I considered three other major body systems: respiratory, cardiovascular and cognitive. After consultation, it was decided that it would be possible to look at one of these adequately. I investigated the possibility of measuring

cardiovascular outcomes, involving blood markers and arterial distensibility. Cardiovascular changes, while important and a major cause of disease burden worldwide, tend to have health effects in middle and older age. While interested in investigating cognitive differences between the groups, we thought it better that this aspect be managed by someone with specialist knowledge, preferably a Nepalese researcher with whom I could work.

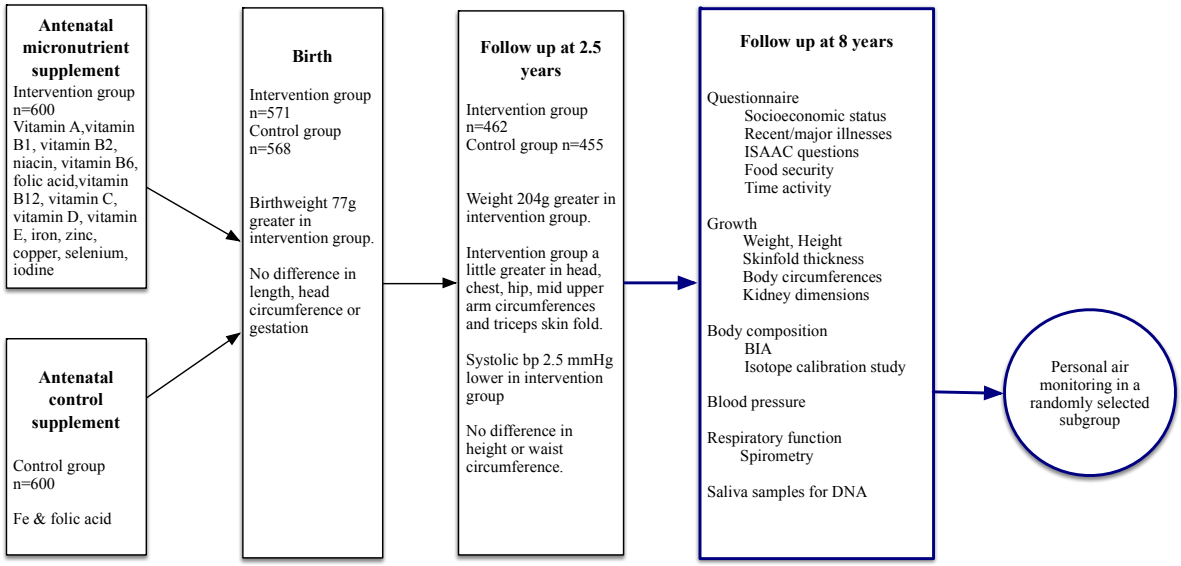

Figure 1: Summary of previous and current work

1.2 Research questions, aims and objectives

1.2.1 Research question

- Does daily multiple micronutrient supplementation in pregnancy, compared with iron and folic acid, lead to a difference in size, body composition, lung function and blood pressure at 8 years of age?

a) Anthropometry, body composition and blood pressure follow-up

The study was designed to investigate the health of children born in the trial, focusing on their growth, respiratory outcomes and blood pressure. The primary focus of the research was to examine the lasting effects of the antenatal MMN supplement to see if differences in anthropometry and blood pressure, previously present, were sustained into childhood. This aspect of the research followed directly from the RCT and last follow-up, but went further by looking at the body composition of the children and their organ (kidney) size, which could provide additional information on long-term disease risk. The study will add to the evidence base for antenatal MMN supplementation, providing information that may help in recommending whether supplements should be given to pregnant women in similar settings. A difference in kidney size could potentially explain the mechanism by which differences in blood pressure might occur. To improve the accuracy of the bioelectrical impedance measurements, a population-specific isotope calibration study was conducted, with an exploration of methodological variations that may help improve the conduct of future studies.

b) Respiratory follow-up

The second aspect of the research question was to investigate whether the antenatal MMN supplements led to differences in respiratory outcomes. The study measured lung function using spirometry and questions about children's history of respiratory illness. I chose to concentrate on the respiratory system as this has immediate consequences for children worldwide. Improved lung function in childhood would be of benefit in coping with respiratory infectious disease and asthma. Increased birthweight has been shown to be associated with better lung function in later life,[23,24] and antenatal micronutrient intake of vitamins A and D has been implicated in the pathways that affect respiratory function and disease.[3]

c) Confounding variables

In an attempt to improve precision, the study collected information on additional variables that were included in multivariable regression models. Using questionnaire data, estimates of food security and socioeconomic status were calculated. The study investigated exposure to

air pollution by microenvironment sampling in the locations in which children resided. Air pollution was measured in detail as it is an important environmental stressor affecting lung function and also growth. Indoor air pollution levels can be very high in countries like Nepal that have a high prevalence of biomass fuel usage.

d) Health of children in Nepal

In addition to the follow-up of the trial, the study was designed to look at the health of the children in southern Nepal and the situation in which they live. By keeping the data together, the study provided information on the growth of the children, their blood pressure and lung function.

e) Secondary analyses

Finally, two secondary analyses were conducted. The first was a cross-sectional analysis of air pollution and asthma symptoms to investigate whether the expected high exposure led to respiratory illness. The second analysis used the data as a cohort study to investigate the effect of socioeconomic status on growth. These secondary analyses do not provide data as robust as the trial results, but do provide additional evidence on these topics in a relatively understudied population, and it would not be possible to conduct randomised controlled trials to answer the questions.

1.2.2 Aims and Objectives

The main aim of the study was to investigate the long-term effects in children born after maternal antenatal MMN supplementation, to see if there are lasting effects in mid-childhood.

The objectives were to:

- Measure anthropometry, body composition, lung function and blood pressure outcomes in the children.
- Investigate whether there was a difference in the measured outcomes between the multiple micronutrient and control groups.
- Measure and create a personal estimate of exposure to air pollution.

1.3 Structure of thesis

Background

The background section starts with a description of the study setting. It then summarizes the previous research and discusses developmental origins of health and disease theory, growth and the effects of antenatal micronutrient supplementation. This is followed by meta-analyses of trials that used the same MMN supplement in pregnancy, and a consideration of the role of causal diagrams in analysis.

Procedures

I summarise the previous trial and the methods used in this follow-up. I describe the process of piloting and conducting the questionnaire, anthropometry, body composition (with population-specific isotope calibration), blood pressure, kidney dimensions, lung function and air pollution measurements. I discuss the ethical factors considered within the study. The variables collected are shown in Box 1.

Analysis and results

These chapters describe assessment of quality of data and technical error of measurement for anthropometry results. WHO reference ranges[25] were applied to the anthropometry data to produce z scores for weight, height and BMI and the new Global Lung Initiative[26] reference ranges were applied to the spirometry data. Bioelectrical impedance values were converted to fat and lean mass using equations derived from a population-specific isotope calibration. Measures of food security and socioeconomic status (multi-dimensional poverty index and principal components analysis) were developed. Particle mass concentrations were calculated for each of the six micro-environments, after which I used the time activity data to model a 24 hour time-weighted average as an estimate of the exposure to air pollution for each child.

I examined summary statistics for the major outcomes and longitudinal trends in growth. I then investigated whether the antenatal multiple micronutrients led to a difference in phenotype and a sustained difference in blood pressure. The primary analysis looked at the main outcome variables and performed independent t tests where appropriate. The secondary analysis performed multivariable regression, restricted the sample to children who were well and looked at effect modification by sex and maternal BMI. A directed acyclic graph was constructed to identify potential confounders that were controlled for using multiple linear regression models. Further analyses were conducted to investigate the association between air pollution and asthma, and socioeconomic status and conditional relative growth.

Public engagement

We held a separate public engagement event, which is briefly summarized.

Discussion

The results of each section are considered separately. The overall results and their implications are considered together in the final section.

Extra material

Appendices include questionnaires and forms used, ethical approval and further reading. In addition, three films were produced that give descriptions of the isotope calibration study (http://youtu.be/pEKxWFhYGag), the main project (http://youtu.be/nFkLOI9UHyw) and the public engagement project (http://youtu.be/dHvlzrVBLOY).

- Questionnaire data
  - Demographic details
  - Recent and major illnesses
  - "International Study of Asthma and Allergies in Childhood" (ISAAC)
  - Socioeconomic status variables to create a Multi-dimensional Poverty Index score
  - Fuels used
  - House characteristics
  - Food security
  - Dietary diversity
- Anthropometry:
  - weight (kg)
  - standing and sitting height (m)
  - triceps, biceps, subscapular and suprailiac skinfold thicknesses (mm)
  - circumferences of head, chest, waist, hip, upper leg and mid-upper arm (cm)
- Bioelectrical impedance (Ohms)
- Renal dimensions: length and anterior-posterior (cm)
- Blood pressure (mmHg)
- Spirometry
  - Forced expiratory volume in the first second (L)
  - Forced vital capacity (L)
  - Forced expiratory flow 25%-75% (L/s)
- Air pollution personal air measurements for particle mass
  - Gravimetric sampling:

Box 1: Indicators investigated in the project

1.4 My contribution

This study involved many people, mentioned in the acknowledgements section, over a number of years in the design of the project, data collection and analysis. A large number of people were also involved in setting up the initial trial and two-year follow-up. In the current project, data collection was carried out by nine members of staff from the non-government organization (NGO) Mother and Infant Research Activities (MIRA). We were lucky to have three of the data collection staff who had been involved in the initial trial and a further team

member who was involved in the 2-year follow-up. Two pairs of staff administered the questionnaire and took the air pollution measurements, two collected the anthropometry and spirometry data, and two were involved in data entry: one inputted the data and the other was in charge of data management for the site. Finally, one person ran the public engagement project. Senior members of MIRA and UCL were also involved in the management of the site, co-ordination of the project with other work, human resources and security considerations. In addition, a number of senior members of research staff at MIRA, UCL and other universities were involved in the design, management and analysis of the study. My contribution, highlighted below, was as coordinator of this large team.

The initial idea for the follow-up came from my primary supervisor, Dr David Osrin, who also coordinated the original trial. I developed the research questions and designed the study in detail under his guidance. I sought specialist advice and input from Professor Jonathan Wells for anthropometry and body composition, Professor Jonathan Ayres for air pollution measurements and Professor Janet Stocks for the spirometry component.

I sought funding and was awarded a Wellcome Trust Research Training Fellowship to conduct the project. I then applied for and received funds from the UCL Beacon public engagement award to run the air pollution art project “Smokescreen”.

I applied for ethical approval from the UCL research ethics board and the Nepal Health Research Council and completed all relevant documentation to register and run the project. I procured the equipment and transported it to Nepal. I then trained the data collection staff and supervised the data collection. This included a piloting and training phase at the start of the project and regular training throughout.

I was present on-site in the first half of the project, when the majority of the children were seen, and less so in the second half. When on-site, I worked daily with all the data collection staff. While not taking the measurements myself, to avoid introducing potential bias from a third observer and because of my inability to communicate effectively for spirometry, I oversaw all aspects of the project and performed regular quality checks on the data. I cleaned the data and assessed all spirograms for acceptability. I conducted all the analyses, seeking statistical advice where necessary.

I conducted regular teaching and training for members of the MIRA staff I worked with, both in relation to the project and wider research skills. I supervised three medical students during the PhD. Two, Suzanne Bartington and Sebastian Roberts, joined me in Janakpur during their medical electives, and the third, Dalvir Kular, helped with secondary analysis of data. Suzanne took detailed assessments of air pollution during cooking to create a calibration

factor for carbon monoxide to particle mass, and conducted qualitative work to explore the issues around fuel choice. Sebastian helped me to conduct the isotope calibration study and led on the creation of the film used for consent and the main project film. Dalvir joined me in a summer attachment to help analyse the food security data and presented a poster on it at the Nutrition Society conference in Lille, France.

Personal training

Anthropometry and spirometry

My training involved one-to-one sessions with Dr Jane Williams in anthropometry and with Dr Jane Kirkby in spirometry, at the Institute of Child Health. I practised these skills during the piloting phase of the Size and Lung function In Children (SLIC) study in schools in north London.

Statistical training

In addition to my supervisors, general statistical advice was given by Dr Andrea Rehman and specific advice on the use of causal diagrams by Dr Rhian Daniel. Professors Caroline Fall and Clive Osmond advised on the creation of the conditional growth indicators.

I also attended a number of statistics courses.

- Introduction to Bayesian Analysis, UCL, 13/1/11
- Analysis of 2x2 tables, UCL, 16/2/11
- Principal Components Analysis and Factor Analysis, UCL, 2/6/11
- Path Analysis and Structural Equation Models, UCL, 9/6/11
- Causal Inference in Epidemiology, LSHTM, 7/11/11- 11/11/11

I attended courses run by UCL on Management Skills for Researchers and Effective Negotiation Skills to help prepare me for managing a research team.

To advise on the project, a group of experts in each aspect of the study was assembled from each of the participating organisations or departments. Meetings were held via teleconference (Skype) every 3 to 4 months.

1.5 Funding

The research project was supported by The Wellcome Trust. I sought funding for a personal fellowship to fund both the project and myself (ref 092121/Z/10/Z; see Appendix 1.1). The public engagement art project on air pollution was funded by the UCL Beacon award.

Chapter 2 Literature review

"The truth is rarely pure and never simple"

Oscar Wilde

Chapter 2 summarises my reading of the background literature. The section starts with a description of Nepal (its land, people and economy) and the study setting, Dhanusha. It then summarizes the Developmental Origins of Health and Disease theory, outlining the epigenetic mechanisms by which it may work. I briefly consider the main outcomes: fetal and child growth, lung function and blood pressure, discussing the role of antenatal micronutrients and air pollution. I try to summarize the main outcomes of trials or meta-analyses and include important observational and animal studies. The chapter then concentrates on multiple micronutrient supplementation in pregnancy. I perform meta-analyses of trials that have used the same supplement in pregnancy as our trial and summarise the follow-up studies. Finally, I consider the role of causal diagrams in epidemiological analysis.

2.1 Literature review introduction

Both macronutrients and micronutrients are important for the short- and long-term health of mothers and children, especially in resource-poor settings in which women may have multiple deficiencies.[2-4] In discussing the role of micronutrients in this chapter, it should be kept in mind that their supply is one aspect in a complex network of causes. Supplementation does not improve the underlying and basic causes of undernutrition. Potentially, if antenatal MMN supplementation has a lasting effect on health, it may be able to improve human capital.

Malnutrition is caused by a lack of or poor quality of food, or disease resulting in increased metabolic demand. In some cases, disease can lead to malnutrition via reduced absorption of nutrients and malnutrition itself can lead to an increase in disease through reduced immune status. The reasons for a woman becoming undernourished are complex, with factors relating to herself, her close environment and the wider community in which she lives. The determinants of undernutrition have been identified by UNICEF (and modified by Black et al) in a framework for nutritional status.[27] This categorises the basic (social, economic and political context), underlying (income poverty leading to food security, inadequate care and unhealthy environments) and immediate (disease and inadequate dietary intake) causes of maternal and child under-nutrition. The conceptual model in Figure 40 expands on the UNICEF model by showing the relationships between the causative variables. Figure 40 is, however, mostly limited to the data assessed in this research, with a focus only on the underlying and immediate causes. The basic causes are likely to have the greatest effect, but may not be related directly to the women and children in the study. An example is the move from civil war to peace within the lifetime of the children. As highlighted by the Food and Agriculture Organization, this resulted in a change in food security (mainly access to food and to a lesser extent the food supply) that was associated with lower proportions of underweight and stunting. In addition to food security, the economy in which food is delivered, societal and possibly family structures, as well as both local and national political organizations, have all undergone - sometimes dramatic - change. After a decline post-war, the country has seen a sharp rise in food prices since 2004. This imposes the greatest burden on the poorest who use a greater share of their income on food (nearly a quarter of the population spend >75% of their budget on food). [28]

Micronutrient deficiency is thought to affect approximately two billion people worldwide. While the diets of women in low resource settings are likely to be deficient in quantity, there is growing acknowledgement that they are also deficient in quality. A contrasting picture of high quantity, but still low quality, diet is seen in poorer communities in rich countries, where the prevalence of obesity in low socioeconomic groups is high, but women may still lack

micronutrients. [29] The WHO considers iron, iodine and vitamin A deficiency to be of particular concern in developing countries. Iron deficiency is the main cause of anaemia, affecting over 30% of the world's population and 38% (32 million) of pregnant women.[30] Iodine deficiency is one of the main causes of impaired cognitive development in children, and vitamin A deficiency is the leading cause of preventable blindness in children and can increase the risk of disease and death from severe infections.[1] More locally, research from Parsa district in the Terai (to the west of Dhanusha) showed that adult diets were deficient in most micronutrients tested, especially vitamin A, riboflavin and iron. Levels tended to be lower in women than in men. [31] In a recent analysis of the global burden of disease, child and maternal undernutrition were responsible for 6.7% (95% CI 5.7, 7.7) of global Disability Adjusted Life Years (DALYs). The largest percentage was due to underweight in children (3.1%). Iron deficiency anaemia made up 1.9% and other dietary risk factors contributed to a further 10% of DALYs. [32]

Current evidence on the developmental origins of health and disease suggests that early-life environment can have lasting effects on growth, physiology and health. The mechanisms involve interplay between environment, genes and hormones, in which epigenetic regulation plays a part.[3,33,34] The evidence for this comes mostly from animal studies. Much of the evidence in humans is based on observational studies due to the difficulties, and sometimes ethical dilemmas, in conducting trials. Human trials have looked at three categories of nutrients: macronutrients, micronutrients (in the form of single nutrients or multiple nutrient combinations) or food. It is likely that both micro- and macronutrients are important, but the degree to which each one is needed would vary across individuals and populations. Micronutrient supplementation can only help to improve the quality of the diet. While important, it cannot address the additional energy requirements of the mother or provide the protein substrates. Macronutrients are particularly important in populations where the availability of food can be inadequate or disrupted, such as in Dhanusha district. An indication of this was given in the meta-analysis of antenatal MMN by Fall et al,[17] which showed an interaction between MMN supplementation and maternal BMI, with a larger effect in women with greater BMI, with the exception on Guinea-Bissau. The extent to which this interaction existed varied across populations. The greatest effect was seen in the two Nepal studies, ours and Sarlahi.[7,35] These two studies had the lowest maternal mean BMI of the 12 studies included. [36]

2.2 Nepal

2.2.1 Geography

Nepal is a relatively small, landlocked country situated between India and China (Figure 2). In terms of its flora and fauna, as well as its people, economy and politics, the country is largely a product of its diverse and spectacular environment. It lies at the boundary between the Indo-Australian and Eurasian tectonic plates. Movements of these plates towards each other push the land mass upwards, resulting in the Himalaya mountain range. This region borders China, contains eight of the world's ten tallest mountains and is home to the highest mountain in the world: Everest (8850m). Nepal's land area of 147 000 km^2 is broadly split into three ecological belts: the northern mountain region, middle hills and plains (Terai) that make up the southern part of the country. The Terai accounts for approximately a quarter of the country's area. It lies at low altitude, making communications relatively easy. The capital of Nepal and its major city is Kathmandu. It is found in the middle hills region in the centre of the country. The country is divided into five development regions made up of 75 districts. Districts are divided into smaller administrative units known as Village Development Committees, which are further divided into wards.

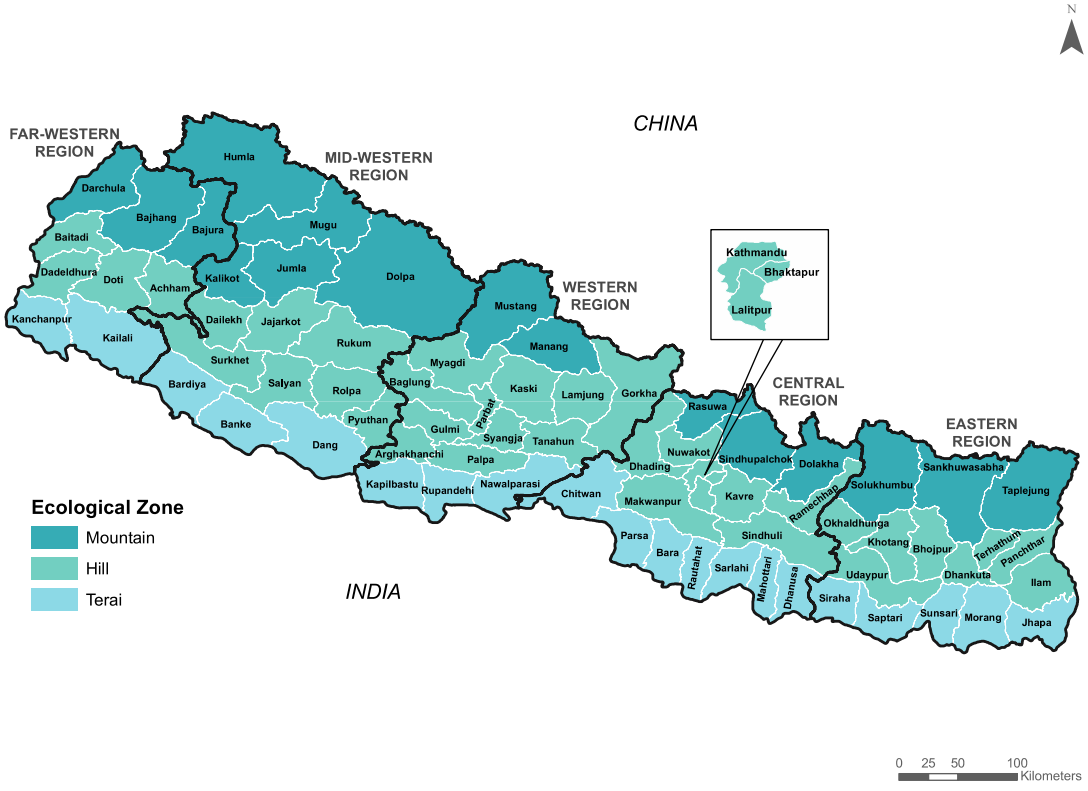

Figure 2: Map of Nepal showing the three ecological regions [37]

2.2.2 People

According to the 2011 census, the population of Nepal was 26.6 million people. Approximately half live in the Terai, while 17% live in cities. Due to the large uninhabitable mountainous areas the population density varies widely. The highest density is 4408 persons/km^2 in Kathmandu, while the country average is a mere 181 persons/km^2. Otherwise, the more densely populated cities tend to be located in the Terai. The demographics of Nepal show a classic pyramid-shaped population and the median age is a low 21. In addition to this, the total fertility rate is 2.36 children per woman, slightly lower than the replacement fertility rate[a] for South Asia of 2.43.[38] Thought to be a marker of family planning, mothers' mean age at first delivery is 20.1 years. [39]

The annual population growth rate over the last ten years has been 1.4%.[40] This is lower than expected and is in part explained by the emigration of people seeking work overseas: the population overseas has doubled since the last census in 2001. The vast majority (97.7%) are men who mostly go to Middle East countries or Malaysia, providing cheap unskilled labour.

The people of Nepal are primarily made up of two ethnic groups: South Asians (a mix of East and West Eurasians) and Tibetans who migrated across the Himalayas. [41,42] The population is subdivided by a caste system that includes over 100 castes. The main ones (over one million people) are the Chhetri (16.6%), Hill Brahman (12.2%), Magar (7.1%), Tharu (6.6%), Tamang (5.8%), Newar (5.0%), Muslim (4.4%), Kami (4.8%) and Yadav (4.0%). In the Terai region the biggest group is the Tharu (12.5%). The caste of a person is closely linked to their wealth and socioeconomic status. The upper castes (Brahmins and Chhetris) tend to hold positions of power in the government and civil services. The "lower" castes have poorer health outcomes, in part related to lower utilization of health services. Children from the Dalit group, for example, have the highest under-five mortality and malnutrition. The causes for this are wide ranging, with a lack of finances being just one problem. In the Terai, 83% of women from the Dalit group were found to have no education, compared to 40% of women nationwide. [43]

Nepali is spoken as the primary language by just under half the population. This is followed by Maithili, the primary language for 3 million people, mostly from the Terai.[40] The main

[a] Replacement fertility is the total fertility rate at which a population remains stable (excluding migration). It takes into account mortality of women in childbearing age and sex ratio at birth.

language in Dhanusha is Maithili, though Nepali is also spoken by many. Maithili is an Indo Aryan language developed from Sanskrit that is widely spoken in eastern India as well as Nepal.

2.2.3 Politics

Nepal has recently emerged from a 10-year civil war. The war broadly pitted a group of Maoist insurgents against the government forces in 1996. It culminated in mass protests in April 2006 that triggered peace talks and the formation of a government following national elections. Elections were held in 2008 and the country became a federal democratic republic. After the deposition of the monarchy, the country is now led by the United Communist Party of Nepal (Maoists) in a coalition government. Led by the Nepal Supreme Court, the next step involves completing a new constitution. This has, however, been postponed on several occasions.

The Nepalese people elected a new prime minister, Dr Baburam Bhattarai, in November 2011. The current president, Ram Baran Yadav, is from Dhanusha. This has the advantage of giving a voice to the people in the region. At the time of writing, Nepal was planning to have a general election, but there was resistance to this from some political parties.

The two major religions, Hinduism and Buddhism, are followed by 81% and 9% of the population, respectively. Religion is generally not a source of conflict and it is not unusual for a Hindu to worship at a Buddhist shrine and vice versa. The third most common religion is Islam, making up 4% of the population, although in the Terai (8%) Islam is second to Hinduism. [40]

Being sandwiched between the two regional super-powers of India and China creates both opportunities and problems for Nepal. Both in population and economic terms, Nepal is tiny compared with its neighbours. While generally unable to resist the influence of these countries, it is not entirely reliant on one or the other.

2.2.4 Economy

Nepal is one of the poorest countries in the world. Its gross domestic product (GDP) is estimated to be US$19.42 billion in real terms or $40.03 billion (US$1500 per capita) in purchasing power parity, ranking it 102nd in the world. [39,44] Agriculture forms the mainstay of the economy, followed by remittances and tourism. Most of the produce is grown in the Terai region as it is flat and relatively fertile. Three quarters of the workforce are involved in

agriculture and the remainder are split between services (18%) and industry (7%). Nepal's main exports are clothing, pulses, carpets, textiles and juice and its main trading partner is India, which takes 62% of its exports and provides 53% of its imports.[39] The World Bank estimates that 25-30% of GDP comes from remittances, either formal or informal.[45] While Nepal is a poor country, it does have a relatively equal distribution of wealth. Its Gini coefficient[b] is 32, ranking it at 100 in the world. By comparison, India has a Gini coefficient of 37, China 47 and the UK 40. [39]

The Nepalese rupee is pegged to the Indian rupee at a ratio of 1.6:1. Recent fluctuations in the Indian rupee have therefore affected Nepal, weakening the rupee from NPR110:£1 at the start of my PhD in 2011 to NPR160 in 2013. This would act to improve the country's exports, but make commodities more expensive to import. That said, as previously mentioned, the majority of trade is with India, so the effect of fluctuations would be reduced.

It is generally appreciated that poverty cannot be adequately expressed in a single indicator such as GDP. Research has moved towards using multiple indicators or conglomerate measures. An example is the annual Human Development Index (HDI) produced by the United Nations Development Programme (UNDP).[c] Nepal's HDI (0.458) is ranked 157th in the world. It has gradually increased over the last three decades, from 0.242 in 1980, to 0.340 in 1990, to 0.398 in 2000. [44] Life expectancy at birth is 68.2 years for women and 65.6 years for men, and children go to school for an average of 3.2 years.[39]

2.2.5 Health

Health expenditure is 5.4% of GDP (2011 figures covering total expenditure on health by institutions or individuals), ranking it 126th in the world. [39] The total per capita expenditure on health is a mere (International)$69. [46] Nepal's public health facilities are made up of 13 central or tertiary hospitals, 15 zonal hospitals, 65 district hospitals and 4054 primary health centres, health posts and sub-health posts. Nepal also has large private for-profit and NGO/not-for-profit sectors of the health service. It is estimated that 50% of patients with acute illness are seen by private health providers. Figures from 2010-2011 show 8000 medical doctors, 2000 of whom were consultants/specialists, and 34 000 nurses or midwives. Only

[b] The Gini coefficient is a scale used to estimate the level of income inequality in a country, where zero is no inequality and 100 is maximum inequality. It is calculated by plotting the cumulative number of families (arranged from poorest to richest) on the x axis against the cumulative share of income on the y axis, producing a Lorenz curve. The index is given by the area between the Lorenz curve and the 45° line and divided by the area of the triangle below the 45° line.

[c] The HDI is a composite score that includes life expectancy, education and per capita income and lies between 0 and 1.

17% of these doctors and 19% of the nurses work in the public sector. In addition, the public sector has 7500 paramedics and 4300 public health workers. [47] Including all registered doctors gives Nepal a ratio of 0.3 doctors/1000 population, well below the threshold limit of 2.28 doctors/1000 population recommended by the WHO to achieve 80% coverage of health services.[48] In addition, these health staff tend to be located in the urban areas. Recruitment to remote areas is a focus of government policy, but difficult to attain.

Child mortality in Nepal is high. Current WHO figures show that the under five mortality rate is 54/1000 live births (equating to approximately 34 000 deaths) compared to the WHO South Asia regional average of 59/1000 livebirths.[46] The infant mortality rate and neonatal mortality rates are 46/1000 and 33/1000 live births, respectively. [49] The pie chart in Figure 3 shows the main causes of mortality in children under five (U5MR) in Nepal in percentage terms.[46] The major causes are diarrhoeal and respiratory diseases and those relating to the neonatal period. According to the Nepal Demographic Health Survey (DHS) 2011, in the two weeks preceding the survey 5% of children under five had symptoms of acute respiratory illness, 19% had a fever and 14% had diarrhoea. [49] An analysis of neonatal mortality was conducted by the Nepal Ministry of Health using the 2011 census data. Predictors of neonatal mortality were maternal education (categorised as no education and primary, OR 1.9), short stature (defined as <145cm and >145cm, OR 4.3) and use of solid fuels or cooking indoors (OR 2.5). Maternal BMI did not seem to be important. [50] Adult obesity prevalence, as defined by BMI>30, was very low at 1.4%. Only Bangladesh and Ethiopia have lower levels.

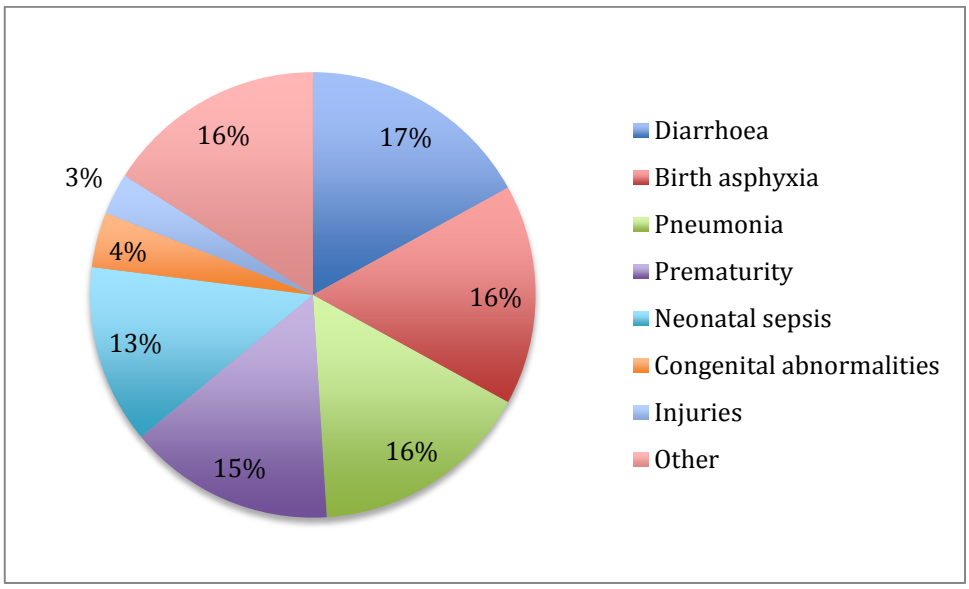

Figure 3: Causes of under five mortality in Nepal [46]

Growth of children

The Nepal DHS 2011 showed that 41% of children under five were stunted (height for age <-2 z scores from the median), 11% were wasted (weight-for-height <-2 z scores) and 29% were underweight (weight-for-age <-2 z scores). In all categories, boys fared slightly worse than girls and rural populations were worse off than urban ones. The Terai had a higher proportion of stunted children. For underweight and wasting, it was similar to the Middle Hills and lower than the Mountain region (Table 1). [49]

	Height-for-age (% children under 5)		**Weight-for-age (% children under 5)**		**Weight-for-height (% children under 5)**	
	-3 z score	-2 z score	-3 z score	-2 z score	-3 z score	-2 z score
Mountains	22.2	52.9	9.9	35.9	3.2	10.9
Middle hills	16.7	42.1	7.1	26.6	1.7	10.6
Terai	14.9	37.4	7.8	29.5	3.2	11.2

Table 1: Proportions of children under five with stunting, underweight and wasting, by region of Nepal [49]

Air pollution

Environmental air pollution is an important cause of ill health. In rural Nepal it is mostly caused by the burning of biomass fuels. Biomass fuel usage is very common, with estimates of use in 75% of households, particularly in poorer areas outside the major cities. [51] The burning of biomass in Nepal has been shown to adversely affect lung function in young adults [52] and exacerbate respiratory disease in children.[53] The link to respiratory illness is particularly important to study as it is estimated that in 2002 there were 4820 acute respiratory deaths in children under five in Nepal due to indoor air pollution. There were a further 2680 deaths from chronic obstructive pulmonary disease in adulthood. This led to 204 000 DALYs lost and contributed to 2.7% of the national burden of disease.[54]

The political situation is particularly important in determining the fuels people use. Nepal currently produces about 60% of its 5.3 billion kWh energy needs. Energy production is mostly from hydroelectric power (about 90%), which currently produces about 600 MW. Nepal has a huge potential for producing hydroelectric power: it is estimated that there is capacity to create 42 000 MW, depending on how efficiently the power plants run, equivalent to a little under 200 billion kWh. This issue leads to a great deal of debate within the country as the inability to realise it is largely seen as being due to ineffective government. The true benefit of maximising hydroelectric power in terms of income to the country is debated, especially as creating power plants would require foreign investment, leading the country further into debt. What does seem clear, though, is that Nepal should be able to service its own energy needs. [39,55]

The lack of electricity creates major problems for the population as electricity needs to be shared (load-shedding). This can result in power cuts of 18 hours a day, and some of the six available hours may well be in the middle of the night. In addition to the loss of productivity and convenience, the lack of clean power leads to the use of other forms of fuel such as firewood and animal dung. This is a problem mostly borne by the poor, who do not have the money to buy and run generators. To explore this further and to highlight some of the issues and impact on the people, I wrote a narrative essay, (which can be seen at: www.thelancet.com/journals/lanres/article/PIIS2213-2600(13)70035-3). [56]

2.2.6 Study setting

The research was based in Janakpur, Dhanusha district, in the Central Terai region south-east of Kathmandu (Figure 2). Dhanusha is made up of 101 Village Development Committee geopolitical units (VDCs) and Janakpur municipality. Table 2 shows the demographics of Dhanusha and the adjoining districts of Mahottari and Siraha. The population of Dhanusha is made up of 674 000 Hindus, 11 200 Buddhists and 63 100 Muslims.[40]

The male to female sex ratio is approximately 1:1, higher than the ratio of 94.4:100 for the country as a whole. The Nepal Central Bureau of Statistics explains this difference as being due to different rates of emigration for men and women. In Dhanusha, 6.8% of the population are thought to be working overseas, a similar figure to the whole country. Other factors would therefore need to account for the equal sex ratio. [40]

The weather in the Terai can broadly be split into four seasons: winter (Nepalese months Push, Magh and Phagun), spring/summer (Chait, Baisakh and Jyesth), monsoon (Ashadh, Shrawan and Bhadau) and post-monsoon (Asoj, Kartik and Mangsir). The winter low temperatures are on average in single figures (degrees Celsius), while summer highs can reach the forties.

	Total population	Number of males	Number of females	Male:female sex ratio	Annual population growth rate (%)	Population density (person/km^2)
Dhanusha	768 404	383 711	384 693	100	1.35	651
Mahottari	646 405	320 886	325 519	99	1.55	645
Siraha	643 136	313 292	329 362	95	1.17	541

Table 2: Demographics of Dhanusha and the surrounding regions

2.3 Developmental Origins of Health and Disease

The developmental origins of health and disease (DOHaD) hypothesis proposes that critical or sensitive periods exist in the early development of an organism in which environmental influences can lead to long-term changes in the child or adult. In humans these critical periods are predominantly in prenatal life, but extend into early childhood. The theory brings together the interplay of both genetic predisposition and environmental influence, building on the idea that chronic disease cannot be explained fully by either.

The initial focus of the DOHaD theory was on the influence of birth outcomes such as birthweight and early mortality on cardiovascular outcomes later in life. In the 1970s Forsdahl showed that cardiovascular disease was associated with infant mortality.[57] Barker and colleagues from the University of Southampton then linked deficits in the intra-uterine environment to coronary heart disease in adults. In a landmark paper, they showed an association between adult ischaemic heart disease and birthweight in a retrospective study from the UK. Those with the lowest birthweights had the highest mortality later in life. [58] In a further study, Barker and Osmond subdivided the country into geographical regions and showed that infant mortality rates were associated with ischaemic heart disease. This association, they felt, was related to nutrition in early life.[59] They hypothesized that, depending on when in pregnancy the deficit in nutrition occurs, the resulting phenotype of the fetus changes, mediated by hormonal changes.[33] Extending the theory from "fetal origins" to "developmental origins" came with the greater understanding that later periods in life are also important. Lucas proposed the idea of programming in which stimuli in "critical periods of development can have lasting significance". In particular, he singled out the first four weeks of life as being one such period.[60] The changes that occur are in these sensitive periods in early life, when the organism is still developing and undergoing rapid change, tend to be maintained throughout life via mitotic cell division. The term canalization,[d] described by Conrad Waddington in the 1940s, has been used to describe traits that are invariant to stresses. [61] As described by Waterland and Michels, during these sensitive periods, genes that would undergo methylation under normal circumstances are prevented from doing so by environmental influences. [62]

Since these first studies, a huge amount of epidemiological research that has supported the hypothesis has been carried out. At first the DOHaD theory was based on observational

[d] Canalization can be defined as "reduced sensitivity of a phenotype to changes or perturbations in the underlying genetic and nongenetic factors that determine its expression". In this context, I refer to environmental canalization where the phenotype shows resistance to environmental changes. 61. Flatt T. The evolutionary genetics of canalization. *The Quarterly review of biology* 2005; **80**(3): 287-316.

retrospective studies, but since then prospective cohorts and randomised controlled trials have been conducted. These human studies have been complemented with experimental work on animals and more recently epigenetic studies that are beginning to decipher the mechanisms by which changes to phenotype and physiology may occur. Box 2 shows examples from famine situations in which dramatic reductions in population nutrition, particularly pregnant women, are associated with adverse heath consequences in future generations.

Inheritance of phenotypic variation can occur via non-gametic processes or across meiosis, resulting in multi-generational changes that affect future offspring. Non-gametic processes are more common, for example poor maternal health during pregnancy (such as insulin resistance) that leads to illness in the offspring. Environmental exposure can also affect the germline cells, this being termed transgenerational epigenetic inheritance. [63] An example from Crews et al showed that exposure of pregnant rats to the fungicide vinclozolin, which acts as an anti-androgen (and endocrine-disruptor), resulted in disease in the offspring. The male offspring then passed this on to the two following generations (although the female offspring did not).[64] In addition to molecular changes, multi-generational inheritance can also be via the phenotype itself; for example, a taller mother can give birth to a larger baby.

Previous animal and human evidence has pointed to the potential for long-term effects of antenatal micronutrients. [2,3] With fifteen micronutrients, and a very large potential number of combinations and interactions, being able to work out a definitive mechanism is difficult. Outcomes such as weight are also multi-factorial. Christian and Stewart have set out potential mechanisms by which micronutrients may affect later disease.[3] The two main pathways are through hormonal changes, often leading to a change in phenotype, and epigenetic changes. In addition to these, micronutrients may improve the immune system, reduce anaemia or improve energy metabolism and anabolic processes in the pregnant woman. [65] For example, animal studies with mice showed that a low zinc diet in pregnancy resulted in reduced immunity in the offspring. This continued to some extent to the two following generations. [66]

Whether these early life changes are adaptive, conferring an advantage to the organism, or maladaptive (or neither) has been debated. Broadly, there are two main evolutionary theories: the thrifty genotype and thrifty phenotype. Both these theories are based on an attempt to explain diabetes risk and prevalence, but have been extrapolated more widely.

1. Dutch Famine, 1944-45

The best evidence for the long-term effects of famines comes from the Dutch famine in World War II. German blockade of food stocks into the Netherlands and the subsequent harsh winter conditions affected food transport and led to a dramatically reduced food supply for the population in the west of the country. At its worst the rations dropped to 400 calories per person per day. Pregnant women were entitled to extra food, but did not always receive it. In addition to the official rations some food was available through the black market and churches, for example. The food supply improved rapidly after liberation in May 1945. [67]

The long-term effects of the famine on the offspring depended on when in gestation the lack of nutrition occurred and the time for which there was a deficit. The effect appeared to be greatest for those in whom the insult was in late pregnancy, but peri-conceptional exposure was associated with hypomethylation of the Insulin-like Growth Factor II gene in adults. [68] This gene is important in a number of growth related processes;[69] for example, regulating placental growth and nutrient transport. It is inherited via paternal imprinting, while the IGF II receptor is maternally imprinted. [70] The detrimental effects of the Dutch famine extended into the next generation, with the offspring of women born in the famine found to have lower birthweight. [71] This may have been due to direct exposure of the mother's ova to poor nutrition while she herself was a fetus. [63]

Cardiovascular effects

Those affected in early gestation were found to have a higher risk of coronary heart disease, while those affected in mid- to late gestation had reduced glucose tolerance. [67] These differences may have been mediated by changes in placental size, which was associated with hypertension, but only in men.[72]

Obesity

Ravelli et al showed that, when following up 300 000 Dutch men in the army who were exposed to the Dutch famine prenatally, they differed in their levels of adiposity depending on the point at which this occurred. Being exposed to the famine in the last trimester was linked to lower levels of obesity later in life, while being exposed in the first half of pregnancy was associated with increased levels of adiposity. [73] Stein et al showed differences in long-term adiposity between men and women. With the exception of the waist-to-hip and waist-to-thigh ratios, all measures of adiposity were greater in female offspring who were exposed to famine in pregnancy. A similar effect was not seen in male offspring. The critical period appeared to be in the middle of pregnancy. [74]

2. Leningrad (St. Petersburg) famine, 1941-44

The siege of Leningrad occurred in World War II when German armies surrounded the city. Food supplies to the city were cut off, leading to the death of approximately 2.4 million people. Contrary to the previous example, Stanner and Yudkin found no association between in-utero exposure and cardiovascular outcomes as adults. In a cross-sectional study those who were exposed to in-utero malnutrition were compared to unexposed groups born before the famine (who were exposed to malnutrition in infancy) or born outside the area. No difference was found in glucose tolerance, lipid profile or blood pressure. The contrast with the findings from the Dutch famine may have been due to the longer time period of the famine, with exposure in childhood and hence reduced catch-up growth. [75,76]

3. Biafran famine, 1968-1970

The Biafran famine occurred during the civil war in Nigeria when food supplies to the region of Biafra were inhibited. Similar results to the Dutch famine were seen on weight and blood pressure in those born during the famine. Hult et al found an increased risk of being over-weight (OR 1.41; 95% CI 1.03, 1.93), having hypertension (OR adjusted for BMI 2.87; 95% CI 1.90, 4.34) and impaired glucose tolerance (OR adjusted for BMI 1.65; 95% CI 1.02, 2.69) in those born during the famine compared to those born after the famine. [77]

4. Chinese famine, 1959-61

The Chinese famine was the result of drought combined with the country's agricultural policies. Here the evidence for an increase in the risk factors for cardiovascular disease is mixed. Li et al showed a trebling in the odds of metabolic syndrome in those who were prenatally exposed to severe famine areas. [78] Zheng et al[79], measuring metabolic disease, and Yang et al[80], measuring BMI in adults, both showed an increased risk in women but not men. However, Huang et al who found evidence to the contrary in a small decrease in the BMI of those exposed prenatally. [81]

Box 2: Case studies on the long-term effects of famine situations on cardiovascular outcomes

2.3.1 Thrifty genotype hypothesis

Described in 1962 by Neel[82], the thrifty genotype hypothesis gives an explanation for how harmful conditions, such as diabetes, can be so common and increasing in prevalence. In this theory, the genotype that predisposes to diabetes was a selective benefit when humans needed to take advantage of the abundant food in preparation for scarce times. This genotype is maladapted to richer countries (and the more affluent populations in low- and middle-income countries) today where there is an abundance of food, leading to conditions such as diabetes. Prentice et al, amongst others, have proposed candidate genes in developing country settings, such as the ApoE gene, but in general the genetic basis for this theory is lacking. [83]

2.3.2 Thrifty phenotype hypothesis

Hales and Barker proposed an adaptation of the hypothesis that they called the "thrifty phenotype". This states that in early life the fetus "sacrifices" growth of certain organs or systems due to a nutritional deficit. Their theory focused on fetal undernutrition, which would cause a reduction in the beta cells of the pancreas, leading to reduced insulin production. Poor nutrition in early life results in permanent changes in glucose metabolism (reduced insulin secretion and increased insulin resistance), leading to changes in both organ size and structure. This produces a phenotype that has a survival advantage in the immediate future, but when environmental conditions change, such as with an excess of food, leads to increased susceptibility to diseases such as II diabetes and metabolic syndrome. [84,85]

The interplay between the thrifty genotype and phenotype has been described by Prentice and Moore as cycles of under- and over-nutrition- see Figure 4. [86] Populations go through cycles of adaptation and maladaptation to their environments. In the current situation of a high incidence of diabetes in South Asian populations, we may currently be in the transitionally dis-adapted cycle with underweight infants and obese adults.

Importantly, the thrifty phenotype needs to be combined with other environmental or behavioural factors such as obesity or reduced physical activity, at least in terms of when and how severely someone experiences disease. In the short-term the thrifty phenotype is adaptive, but it may be maladaptive later. These ideas were developed further by Wells, who proposed the capacity–load theory, whereby stresses, or metabolic loads, challenge the body's homeostatic mechanisms (capacity), leading to chronic disease when the body cannot cope. [87] One example is lower birthweight associated with heat stress. [88]

Gluckman and Hanson attempted to describe fetal changes in light of future adaptations, in their "predictive adaptive response" theory. They proposed that fetal alterations to metabolism or phenotype in relation to environmental cues are designed to confer future advantage. The fetus "sees" the current environment, predicts what the future environment may be like and makes changes that will be beneficial.[89] For this adaptation to current conditions to be beneficial in the long term, it would require that environmental conditions stay similar, but where a mis-match occurs between current and future conditions, disease can result. A counter argument was proposed by Wells, who states that the fetus in fact responds to the physiology and phenotype of the mother, who has herself adapted to cues to her ancestors and her environment. [90] I recently described how previous health and risk factors can combine with current stresses to affect health in the extreme conditions of war.[91]

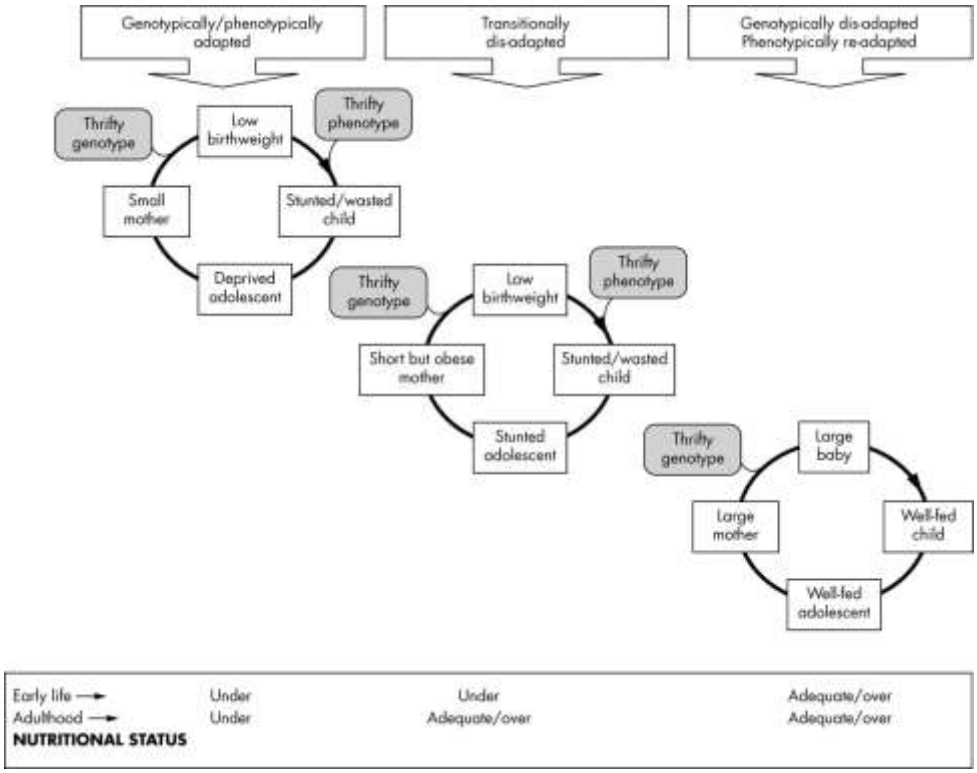

Figure 4: Cycles of under- and over-nutrition.

Reused with permission for use in a dissertation from Prentice and Moore, Archives of Diseases in Childhood, 2005. [86]

2.4 Epigenetics

The primary mechanism for the DOHaD hypothesis is thought to be via epigenetic changes. The term epigenetics was defined by Waddington as "the interactions between genes and their products which bring the phenotype into being".[92] The term is currently thought to refer to heritable changes to DNA that do not alter the base sequence but result in changes in expression, resulting in gene-environment interactions.[34] Epigenetic mechanisms are important in normal physiology, for example in X chromosome deactivation in women, and also in pathological processes. Cancer cells, for example, are found to be globally demethylated, but with hyper-methylation of specific gene promoter regions.[93] Further, epigenetics are believed to control genetic imprinting, where either the maternal or paternal cell lineage is preferentially expressed. This phenomenon occurs in conditions such as Prader-Willi, Angelman and Russel-Silver syndrome.[94] Epigenetics can also help to explain the differences between monozygotic twins. For example, Fraga et al showed that they have differences in DNA methylation, and went on to conclude that these differences arise over the lifetime, although this was based on the comparison of only two pairs of twins.[95]

The actual epigenetic changes are poorly understood, but the three main mechanisms by which gene expression is thought to the be controlled in all eukaryotic cells are:

1. DNA methylation. Hypermethylation halts gene transcription.

2. Noncoding RNAs. These bind to DNA and interfere with transcription.

3. Histone tail modification. The histones make up nucleosomes. These undergo methylation, acetylation and phosphorylation to form a number of "histone codes" that affect chromatin structure and alter gene expression.[96-99]

The best researched of these mechanisms is DNA methylation. This involves addition of methyl groups from the conversion of S-adenylmethionine to cytosine. Most methylation occurs at the CpG dinucleotide, where cytosine is followed by guanine. Methyl groups are attached to the 5' position of the pyrimidine ring of the cytosine base, resulting in 5-methylcytosine. Methyl binding proteins (for example, methyl-CpG binding protein 2) mediate this process by attaching to the methyl groups and initiating chromatin-remodelling co-repressor complexes. The CpG (cytosine guanidine) methyl group prevents the DNA from binding protein. CpG sites are uncommon in humans (about 20% of predicted). They are normally methylated, but the unmethylated 20% tend to be located at CpG "islands", often in promoter regions where they tend to cluster. It is these sites and the surrounding "CpG island shores" that are believed to be important in the transcription processes. [93,98]

The methyl groups for this process are provided by the maternal diet by metabolism of choline, betaine, folate and methionine. In addition to folate, micronutrients are important in this process. Vitamins B_2, B_6 and B_{12} are important enzyme cofactors in the C1 metabolism cycle that converts S-adenosyl-methionine to S-adenosylhomocysteine, releasing the methyl group that is attached to the DNA. [99]

Gametes are highly methylated. After fertilization, demethylation of the genome occurs. The paternally inherited genome undergoes rapid active demethylation (with the exception of imprinted genes) prior to implantation. Passive demethylation occurs in the next few weeks, where methylation is not preserved during replication. This is followed by differential cell lineage-specific methylation in the embryo. The trophoectoderm which goes on to form the placenta remains hypomethylated. [62,98,100] DNA methylation, particularly in early life, was believed to lead to lasting changes in the cells and lineage, but changes can occur throughout life, particularly in early life, puberty and old age. Most methylation changes, however, do occur peri-implantation. [62,98]

Evidence for the effects of epigenetic changes has been shown in Agouti mice given dietary supplements of vitamin B_{12}, folic acid, betaine and choline prior to and during pregnancy. This resulted in a change in hair colour from yellow to brown. The supplements methylated the Agouti gene, switching it off and resulting in the colour change. Mice with brown fur went on to have lower rates of obesity, diabetes and cancer later in life. [101]

The evidence in humans for epigenetic changes is starting to emerge. As described in Box 2, reduction in methylation in the insulin-like growth factor II (IGF2) gene, which is partly responsible for placental nutrient regulation, occurred in the Dutch famine.[68] Changes in fetal growth may have been due to methylation changes in the (maternally imprinted) IGF-II receptor, leading to changes in growth. [98] Recent evidence from a double blind randomised controlled trial of peri-conceptional micronutrient supplementation (UNIMMAP) in The Gambia showed sex-specific changes in methylation, specifically the IGF2 receptor gene in girls and the gene trap locus 2-2 gene in boys. [102,103]

2.5 Growth

Any long-term effects of MMN supplementation may be due to a direct effect, for example a structural change to an organ or system or to its effect on growth in early life. A detailed analysis of in-utero and child growth and the effect of different growth trajectories was beyond the scope of this thesis, but I will briefly outline the effect of both fetal and child growth on later health. The second part of the section looks at the influence of single micronutrient supplementation in pregnancy on growth, with subsequent sections focusing in more detail on the respiratory system and blood pressure, and the effect of multiple micronutrient supplements.

2.5.1 Fetal growth.

Growth restriction in-utero is a cause of both short-term and long-term ill health. For the fetus and neonate, it may lead to increased mortality and morbidity, while in adulthood, particularly when combined with rapid child growth, it may lead to chronic disease. Growth failure, meanwhile, is a composite result of poor nutritional availability at a cellular level and increased metabolic demands.[2]

2.5.1.1 Small for gestational age (SGA)

SGA[e] is usually used as a marker of intra-uterine growth restriction (IUGR)[f] as it takes gestational age into account.[104] It is not ideal as it only compares a fetus with others of the same age, rather than with its own growth potential. A baby on the 50th centile may still be growth restricted if her potential weight was to be on the 90th centile. In 2010, 32 million infants were born SGA (10 million born at term). The situation is particularly common in South Asia, where two-fifths of livebirths were SGA.[105] Another indicator of growth restriction is birthweight. This is less accurate than SGA in that it does not incorporate gestational age. A preterm infant may be low birthweight, but appropriately grown for her gestational age. Commonly, a cut-off of <2500 g is used to define low birthweight. In developing countries the majority of low birthweight is due to IUGR rather than prematurity.[105] In a review of adverse outcomes in pregnancy, Kramer highlighted the main causes as prematurity and IUGR. Prematurity was associated with infection, multiple births, hypertension, low pre-pregnancy BMI, incompetent cervix, heavy work and smoking. IUGR was mainly associated with low energy intake, low pre-pregnancy BMI, short stature, primiparity, hypertension, smoking and, in primiparae, malaria. [106] The nutritional supply to

[e] SGA is defined as weight below the 10th centile for gestational age. It is a usually a population-specific definition.

[f] IUGR is defined as a fetus who has not reached its growth potential. (Mandy, UpToDate 2013)

the fetus depends on pre-existing maternal stores, diet and placental function. These depend in turn on the nutrition that a mother receives and how it is partitioned between herself and the embryo/fetus. Demand-side factors may also increase both maternal and fetal metabolic demands, which may, for example, increase with in-utero infections. [2]

Fetal growth can be split into two periods. Broadly, in the first trimester of pregnancy there is hyperplasia due to rapid mitosis, while in the third trimester growth occurs through hypertrophy. The second trimester involves a mixture of these two processes. Any action of MMN in our study would have been mainly through hypertrophy, as supplementation was started in the second trimester. Weight gain also changes over gestation: from 15-20 g/day at 24 weeks to 30-35 g/day at 34 weeks.[107]

Each organ or system has sensitive periods for growth, so stresses in certain time periods can have an exaggerated effect. A hierarchy is thought to exist between tissues related to the organ's importance to survival, and probably also reproduction. There is preferential protection of the brain, for example, at the expense of other organs such as the kidney and lungs - which have minimal function in-utero. [108] In a situation of poor nutrition, fetal growth reduces, but not uniformly. An in-utero stress is more likely to affect tissues lower in the hierarchy as they are less important for immediate survival. These plastic tissues buffer the ones that are canalized and unable to adapt to immediate pressures.[87]

Growth restriction can also lead to changes in metabolism. The best studied of these is insulin production and resistance. A systematic review of 48 studies, mostly from developed countries, by Newsome et al showed an inverse association with insulin secretion and glucose levels. Some discrepancies in the association with glucose (in glucose tolerance tests) were seen in child studies, but there was greater uniformity in adults. [109]

2.5.1.2 Effect of fetal growth on later phenotype

The associations of birthweight with later outcomes are based on retrospective observational studies, but prospective studies and trials are increasingly being conducted. In a large study of 19 birth cohorts (n = 44 374) from around the world, SGA was associated with an increased odds of stunting of 2.43 (95% CI 2.22, 2.66). The risk almost doubled when combined with being born preterm. Given the high prevalence of SGA, the population attributable risk for stunting in South Asia was 0.18 (95% CI 0.11, 0.24).[110]

While fetal growth is positively associated with child and adult growth, it tends to be negatively associated with fat percentage.[111,112] A review of the literature in developing countries by Yang and Huffman provided evidence for this, showing that low birthweight was

associated with both relatively higher fat mass and lower lean mass. [113] Higher weight gain early in life (infancy) is thought to be related to greater BMI later, but, at least in developing countries, the association is with lean rather than fat mass.[114,115] This idea of low lean mass but relatively high fat mass was termed the Thin-Fat phenotype and is common amongst Indian babies. [116]

In a study of 1012 children from the Netherlands, in multivariable models including current weight, Ay et al showed an inverse association between estimated fetal weight at both 20 and 30 weeks and subcutaneous fat at two years, measured by skinfold thickness. Interestingly, the strongest association was at 30 weeks gestation.[117] Further studies from the same cohort examined the importance of growth in different trimesters of gestation. Jaddoe et al showed an inverse association between first trimester growth and fat mass in children aged six, specifically with waist, but not hip fat mass.[118] Durmuş et al found a negative association between second trimester estimated fetal weight and preperitoneal fat, but not subcutaneous fat at two years of age.[119] Programming of fat has thus been hypothesized to occur at different times in pregnancy: peritoneal fat related to the second trimester and subcutaneous fat to the third.[112] In addition to the association with adiposity, low birthweight is associated with changes in insulin levels and resistance.[120,121]

Studies in rats by Venu et al showed that reduction in vitamins (pyridoxine, B1, B2, folic acid, B12, A, D and E)[122] and minerals[123] (iron, zinc, magnesium and calcium) led to growth restriction in-utero. In both cases, the offspring went on to have greater fat percentage, but in the case of the vitamin restricted maternal diet, if a normal diet was commenced in the pups they did not develop increased fat percentage.

2.5.1.3 Effect on subsequent generations

There is evidence that the growth of a woman as a fetus is associated with that of her children. Understanding of how intergenerational causes can affect growth is complex. Martorell and Zongrone identify many factors that influence child health (Figure 5). Underpinning this is the concept of intergenerational poverty.[124] Growth of a woman in early life affects her final adult size, which in turn has an effect on her offspring. Maternal size, most notably height, is associated with fetal outcomes. The best example of this is disproportion between the mother's pelvic size and the newborn's head (cephalo-pelvic disproportion). In a study in the United States, Collins et al showed that mothers who were low birthweight were more likely to have low birthweight children. After controlling for economic and maternal factors (maternal age, education, marital status, parity, adequacy of prenatal care utilization, and cigarette smoking), they found an increased odds for offspring SGA of 1.8 (95% CI 1.5, 2.3).

[125] Similar results were seen in a cohort study from Brazil by Horta et al, who found that maternal birthweight was associated with the birthweights of her children (P<0.001). Paternal birthweight was, however, not associated. [126]

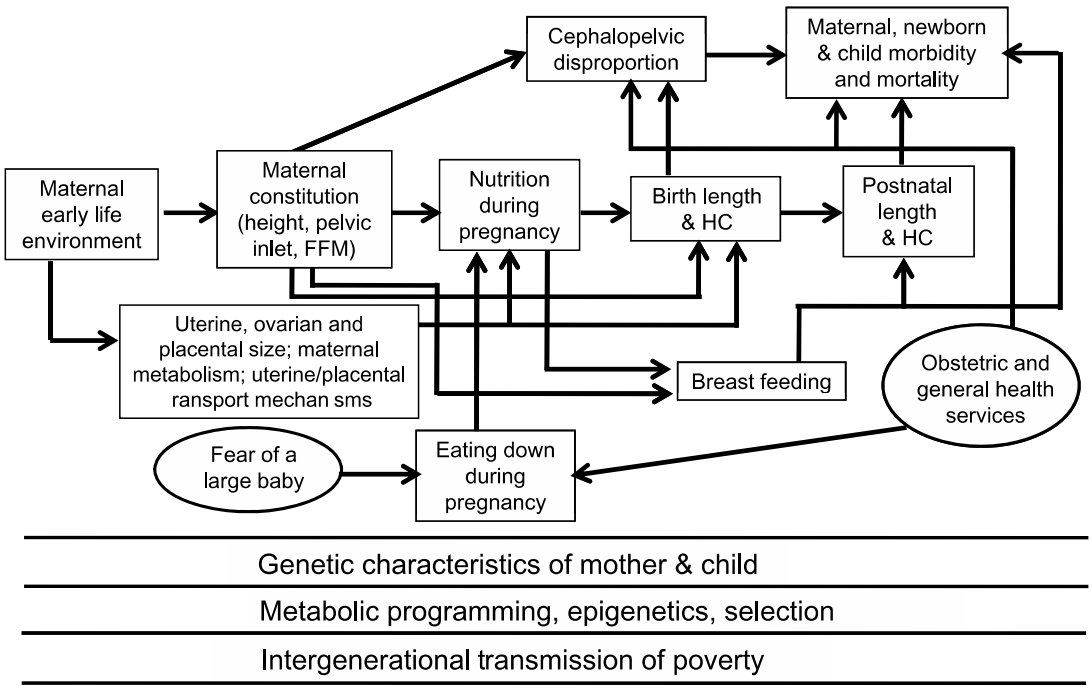

Figure 5: Intergenerational influences on child nutrition

Reused with permission for use in a dissertation from Martorell and Zongrone, Paediatric Perinatal Epidemiology, 2012. [124]

2.5.2 Child growth

Malnutrition in childhood is highly prevalent worldwide. Globally in 2011, 165 million children were estimated to be stunted and 43 million were overweight.[127,128] Karlberg describes how growth can be divided into three phases: infant (a continuation of fetal growth), childhood and adolescent. The lowest musculoskeletal growth rate is in the late childhood period (6 years to puberty). Each phase is controlled by a different set of hormones: insulin and insulin-like growth factor in infancy, growth hormone in childhood and the sex steroids in adolescence. Sitting height (truncal) growth has a greater association with infant growth and puberty, while leg length is more associated with childhood growth (see Figure 4). Growth faltering may be due to problems in moving from one phase to another. Some of the difference between male and female height is explained by different growth in these phases. The female childhood growth period is shorter and leaves females with shorter limbs and overall height.[129]

There are short-term consequences of malnutrition, such as increased risk of infection and mortality, and long-term consequences such as increased risk of chronic disease. Fat mass is a better predictor of long-term, particularly cardiovascular, health. [130] Even in populations with low mean BMI, the relative percentage of fat can be high, making the accurate assessment of nutritional status crucial.[5,27] Obesity is also a growing problem worldwide, with an increasing prevalence in both low- and high-income countries. Obesity in childhood is related to obesity later in life, with a resulting increase in chronic disease. In addition to the high prevalence of underweight, 80% of overweight children are thought to live in developing countries,[127] with many countries experiencing the "double-burden" of under- and over-weight children.[131]

The risk of obesity is multifactorial and includes activity levels, an energy dense diet and a genetic propensity. It is increasingly being shown that early life diet also plays a key role.[113] Again, some recent evidence from epigenetic studies points to an association between methylation states at birth and childhood adiposity. [132]

Children who are obese in childhood are likely to be obese as adults. A review of the literature by Serdula et al found that about a third of obese pre-school children and half of obese school-aged children continued to be obese as adults. [133] Singh et al showed between a doubling and a tenfold increase in risk of an overweight child being overweight as an adult, compared with a normal weight child. In addition, the risk of being overweight increases as childhood weight increases. [134]

Body composition analysis in childhood is one method of measuring nutritional status.[135] Most research on "obesity" is based on BMI, but body composition has the advantage of being able to distinguish fat mass and lean mass. High-precision methods for the assessment

of body composition such as water or air displacement are currently deployed in clinics and research institutions. However, most of these methods are unsuitable for large-scale epidemiological studies as they are either impractical or expensive.[136]

2.5.2.1 Catch-up growth

While growth failure is greatest in early life - the most important determinant of adult stature - there appears to be some scope for catch-up growth. This can occur in-utero. If the growth of an embryo/fetus is affected early in gestation, it is possible that some degree of catch-up can occur, resulting in a normal or near-normal birthweight. Growth may, however, be disproportionate and the child may still have some of the problems resulting from growth faltering.[137]

An attempt to compensate for poor weight gain in infancy is an earlier childhood component of growth. In childhood, there are two main ways in which catch-up growth can occur: through delayed or prolonged puberty.[138] Prentice et al suggest that catch-up growth is due to maturation and adaptation of the child's immune system. They showed that linear growth in puberty can be prolonged, leading to greater adult height. Boys may continue to grow until aged 22-24 and girls until 18-19.[139] Both they and the COHORTS study group investigated growth in populations in Brazil, Guatemala, India, the Philippines and South Africa. They showed that height was mostly lost in early life, with a nadir at the age of two years. By mid-childhood some catch-up growth occurred (height-for-age z scores): Brazil=0.18, Guatemala=0.61, India=-0.01, Philippines=0.15 and South Africa=0.81 z scores. The one exception was India, where z scores continued to reduce. [140]

Catch-up growth in weight (independent of height) in childhood may, however, be detrimental to health. [5] The combination of being born growth restricted and undergoing rapid catch-up growth was associated with insulin resistance in the studies from Pune in India by Yajnik et al.[141]

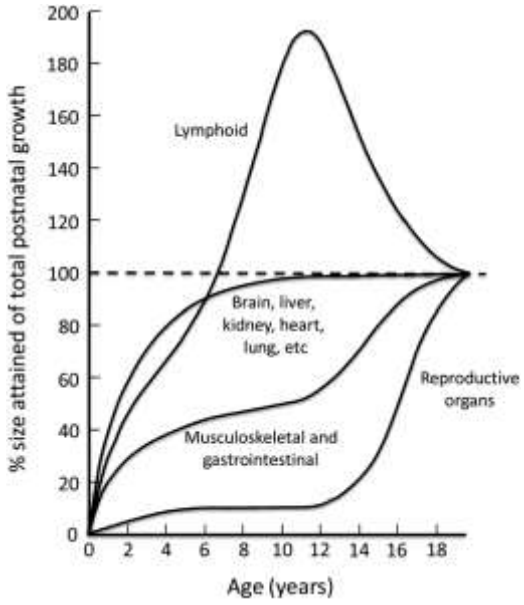

Figure 6: Growth of organ systems in humans.

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2.5.3 Role of micronutrients

Micronutrients are likely to act on fetal growth in a number of ways. Christian and Stewart set out potential pathways based on "hormonal adaptations", particularly via the hypothalamic-pituitary-adrenal axis and "epigenetic regulation". These result in changes in the structure of organs, fat metabolism and deposition and appetite and activity levels. [3]

I describe in detail the effects of multiple micronutrient supplementation in Section 2.9, as the UNIMMAP supplement containing 15 vitamins and minerals was used in the study. I have only briefly summarized the evidence for single micronutrients on growth because it is difficult to know how each micronutrient may act in combination with others. I have also limited myself to descriptions of supplementation trials or meta-analyses. A large amount of observational epidemiological evidence has looked at the effects of antenatal micronutrient supplementation. The results of this work can be misleading, as in addition to the complexity of clearly identifying the nutrient of interest, publication bias and residual confounding may lead to falsely positive results.[143] Blinded randomised controlled trials are generally considered the best forms of evidence because trial evidence is less prone to confounding. The meta-analyses combine the trial data in a systematic way and should provide the most accurate and reliable answer to the question of whether micronutrients lead to an effect on growth.

In terms of fetal and child growth, in general, trials have not found associations between single nutrients given in pregnancy and growth.[2] A discussion of micronutrients for which an association has been postulated follows (iron and folic acid are considered in section 2.8).

Vitamin A and β Carotene (its inactive precursor)

A meta-analysis of three trials in HIV positive women from Malawi, South Africa and Tanzania by Thorne-Lyman and Fawzi showed a small, but not statistically significant, effect on mean birthweight (40g; 95% CI -10, 80g). Two of these studies found no effect of vitamin A on SGA (0.89; 95% CI 0.68, 1.17),[144] and follow-up of one of them found no difference in weight, length, head circumference or mid-upper arm circumference (MUAC).[145]

A recent trial from Indonesia of vitamin A in pregnancy by Prawirohartono et al (n=2173) showed a small increase in birth length, but not birthweight, as compared with placebo (all participants received iron and folic acid). Interestingly, the combination of vitamin A and zinc did not show an effect on birth length. In this trial, zinc alone improved birthweight and length. [146]

Vitamin B6

Vitamin B6 acts as a co-enzyme in numerous metabolic reactions. Meta-analysis by Dror and Allen found a positive effect on birthweight (217g; 95% CI 130, 304g), but this result should be treated with caution. Only three studies (two trials and one cohort) were included, with a total number of 247 participants, and in one study 90% were lost to follow-up. All studies were from developed countries.[147]

Vitamins C and E

Vitamin C is an anti-oxidant involved in the formation of collagen and carnitine, as well as hormones and neurotransmitters. The review by Dror and Allen included eight trials (five from developed countries), six of which also gave vitamin E. The results showed no effect (-26g; 95% CI -0.82, 29). Although the studies showed high heterogeneity (I^2 = 93%), the lack of effect was consistent across all of them.[147]

Vitamin D

A meta-analysis of three supplementation trials (n=507) of vitamin D (two from the UK and one from India) by Thorne-Lyman and Fawzi found a reduction in low birthweight of 60% (95% CI 0.23, 0.71).[148] A Cochrane review by DeRegil et al had similar findings, but the results for low birthweight (n=463) did not attain significance: RR 0.48 (95% CI 0.23, 1.01).[149] The meta-analyses used the same trials but a different result for one. Thorne-Lyman

and Fawzi[148] used the results from publication by Yu et al[150] after personal correspondence with the authors. De-Regil et al referenced a poster from the same study.[151] These results are difficult to verify because neither publication nor poster by Yu et al reports the results for birthweight or low birthweight. Nevertheless, both meta-analyses point to a beneficial effect of vitamin D supplementation on low birthweight.

Zinc

Zinc has wide-ranging effects in the body due to its interactions with numerous enzymes. It has been shown to affect skeletal growth in-utero in animal studies[152] and was presumed to do so in humans. A meta-analysis of 13 trials of zinc supplementation in pregnancy showed no effect on birthweight (-9.9 g; 95% CI -35.7, 16.0) and no difference in SGA. It did, however, report a small reduction in the relative risk of preterm (0.86; 95% CI 0.76, 0.97).[153]

Calcium

A meta-analysis of 11 trials from both richer and poorer countries by Imdad and Bhutta showed that supplementation with calcium could increase birthweight by 85 g (95% CI 38, 134 g). It may also reduce preterm birth (0.76; 95% CI 0.60, 0.97).[154]

Magnesium

A Cochrane review of antenatal magnesium supplementation by Makrides and Crowther showed an increase in birthweight, but the 95% confidence intervals just crossed zero (50.8g; -0.22, 101.9g). The meta-analysis included four trials, all from developed countries (n=1482). Two were included in a meta-analysis, which showed a reduction in the proportion of SGA infants (RR 0.70; 95% CI 0.53, 0.93).[155]

2.6 Respiratory

To my knowledge, the long-term effects of antenatal MMN supplementation on respiratory function have not been investigated, but there is evidence that individual micronutrients are associated with later lung function and lung disease. Micronutrients may have a direct effect on lung function or act via improvements in birthweight and child anthropometry, changes in respiratory disease risk or alterations to the way in which the organism responds to environmental stresses. The effects of these on factors can be cyclical. For example, poor lung function can lead to increased risk of respiratory disease,[156,157] which may itself lead to poorer lung function.[158]

2.6.1 Airway development

Knowledge of the early life determinants of lung development is important as it can determine lung growth and function later in childhood and adult life. Lung development depends on genetic, epigenetic and environmental factors, during both intra- and extra-uterine life. Airway development occurs predominantly in-utero,[159] but infancy and childhood are also important for lung growth. Preterm delivery is a major cause of reduced lung function and later respiratory morbidity.[160]

Lung embryology follows five stages: embryonic, pseudoglandular, canalicular, saccular and alveolar (Box 3). Approximately 100-150 million alveoli form by birth. Alveolar formation ceases in early childhood, but maximum lung capacity is only attained in early adulthood. The alveoli grow in size and the surface area for gas exchange increases by about 20 times from birth.[161] Lung growth is controlled by a large number of growth and transcription factors, and lung development requires movements and fluid. Fetal lung movements, controlled by the phrenic nerve, are required for development. This has been shown in rabbit fetuses, where section of the cord at C1-C3 resulted in reduced lung weight.[162] A number of factors are believed to increase (for example, hyperglycaemia and caffeine) or decrease (for example, hypoxia and maternal alcohol) fetal lung movements.[163] Airway smooth muscle receives innervation from approximately eight weeks' gestation and fetal breathing can be seen from around ten weeks.[161]

Lung fluid, made by epithelial cells in the distal airways, is also needed. If this fluid is drained, as happens during premature rupture of the membranes, this results in hypoplasia. The fetal movements combined with resistance in the upper airways help to maintain some fluid in the lungs.[163]

In people for whom lung development has been interrupted or environmental stresses increase to a destructive level, disease will result. In this sense the trajectory for lung function is set early in life. In the longer-term, micronutrient supplementation could also have an effect on chronic obstructive pulmonary disease (COPD) as this is in part related to lower peak lung function. In a follow-up of children born to mothers who were pregnant during the Dutch famine of 1944-45, Lopuhaä et al produced conflicting evidence of this. They showed that, compared with people conceived before or after the famine, those who were exposed to the famine in early to mid-gestation were more likely to suffer from COPD in adulthood, but their lung function did not differ significantly.[164] Adults with poorer lung function also have increased risk for respiratory morbidity and also mortality from other diseases.[165]

1. Embryonic, 0-7 weeks gestation. Lung tissue develops from the ventral foregut of the endoderm at approximately 24 days. The separate lungs can be seen by six weeks' gestation.
2. Pseudoglandular, 7-17 weeks gestation. Branching and elongation of the airways and blood vessels and formation of the terminal bronchiolus by 17 weeks. Epithelial multiplication is under the influence of hormones such as epidermal growth factor and insulin-like growth factor.
3. Canalicular, 17-27 weeks gestation. Formation of the alveolar ducts and primitive alveoli. Vascularisation of the peripheral mesenchyme also occurs and thinning of the airway walls. The fetal lungs can be said to resemble adult lungs by 24 weeks.
4. Saccular, 28-36 weeks gestation. Enlargement of the peripheral airways and formation of acini.
5. Alveolar, >36 weeks to 2 years of age. Alveoli develop and increase in number.

Box 3: Main embryological stages in the development of the lung [159,161,163]

2.6.2 Birthweight and lung function

Research has shown that increased birthweight is associated with better lung function in later life.[23,24] Being born with a low birthweight, particularly SGA, is a risk factor for pneumonia in childhood,[166] and is associated with reduced airway function in early childhood, after adjusting for birthweight.[167]

Despite their own study finding no association between birthweight and adult lung function, (10 ml per kilogram; 95% CI -20, 40 ml after adjusting for age, observer and height squared), Lawlor et al showed, in a meta-analysis of eight studies, a positive association of 24 ml (95% CI 13, 35 ml) increase in forced expiratory volume in the first second (FEV_1) per 500 g increase in birthweight.[24] One study included from a developing country by Stein et al (south India) found a 90 ml (95% CI 10, 160 ml) increase in FEV_1 per 450 g increase in birthweight after adjusting for height and age. [23] A later study by Canoy et al, not included in the meta-

analysis, found similar results: a 500 g increase in birthweight was associated with a 53 ml increase (95% CI 38, 68 ml) in FEV_1 in 31-year-old adults after controlling for a number of adult and childhood confounders.[168]

Turner et al looked in more detail at fetal anthropometry using ultrasound measures in-utero. They found positive associations between crown-rump length (CRL) in the first trimester and lung function at 5 years of age. Each 1 mm increase in CRL was associated with 5 ml increase in FEV_1 and 6 ml in FVC, [169] and a 6% (95% CI 1, 11%) reduction in the risk of asthma in childhood.[170] Maternal α–tocopherol concentrations were associated with fetal size CRL (p=0.002), but no association was found between α–tocopherol and later lung function.[169]

Overall, the epidemiological studies have found a positive association between birthweight and adult lung function after controlling for current size. Some degree of caution should be maintained, though, as all these studies have been observational and most retrospective and inadequate control of confounding cannot be ruled out. Differences may also be explained by differences in population, setting, disease morbidity and toxin exposure, amongst other things.

The mechanisms by which fetal growth restriction may lead to impaired lung function are through reduced alveolar formation and development, potentially by a reduction in fetal elastin, leading to reduced surface area and lung weight. Gas exchange may also be affected by thickening of alveolar walls.[171,172] IUGR can lead to alteration in methylation of specific, lung-related genes. An example of this is PPAR**γ,** which is important for lung development. Joss-Moore et al showed in a rat model that IUGR led to increased methyl CpG binding protein 2 during alveolarisation. In addition, there were differences in male and female offspring. In males, there was a decrease in H3K9 methylation, while in females there was an increase. In normally grown rats, methylation was the same. [173]

2.6.3 Role of micronutrients

Micronutrients may increase birthweight as described in section 2.5 and consequently improve lung function. Reductions in nutrients can also lead to structural changes in the fetal lung affecting alveolar size and number. Antenatal micronutrients may lead to changes in lung function by altering respiratory disease risk. A review of the effect of maternal and childhood diet by Devereux,[174] for example, highlighted its importance in the development of asthma, a condition suffered by 300 million people.[175] Here, I will outline the main micronutrients involved in determining lung function, not purely mediated by a change in birthweight.

Vitamin A

The best evidence for long-term effects exists for vitamin A. Supplementation in both animals[176] and humans[177] has shown positive effects on lung function. Animal studies in lambs have shown that antenatal administration of vitamin A can improve lung function.[176] This process may in part be due to its role in the modulation of vascular endothelial growth factor, as shown by antenatal vitamin A supplementation in a mouse model.[178] Vitamin A acts through its active metabolite, retinoic acid, binding to nuclear receptors (retinoic acid receptors, α, β, γ and retinoic X receptors α, β and γ), which in turn bind to promoter regions of target genes, leading to a wide array of effects.[179] Retinoic acid acts in the signalling of fibroblast-mediated alveolar septation.[180] It affects gene transcription and inhibits formation of fibroblast growth factor 10, which is involved in a number of cell growth and morphogenic processes.[161] It has also been linked with a reduction in bronchopulmonary disease and is recommended for use in extremely low birthweight infants. [181]

In the follow-up of a trial of vitamin A supplementation versus placebo and beta-carotene in Sarlahi, Nepal, Checkley et al showed that, after adjustment, antenatal vitamin A supplementation led to a greater FEV_1 and forced vital capacity (FVC) in children at 9-13 years of age. The magnitude of the difference was 46 ml in both cases, which corresponded to 3% of the mean difference in the population.[177] While this was the most similar study to ours, it differed in a number of ways. Firstly, the trial design was different: Checkley's study was a cluster randomised trial in which married women were given the supplements over 3.5 years, not just in the second and third trimesters.[182] Secondly, the comparator group was placebo, while ours was iron and folate. Thirdly, there may have been a dose effect: they gave 7000 μg/week of vitamin A, compared to 800 μg/day in our trial.

Vitamin D

A recent trial of antenatal vitamin D supplementation in the UK showed no effect on lung function or in symptoms of wheeze at three years of age. Goldring et al gave vitamin D supplements from 27 weeks gestation and measured lung function using impulse oscillometry (an alternative technique to spirometry useful in young children that measures respiratory impedance using short pulses of air pressure to test airway resistance). This trial does not rule out an effect of vitamin D as it was small and only 28% (n=51) produced acceptable results. The generalizability from the UK is also questionable. [183]

Maternal vitamin D depletion has been shown to alter lung structure and reduce lung volume in mice, independent of their somatic growth.[184] In humans, the evidence for an effect on lung function and respiratory disease is mixed, with associations being seen in some cases,[185,186]

but not in others.[187] There is evidence that deficiency in antenatal vitamin D has been associated with wheeze/asthma in offspring in developed countries.[185,186,188] However, Pike et al did not find such an association between vitamin D levels at 34 weeks and wheeze, asthma or lung function in UK children at age 6.[187] Similarly, a cohort study of 1200 children aged 4-6 years from Spain by Morales et al found no association with wheeze or asthma with maternal 25-hydroxyvitamin D concentrations. There was a possible association with lower respiratory tract infections at one year.[189] Exposure to air pollution may interact with nutritional deficits. One example is a reduction in conversion of vitamin D, as has been shown with an association between PM_{10} and reduced vitamin D levels in infants. [190]

Vitamin E

A cohort study from the UK looked at the association between antenatal vitamin E intake, assessed by a food frequency questionnaire and respiratory outcomes. Comparing the highest tertile of vitamin E with the lowest, it found a difference in FEV_1 of 77ml (95% CI 26, 128 ml) at 5 years of age. There was a negative association with wheeze and asthma.[191]

Selenium

A cohort study of children from the UK showed a negative association between maternal selenium concentrations and wheeze (as defined by maternal recall using questions from the International Study of Asthma and Allergy in Childhood) in children aged 5: adjusted OR 0.86 (95% CI 0.76, 0.97).[192]

2.6.4 Air pollution

Air pollution is amongst the most important environmental determinants of lung function and respiratory disease in children. Particle mass and other toxins such as nitrogen oxides can damage the developing lungs, leading to immediate effects in the child and long-term effects as adults.[193,194] The main source of air pollution is indoor burning of biomass fuel, which was found to adversely affect lung function in adults in Nepal,[52] and has been linked to respiratory infections in children.[195]

2.6.4.1 Biomass

Indoor air pollution is a major cause of ill-health in low-income countries. It is mostly due to the burning of biomass fuels (also referred to as “solid fuels”), a group of organic materials - particularly wood, dung, straw, and charcoal – used as a source of heat and light.[196] Incomplete combustion of biomass fuels in poorly ventilated houses produces domestic levels

of airborne particles hundreds of times higher than commonly encountered outdoors.[197] It is estimated that between one-third and half of the world's population use biomass as a source of energy as it is readily available and usually cheap.[198] Globally, solid fuel use is estimated to cause 3.5 million premature deaths per year, around one million of which are attributed to acute respiratory infections in young children.[32,199] The deaths occur predominantly in poorly resourced settings where an increased susceptibility to illness coexists with reduced access to healthcare. As well as increased mortality, household cooking with solid fuels accounts for 4.3% (95% CI 3.4, 5.3) of DALYs lost worldwide (6% for children under 5 years old), while ambient air pollution accounts for a further 3.1% (95% CI 2.7, 3.4). These figures make indoor air pollution the third leading contributor to global disease burden, and the highest in South Asia.[32] There is limited information on indoor air pollution levels in Nepal, although one study showed a geometric mean 24-hour level of 455 $\mu g/m^3$ in houses where biomass fuels were burnt.[52]

2.6.4.2 Health effects

Children experience adverse health effects from exposure to air pollution. Systematic reviews and meta-analyses show an increased odds of illness with solid fuel use, with odds ratios of 2.80 (95% CI: 1.85 to 4.00) for chronic obstructive pulmonary disease,[200] 1.78 (95% CI 1.45, 2.18) for pneumonia in children under 5,[195] 1.50 (95% CI 1.17, 1.94) for lung cancer,[201] and 1.30 (95% CI 1.04, 1.62) for tuberculosis.[202] There is also weaker evidence for a link with low birthweight,[203,204] anaemia and stunting.[196,197,205] In a study of the association between biomass usage and respiratory infection in Nepal, Bates et al found an increased odds of infection related to not only biomass fuels, but also kerosene and gas usage in comparison with electricity.[53] The incidence of respiratory infections plateaus with increasing particle mass over 1000-2000 $\mu g/m^3$, suggesting that air pollution needs to be reduced to low levels to yield a health advantage.[206 207]

Particle mass can directly damage the respiratory tract through oxidative stress.[208] The smoke produced when biomass burns also contains a number of toxins, such as carbon monoxide, nitrogen oxides and sulphur dioxide.[198] These affect both cell-mediated and humoral immunity.[209] A study from the USA showed that particle mass was associated with airway damage using exhaled nitric oxide as a proxy. These were found to be independent of asthma status.[210]

Exposure to air pollution antenatally can affect the lung function of the children later in life. A study from Poland by Jedrychowski et al measured $PM_{2.5}$ (particles with median diameter less than 2.5 μm) levels over 48 hours in the second trimester and found an inverse

relationship between FEV_1 or FVC and $PM_{2.5}$.[211] There is also some evidence for interaction between genes, for example those coding for anti-oxidants which act to reduce the inflammation produced by the air pollution.[212] Similar to the epigenetic effects of in-utero nutrition, particle mass can lead to changes in DNA methylation. An example is a study of PM_{10}, in which global methylation measured using two techniques (Alu and LINE-1) was inversely associated with long-term exposure to PM_{10} in multi-variable models. Interestingly, short-term (over three days) exposure did not elicit similar changes.[213]

An important air pollution exposure in-utero is tobacco smoke. Independent of birthweight, there is evidence that smoking in pregnancy is associated with poorer lung function in the child.[214] [215] DNA methylation in eight genes in children was also associated with exposure to tobacco smoke in-utero.[216] Bouzigon et al showed an increased risk of asthma associated with early exposure to tobacco smoke, related to the 17q21 region,[217] and tobacco smoke is associated with hyper-methylation in studies of lung cancer.[218] Smoking is not considered in great detail here because the prevalence during pregnancy amongst the women in the study was very low.

2.7 Blood pressure

The previous follow-up at 2.5 years showed a 2.5 mmHg lower mean blood pressure in the MMN allocation group.[8] Blood pressure varies according to age, sex, height and many other temporary factors such as anxiety levels or having just taken a meal. Early changes to the environment in-utero have been shown to be associated with renal disease and hypertension later in life,[219] but the association with later blood pressure is complex (Figure 7). In addition to the role of the kidneys, which I will describe, offspring blood pressure is associated with placental size, maternal blood pressure, child growth, hormonal and nervous system regulation and the structure of the vasculature.[220]

It is possible that the relationship between micronutrients and later blood pressure is mediated by change in birthweight or that birthweight and blood pressure may be the joint results of other processes such as hormonal responses. Birthweight is believed to be inversely correlated with blood pressure later in life, a 1 kg increase being associated with a fall in blood pressure of -2 to -3 mmHg.[221] This is supported by a large meta-regression by Gamborg et al that included nearly 200 000 adults from the Nordic countries. It found a negative relationship with systolic blood pressure at 50 years of age, of -1.52 mmHg/kg (95% CI -2.27, -0.77) in men and -2.80 mmHg/kg (95% CI -3.85, -1.76) in women.[222] Contradictory evidence in a meta-analysis by Huxley et al showed a smaller reduction of -0.6 to -0.4 mmHg, which they felt was accounted for by publication bias.[223] A study of 1570 children from developing countries found negative associations of birthweight with systolic blood pressure in multivariable regression models using data from China, Guatemala and Chile, but not Nigeria.[224]

Hypertension can also result from a combination of growth restriction in-utero and rapid growth in childhood.[225] In a cohort study from Sweden, Leon et al showed that low birthweight and above average adult height were associated with the highest blood pressure. These men were considered to have not reached their full growth potential in-utero.[226]

The mechanism by which birthweight relates to later blood pressure may be via its relationship with kidney size or, more specifically, nephron number, which is hypothesized to be related to essential hypertension.[225] Nephrogenesis occurs in-utero from the 9th to the 36th week of gestation. Nephron number represents the difference between the number formed prior to birth and the number lost during life.[227] In humans, birthweight is positively correlated with nephron number and intra-uterine growth restriction is associated with smaller kidneys relative to body size at birth, and also reduced kidney growth during early life.[228] Nephron number also varies by sex, age and ethnicity. In suboptimal in-utero conditions the fetus will preserve the brain and the heart at the expense of other organs such as the

kidneys.[229] Konje et al showed a difference from 26 weeks gestation onwards between SGA and appropriate-for-gestational-age fetuses. The reduction occurred more in transverse and antero-posterior diameters than in length.[230] As described by Brenner, the reduced number of nephrons may increase capillary pressure, leading to glomerular sclerosis which later results in hypertension.[225] The relationship between nephron number and hypertension is, however, not straightforward as compensatory hyper-filtration can occur, maintaining a normal blood pressure.[231]

The epidemiological studies show a similar magnitude of blood pressure reduction per kilogram to that found in the two-year follow-up of our cohort, but this was only associated with a 77 g increase in birthweight, i.e. a tenfold greater effect. This might suggest a mechanism other than one simply mediated by birthweight. In terms of micronutrient involvement, vitamin A and iron are thought to be the most important, and our trial showed improvements in maternal vitamin A (retinol) and a reduction in maternal anaemia.[7] Retinoic acid regulates the c-Ret receptor for glial-derived neurotrophic factor, which is in turn involved in regulating nephron number.[232] In rat studies, iron has also been implicated in changes in blood pressure and kidney size,[233] but we would not expect to see this relationship in our study as both intervention and control groups received iron supplements (and assuming that the 30 mg of iron in the MMN is truly equivalent to the 60 mg in the control).

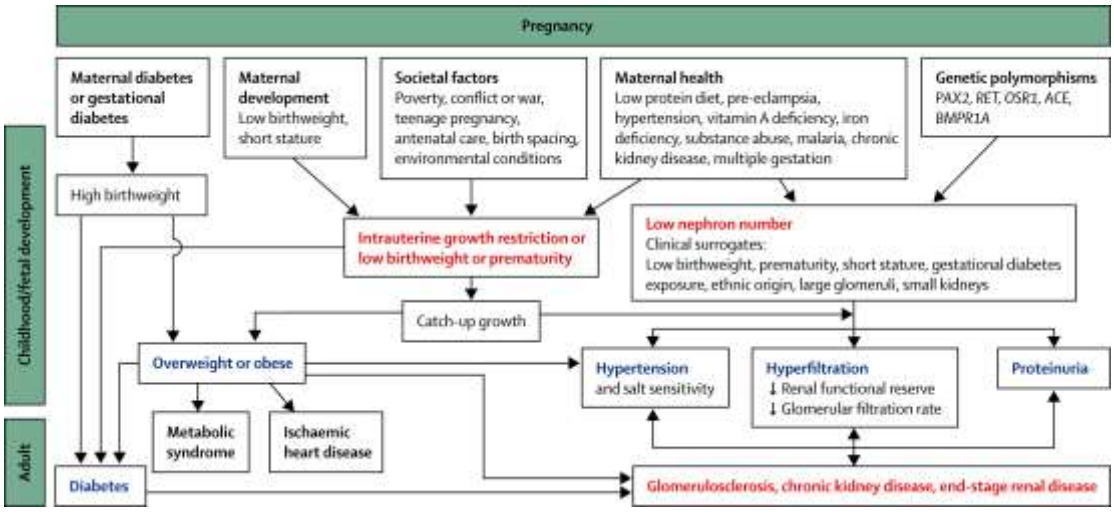

Figure 7: Conceptual diagram for early origins of hypertension and renal disease

Reused with permission for use in a dissertation from Luyckx et al, Lancet 2013.[219]

2.8 Control supplement

The study was not able to investigate the effects of iron and folic acid as they were present in both MMN and control supplements. However, I will briefly consider them as they are both important micronutrients.

2.8.1 Iron and folate together

Pena-Rosas et al performed a Cochrane review of iron and folic acid supplementation in pregnancy in 2012. When comparing trials that used iron and folic acid with supplements that used "the same supplements without iron and folic acid", they found only two trials and one of them made up 97% of the total records. Meta-analysis showed a 57.7 g (95% CI 7.7, 107.8 g) increase in birthweight and no effect on prematurity.[234]

2.8.2 Folate

The use of folic acid is common in pregnancy as it has been shown to reduce neural tube defects[235] and mortality arising from them.[236] Folic acid is recommended for women throughout the world and 53 countries have regulations for fortification. [237] There is evidence, particularly from animal and to some extent human studies, that folate can affect DNA methylation.[238,239] It is an essential co-factor in the synthesis of purines and thymidylate and is a potent DNA methylator through the production of S-adenosyl-L-methionine (a co-factor in one-carbon metabolism). A mouse study found that the offspring of pregnant mice given a high folic acid diet showed greater airway responsiveness, in line with what would occur in asthma. The mechanism by which this occurs is thought to be via a change from a T helper 1 phenotype to a T helper 2 phenotype, related to hypermethylation of the runt-related transcription factor-3 promoter region. Effects were transmitted to the F2 generation via males, indicating an effect on the germ cells. Hollingsworth et al found differential methylation at 82 loci. These effects were reversible when demethylating agents were used.[240]

It is probable that antenatal folic acid alone increases birthweight. A meta-analysis of five trials by Lassi et al showed a non-significant increase of 105.0 g (95% CI -25.0, 235.4 g), but when one trial was removed due to suspected heterogeneity the difference increased to 135.8 g (95% CI 47.9, 223.7g).[241] Analysis of the association between antenatal folic acid intake (measured by food frequency questionnaires) and body composition from the ALSPAC cohort in the UK found no association at 9 years of age.[242]

Though the evidence is mixed, cohort studies in humans have suggested an association between antenatal folic acid consumption and wheeze in childhood. A large study of over 3000 children by Haberg et al showed an increased relative risk for wheeze, defined as chest tightness/congestion and whistling/wheezing, of 1.06 (95% CI 1.03, 1.10) related to folate acid in the first trimester. It also found a positive association with respiratory tract infection, 1.09 (95% CI 1.02, 1.15) in children aged 6 to 18 months, though this was less reliable as it was based on maternal recall. The study controlled for estimated intake of other micronutrients and the sample had a high prevalence of folic acid use amongst all participants.[243] Similar increased risk of asthma was seen in Australian children aged <6 years.[244] Bekkers et al reported an association with wheeze in children at 1 year of age, but not later, in Dutch children.[245] Contrary to this, a cohort of 1499 women and children in the United States did not find an association between folic acid supplementation and asthma at six years of age.[246] There is also evidence that antenatal folic acid does not affect lung function.[247]

2.8.3 Iron

In their Cochrane review, Pena-Rosas et al also looked at iron supplementation alone. They found no effect on birthweight in a meta-analysis of nine trials (n=3953): 16.4 g (95% CI -37.3, 70.1 g). Sub-group analysis also showed no effect, but the effect size was larger when a 30 mg supplement was used and when started in the first half of pregnancy.

They then compared any iron-containing supplement with "supplements without iron or no treatment/placebo", and showed an increased birthweight of 30.8 g (5.9, 55.7 g) in 14 trials. Sub-group analysis also showed a stronger effect if started in the first half of pregnancy: 38.6 g (95% CI 3.3, 74.0 g). While not statistically significant, there was also a larger effect with the 30 mg supplement (52.9 g; 95% CI -11.5, 117.3 g) than the 60 mg supplement (27.6 g; 95% CI 2.6, 52.3 g). The lack of statistical significance was likely to be due to the smaller sample size in the 30 mg trials. The presence of maternal anaemia and malarial settings made little difference to the results.[234] A meta-analysis of antenatal multiple micronutrient supplementation also showed no effect on maternal anaemia. [248]

2.9 Multiple micronutrients

2.9.1 Janakpur trial

The study was the second follow-up of the RCT in Dhanusha district, in which pregnant women received either daily multiple micronutrient supplements in the second and third trimesters or iron and folate supplements.[7] I describe in detail the methods from the trial on which the follow-up was based, in section 3.1. The supplement used in the intervention group was the UNIMMAP multiple micronutrient supplement containing vitamin A 800 µg, vitamin C 70 mg, vitamin D 5 µg, vitamin E 10 mg, vitamin B1 1·4 mg, vitamin B2 1·4 mg, niacin 18 mg, vitamin B6 1·9 mg, vitamin B12 2·6 µg, folic acid 400 µg, iron 30 mg, zinc 15 mg, copper 2 mg, selenium 65 µg, and iodine 150 µg.[6] The control group received iron 60 mg and folic acid 400 µg. The micronutrients in the supplement, developed from US and Canadian recommendations (the National Research Council and the Institute of Medicine), were all at their recommended daily allowance. Only folic acid was given at a different dose that was designed to prevent neural tube defects. The formulation was approved by UNICEF, WHO and the United Nations University in 2000. Consideration was given to toxicity levels, side-effects, potential interactions and deficiencies. In the MMN supplement the dose of iron was reduced to 30 mg. This was believed to be equivalent to the 60 mg dose in the control because the addition of vitamin C improves absorption of iron, resulting in the same bioavailability.

The main outcomes of the trial were birthweight and gestation. The results for both trial and two-year follow-up are shown in Table 3. Babies born in the MMN group were 77 g heavier (95% CI 24, 130 g) at birth, but there was no difference in head circumference or length. The proportion of babies with low birthweight (<2500 g) was correspondingly reduced (OR 0.69; 95% CI 0.52, 0.93). There was no difference in gestation or proportion of preterm births, although gestation was 1.5 days longer in the intervention group. This equates to approximately 30 g, but does not fully explain the difference in weight. There was a suggestion of a increased neonatal mortality, but the study was not powered to detect it.

The first follow-up, conducted at a mean of 2.5 years of age,[8] found 917 children (85% of the number who might have potentially been found from the original trial), with similar numbers in both intervention and control groups. Children in the MMN group were 204 g (95% CI 27, 381 g) heavier at 2.5 years, with larger head, chest, hip and mid-upper arm circumferences and 0.2 mm lower triceps skinfold thickness. Waist circumference was also close to a significant difference. Systolic blood pressure was 2.5 mmHg (95% CI 0.5, 4.6) lower. Similar to the birth findings, there was no difference in height. Control and intervention groups were very similar in terms of the confounding variables measured, but loss to follow-up was associated with residence and occupation.

Table 3: Birth and two-year follow-up outcomes of the Janakpur trial

	Intervention Mean (SD)	**Control** Mean (SD)	**Difference (95% CI)**
Birth			
Birth weight (g)	2810 (453)	2733 (422)	**77 (24, 130)**
Length (cm)	48.9 (2·9)	48.6 (3.2)	0.3 (-0·1, 0.6)
Head circumference (cm)	33.8 (2·2)	33.6 (2.2)	0.2 (-0.1, 0.4)
Gestation at birth (w)	39.4 (1·9)	39.2 (2.0)	0.2 (-0.1, 0.4)
2 years			
Weight (kg)	10.900 (1.54)	10.697 (1.38)	**0.204 (0.027, 0.381)**
Height (cm)	84.07 (4.83)	83.76 (4.68)	0.31 (-0.20, 0.82)
Waist circumference (cm)	46.81 (2.84)	46.48 (2.75)	0.33 (-0.01, 0.68)
Head circumference (cm)	46.64 (1.49)	46.40 (1.43)	**0.24 (0.06, 0.43)**
Chest circumference (cm)	48.28 (2.45)	47.96 (2.26)	**0.32 (0.04, 0.60)**
Hip circumference (cm)	46.34 (2.94)	45.95 (2.68)	**0.40 (0.05, 0.74)**
MUAC (cm)	14.42 (1.07)	14.18 (0.99)	**0.24 (0.11, 0.37)**
Triceps skinfold (mm)	7.15 (1.61)	6.95 (1.45)	**0.20 (0.00, 0.40)**
Systolic blood pressure (mmHg)	99.4 (13.68)	101.9 (17.54)	**2.5 (0.47, 4.55)**

Bold results indicate $p < 0.05$.

2.9.2 Meta-analyses

The Janakpur trial was one of several coordinated trials of the UNIMMAP supplement in pregnancy. The UNIMMAP and similar trials are summarized in Table 4 and Table 5, respectively. The results have been combined in a number of meta-analyses that have looked at birth outcomes, summarized in Table 6. They vary in the trials included, but in general they show a small increase in birthweight with a corresponding reduction in SGA of 10-20%. No difference was found in other anthropometric outcomes or gestation. The largest and most recent was a Cochrane review by Haider and Bhutta which showed a reduction in SGA of 13% (95% CI 0.81, 0.95).[21] Kawai et al[19] included the same trials, but also an earlier trial by Fawzi et al in HIV positive women.[249] They showed a 44 g (95% CI 28, 60g) increase in birthweight and a reduction in relative risk for SGA to 0.85 (95% CI 0.78, 0.93) in the MMN

group. Ramakrishnan et al and Shah and Olhsson found increases of 53 g and 54 g, respectively.

The meta-analysis by Fall et al[17] showed a 22.4 g (95% CI 8.3, 36.4) increase in birthweight and a corresponding reduction in the OR for SGA of 0.90 (95% CI 0.82, 0.99). This meta-analysis included the smallest number of trials which were also the most homogenous (the lowest I^2 values). All were conducted in low- and middle-income countries. Nine used the UNIMMAP supplement and the other three used similar combinations and adjusted for cluster design, sex, maternal age and weight, parity and education. The analysts had access to the original data, enabling them to exclude outliers and create population specific reference ranges from which SGA cut-offs were calculated. The values used for the meta-analyses were therefore sometimes a little different from those in the original trial reports.

The discrepancy in results between the meta-analyses may be due to the different trials included and also to the importance given to the trial by Zagre et al [15] from Niger. This trial reported a difference in birthweight of +48 g (95% CI 33, 62), but the meta-analysis by Fall et al used +53.98 g (95% CI –42.53, 150.50). While the difference in effect size was small, the widening of the confidence intervals reduced the weighting of the trial to 2.12%. If the original data from Zagre et al had been used, it would have had a 28.71% weighting in a random effects model, and would give an effect size of +29.9 g (95% CI 16.5, 43.3). The wider confidence intervals seem to make more sense, given the small sample size. I contacted Professors Fall and Osmond, authors of the meta-analysis, who mentioned inconsistencies between the birthweight and gestation data. Ramakrishnan et al[20] used the original number, giving the trial a 38% weighting. Shah et al[18] and Kawai et al[250] did not specify the weightings, but seem to have used the same figures as Zagre. Haider and Bhutta did not calculate the effect on birthweight.[21]

2.9.2.1 Stratification

Study design

When separating into individually and cluster randomized trials, Shah et al found an effect in only the individually randomized trials.[18]

BMI

Haider and Bhutta indicated that the effects of the micronutrients were greater in mothers with higher BMI ($\geq$20 mg/m^2),[21] and Fall et al found a 7.6 g increase in birthweight for every unit increase in maternal BMI. It may therefore be that a certain macronutrient status is needed for the micronutrients to be effective.

Timing of supplementation and perinatal mortality

Kawai et al found lower perinatal mortality in those who started supplementation in the second half of pregnancy.[250] Their explanation was that longer supplementation would increase the weight of the fetus/infant, increasing complications in labour; it could alter metabolic regulation or might reduce death early in gestation, allowing the fetus to survive for longer, but still resulting in death. Similarly, the single trial powered to look at mortality, by Shankar et al in Indonesia,[13] found lower mortality overall in the MMN supplemented group and a greater reduction in those in whom supplementation was started in the 3rd trimester.

Ramakrishnan et al, however, found that supplementation after the first trimester was associated with higher neonatal mortality.[20] They pointed out that studies with higher mortality were comparing 30 mg of iron in the multiple micronutrient supplement with 60 mg in the control group, and also that trials that started supplementation in the first trimester were considered to be of lower quality. Kawai et al have looked at perinatal mortality, including stillbirths and neonatal mortality, and may have found lower overall mortality than Ramakrishnan et al who looked at neonatal mortality only.

The non-statistically significant increase in neonatal mortality seen in the Janakpur trial was similar to the results from the trial in Sarlahi.[35] A pooled analysis of both these trials did suggest an increase in mortality in the neonatal period: RR 1.52 (95% CI 1.03, 2.25). It was proposed that this may have been due to an increased risk of asphyxia amongst the heavier infants, an effect on uterine sensitivity to oxytocin and to the survival of infants who may otherwise have died in-utero.[251] A meta-analysis of these trials and other similar MMN trials did not find a difference in neonatal mortality. However, exclusion of the trial by Shankar et al, did show an increased early neonatal mortality: OR 1.40 (95% CI 1.08, 1.82).[252]

2.9.2.2 Cumulative micronutrient intake

Additional analysis by Roberfroid et al investigated cumulative micronutrient intake (CMI) in their trial from Burkina Faso. This was possible as supplement intake was directly observed. They divided CMI into tertiles and looked at the odds of SGA. SGA reduced by 21% (OR 0.79; 95% CI 65, 95) per increase in tertile. There was also an interaction with gestation, with greater effects later in pregnancy, and maternal BMI. Interestingly, there was a similar association between CMI and birthweight for both MMN and the control (iron and folate) supplements.[253]

2.9.3 Additional meta-analyses

I performed a search of antenatal MMN trials according to PRISMA guidelines.[254,255] I searched the Medline, Embase and Psychinfo databases for "(micronutrient or multiple micronutrient or UNIMMAP) + (pregnancy or antenatal or prenatal)" and found 1486 records. I then removed the duplicates (n=650) and limited the search to humans, English language and randomised controlled trials, leaving 136 records. I searched these records and found 12 relevant trials of MMN. To find follow-up studies, I searched by the first and last authors of the original trials, by country and by trial name if applicable. In addition, I thought it pertinent to summarize the trials that used a similar combination of micronutrients to the UNIMMAP trials. The numbers of studies considered are shown in Figure 8 and a description of the PRISMA search is given in Appendix 2.1.

I redid the meta-analysis of birthweight with the trials that used the UNIMMAP supplement. Where necessary, confidence intervals were calculated using the following formula:

$$\mu \pm 1.96 \times \sqrt{\frac{\sigma_1^2}{n_1} + \frac{\sigma_2^2}{n_2}}$$

where: μ = mean value, σ = standard deviation, n = number in the trial groups

Using the data used by Fall et al, the combined estimate of increase in birthweight changed by a very small amount to 24.0 g (95% CI 8.6, 39.4) when limited to the UNIMMAP trials (Figure 9). The proportion of SGA did not change. I also re-ran the meta-analysis with the original data from the trials with adjusted values where possible, which produced a difference in birthweight of +44.4 g (95% CI 32, 56g: Figure 10). Finally, I performed a meta-analysis of the nine UNIMMAP trials, plus trials testing similar MMN combinations, as summarized in Table 5, as an update of the previous meta-analyses. This resulted in a birthweight difference of +35.9 g (95% CI 22, 50 g: see Figure 11).

In the trial from Bangladesh (MiniMat), women also had access to energy and protein supplements, given either earlier or later in pregnancy. Removing this trial from the meta-analysis in Figure 11 made only a small difference to the outcome (+36.8 g; 95% CI 21.7, 51.9 g).

Meta-analyses provide a good overall picture of the effects of the micronutrients, and are generally considered the highest form of evidence. While there was a relatively small difference between all the trials, it is also useful to consider whether the differences between the studies played a part in the different effect sizes. I could find no obvious difference by region of the world or by study design (individually or cluster randomised). Other factors

such as the gestation at which supplementation was commenced and maternal BMI have been considered in the meta-analyses by others. It is useful in this respect to have studies from the same country, as is the case in Nepal and Indonesia. The Indonesian trials by Shankar et al[13] and Sunawang et al[14] showed similar, non-statistically significant, results. The trial by Christian et al, from the adjoining district in the Terai region of Nepal, produced a quite different result of +7 g (95% CI 40.26, 54.26).[35] The difference may be due to chance, but the lack of a clear explanation would point to the need for much more detailed analysis of the trial methods and participants (for example, knowledge of diet before and during pregnancy), much of which is not possible.

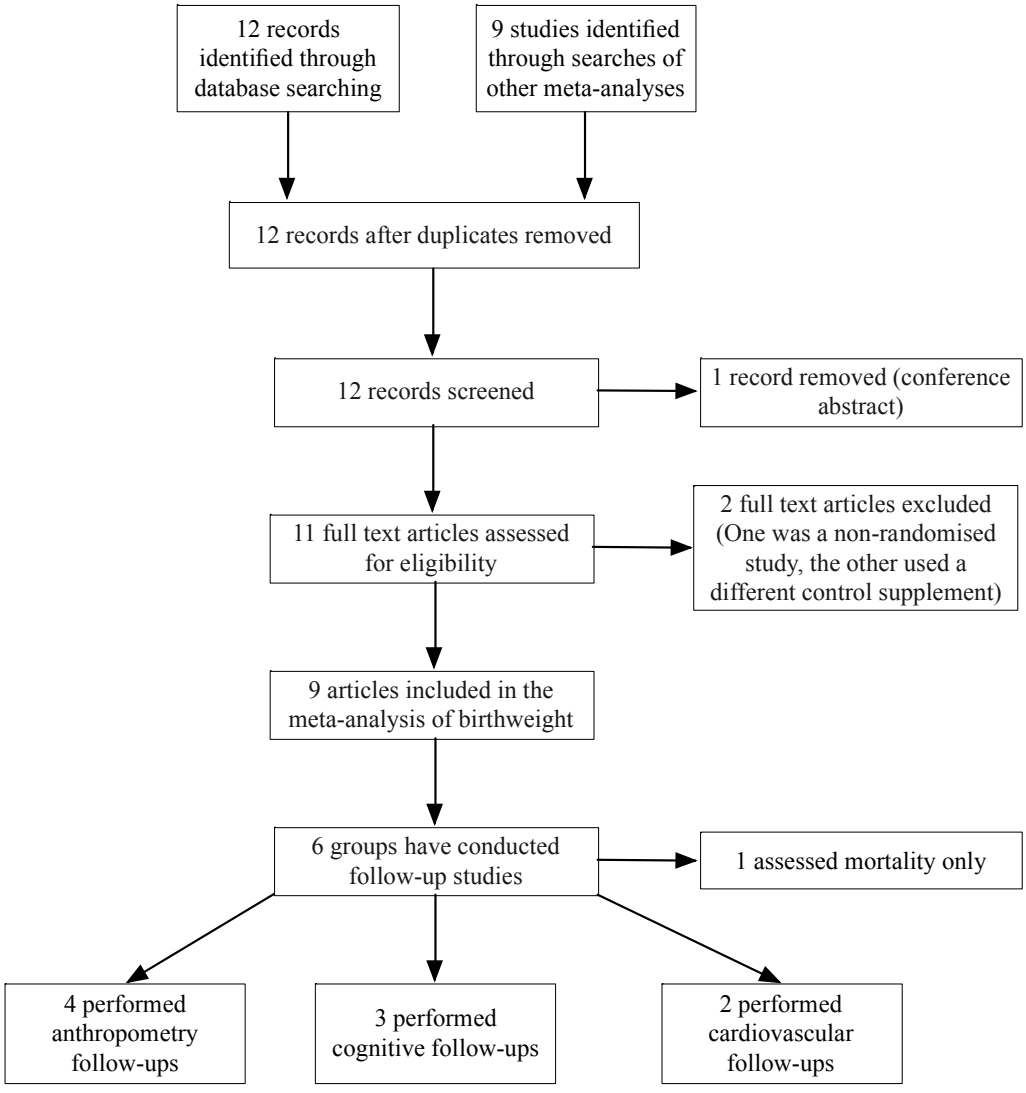

Figure 8: Numbers of studies found at each stage of the search

2.9.3.1 Other trials not included in the meta-analysis

A non-randomised, non-blinded "side-by-side effectiveness trial" in Vietnam by Huy et al showed an increase of 166 g in the region that received antenatal UNIMMAP supplementation. While the effect size was larger than in most of the trials mentioned, the study design and the lack of adjustment for potential confounders raises uncertainty about the results. In this study, a third district provided gender training that was designed to improve maternal care practices. This district had similar results to the MMN district, so there appeared to be no additional advantage.[256]

A large trial carried out by Liu et al compared the UNIMMAP supplement (n=6262) to folic acid alone (n=6261) and iron 30 mg and folic acid (n=6252). Participants were individually randomised, but stratified by geographical location. Comparison of the MMN with the iron and folic acid group showed no difference in birthweight (6.43 g; 95% CI -7.65, 20.51g), birth length or gestational age. There was a lower relative risk of neonatal, but not perinatal, mortality. This trial was not included in my meta-analyses because of the different control sample. If it is included in a meta-analysis of UNIMMAP trials (using original trial data, Figure 10), the effect size falls a little to 38.20 g (95% CI 20.09, 56.30). If included in the analysis of UNIMMAP plus similar trials (Figure 11), the effect size is 31.58 g (95% CI 15.98, 47.18), I^2 57.8%. Due to its large size, this trial had a 13.5% weighting in the meta-analysis.

The Antenatal Micronutrient Supplementation and Infant Survival (JiVitA-3) trial is currently ongoing in Bangladesh. At the time of writing, only a conference abstract of the findings had been published. The constituents of the supplement were the same as UNIMMAP, but the doses were a little different. Both the control and MMN supplement had slightly different iron (27 mg) and folate (600 μg) levels. The MMN supplement differed in the following micronutrients: vitamin A (770 μg), vitamin E (15 mg), riboflavin (1.4 mg), vitamin C (85 mg), zinc (12 mg), iodine (220 μg), copper (1 mg), selenium (60 μg). The trial was a cluster RCT involving 44 567 pregnant women. Supplementation was from the 1st trimester to 12 weeks postpartum. MMN were associated with slightly fewer stillbirths (0.89; 95% CI 0.81, 0.99) and preterm births (0.87; 95% CI 0.82, 0.92) and reduced prevalence of low birthweight (0.88; 95% CI 0.85, 0.91). There was no difference in SGA or overall mortality, though mortality was lower at 6 (0.87; 95% CI 0.74, 1.01) and 12 months (0.85; 0.73, 0.97) of age in girls, but not boys. The increase in birthweight (55 g), length (0.21 cm), and head circumference (0.21 cm) was attributed to a 0.3 week longer gestation.[257] There were insufficient details in the abstract to be able to add this trial to my meta-analyses.

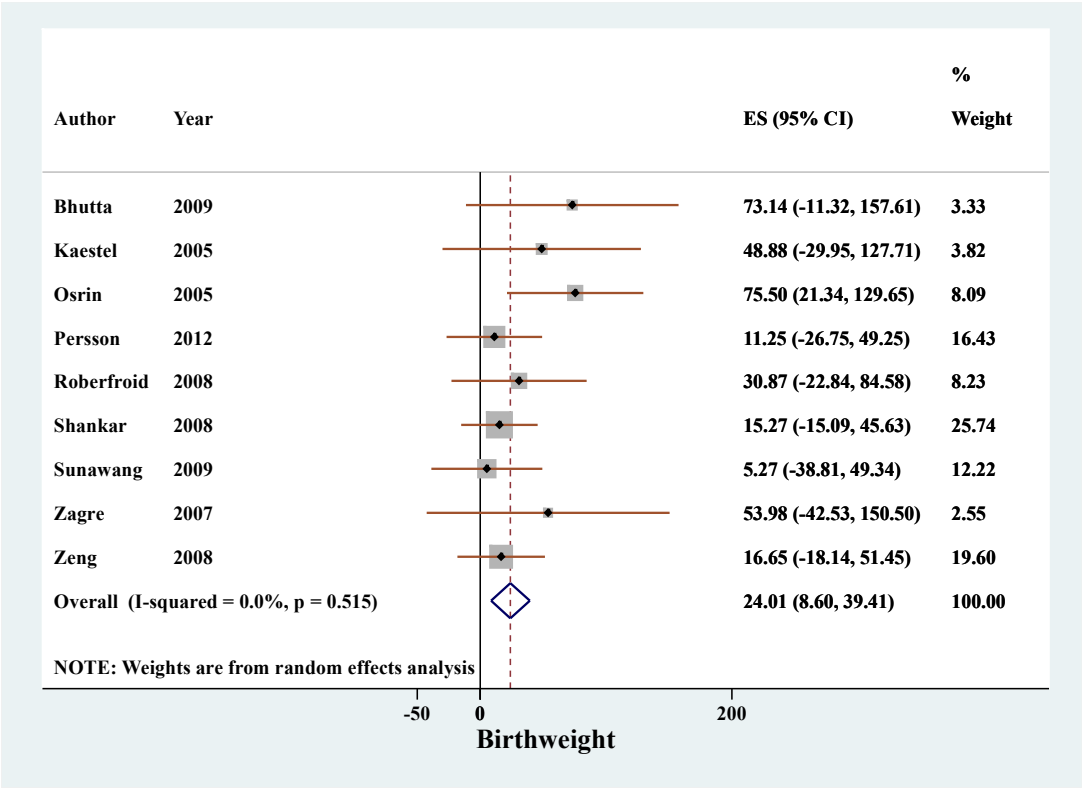

Figure 9: Meta-analysis of UNIMMAP trials only, using data from Fall et al, Food Nutrition Bulletin, 2009 [17]

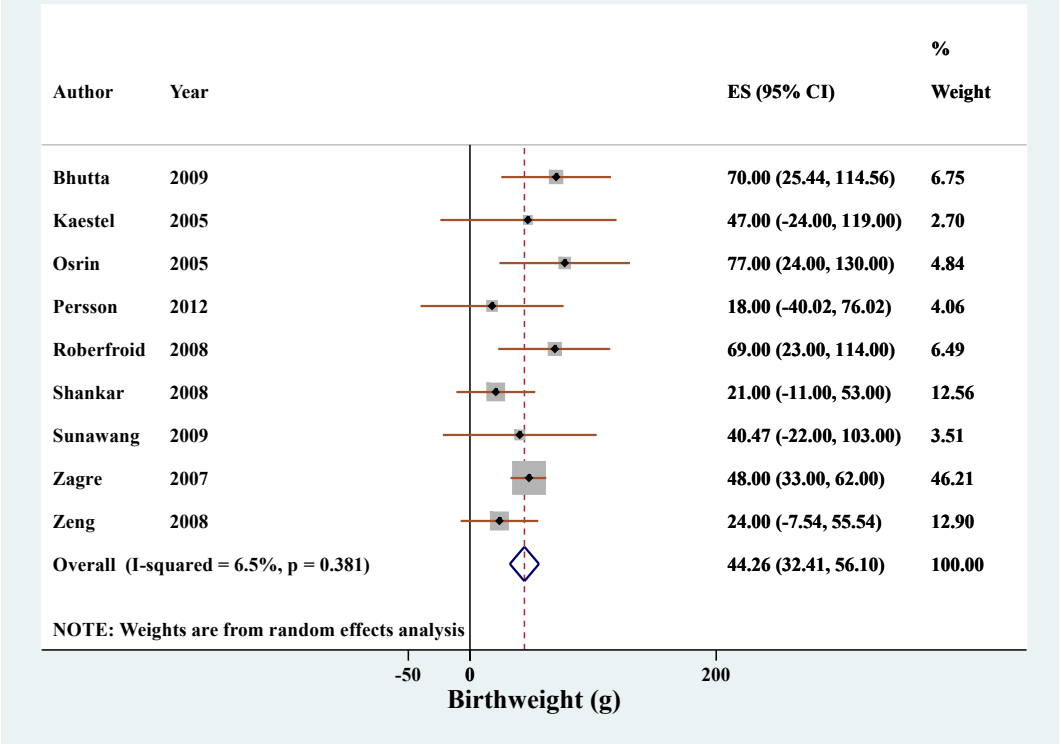

Figure 10: Meta-analysis of UNIMMAP trials only, using original data from the trials.

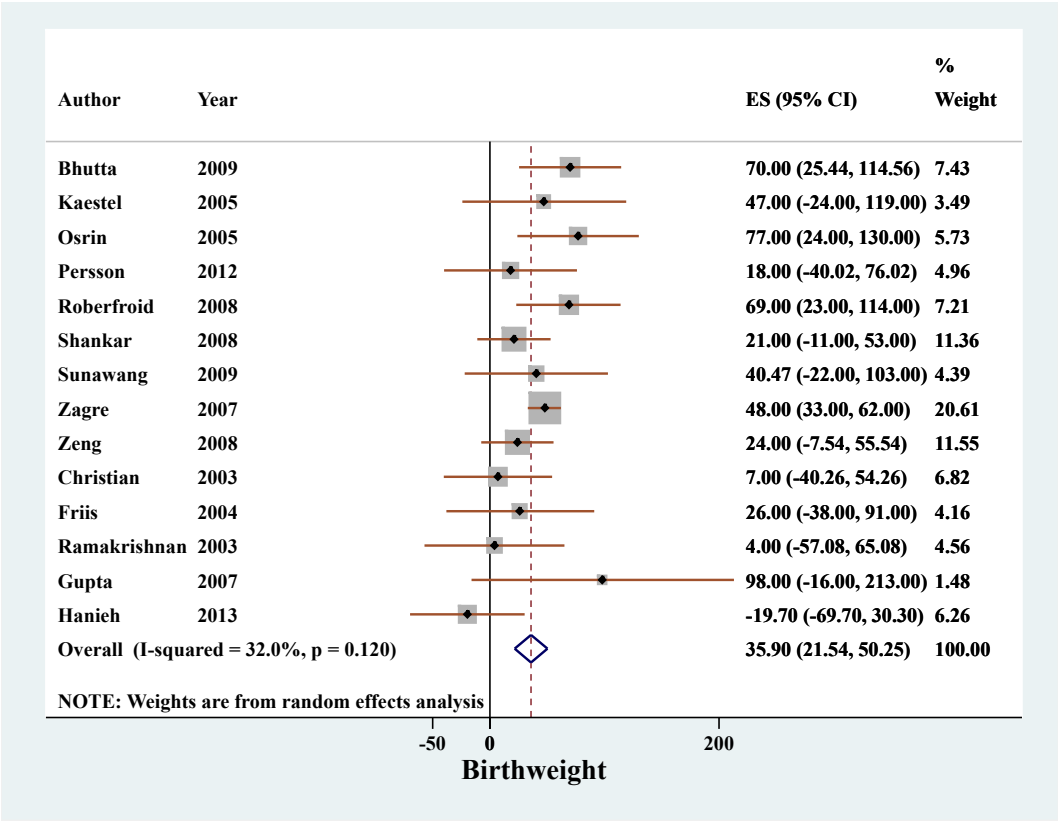

Figure 11: Meta-analysis of UNIMMAP trials and five other similar multiple micronutrient trials, using original data from the trials.

2.9.4 Long-term effects of antenatal multiple micronutrient supplementation

Six groups who conducted UNIMMAP trials have carried out longer-term follow-ups. Andersen et al looked only at mortality, finding no difference in overall mortality up to two years of age.[258] In addition to the Janakpur trial, three groups have done follow-up assessments of child growth: Roberfroid et al in Burkina Faso,[259] Khan et al in Bangladesh,[260,261] and Wang et al in China.[262] Three groups have done cognitive follow-ups: Li et al in China,[263] Tofail et al in Bangladesh,[264] and Prado et al in Lombok, Indonesia.[265] Hawkesworth et al have looked at cardiovascular outcomes in Bangladesh.[266] In addition to these, Ramakrishnan et al did an anthropometric follow-up,[267] and Christian et al did follow-ups of anthropometry, cardiovascular and cognitive outcomes.[268-270]

2.9.4.1 Anthropometry

In a factorial study design, Khan et al gave MMN, 30 mg of iron and folic acid and 60 mg of iron and folic acid to pregnant women in Bangladesh. Each of these three groups received

micronutrients early or later in pregnancy. They followed up the children until they were 54 months of age. MMN supplementation resulted in lower linear growth compared to the 60 mg of iron and folic acid group, with a mean percentage of stunting of 36.1% (95% CI 33.3, 39.0) versus 31.8% (95% CI 28.8, 34.7). Compared to the 30 mg of iron and folic acid, there was no difference. Children of mothers who received early food supplementation in pregnancy were less likely to be stunted.[260]

Wang et al found no difference in wasting, stunting and underweight between the MMN group and the controls of folic acid or iron and folic acid at 30 months of age in China.[262] Roberfroid et al showed small increases in length for age z-score (β = 0.13; 95% CI 0.02, 0.24) and weight for age z-score (β = 0.13; 95% CI 0.04, 0.23) at one year of age, but this difference had disappeared by 2.5 years.[259]

In Sarlahi, Nepal, Stewart et al investigated the anthropometry of children at 6-8 years of age. They measured height, weight, mid-upper arm circumference, triceps and subscapular skin-fold thickness and waist circumference. They found no difference between the MMN group and the control group. The folic acid, iron and zinc group, however, did show an increase in height of 0.64 cm (95% CI 0.04, 1.25) and small reductions in skinfold thicknesses.[268]

Ramakrishnan et al investigated growth in children born in a MMN trial in Mexico. The children born in the original trial were subsequently randomised to receive postnatal micronutrients or iron supplements. Using intention to treat analysis they found no difference in height from the postnatal micronutrients. Though not explicitly analysed in their paper, there did not appear to be a difference between antenatal micronutrient and control groups in later height.[267]

Overall, the follow-up studies do not show a difference in anthropometry in those born to mothers taking MMN antenatally.

2.9.4.2 Cardiovascular outcomes

Hawkesworth et al followed up children from the MINIMat trial in Bangladesh at 4.5 years of age. Compared to a control sample who received 30 mg iron and 400 μg folate, they found no difference in systolic blood pressure. For diastolic blood pressure, they used three models to adjust for potential confounders. The basic model (model 1) adjusted for maternal food intervention (this was a multi-arm trial that also tested food supplementation), another model adjusted for factors relating to the outcomes (model 2), and another for factors associated with the maternal interventions (model 3). Models 1 and 2 showed no effect. Model 3 showed a small increase in diastolic pressure of 0.87 mmHg (95% CI 0.18, 1.56). My feeling is that

model 3 was over-adjusted: there were three correlated measures of anthropometry (BMI, lean mass and height) and markers of child illness that should not be associated with the randomized interventions. Also, comparing 60 mg iron to 30 mg iron showed a small, non-significant increase of 0.42 (95% CI -0.27, 1.11) in diastolic pressure, and I would assume that comparing with 60 mg iron would also produce a null effect.

One explanation of the lower blood pressure results in the Janakpur follow-up was that the higher dose of iron in the control sample (60 mg) may have led to a higher blood pressure: the 30 mg lower dose in the MMN had no advantageous or detrimental effects. This theory was contradicted by Hawkesworth et al, who showed no difference in systolic blood pressure when comparing the two doses of iron.[266]

The team in Sarlahi also went on to investigate metabolic syndrome. They tested for a number of outcomes: “Blood pressure, BMI, waist circumference, glycated haemoglobin, cholesterol, triglycerides, glucose, insulin and urinary microalbumin:creatinine ratio”. They found no difference in any of these variables.[269]

2.9.5 Summary

A number of trials have tested the antenatal UNIMMAP supplement and many others have examined similar combinations of micronutrients. The meta-analyses overall have shown a small effect on birthweight, with a corresponding fall in the proportion with SGA. There does not appear to be an effect on other anthropometric measurements at birth. The follow-up studies have tended not to find a lasting effect of the antenatal MMN supplement in children. The Janakpur trial is unusual compared to the others in that there was a greater difference in birthweight, which was sustained until two years, and a difference in blood pressure was seen.

Table 4: Trials using the United Nations International Multiple Micronutrient Preparation (UNIMMAP) supplement

Trial	Trial details	Main trial results*	Long-term results	
Bhutta et al, [9] Pakistan	Cluster randomised. n=2378 Enrolled in 2nd trimester Included nutrition education Supplementation till birth.	70g heavier (95% CI -25, 115g) SGA: 0.73 (95% CI 0.39, 1.38)	Nil	
Kaestel et al, [10] Guinea Bissau	Individually randomised. n=2100 Enrolled <37 weeks Supplementation till birth Allocation was probably not concealed.	47g heavier (95% CI -24, 119g) adjusted for gestation, malarial parasitaemia, anaemia, sex, season. SGA: 0.74 (95% CI 0.42, 1.28)	Andersen et al.[258] 2 years	There was no effect on under-2 mortality. However, when looking at the interaction of age and mortality, there was some evidence of a negative effect on late-infant (92-365 days) RR 2.1 (95% CI 0.99 to 4.46). There was no effect in other age groups.
Osrin et al. [7] Dhanusha, Nepal	Individually randomised. n=1200 Enrolled in 2nd trimester Supplementation till birth	77g heavier (95% CI 24, 130g) SGA: 0.75 (95% CI 0.49, 1.14)	Vaidya et al, [8] Age 2.5 years 83% followed up.	Growth Intervention group was 204g heavier (95% CI: 27-381g) and systolic blood pressure was 2.5 mmHg (95% CI: 0.5-4.6) lower.
Persson et al. [11] Eneroth et al. [271]Bangladesh	Individually randomised. n=4436 Enrolled in first trimester Supplementation till birth Factorial design- MMN, 30mg of iron and folic acid and 60mg of iron and folic acid. Each of these three groups received micronutrients early or later in pregnancy.	18g heavier (95% CI -40, 54g) SGA: 0.95 (95% CI 0.66, 1.22)	Khan et al, [260] 54 months	Growth MMN resulted in lower linear growth compared to the 60mg of iron and folic acid group, with a mean percentage of stunting of 36.1% (95% CI: 33.3 to 39.0) versus 31.8% (95% CI: 28.8-34.7). Compared to the 30mg of iron and folic acid there was no difference.
			Khan et al, [261] 54 months 70% followed up	Body composition Measured by leg-to-leg bioelectrical impedance. No difference between early or late prenatal supplementation and body composition, weight, height, MUAC, head circumference and skinfold thickness.
			Tofail et al, [264] 7 months of age	Cognitive ability Assessed problem solving, motor development and behaviour. They stratified the groups into to whether the mother had a low BMI (<18.5kg/m^2) or higher BMI. They showed that antenatal MMN was associated with

				higher motor score and activity levels in both early and late food supplemented groups in mothers with a low BMI. No other differences were found in problem solving or behaviour.
			Eneroth et al, [271] 6 months	Micronutrient status Blood was collected for haemoglobin, ferritin, zinc, retinol, vitamin B12 and folate. There was a higher B12 concentration in the MMN group in girls.
			Hawkesworth et al, [266] 4.5 years	Blood pressure, renal dimensions and kidney function (glomerular filtration rate). There was a small increase in diastolic blood pressure. 0.87 mmHg (95% CI 0.18, 1.56)
Roberfroid et al, [12] **Burkina Faso,**	Individually randomised. n=1426 Enrolled <37 weeks Supplementation till 12 weeks after birth	69g heavier (95% CI 23, 114g) after adjustment for maternal Hb, type of malaria prevention, maternal education, season and area. SGA: 0.89 (95% CI 0.62, 1.44) Possibly increased peri-natal mortality OR 1.78 (95% CI 0.95, 3.32)	Roberfroid et al.[259] 2.5 years	Growth Greater length-for-age z score β = 0.13 (95% CI 0.02, 0.24) and weight-for-age β = 0.13 (95% CI 0.04, 0.23) after one year, but this difference had disappeared by 2.5 years. No difference in mortality.
Shankar et al, [13] **Lombok, Indonesia,**	Cluster randomised. n=31 290 Enrolled <37 weeks Supplementation till 12 weeks after birth	21g heavier (95% CI -11, 53g) adjusted for clustering SGA: 0.97 (95% CI 0.83, 1.13) Reduced fetal and neonatal mortality in the MMN group RR 0.89 (95% CI 0.81, 1.00) There was a greater reduction (28%) in those in whom supplementation was started in the 3rd trimester.	Prado et al. [265] 42 months	Cognition Tested a subgroup of 487 children. In children of undernourished mothers (MUAC <23.5cm), MMN improved motor ability β= 0.39 (95% CI 0.08, 0.70) and visual attention/ spatial ability β = 0.37 (95% CI 0.11, 0.62) In children of anemic mothers (Hb <110 g/L) MMN improved visual attention/ spatial ability β= 0.24 (95% CI 0.02, 0.46)
Sunawang et al,[14] **Indra**	Cluster randomised. n=843 Enrolled in 2nd trimester Supplementation till 4 weeks	40.5g heavier (95% CI -22, 103g) SGA: 0.87 (95% CI 0.61, 1.24)	Nil	

	after birth			
Zagre et al,[15] **Niger**	Cluster randomised. n=2550 Included nutrition education Enrolled in 1st trimester Supplementation till birth	48g heavier (95% CI 33, 62g) adjusted for household size, education, diet morbidity during pregnancy, preventive measures against malaria and season SGA: 0.79 (95% CI 0.53, 1.18)	Nil	
Zeng et al,[16] **Zeng et al, [272]** **China,**	Cluster randomised. n=5828 Enrolled <28 weeks Supplementation till birth	24g heavier (95% CI -8, 56g) SGA: 0.92 (95% CI 0.72, 1.18) Possibly a greater effect in the poorer households.	Li et al, [263] 1 year	Cognitive Assessed mental and psychomotor development, using age-appropriate Bayley Scales of Infant Development. The age-adjusted scores at one year were higher in the MMN group than the folic acid + iron or the folic acid groups for mental development. There was no difference at three months or six months and also no difference in the psychomotor scores.
			Wang et al, [262] 2.5 years	Growth No difference in wasting, stunting and underweight between the MMN group and the control of folic acid or iron and folic acid.

*Birthweight and small for gestational age (SGA). SGA values taken from the meta-analysis by Fall et al [17], who defined this as below the "within-population 10th centile".

Table 5: Similar supplements to UNIMMAP

Trial	Number	Supplement	Main trial results at birth	Long-term results	
Christian et al, [35] **Sarlahi, Nepal**	Cluster randomised Enrolled in 1st trimester Supplementation till 12 weeks after delivery n = 4926	Folic acid 400 µg, iron 60 mg as ferrous fumerate, vit A, Zn 30 mg, vit D 10 µg, vit E 10 mg, vit B1 1.6 mg, vit B2 1.8 mg, niacin 20 mg, vit B6 2.2 mg, vit B12 2.6 µg, vit C 100 mg, vit K 65 µg, copper 2 mg and Mg 100 mg. Control group had vitamin A as well.	7g heavier (95% CI -40, 54g) SGA: 1.05 (95% CI 0.70, 1.57)	Stewart et al, [268] 6-8 years	Growth They measured height, weight, mid-upper arm circumference, triceps and subscapular skin-fold thickness and waist circumference. They found no difference between the MMN group and the control group.
				Stewart et al, [269] 6-8 years	Metabolic syndrome. They tested for blood pressure, BMI, waist circumference, glycated haemoglobin, cholesterol, triglycerides, glucose, insulin and urinary microalbumin:creatinine ratio. They found no difference in any of these outcomes.
				Christian et al, [270] 7-9 years	Cognitive They used a number of tests: Intellectual functioning: Used the Universal Nonverbal Intelligence Test and tests of executive functioning. Motor: Movement Assessment Battery for Children and finger-tapping test. They did not find any find any difference between the MMN group and control groups.
Friis et al, [273] **Zimbabwe,**	Individually randomised HIV +ve and HIV –ve women Enrolled in 2nd and 3rd trimesters Supplementation till birth n = 1169	Vit A 3000 µg, beta carotene 3.5 mg, thiamine 1.5 mg, riboflavin 1.6 mg, B6 2.2 mg, B12 4 µg, niacin 17 mg, C 80 mg, D 10 µg, E 10 mg, Zn 15 mg, Cu 1.2 µg, Se 65 µg. Control = placebo No iodine, iron or folate- these part of the routine antenatal care	26g heavier (95% CI -38, 91g) in HIV –ve women. 101g heavier (95% CI -3, 205g) in HIV +ve women. SGA: 0.79 (95% CI 0.47, 1.31)	Nil	

Gupta et al. [274], India	Enrolled in 2nd and 3rd trimesters Supplementation till birth n = 200 BMI of women <18.5 and/or a haemoglobin of 7-9 g/dL	Vit A 1500 IU, B1 1 mg, B2 1.5 mg, B6 1 mg, B12 1 µg, C 50 mg, D3 200 IU, E 7.5 mg, calcium pentothenate 5 mg, folic acid 0.15 mg, nicotinamide 20 mg, biotin 30 µg, Zn 15mg, potassium iodide 0.15 mg, ferrous fumarate 10mg, magnesium oxide 100 mg, manganese sulfate 2.5 mg, copper 2 mg, calcium 162 mg, phosphorus 125 mg, potassium 40 mg, chloride 36.3 mg, chromium 25 µg, molybdenum 25 µg, sodium selenate 30 µg, nickel 5 µg, silicon dioxide 2 mg, vanadium 10 µg, boron 150 µg. Placebo consisted of calcium All subjects received iron and folic acid	98 g heavier (95% CI −16, 213 g) 0.8 cm (95% CI 0.03, 1.57 cm) longer 0.2 cm (95% CI 0.04, 0.36 cm) larger MUAC SGA RR = 0.45 (95% CI 0.21, 0.97) Adjusted for maternal age, family income, parity, pre-pregnancy weight, height, hemoglobin, sex and gestation. Early neonatal morbidity declined RR= 0.42 (95% CI 0.19, 0.94)	Nil	
Hanieh et al.,[275] Vietnam	Cluster randomised Enrolled <16 weeks gestation Supplementation given twice weekly n = 833	Fe 60 mg, folic acid 1.5mg, vit A 1.6mg, vit D 400 IU, vit E 20mg, thiamin 2.8 mg, riboflavin 2.8 mg, niacin 36 mg, vit B6 3.8mg, vit B12 5.2 µg, vit C 140 mg, Zn 20 mg, iodine 300 µg, Cu 4mg, Se 130 µg Control 60 mg Fe	19.7g lighter (95% CI -70, 30g) adjusted for clustering, maternal age, parity, sex and gestation. Low birthweight (SGA not reported) 1.53 (0.23, 10.1)	Hanieh et al, [275] 6 months	No difference in length-for-age z scores. Mean difference -0.04 (95% CI -0.2, 0.1). No difference in any parameter of "Bayley scales of infant and toddler development".[276]
Ramakrishnan et al.,[277] Mexico	Individually randomised Enrolled in 1st trimester Supplementation till birth n = 873	Iron 60 mg as Fe SO_4, folic acid 215 µg, vit A 2150 IU, vit D3 309 IU, vit E 5.73 IU, thiamin 0.93 mg, riboflavin 1.87 mg, niacin 15.5 mg, vit B6 1.94 mg, vit B12 2.04 µg, vit C 66.5 mg, Zn 12.9 mg, Mg 252 mg. Control 60 mg Fe SO_4	9g heavier (95% CI -54, 73g) SGA: 0.82 (95% CI 0.47, 1.41)	Ramakrishnan et al. [267] 2 years 25% loss to follow up	The children born in the original trial were randomised to receive postnatal micronutrients or iron supplements. Using intention to treat analysis they found no difference in height from the postnatal micronutrients. Though not explicitly analysed in their paper, there does not appear to be a difference between antenatal micronutrient and control groups in later height.

Table 6: Meta-analyses of multiple micronutrient supplementation trials (* developed countries)

	Trials included	Comments	Results
Fall et al [17] 2009	Bhutta, Christian, Friis, Kaestel, Osrin, Ramakrishnan, Roberfroid, Shankar (SUMMIT), Sunawang, Tofail, Zagre, Zeng	Contains trials that used the UNIMMAP supplement plus Christian, Friis and Ramakrishnan HIV-ve women only. I^2 = 0%. Used the raw data from the trials rather than published results.	Increase in birthweight of 22g (95% CI 8, 36g). Increase of 7.6g (95% CI 1.9, 13.3g) per unit increase in maternal BMI. There was a right shift in the entire birthweight distribution. Reduction in SGA OR = 0.90 (95% CI, 0.82, 0.99) Increase in large for gestational age OR= 1.13 (95% CI, 1.00, 1.28) No interaction with maternal height, parity or maternal age.
Shah and Ohlsson [18] 2009	Christian, Fawzi (1998), Fawzi (2007), Friis, Gupta, Hininger*, Kaestel, Osrin, Ramakrishnan, Roberfroid, Shankar (SUMMIT), Zagre, Zeng	Fawzi 1998 was in an HIV +ve population. Control = iron and folate Excluded studies that included macronutrients. I^2 = 55-64%	Birthweight was 54g greater (95% CI 36, 72g) Greater effect in individually randomised rather than cluster randomised trials. Individual SGA = 0.82 (95% CI 0.73, 0.92) Cluster SGA = 0.98 (95% CI 0.84, 1.14)
Kawai et al [21] 2011	Bhutta, Christian, Fawzi (1998), Fawzi (2007), Friis, Gupta, Kaestel, Osrin, Ramakrishnan, Roberfroid, Shankar, Sunawang, Tofail, Zagre, Zeng	Contains trials that used the UNIMMAP supplement plus Christian, Fawzi (1998), Fawzi (2007), Friis, Gupta and Ramakrishnan I^2 for SGA= 0%	Increase in birthweight of 44g (95% CI 28, 60g) Reduction in SGA RR 0.85 (95% CI 0.78, 0.93) Micronutrient supplementation had no overall effect on perinatal mortality although subgroup analysis suggested a reduction when >50% of mothers had formal education (RR 0.93; 95% CI 0.82, 1.06) or when supplementation was initiated after a mean of 20 weeks gestation (RR 0.88; 95% CI 0.80, 0.97).
Ramakrishnan et al [20] 2012	Bhutta, Christian, Fawzi (1998), Fawzi (2007), Friis, Gupta, Hininger*, Kaestel, Osrin, Ramakrishnan, Roberfroid, Shankar (SUMMIT), Sunawang, Tofail, Zagre, Zeng	Contains trials from developing countries plus Hininger. Fawzi 1998 was in an HIV +ve population. I^2 = 46% for birthweight and 71% for SGA.	Reduction in SGA RR 0.83 (95% CI 0.73, 0.95). The effect was greater in trials that used 60mg of iron. Increase in birthweight 53g (95% CI 43.2, 62.0g). There was no difference between starting supplements in the 1st trimester compared with later. Increased risk of neonatal death in trials that began after the first trimester RR 1.38 (95% CI 1.05, 1.81). Overall risk of neonatal death 0.97 (95% CI 0.87, 1.09).
Haider and Bhutta [21] 2012	Bhutta, Brough*, Christian, Diekmann*, Fawzi (2007), Friis, Gupta, Javenpaa*, Kaestel, Osrin, Ramakrishnan, Roberfroid, Rumiris, Shankar (SUMMIT), Sunawang, Tatala, Theobald*, Tofail, Vadillo-Ortega, Zagre, Zeng	Contains multiple micronutrient trials from both developing and developed countries. I^2 = 57% for birthweight and 47% for SGA	Reduction in SGA RR 0.87 (95% CI 0.81, 0.95). Maternal BMI <20 kg/m^2 RR 0.90 (95% CI 0.83, 0.98). Maternal BMI ≥20 kg/m^2 RR 0.85 (95% CI 0.80, 0.91). Maternal height <154.9 RR 0.91 (95% CI 0.85, 0.98). Maternal height ≥154.9 RR 0.82 (95% CI 0.76, 0.89). Supplementation started before 20 weeks RR 0.90 (95% CI 0.84, 0.96). If started after 20 weeks RR 0.83 (95% CI 0.76, 0.91) Neonatal mortality RR 1.01 (95% CI 0.89, 1.15).

2.10 Causal diagrams

"felix qui potuit rerum cognoscere causas"

(fortunate is he who can understand the cause of things) - Virgil, Georgics

Associations are common in epidemiology, but determining causation is difficult, some might say impossible. Identifying associations or predictors of an outcome are useful in their own right, but to effect change, particularly in public health contexts, causal inference is needed. Public health actions and policies are generally based on observational research (if any). Causal inference is therefore assumed at this later stage and can be defined as "the thought process that tests whether a relation of cause to effect exists". [278]

2.10.1 What is causation?

Causation has been discussed by philosophers and scientists for centuries. Aristotle for example described the "four causes" model (efficient, final, formal and material). While flawed in many applications, his theory highlighted the multi-factorial nature of causation. David Hume defined a cause as "an object followed by another ... where, if the first object had not been, the second never had existed." [279] Historically, assumptions about causation were based on induction, as described by Francis Bacon in the 16th century. Induction is a process that involves making inferences about what will happen based on observation of previous events. Karl Popper instead proposed that experiments should be used to test or refute hypotheses. The "truth" can change and is falsifiable by a single experiment. A scientific theory cannot be verified, i.e. proved undeniably true. In fact, falsifiability was Popper's main criterion for a scientific theory itself. Scientists should test hypotheses attempting to refute their theories. Popper describes the assumption that all swans are white until the observation of a black swan that refutes this. [280]

Scientific truth is not just based on scientific facts, rather, as described by Thomas Kuhn in his 1962 book The Structure of Scientific Revolutions, it is defined by the scientific community and is therefore subjective. Kuhn said that science progresses non-linearly and is prone to revolutions, also referred to as paradigm shifts. Famous examples may be Darwin's theory of evolution or Thomas Bayes' theorem (though neither had immediate traction within the scientific community). Kuhn defines three stages of science: pre-, normal and revolutionary. In pre-science an idea emerges which is then worked on in the normal science stage. At this stage results that may differ do not refute the hypothesis because they may be mistakes or special circumstances. Only when there is a critical mass of contradictory evidence would a theory change in the revolutionary phase.

Causal factors are often required to be both necessary and sufficient. A necessary factor is required for the effect to occur, but may require additional factors to act. A sufficient factor implies that the effect will occur if the factor is present. [278] [281] The following criteria for causality were proposed by Austin Bradford-Hill in 1965.[282] While not intended to be all-encompassing, they have been widely adopted by epidemiologists and scientists. At the very least, they form an initial framework.

1. Strength of the association. Stronger associations are more likely to be causal in nature, but this does not rule out a weak causal influence. This is particularly important in multi-factorial diseases where each factor may only contribute a small effect. Strong causal assumptions assume that parameters take particular values. Weak assumptions allow a range of values to be taken. [283]

2. Consistency of findings in other settings. Other research in different places and populations should give similar results. This has been questioned because causal effects may be population-specific.

3. Specificity of the association. A single cause and single effect. This criterion does not always hold as many effects may arise from a single cause or multiple causal factors may be needed for a single effect.

4. Temporal sequence of association. The cause should precede the effect. This is said to be the only fundamental criterion.

5. Biological gradient (dose-response). While generally true, there may be causes that require a threshold level to be reached before an effect is produced.

6. Biological plausibility. By definition, this needs to be in line with current knowledge.

7. Coherence with current knowledge. A similar criterion to biological plausibility. However, evidence may arise that is counter to current wisdom.

8. Experiment. Can be proven by experiment. This is an ideal situation, but is not always possible. Some argue that causation can only be interpreted from interventional studies, such as trials. [284]

2.10.2 Counterfactual theory

Causal theory is based on the idea of counterfactuals. These are defined as “parameters that describe events under a hypothetical alternative to actual conditions”. [285] These counterfactual outcomes do not occur, so are always assumed. The true counterfactual effect is therefore

always inferred. For example, if a person received a drug (exposure) and developed an allergic reaction (outcome), the counterfactual would be the outcome if he had not received the drug. The counterfactual is an assumption that is usually a substitute, an imperfect representation of the true counterfactual, such as a similar population or the same population at a different time. A quantitative counterfactual model was developed by Jerzy Neyman in 1923 and was applied to observational studies by Donald Rubin in the potential outcomes model.[285] Using counterfactual theory, causation is assumed when the counterfactual (or potential) outcomes differ, with the assumption that the exposures and individuals are independent.[7] [286] An association represents a different risk in separate subsets of a population using actual measured variables. Causation, using the counterfactual approach, represents a different risk in the same population with two exposures, the actual and the counterfactual.

Glass et al argue that this framework is better suited to a public health context as it seeks to compare outcomes under different hypothetical interventions in complex networks of causal factors. It can also help to highlight where important (often distal) interventions may lie that may not lend themselves to straightforward study designs such as the randomised controlled trial. [287]

2.10.3 Causal inference in populations

At its most basic, causation is straightforward, but identifying causal factors in populations is complicated and some, like RA Fisher, who famously argued that smoking was not a causal factor for lung cancer, [288] say it is impossible: we can only look at associations. Judea Pearl draws the distinction between associations and causation by defining an associational concept as any relationship that can be defined in terms of a joint distribution of observed variables". [289] A causal relationship, however, is not just determined by the distribution alone and has directionality.

At a population level non-determinism is a problem. This is where a treatment does not always cause an outcome, but increases its probability. An "average causal effect" in a population is sought that is both population- and time-specific. Absence of an average causal effect does not mean the absence of an individual effect, though. [286] In the situation where there is no average causal effect and no effect in any individual, there is said to be a "sharp" null hypothesis. While Popperian falsification remains valid, the complexity of a population

[7] In mathematical notation, on an individual level $O^{a=1} \neq O^{a=0}$, and a population level $E[O^{a=1}] \neq E[O^{a=0}]$, where O is the outcome, $O^{a=1}$ and $O^{a=0}$ are potential outcomes and E is the average causal effect in the population.

and system render the single "black swan" insufficient. Falsification therefore cannot apply in an absolute sense in epidemiology. This is not to say that research should not be designed to falsify current hypotheses. Using Bayesian language, it may change the probability of an outcome.

A multi-causal model has been proposed in epidemiology. This theory held sway in the second half of the 20th century, particularly in relation to chronic diseases.[278] Susser and Susser argue for a new multi-level epidemiological model of causation involving what they term eco-epidemiology. The new "paradigm" they suggest is Chinese boxes. This combines a focus on both the small-scale individual molecular level and the wider societal level. [290] A model that was more applicable to chronic diseases was described by Rothman et al. In their component model, a number of components act together to produce an effect. For this, a number of sufficient causes that work together are needed, but each by itself may not be sufficient.[291] Similar to this, Parascandola and Weed describe a probabilistic model of causation in which causes can exert degrees of influence on an effect. The cause may therefore not be necessary or sufficient, but this they feel may be more appropriate for a population, where determinism is difficult. This would be my preferred model of causation. [281]

2.10.4 Causal diagrams

Causal diagrams are networks of variables that form a subset of Bayesian networks and are akin to a graphical presentation of counterfactual theory. Causal diagram theory stemmed from the work of Sewall Wright in 1921 on path analysis, which later developed into structural equation models (SEM). The mathematical basis was defined by Judea Pearl using do-calculus. [289,292] While normally parametric in nature, it was extended into non-parametric forms. According to Pearl, these require us to "set out clearly our assumptions about causal relationships and they attempt to go further than normal epidemiological associations to describe causality". [289] They are considered to be less prone to error, especially in complex systems. [293]

Causal diagrams go beyond the normal depiction of exposure, outcome and confounders, to show how variables are related. The diagrams are constructed using the author's *a priori* knowledge of the relationships between variables which are connected together by edges (arrows or lines) that indicate a causal inference. These edges normally just denote the direction of causality rather than whether it is positive or negative or its strength. The diagrams tend to be hierarchical in nature, with parents and descendants. Variables are linked together to depict more than single relationships between them, enabling the mapping of

systems, depicting direct and indirect effects. [293] They also show what variables are not related in a system.

Following the rules of causal diagrams provides a guide to analysis. They provide transparency in the analysis method, allowing others to critique and amend if necessary. Causal diagrams set out explicitly the assumptions and methods that follow them. They highlight backdoor pathways that need to be controlled for, and mediators that should not (unless the intention is to find a direct effect between two variables). A backdoor path is one that goes from one node to another against the direction of an arrow. I believe such transparency is crucial for research to advance as it sets out clearly what has been done and the justification for it.

A criticism of causal diagrams is that they can constrain analysis and hypothesis generation. The counter-argument is that the approach does not preclude alternative hypotheses, but challenges researchers to set them out clearly. The diagrams help to avoid multiple analyses by specifying what analyses should be done. Another criticism of causal models, voiced by Philip Dawid amongst others, is that they are "just an ambitious associational model" and questions whether the associations can equate with causal relationships. [294] The difference between the links in a SEM and a regression between two variables is that the former refers to a causal link, while the latter "represents the conditional mean of a dependent variable as a function of explanatory variables." [295] Bollen and Pearl describe SEMs as "an inference engine that takes in two inputs, qualitative causal assumptions and empirical data, and derives two logical consequences of these inputs: quantitative causal conclusions and statistical measures of fit. Fitting the data does not "prove" the causal assumptions, but it makes them tentatively more plausible." [283] SEMs cannot create causation from association. They rely on the prior knowledge and assumptions of the researcher, combined with data analysis. [284]

The most common form of causal diagram is the Directed Acyclic Graph (DAG), defined as "a graph whose nodes (vertices) are random variables with directed edges (arrows) and no directed cycles".[286] DAGs use the Causal Markov assumption that "the probability of each variable or node is independent of its non-descendants conditional on its parents". To construct a DAG, we start with the exposure and outcome and then add variables that are causes of both exposure and outcome. Common causes of any two variables in the model are then added, until there are no more variables to add. Unmeasured variables are normally also included. With the assumption that the model is correct, the causal effect can be found if there are no common causes (confounders) or the backdoor paths can be blocked from the exposure to the outcome. In this scenario, the exposure and outcome are said to be "D-separated".[296]

2.10.5 Randomised controlled trials and observational studies

In randomized controlled trials, association can equal causation. Appropriate randomization ensures that missing counterfactual values occur by chance and that "exchangeability" (defined as "independence between the counterfactual outcome and the observed treatment") is present. [286] The outcome could equally have occurred in either counterfactual group. If two groups have the same treatment, the outcome would be the same in both, with the (independent) predictors equally distributed between both groups. Randomization does not guarantee exchangeability, but any departures are due to random variability rather than systematic bias: the missing counterfactual information is missing completely at random. A potential problem with RCTs is that there may be a difference between assigned treatment and received treatment. Blinding attempts to ensure that this does not happen, by preventing an independent effect of assigned treatment on outcome.

Exchangeability does not exist in observational studies, but causal inference can be assumed if they are treated as conditionally randomized trials. This requires the three following criteria:

1. Well defined interventions (or exposures).

2. The conditional probability of receiving every value of treatment - depends only on the measured covariates.

3. Positivity: the conditional probability of receiving every value of treatment is greater than zero. [286]

In some observational research a variable may be randomly assigned, for example studies looking at the effect of birth order. In observational studies, the distribution of outcome is conditional on another factor C. The assumption is that the exposure was "assigned" randomly in the groups of C. Standardization (or alternatively inverse probability weighting) can be applied in such a scenario to get the population effect. In this, the marginal counterfactual risk is the weighted average of the stratum-specific risks with weights equal to the proportion of individuals in the population with C = 0 and C = 1, respectively. Stratification would produce a conditional effect measure, rather than the marginal one, for the whole population. [286]

2.10.6 Bias and Confounding

A bias is "any association between treatment and outcome that does not arise from the causal effect of treatment on outcome". [286] An example is confounding when exposure and outcome

share the same cause. The counterfactual definition of a confounder is a variable that can block the backdoor paths between exposure and outcome. This contrasts with the traditional definition of a confounder as a variable that is associated with the exposure and outcome (conditional on the treatment) and is not on the causal pathway between treatment and outcome. These two definitions normally coincide, but there can be situations when they do not (see Appendix 2.2). The counterfactual definition takes an absolutist stance that assumes confounding exists or does not, regardless of whether the variables are measured. The backdoor paths can be open, which allows confounding, or closed due to a collider (a node at which a causal path is blocked- see Appendix 2.2 for an example). Conditioning on a non-collider closes the path, whereas conditioning on a collider opens up the pathway through it, thus allowing confounding. In this case unnecessary adjustment leads to confounding when it does not exist. [286] An alternative approach to using a causal diagram to control for confounding is to look for a change in the effect measure. This is not adequate as adjustment will usually change the effect measure and if done inappropriately can introduce bias as described. Adjustment for confounding requires controlling for a "sufficient set" of variables (S) for exposure E and outcome O. This requires that:

1. "No element of S is a descendant of E, (i.e. not on a causal path emanating from E)

2. The elements of S "block" all "back-door" paths from E to O."[284]

2.10.7 Effect modification and interaction

Effect modification occurs when the effect of the exposure on the outcome varies with levels of the effect modifier variable. The exposure variable is not affected by the effect modifier. Effect modification can be additive or multiplicative. It can be identified using stratification: exchangeability would exist within the different levels of the effect modifier.

Interaction is similar to effect modification, but in contrast implies a causal effect of the interacting variable. The effect of exposure on outcome varies in levels of the interacting variable, due to a joint effect of exposure and interacting variables. Both variables work together to cause the outcome. Effect modification and interaction are independent of causal diagrams. [286]

Chapter 3 Procedures

"Eighty percent of success is showing up"

Woody Allen

This section summarises the methods used in the study. I begin with a detailed description of the previous trial methods and then the preliminary work prior to the start of data collection: the sample size calculations, piloting, training and the calibration of the equipment. I then describe the methods used in conducting the questionnaire, anthropometry, body composition, kidney dimensions, blood pressure, spirometry, isotope calibration study and air pollution measurements. Finally, I discuss some of the safety and ethical considerations within the study.

3.1 Method of original trial

A detailed description of the original trial is provided by Osrin et al. [7] and described with the results in more detail in section 2.9.1. The trial was designed to test the effect of multiple micronutrient supplementation in the second and third trimesters of pregnancy. In total, 1985 women attending the obstetric unit at Janakpur zonal hospital for antenatal care were screened for eligibility and 1200 were recruited. The trial was powered to detect a difference in birthweight of 100 g. At a power of 90%, with a two-sided significance level of 5%, allowing for 30% loss to follow-up, the sample size was 600 participants per arm.

The eligibility criteria were: being within 12-20 weeks of gestation (measured by ultrasound), singleton pregnancy, no fetal abnormality on obstetric ultrasound (anencephaly, occipital meningocele, encephalocele, duodenal atresia and a grossly dilated pelvicalyceal system), no existing maternal illness "of a severity that could compromise the outcome of pregnancy" (cysticercosis, need for chlorpromazine or anticoagulant drugs with changing doses and symptomatic mitral stenosis or multivalvular heart disease) and residence within Dhanusha or the adjoining districts.

Participants received the routine antenatal healthcare provided by the hospital, but were helped to access services if needed. They were randomized into either the MMN or the control groups. Participants were given identification (ID) numbers generated randomly by someone independent of the trial and allocated in blocks of 50. The supplements were placed in containers in Kathmandu and labeled with the ID numbers, again by someone not involved in the trial. Both MMN and the control supplements were manufactured by Danish Pharmaceutical Industries (Ballerup, Denmark) and were designed to look identical. Women were asked to take one tablet per day, preferably after food and at the same time. If other medications were needed, these were recommended by the study obstetrician. The MMN supplements were tested for their constituents. It was found that vitamin E levels were 25% higher and vitamin A (retinol), vitamin C and iron were approximately 10% lower than projected levels.

The discrepancy estimate method was used to assess compliance. Supplements were given to participants in varying numbers. When new supplements were given, the remaining number was counted. Supplements were consumed for a median of 98% of days in the control and 97% in the intervention group.

Birthweight was measured with Seca 835 electronic scales (Hamburg, Germany) accurate to 10 g, within 72 hours of birth, and infant length on a Kiddimetre board accurate to 1 mm (Raven Equipment, Castlemead, UK) or a Rollametre (Raven Equipment) if weather

conditions did not allow, and occipitofrontal head circumference with a plastic length tape accurate to 1 mm. Three measurements were taken and the median value chosen. Only the anthropometry of liveborn infants was assessed.

Loss to follow-up was defined as failure to attend the antenatal clinic for 3 months and to meet the participant after three home visits. Miscarriage was defined as "cessation of confirmed pregnancy before 23 weeks' gestation" and stillbirth as "delivery of an infant showing no signs of life after 23 weeks gestation". Neonatal deaths were categorized as early if within the first 7 days and late if 8-28 days.

Maternal blood samples, taken from a subsample of participants, showed that retinol levels (vitamin A) and α tocopherol (vitamin E) were significantly higher in the MMN group. The prevalence of anaemia (defined as haemoglobin <110 g/L) fell in both groups. An extra 60 mg of iron and antihelminthic drugs were given if the woman had a haemoglobin level < 70 g/L. If she had symptoms of night blindness, she was given 2000 μg of vitamin A. Participants were followed up every two weeks either in their home or the clinic.

1069 mothers and infants completed the trial and were seen at birth and one month of age. The intervention and control groups were comparable for all baseline measures, implying, at least for the variables measured, that the randomization process had been successful. Most women came from families in which the husband had a salaried income (43%) and the family owned land (94%). Approximately equal numbers of women had secondary or higher education as women who did not have any education. The sample was therefore not from the very poor group, although richer people were more likely to attend private clinics for their antenatal care.

3.2 Sample size calculation

The sample size available for follow-up was constrained to infants available from the initial trial, of whom there were 1064. From the 2-year follow-up we had contact details for 917, making this the likely maximum number. Members of the research team who performed the 2-year follow-up estimated that we could find approximately 800 children. Power for the primary outcomes was calculated at this sample size. Statistical power is the likelihood of detecting an effect if one does exist. Greater power reduces the chance of a type II error. Figure 12 shows the relationship between sample size and power. Increasing sample size has diminishing returns in terms of power.

3.2.1 Weight

The difference observed in weight at birth and at 2.5 years was 0.2 z scores. With an estimated sample size of 400 in each group, at 5% significance level, the study was powered at 81% to detect a difference of 0.2 z scores between the groups. This was calculated using Stata software (`sampsi` command). An approximation of the power can be calculated as shown in Appendix 3.1.

3.2.2 Forced expiratory volume in 1 second (FEV_1)

Calculating the power for lung function was more complicated as reference ranges for Nepalese children do not exist. Power estimates were calculated using data from rural Nepalese teenagers and young adults after personal correspondence with Professor Graham Devereux[297], and using US data for children (National Health and Nutrition Examination Survey III [298]). With a sample size of 400 in each group, at 5% significance level, the study was powered at 80% to detect a difference between the groups of 0.2 z scores, equivalent to a 4.0% difference in FEV_1 using the Nepal adult data, or 2.6% using the US child data A similar study of antenatal vitamin A supplementation by Checkley et al found a 3% difference.[177] These calculations were made before the Global Lung Initiative equations described in Section 6.4 were produced. [26]

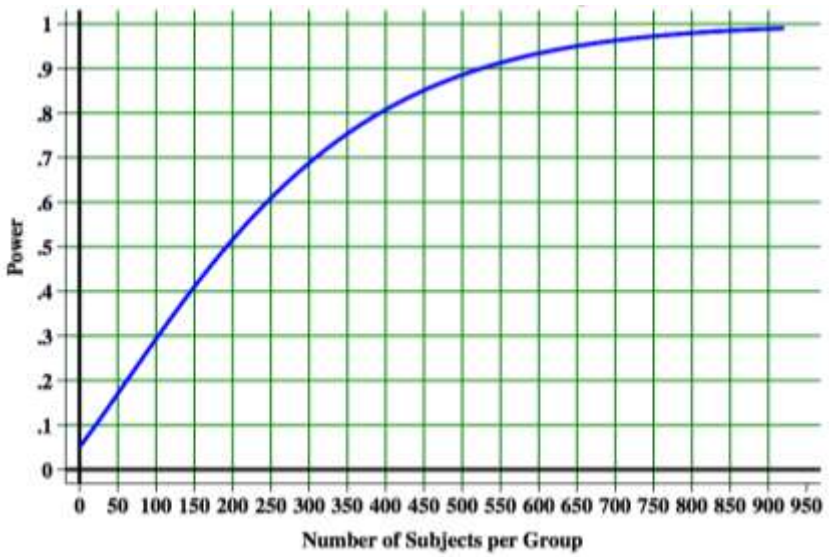

Figure 12: Power as a function of sample size

Adapted from Stata commands produced by GJ Stoddard.[299]

3.3 Piloting and Training

The pilot phase ran for four weeks prior to data collection. Piloting was done in two stages: after initial training, the instruments were piloted with adults and friends of the staff in the office; the instruments were then piloted in the field with families in areas where MIRA already worked. The instruments or methods were adapted at each stage.

3.3.1 Questionnaire

Piloting of the questionnaire ran from 25/8/11 to 14/9/11. The questionnaire was developed in English and translated into Maithili and Nepali. The final questionnaire was trilingual, with Nepali as the main language and English and Maithili translations. Translation was done by seven staff members of MIRA and UCL. Each question was back-translated to make sure the three versions were equivalent.

The questionnaire was given to the field team for consideration. Each question was considered in detail, with an explanation of why it was being asked. It was piloted with other members of staff and friends. Each questionnaire was scrutinised in detail, with feedback provided individually to each member of staff conducting the interview. It was then piloted in field sites where MIRA had been working, both urban and rural. Each field team member conducted 12 interviews. The data entry officer was also involved in this process and did two field interviews to help make him aware of the questions being used. Modifications were made at each stage of the process. Mock interviews were conducted with the field team monthly to check their understanding and the consistency with which questions were asked.

3.3.2 Anthropometry and spirometry

The times at which each component of the project occurred during my PhD are shown in the Gantt chart in Appendix 3.2. Piloting of anthropometry and spirometry ran from 25th August to 20th September 2011. Anthropometry training was first done by measuring adult members of staff within the MIRA office, then with children 7-9 years old in two schools. A consent form was sent to parents (Appendix 3.3). In total, 53 children whose parents or guardians consented were assessed in the schools. A brief report detailing their measurements was given to parents along with a small gift for the child (an exercise book and pencil). A summary report was given to the school.

3.3.3 Air pollution

During a pilot phase, the four field team members visited ten families each with children aged 7-9 years to establish the main locations in which children spent their time. The parent or guardian was taken through an "average" school day and asked to say where the child would be for most of the time, in half-hour blocks over a 24-hour period. Children divided their time between five locations: bedroom/living room, kitchen, verandah, in school and outdoors. Periods in the kitchen were subdivided into time when cooking was taking place and time when it was not.

The DustTrak II (TSI Inc, St Paul MN, USA) was used to take measures in kitchens of MIRA staff members to help determine the time needed between cooking and non-cooking samples. It was also used in schools to help decide where to place the instrument. It was noticed, for example, that very high exposure levels were recorded if the DustTrak was placed near a blackboard.

3.4 Calibration

Each instrument was calibrated according to the manufacturer's instructions. Table 7 shows the method used to calibrate all the instruments used in the study and reported accuracies. Calibration was performed fortnightly unless stated otherwise.

Table 7: Accuracy and calibration method of all instruments used

Device	Calibration method	Accuracy
Air pollution		
Casella Apex pumps	Calibrated before and after each use with a portable flow meter (Casella Rotameter) range 0.5 L/min to 5 L/min; calibrated in turn with a Bios Dry Cal DC-Lite Primary flow meter.	0.1 L/min
TSI DustTrak II 8530 photometric device	Photometric readings were calibrated against the Casella Apex.	Gravimetric readings accurate to 0.00001 g
Weighing balance for filter samples	Professionally calibrated annually.	0.00001 g
Anthropometry		
Harpenden callipers	Callipers were zeroed before each use. They were calibrated against Vernier callipers, accurate to 0.05 mm.	2 mm
Leicester stadiometer	Calibrated against a 50 cm metal calibration rod	1 mm
Seca 201 measuring tape	Measuring tapes were inspected and calibrated against the 50 cm metal calibration rod daily. If the tape was found to be inaccurate, it was replaced.	1 mm
Tanita 418 bioelectrical impedance machine	Body composition was calibrated against deuterium total body water. Weighing scales were calibrated with calibration weights of 5 kg, 10 kg, 15 kg and 20 kg.	0.1 kg
Kidney measurements		
Aloka SDD-500 ultrasound machine	The machine was checked by a qualified ultrasound technician prior to the start of the study.	Measurements accurate to 1 mm.
Blood pressure		
Omron M6 Blood pressure monitor	Instruments were calibrated before purchase. The manufacturer stated that they would last the course of the study without requiring recalibration.	Measurement range: Pressure = 0 to 299 mmHg Accuracy: Pressure = +/- 3 mmHg
GPS		
Garmin eTrex Summit	Calibrated every time the batteries were changed, as per the manufacturer's instructions.	15 m The accuracy varies according to the number of

		satellites the instrument is able to pick up. It is reportedly accurate to the nearest 15 m, but this sometimes increased to 50 m.
Spirometry		
EasyOne spirometers	Auto calibration	FEV_1 and FVC are accurate to 0.01 L

3.4.1 Weighing scales

We were unable to get accurate calibration weights until halfway through the project. The scales were subsequently calibrated fortnightly. The Tanita 418 was accurate for every calibration measurement throughout.

3.4.2 Harpenden Callipers

The callipers were calibrated against gold standard Vernier callipers using measurements of 12 One Rupee coins (each measuring approximately 1.8 mm). The standard deviation of the measurements did not appear to vary with increasing distance. As shown in Figure 1, Appendix 3.4 neither of the Harpenden callipers showed a bias over time, implying that children at the end of the study could be compared with children at the start. The variability seen can be considered consistent with measurement error. While the overall difference was minimal (approximately 5-10%) and unlikely to affect the conclusions, in an attempt to maximise accuracy, I calibrated the results according to Equation As and Equation B. The difference between the Vernier and each Harpenden calliper was calculated, see Table 8. The mean difference for Calliper 1 was greater than for Calliper 2 and increased with increasing distance. The standard deviation did not increase. This is shown graphically in Figure 2, Appendix 3.4. Calliper 1 showed a gradient of 1.036 (95% CI 1.031, 1.040) and calliper 2 a gradient of 0.9893 (95% CI 0.985, 0.994).

Equation A

Calliper 1 $y = 1.036x + 0.1451$

Equation B

Calliper 2 $y = 0.9893x + 0.1577$

Table 8: Calibration of Harpenden callipers compared to the Vernier calliper

	Calliper 1	Calliper 2
Number of coins	**Mean difference from Vernier calliper (SD) (mm)**	**Mean difference from Vernier calliper (SD) (mm)**
2	0.31 (0.08)	0.17 (0.08)
4	0.37 (0.10)	0.06 (0.16)
6	0.47 (0.11)	-0.01 (0.11)
8	0.64 (0.16)	-0.01 (0.17)
10	0.73 (0.14)	-0.05 (0.11)
12	0.95 (0.08)	-0.03 (0.10)

3.5 Consent

Consent was obtained from parents or guardians in their homes in their native language. Children entered the study only after informed consent. The consent process is considered further in section 3.18.

3.6 Questionnaire

Children were identified through contact details obtained from the initial trial and previous follow-up. After obtaining informed consent, the questionnaire was administered at the respondent's home in their native language by our local staff. The questionnaire can be seen in Appendix 3.5.

I developed the questionnaire after extensive discussion with members of the MIRA and UCL research teams. It was based on the one used in the 2-year assessment, and was designed to contain similar information for longitudinal comparisons and new information to help answer the research questions. It had four sections:

A: Demographic details

B: Health status and "International Study of Asthma and Allergies in Childhood" (ISAAC) questions on rhinitis and asthma. To assess the presence of asthma, we used the ISAAC questionnaires (our study was not part of the "ISAAC collaboration"),[300] which have been validated for use throughout the world. [301] The questionnaires were translated and back-translated into Nepali and the local language, Maithili. The main outcomes were wheeze, dry cough and runny nose in the last 12 months. We did not

use the ISAAC eczema questions as we felt they we would not be able to adequately distinguish eczema from other rashes. Two open questions about major and chronic illnesses were included. I went through each questionnaire with the data collectors and coded these questions into "no illness, major, chronic or major and chronic".

C: Socioeconomic level. This included information about the child's home that was used in relation to their exposure to air pollution.

D: Food security. Food security was assessed using the Household Food Insecurity Access Scale (HFIAS),[302] the Household Dietary Diversity Score (HDDS),[303] and the Months of Adequate Household Food Provisioning (MAHFP)[304] tools developed by the Food and Nutrition Technical Assistance Project. The questions were developed in the United States, but have been validated in other countries. The tools have been used successfully in other research carried out by MIRA in the district.[305] The HFIAS and MAHFP indicate the degree of food insecurity perceived in a household over the last year, in terms of access to food and time without food. The HDDS was used to examine the breadth of the child's diet in the preceding week.

Questionnaires were checked daily for missing data or inconsistencies when the field team returned to the office. A further check was carried out by the data inputter before entering.

A question on commencing puberty was included as it will affect growth and other changes in body dimensions. As it was unlikely that the children would have started puberty and this is a sensitive topic, the data collectors were asked not to press the parents. It was felt that the data collectors should not formally assess children to avoid the placing them or their parents in an uncomfortable position.

3.7 Global Positioning System (GPS) recordings

GPS recordings were taken at each person's house using the Garmin eTrex Summit Handheld GPS Navigator (Garmin ltd., USA). Waypoints were downloaded daily to MapSource software (Garmin ltd., USA).

3.8 Anthropometry

Anthropometry was conducted in accordance with guidelines produced by the UCL Institute of Child Health (based on an Anthropometric Standardization Reference Manual)[306] – see Appendix 3.6 - and WHO.[307] We attempted to minimise biological variation by taking

measurements at a similar time of day, but it was not possible to schedule appointments. All measurements were recorded on a proforma (Appendix 3.7).

Standing Height (trunk length)

Height was measured in duplicate with a Leicester stadiometer. The child's shoes/sandals and hair bands were removed. She was positioned with her head and back touching the stadiometer. Knees were extended and feet were placed together with their heels touching the base of the stadiometer. The head was placed in the Frankfort plane. The child was asked to take a breath in just prior to the reading. The observer read at the appropriate height to avoid parallax.

Sitting Height

Sitting height was measured with the child seated on a custom-made stool. The legs were supported so that the knees were bent at 90°. The measurement was taken in a fashion similar to standing height.

Weight

Weight was measured with a Tanita BC-418 scale (Tanita Corp, Japan). Children were given a standard set of clothes - underwear, vest and sarong - weighing 200 g. Before assessment, the child was asked to pass urine and wash her hands and feet. As a back-up, in case of damage to the Tanita BC-418, body weight was also measured with a Tanita Solar standing scale. The scales were zeroed and the child stepped on the predefined footmarks.

Skinfold thickness

Biceps, triceps, subscapular and supra-iliac skin fold thicknesses were measured in triplicate on the left side of the body. Two pairs of callipers were used and alternated fortnightly. Callipers were zeroed before each use. For all measurements an eyeliner pencil was used to mark the points to measure. Using the thumb and forefinger of the non-dominant hand, the observer pulled a fold of skin and subcutaneous fat about 1 cm from the pre-marked measurement point. The callipers were placed on the skinfold at 90° to the long axis approximately halfway up. The reading was taken when the dial almost stopped, after not more than four seconds. The callipers and skinfold were released. Observers positioned themselves to avoid errors due to parallax.

The landmarks were identified as:

Biceps: anterior aspect of the mid-upper arm.

Triceps: posterior aspect of the mid-upper arm.

Subscapular: inferior angle of the lower margin of the scapula. The skinfold was grasped diagonally with the fold inclined infero-laterally at approximately 45°.

Supra-iliac: a cross was marked above the top of the iliac crest along the mid-axillary line. An oblique skinfold was grasped 1 cm above the cross following the natural cleavage line of the skin at 45°.

Body circumferences

Body circumferences were measured in duplicate using Seca 201 measuring tapes, accurate to 1 mm. Our intention was to alternate two measuring tapes fortnightly, but we needed to use nine tapes as they would tear easily. The tape was inspected and calibrated daily. For the measurements the child remained standing in the anatomical position while the observer took the readings.

Head: if the child had long hair, it was lifted up and the measuring tape placed underneath. The head was positioned in the Frankfort plane. Maximum horizontal head circumference was measured around the forehead and occiput.

Chest: chest circumference was measured with a vest on to avoid embarrassment. This was standardised across all children. The measurement was taken at the nipple line when the child was relaxed at the end of normal expiration.

Waist: the child faced forward with her abdomen relaxed and arms hanging beside her. The child was asked to bend from one side to the other and the observer marked the point of flexure on both sides. The measuring tape was placed around the abdomen at these points, when the child was relaxed at the end of normal expiration.

MUAC: the left arm was flexed to 90°. The observer marked the point mid-way between the olecranon process and the tip of the acromion. The arm was straightened and relaxed while the measurement was taken.

Upper leg circumference: the observer marked the point mid-way between the greater trochanter and the lateral epicondyle of the femur, and measured the circumference at this point.

Hip circumference: the measurement was made horizontally at the widest girth of the hips.

3.9 Bio-electrical impedance (BIA)

Body composition was estimated using BIA with a population-specific calibration study using isotope dilution. BIA measures the electrical impedance of a person and uses calibration equations to convert impedance values to an estimate of total body water and then lean mass. The relative benefits of different methods of body composition estimation have been reviewed in detail.[135] BIA measures the conductance of a small alternating current through the body. It works on the premise that different body tissues have different and consistent impedance to current. In simple terms, fat mass has high impedance (is a good insulator) while lean mass (LM), with its high water and electrolyte content, is a good conductor. The conductivity of tissues varies according to water content, and BIA assumes consistent hydration. The relationship between the resistance of an object and its dimensions is dictated by Ohm's law - "a volume of constant section is proportional to the square of the length divided by the resistance" - so that there is a linear relationship between the height^2/resistance (impedance index) and the LM. [308] [309] Impedance is made up of two components: resistance and reactance. Reactance tends to be about 10% of resistance. The importance of reactance is poorly understood, but it is thought that it may relate to the intracellular to extracellular ratio. [310] It is assumed that the reactance of the body is small, and that the only barrier to the flow of current is the resistance. These assumptions are not strictly true as the body does have some reactance and the limbs are not perfect cylinders.

BIA was performed using the Tanita BC-418. This has 8 electrodes: two on each hand and foot plate. It passes an imperceptible alternating current (frequency 50-60 Hz) from one electrode to another, measuring impedance from arm-to-arm, arm-to-leg and leg-to-leg. Segmental impedance measures were not used in our study. This method assumes the body is made up of uniform cylinders that have uniform resistance. The reported age range is 7-99 years old, but we were not able to get readings from some children because they were too small, with impedance values above the 1200 Ohm cut-off. Flexing the elbows to 90° lowered the impedance and enabled us to take measurements from these smaller children. Two recordings were taken, one with the child's arms at 90° and one with elbows straight (at 180°). Initially, all measures were taken at 90°, and then later in the study measures were taken at both 90° and 180°. We tested this method to see if it would produce adequate results and to see if the error was acceptable.

Children were given a standard set of clothes weighing 200 g. At the start of the assessment they were asked to pass urine and to wash their hands and feet. The standard male or female setting was used and a 200 g weight adjustment was applied. The child stood on the metal plates with legs apart and arms not touching the body. Measurements were conducted at a

similar time of the day, but it was not possible to conduct fasting measurements due to the logistical difficulties of getting children to the office.

3.10 Ultrasound measurements of the kidney

Ultrasound measurements of kidney size were taken by a local doctor trained in ultrasonography, using an Aloka SDD-500 machine with a 2-8 MHz convex probe (Aloka Co ltd, Japan). The maximum length and antero-posterior dimensions were found, making sure that sinus and parenchyma were visualised. Table 9 shows the landmarks used to identify the kidney. Due to time and logistic constraints no other organs were measured.

Table 9: Landmarks used to identify the kidney

	Right	Left
Superior medial	Right adrenal gland	Left adrenal gland Stomach
Superior lateral	Right lobe of the liver	Spleen
Medial	Duodenum	Pancreatic tail
Inferior/Inferior lateral	Hepatic flexure	Splenic flexure

3.11 Blood pressure

We used an Omron M6 (Omron Healthcare ltd, Japan) electronic blood pressure monitor with a paediatric cuff. This monitor has a dual check system that confirms the blood pressure by a second sensor and "intellisense" technology, which according to the manufacturer "assures an accurate and comfortable measurement". An appropriate cuff size was used for children aged 7-9 years (17-22 cm cuff length). A larger cuff (22-32 cm) was available if required. While generally considered more accurate, ambulatory blood pressure measurements would not have been practical in this setting. Two identical monitors were used and alternated fortnightly.

Measurements followed Great Ormond Street Hospital for children guidelines.[311] Blood pressure was recorded after the child had been seated for at least one minute with her legs uncrossed. She was told to relax with her head back and right arm on the armrest at the level of the sternum. The brachial artery was palpated and the cuff positioned so that the "artery" marker lay just superior. Data collection staff avoided standing over the child during recording. Two readings were taken - the cuff deflated fully and one minute between them - at the start and end of the assessment. The two lowest values were recorded.

It was likely that a child coming to the office for measurements for the first time would experience "white coat hypertension". This is a brief period of higher blood pressure due to anxiety. We tried to minimise this by doing the measurement later in the assessment. Occasionally, repeat measures were made when we suspected abnormally high readings due to anxiety.

If the blood pressure was high, the paediatrician linked to the project was informed. The cut-off for what was considered a high blood pressure was taken from the National Institute of Health 95% percentile cut off for a 7 year old girl of average height of >113 mmHg. [312 313] The parents were reassured that, while the recording was high, a one-off reading did not imply a problem. In one case, the systolic pressure was 150-160 mmHg. This child had a long-standing renal disease, the history being consistent with nephritic syndrome.

3.12 Spirometry

Lung function was measured using one of two EasyOne WorldSpirometers (NDD Zurich, Switzerland), alternated fortnightly. These spirometers were chosen because of their ease of use in similar environments and automatic calibration. Spirometry was done online, connected to a computer, to allow real-time assessment of each curve by the data collector. The criteria for the acceptability and repeatability of the spirometry curves were taken from "ATS/ERS task force: standardisation of lung function testing" by Miller et al. [314] These were adapted slightly after discussion with the Portex respiratory physiology team to accept a 10% difference between spirograms in accordance with guidelines used by Kirkby et al in the 11-year follow-up of children born in the Epicure study. [315] While still aiming for a 5% difference, this was done in recognition of the age of the child and the difficulties in performing the test. Bronchodilators were not used. Parents were requested only to bring their children for assessment when well.

Three members of staff were trained to conduct spirometry. Most assessments were carried out by two investigators, with a third assisting if there was difficulty in achieving acceptable results. The success of spirometry, particularly in children, is very dependent on the communication skills of the investigator. A lot of time was spent discussing communication and trying to develop techniques to put the child at ease and to encourage a maximum blow.

Prior to starting, the investigator explained and demonstrated the procedure to the child and their parent or guardian, who sat beside them to help. They initially practiced with the spirette by itself and with a balloon. The child was seated on a chair with her back straight, wearing a nose clip. Repeated blows were made until the criteria for acceptability and repeatability were met. If the child became tired, the test was stopped or postponed. If the child was unable to understand the instructions, another member of staff helped. A biological control (a member of staff) was tested every fortnight to monitor changes in spirometry readings over the year.

All spirographs were assessed and interpreted by myself. One-in-ten were then over-read by Dr. Jane Kirkby, a respiratory physiologist from UCL, Institute of Child Health and Great Ormond Street Children's hospital, UK.

Individual spirograms are ‘‘acceptable’’ if

- They are free from artefacts
  - Cough during the first second of exhalation
  - Glottis closure that influences the measurement
  - Early termination or cut-off
  - Effort that is not maximal throughout
  - Leak
  - Obstructed mouthpiece
- They have good starts
- Back extrapolated volume
  - <5% of FVC
- They show satisfactory exhalation
  - Duration of 3s for children or a plateau in the volume–time curve or if the subject cannot or should not continue to exhale

Between-manoeuvre criteria

- After three acceptable spirograms have been obtained.
- The two largest values of FVC must be within 5% (or 10%) of each other
- The two largest values of FEV_1 must be within 5% (or 10%) of each other

Box 4: Criteria for acceptability and repeatability of spirometry [314]

3.13 Isotope calibration study

An isotope calibration study was conducted to provide the calibration factor for the bioelectrical impedance measurements. It has been shown that population-specific calibration studies are needed to convert BIA impedance into an estimate of lean mass. Isotope calibration studies of BIA in children have been carried out in a number of low and middle-income countries and have yielded different prediction equations. [316-319]

3.13.1 Participants

The isotope calibration study was conducted over three weeks in month 4 of the project. At the time of the isotope calibration study, we had seen 200 children in the micronutrient trial follow-up. Based on the weights of these children (14 kg to 34 kg), we recruited children for the calibration study from both the trial cohort and other children in Janakpur. We began by contacting parents of children seen in our follow-up study as calibrating the BIA on the children in our cohort would be the ideal scenario. We then extended the sample by enrolling children from three local schools and neighbourhoods. To optimise the accuracy of the resulting prediction equation, we attempted to selectively sample 50 boys and 50 girls, with equal numbers in 2 kg weight bands. The children were all aged between seven and nine years and pre-pubertal. There was no difference between rural and urban children in mean weight or relative leg length in the first 200 children seen, and we did not stratify the sample on this basis.

3.13.2 Isotope administration

All children were invited to our office in the morning and time of entry was recorded. Each child was given a bracelet with her ID number and was monitored by a member of staff until she left the building. The first of two saliva samples was taken at least half an hour after entry. The child was advised to collect some saliva in her mouth and was given a salivette to roll in her mouth for approximately two minutes. She was asked not to chew the bud and to make it as wet as possible. When the salivette appeared wet enough, the child replaced it in the container and it was centrifuged immediately at 3000 rpm for at least three minutes. The process was repeated if more saliva was needed. The saliva was then pipetted into pre-labelled 2 ml microtube bottles.

The child was given a pre-prepared and pre-labelled drink of approximately 125 ml of bottled water with 1.2 ml of 99.8% sterility tested deuterium oxide (CK Gas Products Ltd, Hampshire). Deuterium (^2H_2O) was chosen because it is the mostly widely used isotope for

this purpose and is cheaper than the alternative ^{18}O-labeled water. The drink was made to a concentration of 0.06 mg/kg, with the assumption that the average child weighed 20 kg. It was shaken for one minute and a sample taken for analysis of the dose concentration.

The bottle with deuterium, straw and bag were pre-weighed before giving them to the child and post-weighed after the child had taken the drink, using an Ohaus Traveler TA152 weighing balance (Ohaus Corp, USA) accurate to 0.01 g. In calibrating this balance we found that the weight drifted downwards up to 0.1 g over a 10 minute period. This drift was compensated for by zeroing the balance, nevertheless it was recalibrated every five minutes.

The child was asked to drink the deuterated water through a straw and the bottle was kept in the bag to catch any droplets. Drinks were made in batches and stored no longer than one week in airtight bottles in sealed bags. The bottle, straw and bag were weighed after the procedure as before.

After the measurements were taken, children were taken into a separate room where they were shown a film, given biscuits and a standard drink (totalling 230 ml). They were not allowed to take other food or drink from 3½ hours after the procedure until the second saliva sample, which was taken in a similar way to the first, four hours after isotope drink consumption. The saliva samples were stored in a freezer and transported frozen to the UK. The full protocol is provided in Appendix 3.8.

To limit inter-observer variation, measurements were carried out by only two trained data collectors. Standing height, sitting height, weight and BIA were measured as in the rest of the study (see section 3.8 and 3.9).

3.13.3 Total body water estimation (TBW)

The samples were analysed by Iso-Analytical (Iso-Analytical ltd., Crewe, UK). Total body water was estimated using continuous flow isotope ratio mass spectrometry (Thermo-Fisher Gasbench-Delta XP system, Germany). The Hoffman method was used. This relies on the property that different isotopes have different degrees of deflection within a mass spectrometer. The samples were tested in duplicate using the equilibration technique. A sample was pipetted into Exetainer tubes containing 5% platinum on alumina. The tubes were sealed, filled with pure hydrogen and left to equilibrate for at least eight hours. The hydrogen gas enrichment would then be proportional to the water. The samples were measured against three reference standards, which were all prepared in the same manner.

3.14 Air pollution

Despite its high prevalence and adverse health effects, most research takes fuel usage as a proxy for true exposure. In this study, we sampled the particle mass concentrations for children aged 7 to 9 years in the microenvironments in which they spent time. We performed size selective sampling to differentiate the particles that collect in the different regions of the respiratory system. We chose to measure the respirable fraction of particle mass (PM_4), the reasons for which are described in Box 5. We also collected data on fuel usage, household characteristics and children's time-activity patterns, to produce a 24 hour time-weighted average (TWA) exposure.

Particulate matter concentrations were measured in a subsample and the data were used to model the likely exposure for all children based on household fuel usage and time-activity information. A 24-hour TWA exposure estimate was created for each child in relation to respirable particulate (<4 μm median aerodynamic diameter PM_4). Sampling was carried out from December 2011 to December 2012. Questionnaire data were collected for all children in the cohort. The only difference between the subsample and the rest of the children was that they could have been older. However, this was tested and found not to be the case.

Due to the logistics of staff working hours and not being able to travel in the dark, we were not able to start the Apex at the time the child would normally sleep. One member of staff would start work early to go and pick up the Apex in the morning.

We measured particles that have 50% penetration of the respirable fraction, with a mean aerodynamic diameter (defined as "a unit density sphere that has the same terminal settling velocity as the given particle"[320]) of 4 μm in diameter (PM_4). The European Committee for Standardization have defined the following four categories of particles, depending on where they reach in the respiratory tract:

- "Inhalable fraction – mass fraction of total airborne particles which is inhaled through the nose and mouth.
- Extrathoracic fraction – mass fraction of inhaled particles failing to penetrate beyond the larynx.
- Thoracic fraction – mass fraction of inhaled particles penetrating beyond the larynx.
- Respirable fraction – mass fraction of inhaled particles penetrating to the unciliated airways." [321]

The trend has been for PM_{10}, or more recently $PM_{2.5}$, to be used in studies of health, whereas PM_4 has traditionally been the standard used to assess occupational exposures. The PM_{10} cut-off was recommended as the particle size that penetrates the thoracic region by the American Conference of Governmental Industrial Hygienists.[322] This cut-off was designed to be protective of workers, rather than to accurately define the fraction that passes to different parts of the respiratory system. $PM_{2.5}$ was described by Miller et al[320] as indicating particle mass that penetrates to the gas exchange region. The choice of PM_4 was in part related to historic norms, in that the measurement of $PM_{2.5}$ is less standardised and more expensive. In addition, we wanted to consider damage to the airways as well as impairment to the gas exchange area and there is also evidence that in children the particle size is a little greater. A 50% cut-off of 4 μm was found to reach the tracheobronchial region compared to 3 μm in adults. [323] In reality there is probably not much difference as the fine particles created from combustion tend to be less than 1 micrometer, so the $PM_{2.5}$ and PM_4 are very similar. Additionally, simply by changing from nose to mouth breathing increases the particle size inhaled to the alveolar region to 5-10 μm. [320]

Box 5: Particle size choice

3.14.1 Setting

The measurements were carried out in Dhanusha district. Urban samples were taken in Janakpur, which houses about one-eighth of the district population. [40] Rural samples were taken from nearby villages. There are few asphalted roads, even in the city. Mechanized traffic consists mostly of motorbikes and small numbers of cars, tractors, trucks and buses.

3.14.2 Sampling strategy

We sampled air pollution concentrations in the six microenvironments in which the children resided: the bedroom, verandah, outdoors, the school and the kitchen when cooking was taking place and when it was not. The initial plan was to take repeat samples from 40 bedrooms (5% of the expected total), eight verandah samples, eight schools, eight outdoor samples stratified by urban and rural, and eight kitchen samples stratified by biomass and non-biomass users (1% of the expected total). The bedroom is the location where children spend the most time during the day, so it was felt that it should make up the majority of the samples. We stratified the sample by urban or rural location as we thought the dust and traffic and the close proximity of air pollution from neighbours would make these locations different. We also stratified bedroom samples by ceiling type (cement, tiles and straw) as different roof

types were believed to allow different degrees of ventilation. We stratified kitchen samples by the type of fuel used: biomass and non-biomass. The initial sampling strategy can be seen in Appendix 3.9. We attempted to make the sample representative of all the children in our study. We chose houses to sample in by randomly ordering the first 100 children seen in the study, and proceeding down the list until the required number in each stratum was found. To take account of seasonal variation in air pollution levels, we repeated the measurements three times over a year during the winter season (December to March), the monsoon season (June to September), and the hotter spring and autumn seasons (April, May, October and November). After assessing the results from the first season, the sampling schedule was adapted. Due to the lack of variation by roof type, the number of bedroom samples was reduced and the number of outdoor and kitchen samples increased for the second and third seasons.

Samples were taken at the following locations:

Bedroom**.** A Casella Apex gravimetric sampler was placed in the room where the child slept, set to sample from late afternoon and collected first thing the following morning.

Verandah. The TSI DustTrak monitor was placed in the verandah, approximately equidistant from the inside of the building and the outside. Sampling was done in the evening to coincide with the time that the child was normally on the verandah. The DustTrak was chosen because it can be programmed for a set time. Using this, we were able to do evening samples that would otherwise have been difficult to complete.

Kitchen. An Apex sampler was placed in the kitchen about 1 m away from the stove for the period of cooking, and also when there was no cooking for a three-hour period (at least one hour after cooking had ceased). The DustTrak was also used to collect seven 12-hour samples to look at changes in air pollution concentration over time.

School. Schools were chosen according to their accessibility and willingness to participate. An Apex sampler was placed in a classroom advised by the principal, during school hours, which varied from school to school and by season. Care was taken not to place it close to the door, windows, or blackboard. All classrooms were on the ground floor.

Outdoors. Outdoor samples were taken by members of staff, close to their homes in eight rural and urban areas. An Apex sampler was kept in the garden or compound, as far as possible from the house or adjacent houses.

3.14.3 Time activity

Time activity was recorded in the questionnaire. The data collector went through an average day with the parent or guardian, who said where the child was likely to be in every half-hour period. A table was created for each child, summarizing the amount of time in each of the pre-determined locations.

3.14.4 Gravimetric sampling

Gravimetric sampling gives the average particle mass per hour that the children are breathing in, and is considered the gold standard. It was conducted in accordance with "Methods for Determination of Hazardous Substances (MDHS) no. 14/3 (Health and Safety Executive 2000) guidelines". [324] New glass fiber 37 mm filters (Casella, Bedford, UK) were weighed on a Sartorius balance (Sartorius Ltd, Epsom, UK) accurate to 0.00001g. The filters were pre-weighed in the UK as close as possible to departure for Nepal. Post-sample weighing was done in four batches over a nine-month period, determined by the logistics of travel to the field site. Filters were kept in the laboratory overnight to acclimatize, and then weighed twice over two consecutive days and the average calculated. The weighing balance was zeroed and the filter was placed onto the weighing scales using a pair of flat-tipped tweezers. Care was taken not to keep electronic equipment, such as laptops, close to the balance when weighing as they may interfere with the reading. The filter was discarded if the two weights differed by more than 100 μg. Each filter was placed in a plastic "filter keeper" (SKC ltd., Dorset, UK) and transported to Nepal in an airtight box.

Air sampling was conducted using an Apex air pump (Casella, Bedford, UK) attached to a cyclone sampling head (Casella, Bedford, UK) to collect respirable sized particulate in accordance with MDHS guidelines. [324] The head and cassette were cleaned before every use and the filter inserted using flat-tipped tweezers. Flow rates were set to 2.2 L/min using a portable flow meter (Casella Ltd. Rotameter, range 0.5 to 5 L/min). The pump was allowed to run for one or two minutes to allow it to stabilise before calibrating.

The time, volume, temperature and flow rate were recorded for each sample taken (see Appendix 3.10). The sampling head was placed at 1.1 m from the ground, attached to a portable Leicester stadiometer. This height was chosen because it was thought to be approximately the height of the child's mouth and nose. Time was recorded from data collectors' mobile phones, synchronized weekly. The sample was discarded if the total sampling time differed by more than 5% from the time recorded on the Apex pump. After sampling, the Cyclone head was kept upright until the filter was removed. The Apex was re-calibrated after use. If the flow rate of the pump had changed by more than 0.1 L/min, the

sample was discarded. Each filter was placed in the plastic filter keeper and airtight box, and taken to the United Kingdom where it was reweighed using the same balance and weighing protocol. Filters were examined and discarded if loss of material was thought to have occurred. One in ten filters were used as field blanks to correct for changes in filter weight.

3.14.5 Photometric sampling

A TSI DustTrak II 8530 monitor was used to measure particulate concentrations in the verandah and some of the kitchen microenvironments. This device provided 1-minute resolution of respirable dust concentrations. Sampling was performed at 1.7 L/min using a Dorr-Oliver cyclone attachment (TSI Inc, St Paul MN, USA). The DustTrak was zero calibrated, pre-programmed to run for the appropriate time, and placed in the location. Data were downloaded via TSI Trakpro software.

3.15 Gesture of thanks

All children who attended for anthropometry and spirometry were given a T-shirt and refreshments (tea, a packet of biscuits and fruit juice). Parents were compensated for the cost of transport to the MIRA office. They were also given a voucher to be seen by a local paediatrician (see Appendix 3.11). The local doctor was not part of the research and was paid at a local rate for all consultations and the costs of minor acute treatments.

3.16 Safety

The project was approved by UCL after a risk assessment.

3.16.1 Safety of the children

The research inconvenienced the participants in terms of time, but none of the procedures was invasive or potentially harmful. The only substance given to the children was deuterium, in a sub-sample of 109 children as part of the isotope calibration study. The protocol for isotope dilution is widely used in medical research in all age groups and has been approved by the International Atomic Energy Agency. Deuterium occurs naturally in the body and in all foods and fluids consumed at natural abundance levels. It is a stable isotope of water and is non-toxic, non-radioactive and has not been associated with any risk in human studies. “Almost

half a century of stable isotope usage in human metabolic studies has occurred without documented significant adverse effect". [325]

The investigators performing the spirometry were trained to recognise wheezing and signs of respiratory distress and to inform a clinician immediately. If the child was suffering from acute respiratory distress, they were to contact a local paediatrician. A new salbutamol inhaler was kept on site, but no acute situations occurred. If necessary, referrals for further non-acute assessment were made to a local paediatrician.

3.16.2 Infectious diseases

Precautions were taken to prevent infection, including drinking clean water and using a bed-net. Appropriate clothing and footwear were worn. Members of staff from the UK working long-term in Nepal were appropriately immunised prior to departure. These staff were covered by comprehensive health and travel medical insurance, which included cover for medical evacuation.

3.16.3 Security

The security of participants and staff was paramount. The project ran according to advice from our Nepalese partners, MIRA, under the guidance of the site manager. All operations were subject to his agreement and he was aware of all movements. Staff carried mobile phones with emergency contact numbers. Data collection staff were aware of the local culture and customs and were able to converse with the local population. Foreign staff were accompanied by Nepalese staff where appropriate. On days on which strikes occurred, the field staff went to other areas or remained in the office.

3.16.4 Equipment

The instruments and devices were new and were used according to their instructions only by those trained to do so. They were stored appropriately indoors. The instruments were serviced as recommended by the manufacturer. All electrical appliances were used according to their purpose with surge protectors. All staff were obliged to wear helmets when riding on motorbikes for work-related activities.

3.17 Challenges: strikes, festivals, and more strikes

3.17.1 Finding the children

The most difficult part of the project was finding the children. While we did have addresses from the previous follow-up, they were often quite vague: a name and village. We relied heavily on the local knowledge of our field staff. We were very lucky to have three members of staff who were involved in the trial and another person who took part in the last follow-up. It became progressively more difficult to find the children as the project went along.

3.17.2 Strikes

Strikes, known locally as *bandas*, happened often. The importance of the strike and its relevance to the study depended on who was striking and for what reason. An example was a strike called by medical students when one of their member was killed in a road traffic accident by a bus. While not particularly threatening for us, it did mean that all local public transport ceased, and that children could not come for anthropometry and spirometry measurements. Strikes leading to a ban on mechanised transport and stopping our field team from working were very common. In the middle of the project, all activities stopped for about a month after two bombs were set off close to the office. The first one exploded in a rally, killing five people. The second thankfully caused no harm. The rally was run by a separatist group from the Terai, who wanted Nepal to split into two countries, the hills and the plains. The group that planted the bomb was thought to be from a different separatist Terai group with similar but slightly different goals. Thankfully, these events did not lead to further violence. The tense nature of the strikes was often interspersed with holidays and festivals. While also calling a halt to our work, they were altogether more pleasant.

3.17.3 Weather

The weather was a constant source of problems. While normally very hot, the Central Terai does have cold and rainy seasons. This mainly caused transport problems for the participants and for us. The main problem was a spell of heavy rain for 24 hours that led to flooding of our office. Luckily, the water did not damage the equipment.

3.18 Ethics

The research project was approved by both the University College London research ethics board (UCL Ethics Project ID 2744/001) and the Nepal Health Research Council (Reference number 51/2011; Appendix 3.12) A later amendment to conduct epigenetic analysis of DNA samples was approved by both boards.

The research project was completed as planned. We did not perform an intervention and no adverse effects related to the children or staff were reported.

3.18.1 Main study

Informed consent from the parent or guardian of the child was taken at the start of the research when the family was first contacted. Participants were given a detailed information sheet describing the research project in their native language (information sheet and consent form in Appendix 3.13). In addition, the project was explained to them orally by a member of the research team. They were given an opportunity to discuss the project with the member of staff or to discuss it with other members of their family before deciding whether to participate. As part of the consent process, families were assured that they did not have to participate or could consent to part of the project and had the right to withdraw at any point. Parents or guardians were advised that the study was a follow-up of the one the mother and child were previously involved in. Families were fairly familiar with what we were planning to do and there did not seem to be major concerns. Oral and written consent (with thumbprints if unable to write) were obtained from the parent or guardian.

Participants were advised that the information collected would be stored securely and treated confidentially and anonymously. In an example of different attitudes towards confidentiality to the UK setting, one guardian was concerned that the data would be kept confidential. He was reassured when told that it was for the benefit of his child rather than to hide something.

Of the 852 children we found, one person refused to take any part in the research because of a long-standing issue she had with the organisation. Three others were happy to complete the questionnaire, but did not want their child to come in for the anthropometry and spirometry, mostly claiming a lack of time or benefit to them.

3.18.1.1 Children with an illness or malnutrition

During our assessment we found children with acute or chronic illnesses. This most commonly occurred because of high blood pressure, an abnormality on kidney ultrasound or if the child was found to be underweight. In the case of lung or kidney problems, these were

sometimes discovered for the first time by us. A formal letter of referral was made to the local paediatrician. Any further referral would be co-ordinated by him, but we provided help where possible. Occasionally, further ultrasound scans were needed and we covered their costs. In a small number of cases we tried to help organise further specialist care for the child. This medical care was within the government system, but we considered helping with transportation costs. The decisions were ultimately made by our partner organisation, MIRA.

We regularly found children with malnutrition and referred them to the local malnutrition unit. A copy of the referral form can be seen in Appendix 3.14. No action was taken for children who were stunted as this is due to multiple causes over a long period and not obviously ameliorable to interventions. Children with low weight-for-age, on the other hand, may have been suffering from an acute episode that could be related to high morbidity and mortality, and potentially treatable. A decision was made to refer the child if their weight for height score was <-2 z scores in accordance with their referral guidelines (I believe adapted from Gorstein et al, 1994[326]). After discussion, we agreed that we would also refer children with BMI-for-age z score <-3. For children whose BMI-for-age z scores were -2 to -3, a referral was made to the local paediatrician who would refer on if needed.

3.18.1.2 Time

The assessments generally took a little longer than anticipated. On some occasions they would take a few hours due to difficulty in coordinating ultrasound measurements. We attempted to complete each assessment as quickly as possible and occasionally needed to reschedule. While not ideal, the participants did not seem overly concerned.

3.18.2 Air pollution

Approximately 80% of households in the follow-up used biomass fuels producing high levels of air pollution. Families and schoolteachers were sometimes concerned about the level of air pollution in their residences. When we reported the results back to them, our perception was that, while some appreciated that air pollution could be a problem to their health, others were not aware of it. In addition to the primary questionnaire, I supervised a medical student who conducted semi-structured interviews with some of the mothers in the study. In general, air pollution was thought to be detrimental, but understanding of its health risk was low. To explore these issues further, we ran a public engagement project with women from the families in which we were measuring air pollution levels. We did this by bringing together local women, our researchers and a well-known group of neighbouring artists from the Janakpur Women's Development Centre, to look at problems and potential solutions in a creative environment. More information on this is in Chapter 7.

3.18.3 Isotope calibration study

Separate consent was taken for this part of the study (Appendix 3.13). A study such as this that involved giving children an "isotope" was a departure from the type of work we had done in this region and we were apprehensive about how it would be perceived. We were worried that it would cause concern to the participants and their families, be they children within our cohort or other children within the region.

Despite it being safe, especially in locations such as ours where illiteracy rates are high, written information is less useful. To give the parents as much information as possible about what we were doing, a film detailing the project was created. The film explained the study in greater detail, showing what would happen, the purpose for doing it and some background information about our organisation. It did not replace other aspects of the consent process and parents or guardians were still able to discuss the issues. Our participants all chose to watch the film when it was offered to them and we received positive feedback on it. The film was produced by a medical student I was supervising and included the research team as a camerawoman and actors. We think that media such as this can be useful in low resource settings to help in the understanding of research. The main drawback of the film was the time and technical difficulty involved in producing it. To be fully effective, a film needs to be specific to the population and at the very least in the local language.

3.18.4 Photographs

The issue of how photographs of children were taken was considered at length. Within a research setting it is common for children to be photographed, either as a fundamental aspect of the research - for example, to portray the effectiveness of a nutrition intervention - or to set the scene. Photos are also commonly used to aid in teaching, advocacy and fundraising. In this sense, photographs can be valuable.

In this study, written consent for the taking of photographs was specifically mentioned in the consent form. If a photo was to be taken, verbal consent, with an explanation of what the photo will be used for, was also taken. A separate consent process and form was completed for participants who were included in filming.

To explore the issue of consent further, I conducted some qualitative research that considered the question of what ethical photography of children in low-resource settings is. [327] The research involved focus groups with UK doctors or researchers who have worked in low-resource settings. The groups concentrated on medical as well as research settings. The discussions centred on the problem of what informed consent is and whether children, parents

or guardians really understand the extent to which a photograph may be shown. From this, we proposed a "ladder of dissemination" that links how widely the photograph is disseminated with how detailed the consent should be (http://www.biomedcentral.com/1472-6939/14/27).

4 Data management and analysis

Chapter 4 describes the data collected in the study. It summarises how they were managed and cleaned, with a discussion of measurement error and bias. It then documents the global positioning system data and the analysis method for the isotope calibration study, with an investigation of methodological variations.

4.1 Data storage

While work in Nepal does not fall officially under the UK Data Protection Act, we attempted to abide by it. All samples were anonymised and stored securely and no analyses or shared data included the names of participants. Questionnaires were stored in an office that was locked at all times when not in use. Outside normal working hours, the building was guarded by a security guard. All electronic data were stored in the research project computer. The computer was password protected and stored in a locked cupboard in the office. All data files were password protected and backed up daily on to two external hard drives. One was kept with the computer and the other in a locked cupboard in a separate location.

4.2 Data cleaning

4.2.1 Data management

All questionnaires were checked by myself or the MIRA data manager daily when the field staff returned to the office. Data were entered as soon as possible, normally within one or two days, into an Open Office database. If there was a discrepancy, the data manager would alert the field staff member. If necessary, parents were called or visited again to clarify the data or complete the missing data point. The database containing information from the questionnaires was checked weekly basis by the data team manager. The data that were collected are shown in Table 10.

Anthropometry, spirometry and air pollution data were entered into an Excel spreadsheet on the day in which the measurements were taken. All the data points in the spreadsheet were checked for accuracy against the original records. All spirometry curves were checked by myself. Any problems or queries were reported back to the respiratory team at Great Ormond Street hospital, who over-read one in ten spirograms.

Data from the EasyOne (Easyware, ndd Zurich, Switzerland), GPS waypoints (Garmin Map Source programme, Garmin ltd., USA) and DustTrak II (Trakpro software, TSI Inc, St Paul MN, USA) were downloaded directly into their specialised software. Statistical analyses were carried out using Excel (version 14.3.2, Microsoft Corp, USA), Prism (version 6.0a, Graphpad Software Inc, USA) and Stata (version 12.1; Stata Corp, USA) software.

Table 10: Data collected in the study

Data type			Type of variable	Description
Questionnaire data	Illness	History of recent or major illnesses	Binary	Illness questions that covered the last seven days and year
		"International Study of Asthma and Allergies in Childhood"	Binary	
	Socio-economic status	Multi-dimensional poverty index	Binary questions combined into an ordered categorical MPI score	10 pre-specified indicators
	Air pollution	Time activity	Continuous (or more precisely, ordered categorical)	Number of hours in six different locations.
		Fuel usage	Categorical	
		House characteristics	Ordered categorical	Number of rooms, floor, windows, doors, roof, walls.
	Food security	HFIAS questions	Binary	Ten questions
Anthropometry	Weight	Tanita 418	Continuous	
		Tanita Solar	Continuous	
	Height	Sitting height	Continuous	Duplicate
		Standing height	Continuous	Duplicate
	Skinfold thickness	Triceps	Continuous	Triplicate
		Biceps	Continuous	Triplicate
		Subscapular	Continuous	Triplicate
		Suprailiac	Continuous	Triplicate
	Body circumference	Head	Continuous	Duplicate
		Chest	Continuous	Duplicate
		Waist	Continuous	Duplicate
		Hip	Continuous	Duplicate

		Upper leg	Continuous	Duplicate
		Mid-upper arm	Continuous	Duplicate
	Body composition	Bioelectrical impedance	Continuous	Whole body impedance values
	Kidney dimensions	Length	Continuous	Ultrasound measurements
		Antero – Posterior distance	Continuous	
	Blood pressure	Systolic	Continuous	Lowest pressure recorded
		Diastolic	Continuous	Lowest pressure recorded
Spirometry		Forced expiratory volume in the first second	Continuous	Best value from acceptable curve
		Forced vital capacity	Continuous	Best value from acceptable curve
		Forced expiratory flow 25%-75%	Continuous	Best value from acceptable curve
Air pollution		Average respirable fraction particle mass	Continuous	Gravimetric PM_4
		Real-time readings	Continuous	Photometric PM_4

4.2.2 Assessment of outliers

Bland-Altman plots were created for all repeat measures to look at reproducibility. [328] These plot the mean of two repeated measures against the difference between the measures. They are normally used to look at the correlation between two techniques or repeated measurements. In this example they are useful in identifying outliers, which were outside the 95% confidence intervals. These data points were checked against the hard copies to look for data entry errors if considered beyond measurement error. For skinfold thickness, an erroneous data point did not make a difference because the median point was used rather than the mean.

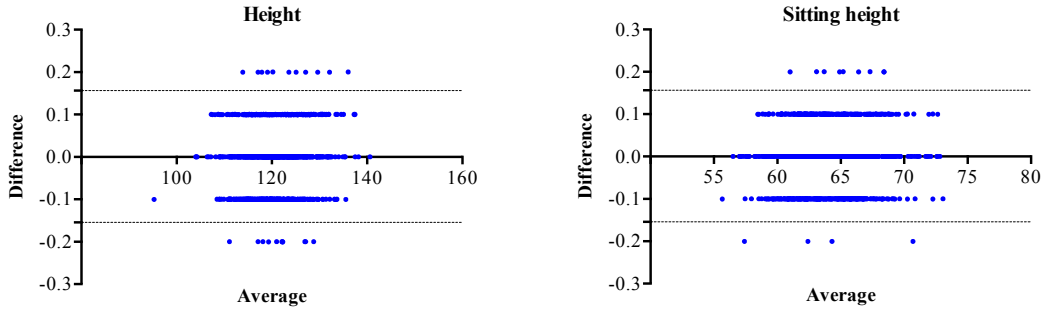

Figure 13: Bland-Altman plots for standing and sitting height

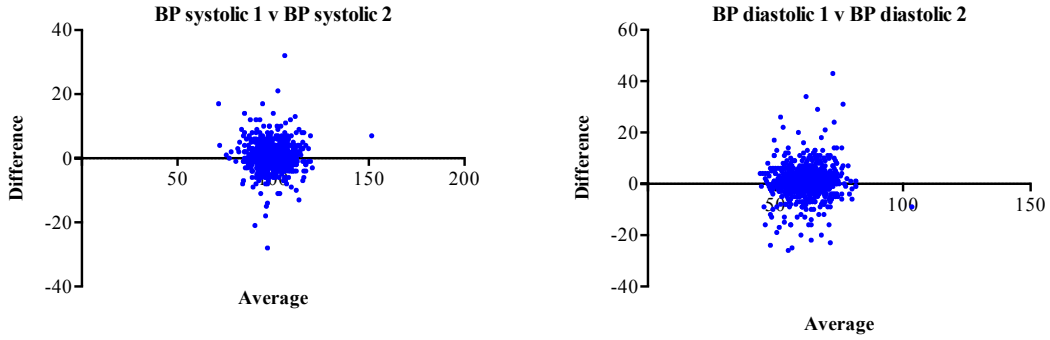

Figure 14: Bland-Altman plots for systolic and diastolic blood pressure

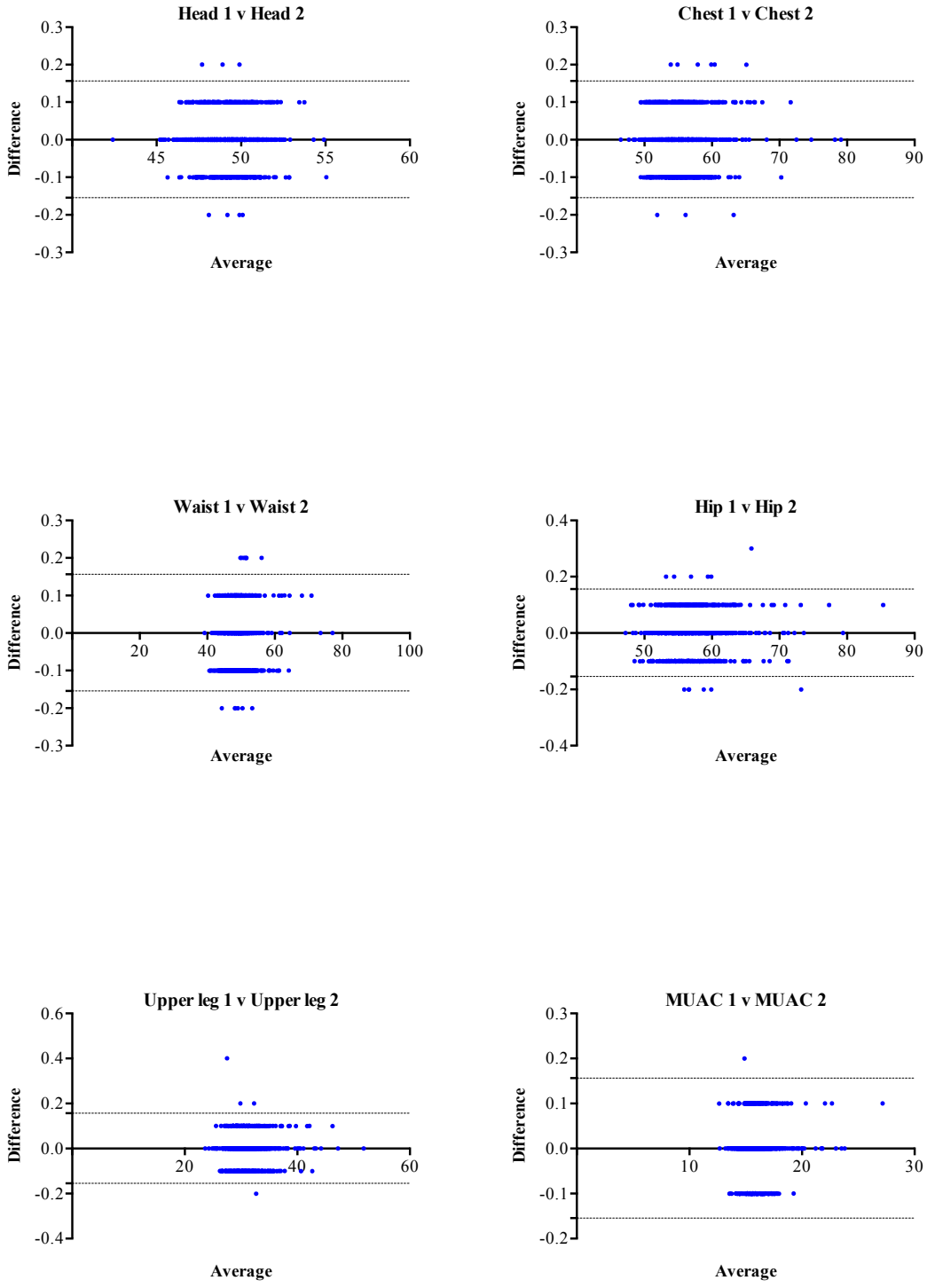

Figure 15: Bland-Altman plots for body circumferences

4.3 Missing data

4.3.1 Main study

Data were examined throughout the study to identify missing variables. Missing data are summarised in Table 11. With the exception of bioelectrical impedance (BIA), the number of missing data points was very small and unlikely to make a difference to our outcomes. The children who were missing for BIA were a little younger and lighter than those for whom we had measurements. Heights were the same. This difference may be partly explained by these children coming from families with a lower socioeconomic status (defined using principal components analysis- see Section 5.2). For bioelectrical impedance, measures for all children were present, but with different arm positions. This is discussed in greater detail in section 4.8. As described, arm position-specific calibration equations can be used. Importantly, there was no difference by trial group, so the main analysis of antenatal MMN would be unaffected.

4.3.2 Birthweight

From the initial trial only birthweights taken within 72 hours were used in analyses involving birthweight. This excluded 53 birthweights. Babies who were weighed after three days were a mean 82 g heavier than those weighed within 72 hrs.

4.3.3 Child deaths

Three children died between the ages of two and eight. Questionnaire data were collected from their parents. It is possible that they would have had lower values for their anthropometry and spirometry because these may have been related to their death (for example, chronic illness may lead to malnutrition and stunting), but as the number was so small it was unlikely to affect the main analyses.

Table 11: Missing data

Variable	Number of missing data points	Category of missing data	Comments
Bioelectrical impedance	213	MNAR	The children were 1 month younger (p=<0.001) and weighed 0.2 z scores less (p=0.02) than the other children.
Spirometry	6	MNAR	We could not get any data from 6 children. Five had developmental delay and 1 child could not coordinate a blow.
Suprailiac skinfold thickness	1	MNAR	One child refused.
Renal ultrasound	1	MCAR	Ultrasound technician not available to do the measurement.

MNAR = missing not at random, MCAR = missing completely at random

4.4 Error

We made every effort to increase precision in data collection. The variation is made up of a combination of biological variation and measurement variation (error). By minimising measurement error, true biological variation between subjects can be identified, reducing the chance of a type II error.

4.4.1 Measurement error

Measurement error is made up of two components: accuracy and repeatability. Accuracy, or validity, refers to how close the value is to the "true" measure. To maximise accuracy we used all instruments according to their instructions and conducted the measurements according to pre-defined approved standards, described previously. Instruments were calibrated and adjustments made accordingly. Repeatability, also referred to as reliability and precision, is how close repeat measures are to each other. [329,330] We attempted to reduce biological variation by taking measurements at a similar time of day, although this was not always possible. We could not account for other forms of biological variation, for example from season to season, but assumed these were balanced between the trial groups. Children were weighed post-micturition as a full bladder can contribute 250-300 g.[331] Training was given prior to and during the data collection period on a regular basis. Only two data collection staff conducted the measurements and they remained blind to the original allocation.

At the end of the piloting phase and periodically through the project, the technical error of measurement (TEM) was calculated for intra-[332] and inter-observer [333] variability. TEM is the standard deviation of sets of repeated observations, with 2n as the denominator to account for two sets of observations. [334] It is a measure of error variability that has the same units as the variable, with 95% of the difference between two replicated measurements expected to lie wihin twice the TEM value.[307,330] To allow a comparison between different variables, relative TEM (TEM%) was calculated. [335] The coefficient of reliability is also used in population studies. It gives the proportion of variation that is "free from measurement error". A value of 0.95 means that 5% of the error is due to measurement error. TEM and coefficient of reliability were calculated for both observers conducting repeat measurements on a group of between five and ten children.

We attempted to keep all coefficient of reliability values ≥ 0.95. [330] In the event of a lower value, further training was conducted, the tests were repeated on five different children and the process was repeated.

The following formulas were used, where necessary, simplified for two observers: [307,330,332,333]

Equation C

$$\text{Intra-observer variability} = \sqrt{\frac{\sum_{i=1}^{N} (M_{i1} - M_{i2})^2}{2N}}$$

Where M_{i1} and M_{i2} are repeat measurements of the i^{th} child and N is the number of children.

Equation D

$$\text{Inter-observer variability} = \sqrt{\frac{1}{N} \sum_{i=1}^{N} \sum_{j=1}^{2} Y_{ij}^2 - \sum_{j=1}^{2} Y_{ij} \div 2}$$

Where Y is the duplicate measurements from each observer j.

Equation E

$$\text{TEM\%} = \frac{TEM}{\mu} \times 100$$

Equation F

$$\text{Coefficient of reliability} = 1 - \frac{\left(TEM(Inter)\right)^2}{\sigma^2}$$

Where μ = mean and σ = standard deviation.

The data shown in Table 1, Appendix 4.1, show that intra-observer TEM was adequate throughout the project. With the exception of skinfold thicknesses, TEM% was <0.3%, with most values <0.1%. Skinfold thicknesses ranged up to 3.6%, but absolute TEM values were very small. Inter-observer TEM was generally good throughout, with R values >0.95. It was substantially less than 0.95 for chest and waist measurements in February 2012 (values of 0.13 and 0.18, respectively). Head circumference TEM was also a little low on this occasion, at 0.82. After further training, R values rose to ≥0.90 and stayed high for the rest of the project. It is possible, however, that TEM values of 1-1.27 cm existed for these variables for the first five months of the project. This should not make a difference by trial group because the intra-observer TEM was still high, but means that a combined result could be unreliable. We can control for the effect of the observer when looking at the association between antenatal multiple micronutrient supplementation and waist and chest circumference. Table 13 shows that adjustment for observer makes only a 0.0003 cm difference to head

circumference, a 0.003 cm difference to chest circumference and a 0.001 cm difference to waist circumference. This does not change the overall conclusions.

The overall results for TEM values for each variable are shown in Table 12. For kidney dimensions, TEM values were calculated from a subsample of approximately 5% of the total. Kidney AP distance had the highest TEM% at around 5%. Skinfold thickness TEM% was 1-2%, equating to ≤0.1 mm. The other anthropometry measurement TEMs were generally <0.1% with values ≤5mm. These were within the generally agreed norms for anthropometry of TEM <3 mm for height, <2 mm for body circumferences and TEM% <1% or <5% for skinfold thicknesses. [336,337]

TEM% values for the kidney were higher than for other anthropometric measurements. Our study was consistent with an assessment of error in renal measurements by Bakker et al. Using the standard deviation of the difference as a percentage (SDD%) of kidney length to measure intra-observer error, their observers reported SDD% of 5.1% and 6.1%. In our study the SDD% was up to 4.3% and 2.7% for the kidneys, making it consistent with the findings of radiologists assessed by Bakker et al. The AP diameter SSD% was a little higher at 7.5 and 8.2%, but this was not assessed in the Bakker study. [338]

4.4.2 Biological variability

Biological variability is a combination of total variability and measurement imprecision. It can be calculated with Equation G. [339] By removing imprecision, the confidence intervals should get smaller, the results of which are shown in Table 2, Appendix 4.1. The difference this made to anthropometry outcomes between intervention and control groups is shown in Table 12. Measurement error was very small, so confidence intervals changed by <0.0001 and the overall conclusions did not change. This difference is not important in a practical sense, but does highlight that we can be confident of our results and that any differences are unlikely to be the result of measurement error.

Equation G

$$\text{Biological variability} = \sqrt{(\sigma_{Total}^2 - \sigma_{Methods}^2)}$$

Where σ_{total} is the total variability (standard deviation) and $\sigma_{methods}$ is the measurement imprecision, or TEM.

Table 12: Technical error of measurement for anthropometry variables

		TEM	TEM %	Difference in mean values	95 % confidence intervals	95% confidence intervals after adjustment for error
Height (cm)	Standing	0.06	0.05	0.00	-0.81, 0.81	-0.81, 0.81
	Sitting	0.05	0.05	0.02	-0.38, 0.41	-0.38, 0.41
Skinfold thickness (mm)	Triceps	0.08	1.08	-0.03	-0.37, 0.31	-0.36, 0.31
	Biceps	0.07	1.91	-0.01	-0.19, 0.18	-0.19, 0.18
	Sub-scapular	0.07	1.46	0.01	-0.18, 0.20	-0.18, 0.20
	Supra-iliac	0.08	1.44	-0.19	-0.52, 0.14	-0.52, 0.14
Circum-ferences (cm)	Head	0.04	0.09	0.18	-0.02, 0.38	-0.02, 0.38
	Chest	0.05	0.09	0.15	-0.32, 0.63	-0.32, 0.63
	Waist	0.06	0.11	0.19	-0.33, 0.71	-0.33, 0.71
	Hip	0.05	0.09	0.07	-0.48, 0.61	-0.48, 0.61
	Upper leg	0.04	0.14	0.10	-0.23, 0.49	-0.23, 0.49
	Mid-upper arm	0.04	0.25	0.04	-0.14, 0.23	-0.14, 0.23
Kidney dimension (cm)	Right length	0.21	2.61	-0.01	-0.08, 0.07	-0.08, 0.06
	Right AP	0.14	4.73	0.02	-0.01, 0.06	-0.01, 0.05
	Left length	0.16	1.88	-0.03	-0.11, 0.05	-0.11, 0.05
	Left AP	0.19	5.66	0.02	-0.02, 0.06	-0.02, 0.06

Table 13: Waist and chest circumference, by allocation, with adjustment for observer

	Control mean	Intervention mean	Unadjusted difference (95% CI)	Difference adjusting for observer (95% CI)
Head circumference (cm)	49.37	49.19	0.18 (-0.02, 0.38)	0.18 (-0.02, 0.38)
Chest circumference (cm)	55.59	55.74	0.15 (-0.33, 0.63)	0.15 (-0.32, 0.63)
Waist circumference (cm)	49.01	49.20	0.19 (-0.33, 0.71)	0.19 (-0.33, 0.71)

4.5 Bias

4.5.1 Selection bias

Selection bias should have been minimal for the follow-up as we attempted to assess all children born in the original randomised controlled trial. The analysis compared intervention and control groups, the initial allocation of the supplement was concealed and participants and data collection staff remained blind to allocation. The main bias was likely to be from differential loss to follow-up. If we wish to generalise from our cohort to Dhanusha district or further, we need to consider whether the sample was representative of the general population. It is possible that mothers who chose to participate differed systematically from those who did not; for example, in wealth or educational status. Using an absolute scale of poverty such as the Multi-dimensional Poverty Index could allow us to compare the sample with others in the region, country or other countries. This is discussed in section 5.2.

4.5.2 Loss to follow-up

Differential loss to follow-up may induce bias as it affects both the outcome and the likelihood of being followed up. We used the same definition of loss to follow-up as the previous study at two years of age: "confirmed information that a participant had moved beyond the possibility of visiting." If we had information confirming that a child had moved to a different location outside the region, we made all possible attempts to find them (questionnaires and measurements were conducted in Kathmandu and Hetauda). The trial profile can be seen in Figure 16. Table 14 shows the characteristics of the children found within the 8 year follow-up and those not found.

Comparing those included with those lost to follow-up, there were differences in maternal education and residence. Children lost to follow-up were more likely to have mothers with some education and to be from an urban residence. Maternal education and residence were included in the multivariable model.[340] Women lost to follow-up were also more likely to have had a preterm birth, but this was unreliable because data were missing for 61 individuals and the lost category included infants who had died. Two-by-two tables were created to show the numbers within the study and lost to follow-up (Table 15). Even though the appliance score was very similar, these results point to those lost to follow-up being more affluent.

Comparing intervention and control groups, the intervention group were a little more likely to have delivered in hospital (Chi-square p value = 0.006), but were otherwise similar. This factor was not included in the adjusted analyses in section 6.3. As discussed by Assman et al, a significant difference in baseline characteristics does not necessarily mean that it should be adjusted for. More important is whether it is a strong predictor of the outcome. [341]

Table 14: Characteristics of 841 children retained and 359 lost to follow-up

	8 year follow-up		Lost to follow-up	
	Control n=422 Frequency (%)	Intervention n=419 Frequency (%)	Before end of trial [a] n=69 Frequency (%)	After end of trial [b] n=290 Frequency (%)
Residence				
Urban	199 (47.2)	197 (47.0)	47 (68.1)	184 (63.5)
Rural	223 (52.8)	222 (53.0)	22 (31.9)	106 (36.6)
District				
Dhanusha	348 (82.5)	336 (80.2)	59 (85.5)	245 (84.5)
Mahotari	74 (17.5)	79 (18.6)	10 (14.5)	43 (14.8)
Siraha		2 (0.5)	0	1 (0.3)
Sarlahi		2 (0.5)	0	1 (0.3)
Mother's age at enrolment				
<20	126 (29.9)	130 (31.0)	20 (29.0)	85 (29.3)
20-29	273 (64.7)	272 (64.9)	44 (63.8)	196 (67.6)
≥30	23 (5.5)	17 (4.1)	5 (7.3)	9 (3.1)
Maternal education				
None	206 (48.8)	210 (50.1)	27 (39.1)	101 (34.8)
Primary	37 (8.8)	33 (7.9)	16 (23.2)	37 (12.8)
Secondary or higher	179 (42.4)	176 (42.0)	26 (37.7)	152 (52.4)
Maternal Height mean (SD)	151.0 (5.7)	150.4 (5.4)	150.4 (5.0)	151.0 (5.6)
Ethnicity				
Terai Brahmin	55 (13.0)	50 (11.9)	10 (14.5)	45 (15.5)
Terai Chhetri	7 (1.7)	15 (3.6)	3 (4.4)	17 (5.9)
Terai Vaishya	296 (70.1)	276 (65.9)	38 (55.1)	176 (60.7)
Terai Sudra	10 (2.4)	14 (3.3)	2 (2.9)	3 (1.0)
Hill Brahmin	11 (2.6)	13 (3.1)	4 (5.8)	14 (4.8)
Hill Chhetri	13 (3.1)	10 (2.4)	2 (2.9)	5 (1.7)

Muslim	23 (5.5)	29 (6.9)	8 (11.6)	17 (5.9)
Newar	4 (1.0)	5 (1.2)	1 (1.5)	3 (1.0)
Tibeto-Burman	3 (0.7)	6 (1.4)	1 (1.5)	9 (3.1)
Other	0	1 (0.2)	0	1 (0.3)
Main household livelihood				
No work	49 (11.6)	46 (11.0)	1 (1.5)	34 (11.7)
Farming	72 (17.1)	72 (16.2)	7 (10.1)	34 (11.7)
Salaried	153 (36.3)	178 (42.5)	34 (49.3)	148 (51.0)
Small business	82 (19.4)	76 (18.1)	19 (27.5)	46 (15.9)
Waged labour	53 (12.6)	43 (10.3)	5 (7.3)	18 (6.2)
Student	7 (1.7)	3 (0.7)	3 (4.4)	4 (1.4)
Out of country	6 (1.4)	5 (1.2)	0	6 (2.1)
Land ownership				
0	47 (11.1)	44 (10.5)	8 (12.1)	30 (10.3)
<30 dhur (~ 500 m^2)	290 (68.7)	294 (70.2)	45 (65.2)	196 (67.6)
>30 dhur	85 (20.5)	81 (19.3)	16 (23.2)	64 (22.0)
Appliance score				
Motor vehicle, TV or refrigerator	217 (51.4)	214 (51.1)	36 (52.9) [c]	146 (50.3)
Sewing machine, cassette player, camera, fan, bullock cart, wall clock, radio, iron, bicycle	147 (34.8)	138 (32.9)	21 (30.9)	95 (32.8)
None of the above	58 (13.7)	67 (16.0)	11 (16.2)	49 (16.9)
Parity				
0	185 (43.8)	182 (43.4)	33 (47.8)	140 (48.3)
1-2	183 (43.4)	202 (48.2)	28 (40.6)	124 (42.8)
≥3	54 (12.8)	35 (8.4)	8 (11.6)	26 (9.0)
Preterm	29 (6.9)	28 (6.7)		41 (14.1)
Delivery site				

Hospital	218 (51.7)	252 (60.1)	176 (60.7)
Home	194 (46.0)	165 (39.4)	100 (34.5)
On the way	10 (2.4)	2 (0.5)	14 (4.8)
Child sex			
Female	210 (49.8)	196 (46.8)	152 (53.5) [c]
Male	212 (50.2)	223 (53.2)	132 (46.5)
Birthweight (kg)	2.74 (0.41)	2.81 (0.43)	2.75 (0.50)

[a] 14 withdrew from trial, 1 dropped out with clinical problem, 19 moved beyond study area, 28 untraceable, 7 miscarriages.

[b] 4 refused to attend, 2 moved beyond study area, 2 not assessable within follow-up period, 204 untraceable, 33 stillbirths, 29 neonatal deaths, 16 postneonatal deaths.

[c] Original trial data incomplete.

Table 15: Two-by-two tables showing the numbers included in the study and lost to follow-up, for maternal education, residence and preterm births

Maternal education	Study	Lost	Total
No education	416	128	544
Primary	70	53	123
Secondary	355	178	533
Total	841	359	1200

Chi-square test for difference = <0.0001

Residence	Study	Lost	Total
Urban	396	231	627
Rural	445	128	579
Total	841	359	1200

Chi-square test for difference = <0.0001

Preterm births	Study	Lost	Total
Normal gestation	784	254	1038
Preterm	57	44	101
Total	841	298	1139

Chi-square test for difference = <0.0001

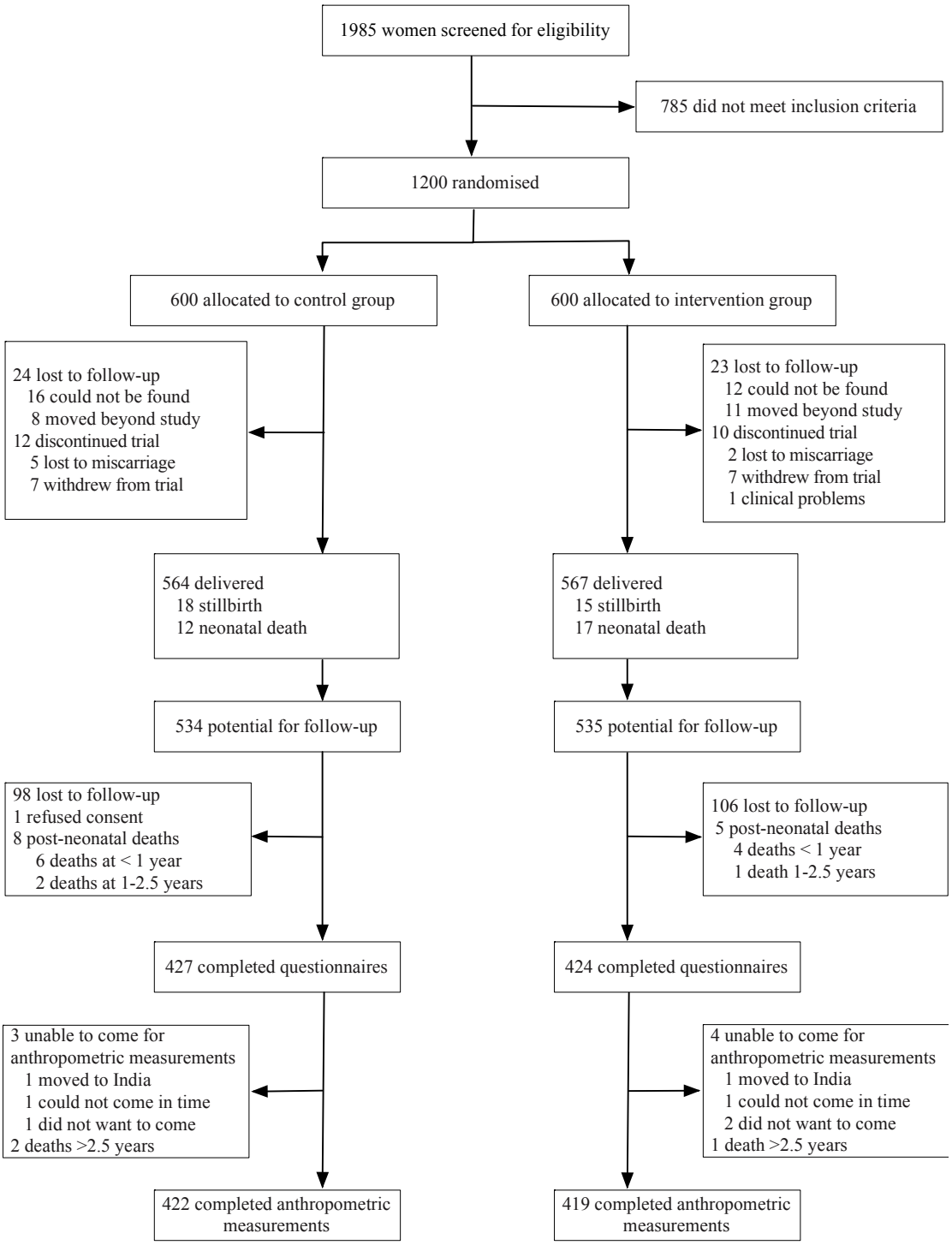

Figure 16: Trial profile

4.5.3 Measurement bias

In addition to measurement error, recall bias may have existed, particularly when asking questions about recent illness. It is possible that major illnesses were over-estimated and it was difficult to corroborate health information in this setting with medical records. It is expected that both the intervention and control groups would have a similar level of recall bias.

Measurement bias may also occur with spirometry. Spirometry is more difficult to standardise because it depends very much on the communication between the observer and the child and the understanding of the child. We followed approved guidelines for the acceptability and repeatability of curves. Assessments were limited to three people, two of whom conducted the majority. All three received the same training.

4.5.4 Air pollution sampling

Due to logistic difficulties we were unable to measure each child continuously for long periods. We therefore had to model the data based on a subsample. This led to a generalisation as we reduced a child's exposure to three measurements with the assumption that it was similar throughout life. This is inherently problematic, but a compromise is necessary as we were unable to do more sampling. This is discussed in more detail in section 5.1.

4.6 Assessment of normality

Histograms were created for the main outcomes variables (weight, height, blood pressure, FEV_1 and FVC) to check for normality (Figures 17-19), with normal curves superimposed. The variables were deemed adequate for use in further analyses. Normality is required when testing an hypothesis using independent t tests, although they are robust to a degree of departure.

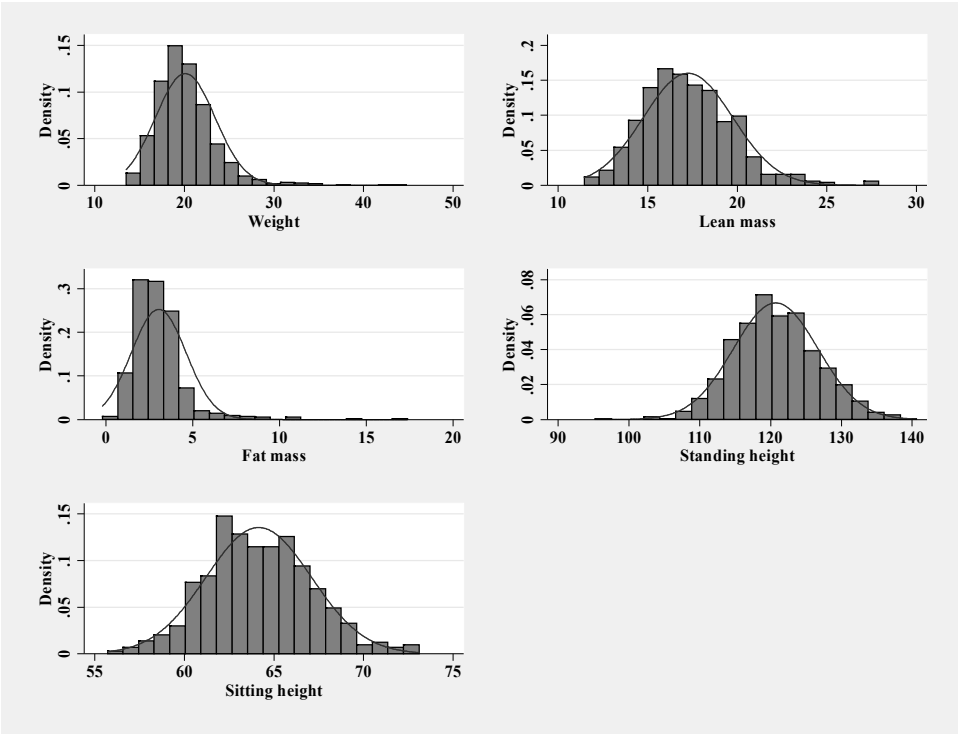

Figure 17: Histograms showing distributions of weight, fat mass, lean mass and height variables

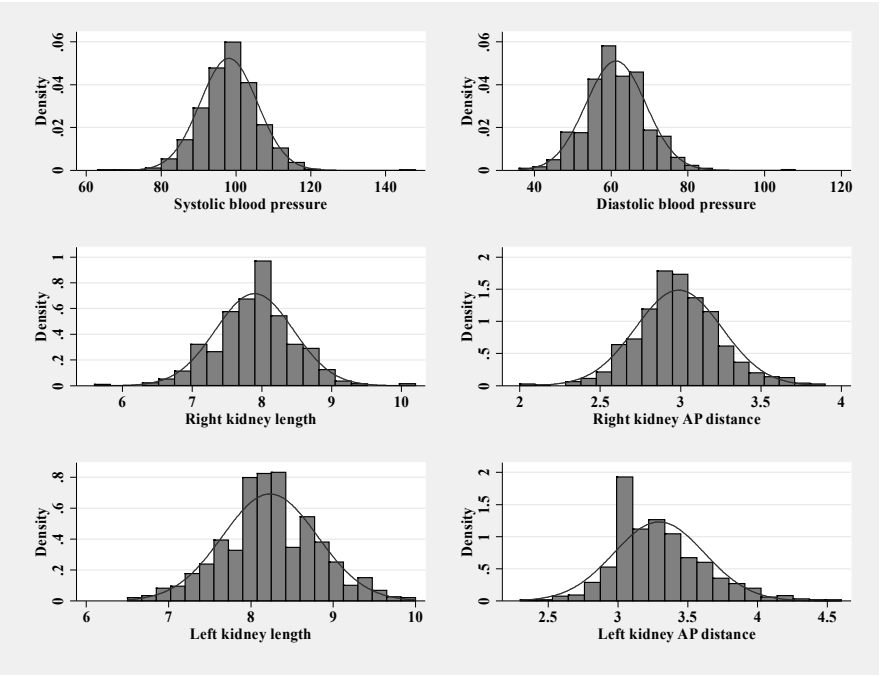

Figure 18: Histograms showing distributions of blood pressure and kidney dimensions

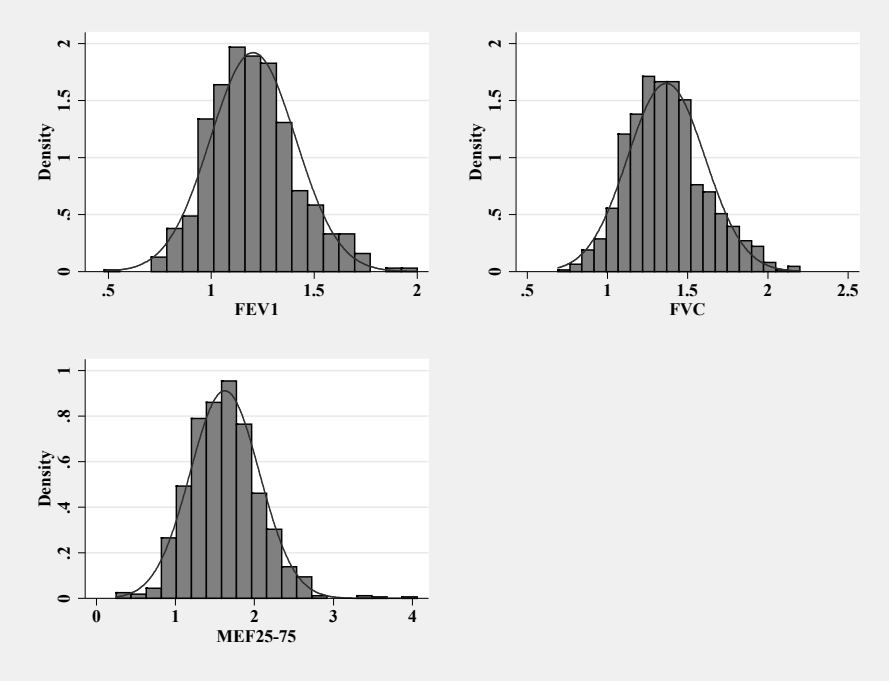

Figure 19: Histograms showing distributions of lung function

4.7 Global positioning system (GPS)

GPS waypoints were taken at the houses in which children lived, downloaded to MapSource software (Garmin ltd., USA) and maps were created using GPSVisualizer and Google Earth (Google Inc., USA). Examples of two of the maps are shown in Figure 20 and Figure 21 using Google's "terrain" and "hybrid" backgrounds, respectively.

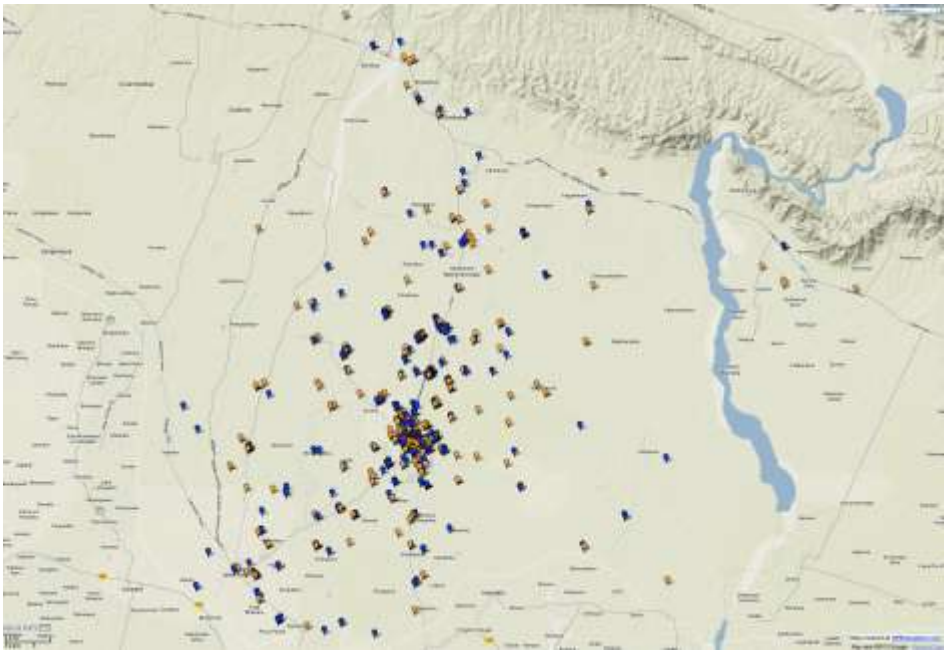

Figure 20: GPS map of Dhanusha district showing intervention (orange) and control group (blue) households

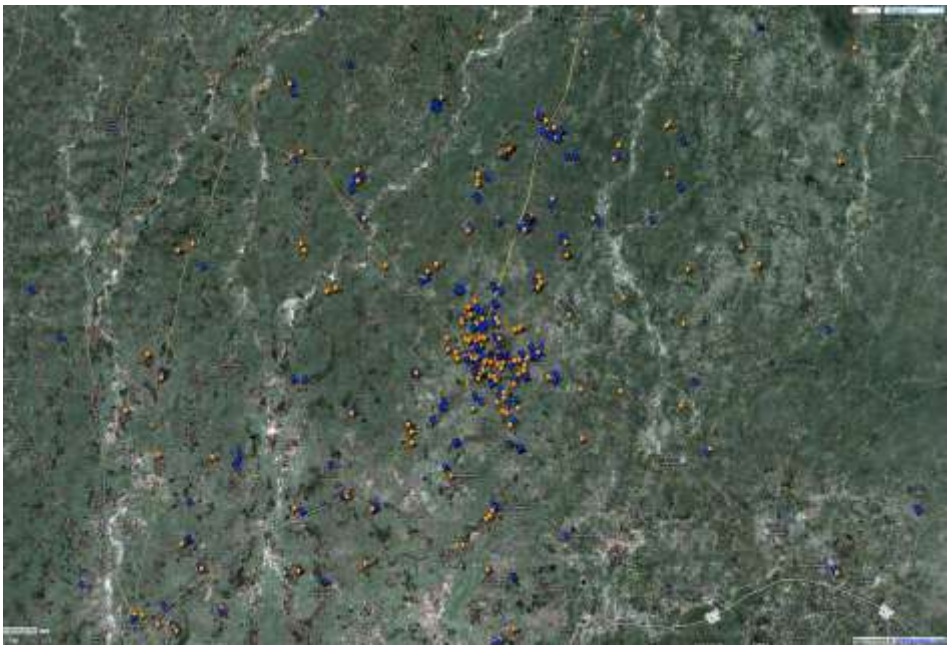

Figure 21: GPS map of Janakpur city and surroundings showing intervention (orange) and control group (blue) households

4.8 Isotope calibration study

The study set out to calibrate the Tanita BC-418 by measuring total body water using deuterium oxide, in a sample of children aged 7-9 in Nepal that was used to calculate body composition in a larger cohort. In addition, I investigated methodological variations, such as alteration of arm position to extend the range of the device and the effect of changes to the number needed for accurate prediction equations.

4.8.1 Analysis

4.8.1.1 Total body water estimation (TBW) and prediction equations

TBW is proportional to the impedance index (height^2/impedance). The derivation is shown in Equation H and Equation I. This assumes the body is a cylinder and that the conduction material is mostly water, so volume is equivalent to total body water:

Equation H

$$a = \frac{v}{l} = k \times \frac{l}{r}$$

therefore

$$v = k \times \frac{l^2}{r}$$

where, a = cross-sectional area; v = volume; l = the length or height of the cylinder; k = conductance per unit length; and r = resistance

TBW and lean mass (LM) were calculated with Equation I and Equation J respectively. Fat mass (FM) was calculated as the difference between LM and total mass.

Equation I

$$N = \frac{TA}{a} \times \frac{(Ed\text{-}Et)}{(Es\text{-}Ep)}$$

where N is the total body water, T is amount of tap water in which a is diluted =100 ml, A is the mass of drink given to the child, a is the portion of the dose diluted in T, and E values are isotope enrichments in delta (δ) units: Ed = dose, Ep = pre-dose, Es = post-dose, Et = tap water

Equation J

$$\text{lean mass} = \frac{\left(\frac{\left(\frac{N}{1.044}\right)}{1000}\right) - 0.23}{h}$$

where 1.044 is the correction factor to account for hydrogen ion exchange[h],[342-344] 1000 is needed to covert the figure into litres, 0.23 is the amount of fluid drunk by the children in litres during the equilibration period, *h* = age and sex-specific hydration factors. The choice of hydration factors is described in Box 6.

Based on the chemical analysis of cadavers, a hydration factor for LM of 0.773 is normally taken, [345,346] but age-specific hydration factors are needed in children. A normal healthy newborn baby, for example, has a much higher water content (80-83% of their LM) and this reduces to adult levels by 3 to 5 years.[347] Hydration of LM can also change with the level of obesity. [348] The age and sex specific hydration factors used are: 0.761 for boys and 0.753 for girls aged 6 to 7.99 and 0.758 for boys and 0.752 for girls aged 8 to 9.99. [349]

Box 6: Choice of hydration

Prediction equations

The calibration prediction equations for BIA were generated using stepwise multivariable linear regression models with lean mass (LM) as the dependent variable. While impedance index predicts LM, the coefficient of determination (R^2) can be improved by adding other variables. Predictor variables were added to the model with assessment of goodness of fit.

4.8.1.2 Comparison of our results with the Tanita system

Agreement of our results with the Tanita system's built-in equations was assessed using the Bland-Altman method.[328]

[h] The dilution volume is larger than the actual TBW volume obtained because the labelled hydrogen atoms exchange with hydrogen atoms associated with carboxyl, hydroxyl and amino groups.

4.8.1.3 Assessment of the effect of changing arm position

Separate equations were created for the 180° and 90° arm positions and we assessed the difference these two methods made when applied to the whole cohort and also when limited to children <16 kg, in whom the 90° arm position may be useful. This is needed because the Tanita BC-418 software allows a maximum impedance of 1200 Ohms. Lighter children may have impedance values above this. To see if specific calibration equations for each arm position needed to be developed, I compared the effect of using the specific calibration equations against using the same 180° equation for both arms.

4.8.1.4 Reduction of the sample.

We attempted to find children in all weight categories in our larger cohort in order to optimise regression lines across the range of body weights. Finding extra children adds to the time and cost of conducting an isotope calibration study, and it would be useful to know how much of a reduction in the sample would be tolerable in practice. To explore the difference this would make, I looked at the effect a reduction in numbers would make to our equations and LM outcomes. I did this in two ways:

i) Reduction of the weight range to 1.5, 1.25, 1 and 0.75 standard deviations from the mean weight.

ii) Reduction of data points at the tails. I first identified data >1SD from the mean and assigned a number to each data point, from 1 to 34. I then used a random number generator to choose half of the numbers, which were removed (www.randomizer.org). The process was repeated four times to produce four datasets with 87 data points each.

Prediction equations were then created for the new datasets, applied to our larger cohort of 628 children, and summarized with Bland Altman plots.[328]

4.8.2 Results

One hundred and nine children participated in the isotope calibration study. In two cases we were unable to complete the study: one child had learning difficulties and was unable to provide a saliva sample; the other was found to be 10 years old after entering the study. Of the remaining 107 samples, 102 were sent for mass spectrometry analysis (five were removed when we had other children in their weight categories). We were unable to get a BIA reading in the 180° arm position in two cases. Both these children were light and their whole body impedance with arms at 90° was close to the Tanita BC-418's maximum impedance. Table 16

shows characteristics of the sample. Boys were heavier than girls (difference in mean weight 2.2 kg; 95% CI 0.3, 4.2), but not different in height or BMI. The intra-observer TEM% was 0.05% for both observers and the inter-observer coefficient of reliability was 0.97 for height.

Table 16: Characteristics of the sample

	Boys	Girls
Number	50	52
Age (years)	8.7 (0.6)	8.6 (0.6)
Weight (kg)	23.3 (5.4)	21.0 (4.7)
Height (cm)	124.2 (9.4)	121.3 (8.9)
Trunk height (cm)	111.8 (4.4)	110.1 (4.4)
BMI (kg/m^2)	14.9 (1.9)	14.4 (2.1)
TBW (L)	13.9 (2.8)	12.5 (2.5)
Lean mass (kg)	18.4 (3.7)	16.7 (3.3)
Fat mass (kg)	4.9 (2.5)	4.3 (1.9)
Fat mass %	20.4 (6.5)	20.1 (6.4)
180° arms impedance (Ohms)	903.3 (96.5)	990.0 (100.8)
180° arms impedance index (cm^2/Ω)	17.5 (3.7)	15.3 (3.2)
90° arms impedance (Ohms)	844.1 (91.3)	934.7 (104.6)
90° arms impedance index (cm^2/Ω)	18.7 (3.9)	16.1 (3.6)

4.8.2.1 Prediction equations

The prediction equations generated for TBW and LM (with height in cm and impedance in Ohms) with a 180° arm position were:

Equation K

$$TBW\ (arms\ 180°) = 0.715 + 1.596\ ((height^2/impedance)$$

Root mean squared error = 0.78, $R^2 = 0.92$

Equation L

$$LM\ (arms\ 180°) = 2.202 + 0.941\ (height^2/impedance)$$

Root mean squared error = 1.05, $R^2 = 0.91$

I added sex (coded 0 for females, 1 for males) and weight (in kg) to produce the final prediction equation, Equation M. Previous research has shown these to be the best predictors to add to the model (height and BMI tend to be collinear with weight).[317] The R^2 and root mean squared error results are shown in Table 17. Adding in weight and sex increased R^2 from 0.91 to 0.93 and reduced the root mean squared error from 1.05 to 0.95. Root mean squared error is a composite of the residuals and is a measure of model fit. The closer to zero, the better.

Equation M

LM (arms 180°) = 1.946 + (0.681× height^2/impedance) + (0.211×weight) + (-0.363×sex)

Root mean squared error = 0.95, R^2 = 0.93

Prediction Equation M and Equation P are shown in Figure 22 as scatterplots of impedance index against LM for the 180° and 90° arm positions. The graphs show the line of best fit with 95% confidence intervals (showing where the true line would be 95% of the time) and 95% prediction lines (showing where future data points are likely to be 95% of the time.

Table 17: Alternative models to predict lean mass

	Arms 180°		Arms 90°	
Predictors	R^2	Root mean squared error	R^2	Root mean squared error
Impedance index	0.91	1.053	0.92	1.044
Impedance index, sex of child	0.92	1.025	0.92	1.014
Impedance index, weight	0.93	0.960	0.93	0.954
Impedance index, sex of child, weight	0.93	0.949	0.93	0.942

4.8.2.2 Comparison of our results with the Tanita system

Figure 23 shows the level of agreement for derived TBW and LM obtained using the equipment's built-in equations and deuterium dilution. The built-in equations resulted in a mean error of 385 g (SD 1018 g) in lean mass, corresponding to a 2.2% error. The greatest error was 4.54 kg (25.8%).

4.8.2.3 Assessment of the effect of changing arm position

The prediction equations generated for TBW and LM using the 90° arm position were:

Equation N

TBW (arms 90°) = 0.667 + 1.632 (height^2/impedance)

Root mean squared error = 0.78, R^2 = 0.92

Equation O

LM (arms 90°) = 2.254 + 0.877 (height^2/impedance)

Root mean squared error = 1.04, R^2 = 0.92

Equation P

LM (arms 90°) = 1.980 + (0.638×height^2/impedance) + (0.208×weight) + (-0.378×sex)

Root mean squared error = 0.94, R^2 = 0.93

The impedance values obtained with arms at 90° were a mean 63.9 Ω (95% CI 58.8, 68.9) lower for girls and 59.2 Ω (95% CI 54.0, 64.4) lower for boys than with arms at 180°. When using calibration Equation M and Equation P to calculate LM values in the larger cohort, the 180° and 90° arm position gave a mean LM of 17.32 kg (SD: 2.5 kg) and 17.25 kg (SD: 2.4 kg) respectively, with a difference of 70 g (95% CI 55, 86 g; p <0.00001). The largest difference was 321 g, equivalent to a 1.9% error in LM. When limiting this to children <16 kg (n=32), the mean difference was 19 g (95% CI -29, 67g; paired t test p 0.42).

If BIA is conducted with arms at 90°, but calibrated with the standard 180° equation, the resulting error in LM is 694 g (95% CI 676, 711 g), with the largest difference being 1.55 kg (8.9% error). This is illustrated in Figure 24, in which the first graph shows the same standard 180° calibration (Equation M) applied to both 180° and 90° data, producing an error that increases with mean LM value. When using a specific calibration, the error reduces and is consistent across LM values.

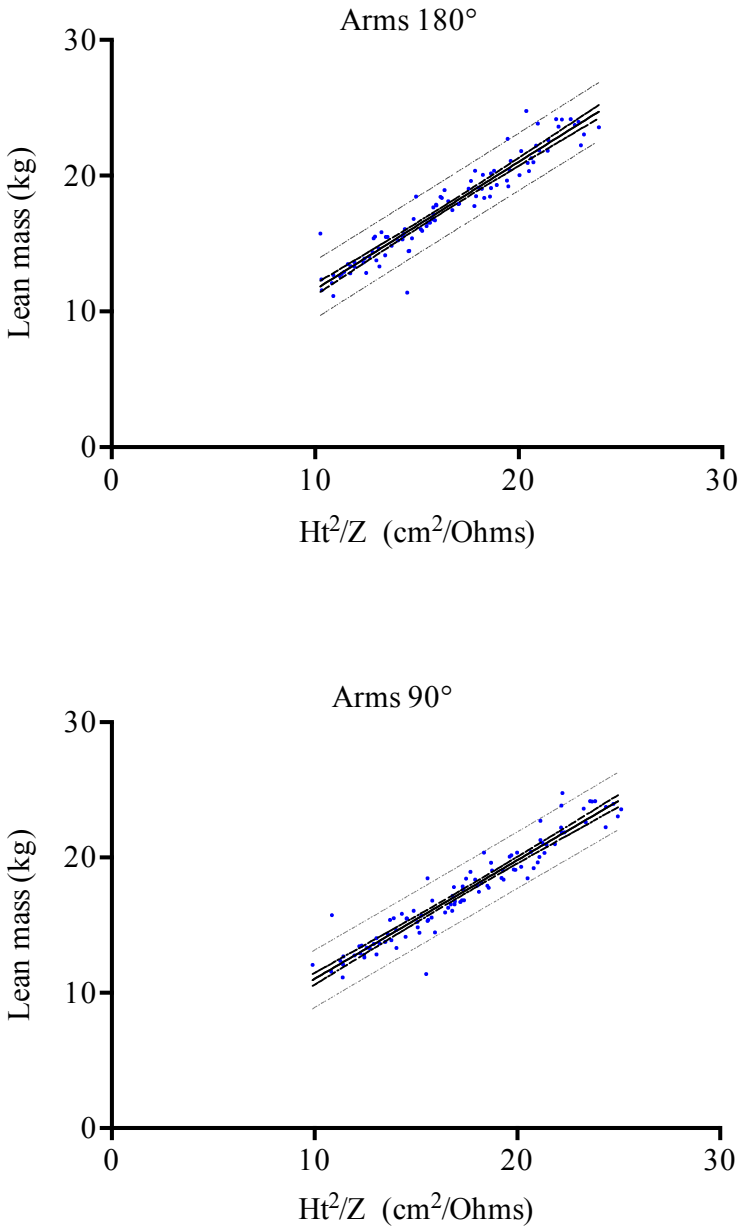

Figure 22: Scatter plots, with regression lines, 95% confidence intervals and 95% prediction lines for lean mass from isotope dilution and impedance index (height^2/impedance)

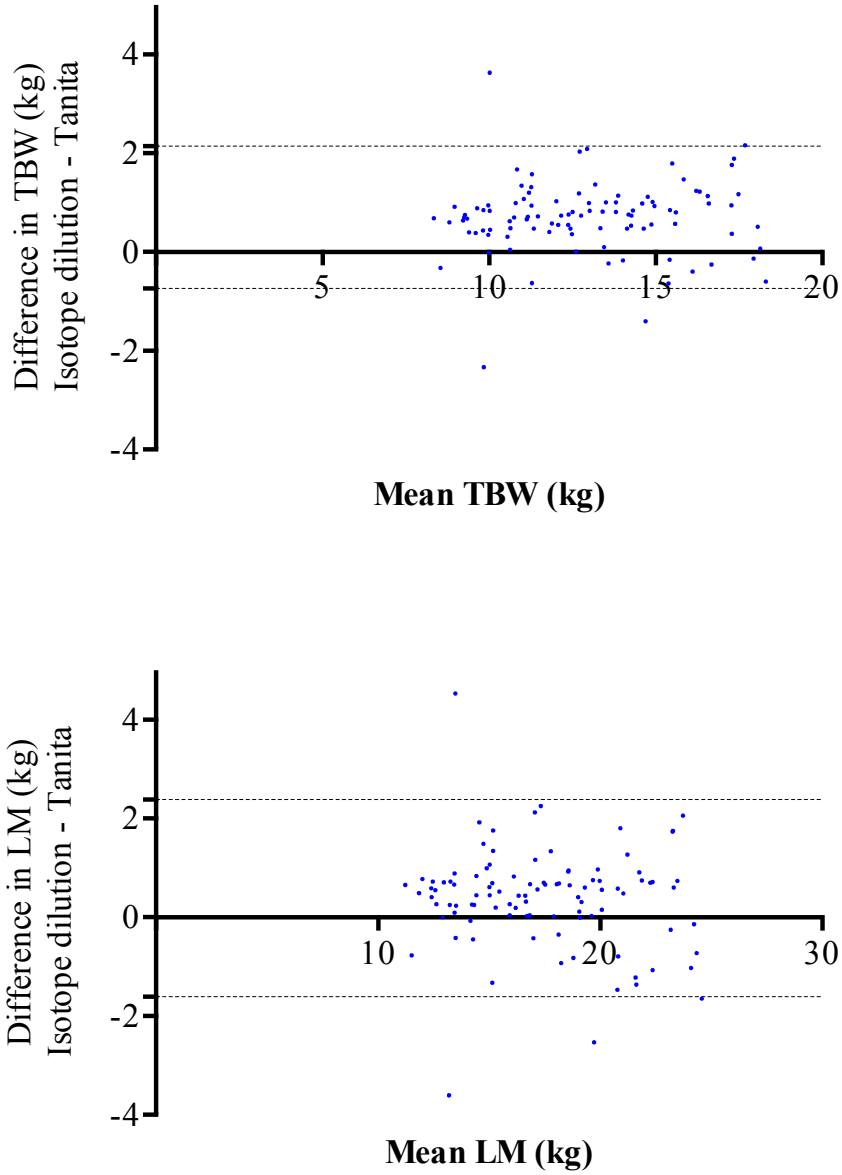

Figure 23: Bland-Altman plots showing level of agreement between LM and TBW for isotope dilution and the Tanita system methods

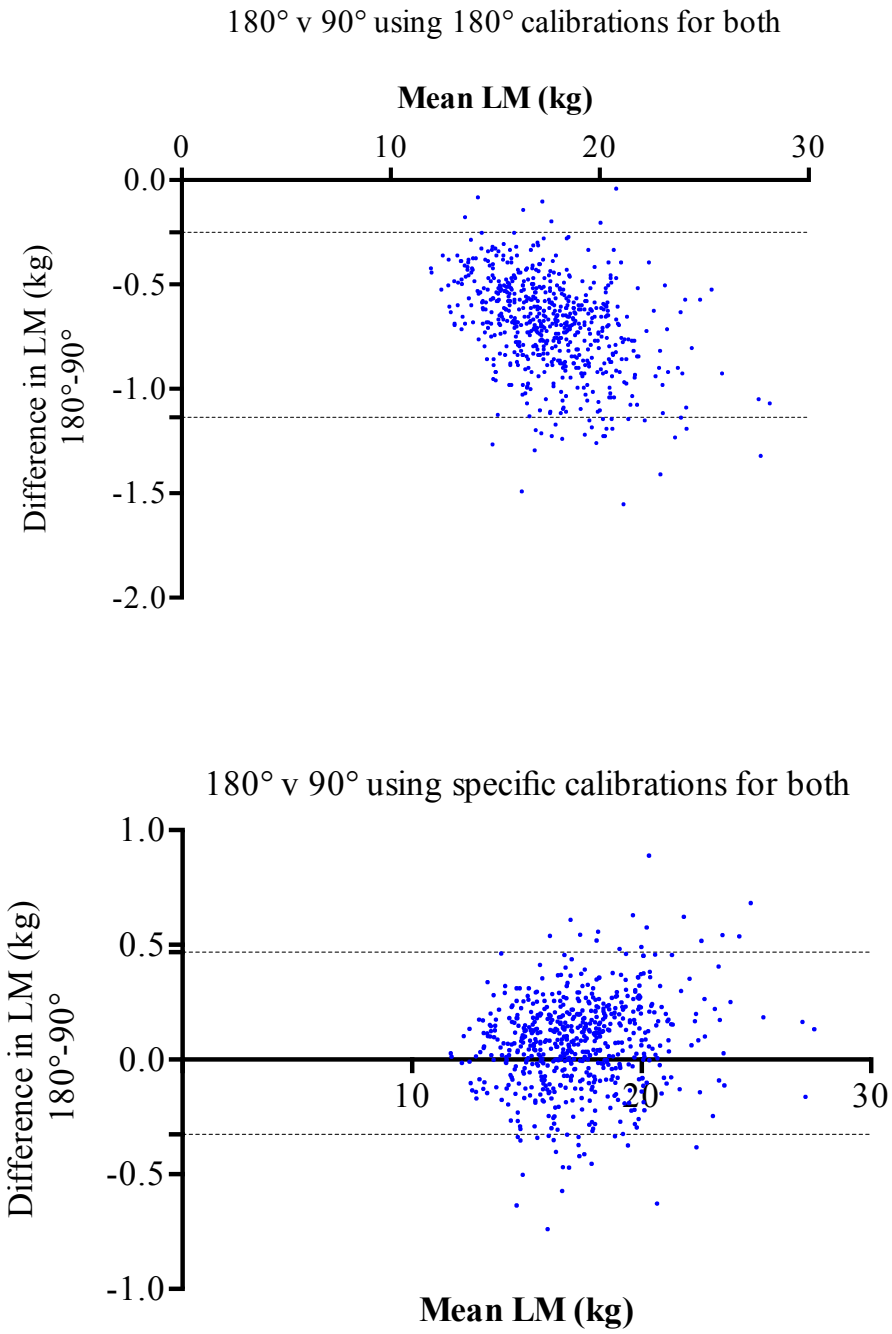

Figure 24: Comparison of lean mass results using standard (180°) calibration versus specific calibration equations

4.8.2.4 Reduction of the sample

Figure 25 shows the time required to find the target number of children. I looked at whether reducing the number of children included in the study made a difference to the prediction equations. In the first scenario the data range was limited, from all the data (n=100) to 0.75 SD from the mean (n=50), see Table 18. The prediction equations were similar until the data were limited to <1SD. The error in LM increased from 0.7% to 1.3% as the numbers fell. In the second scenario, the central data remained, but data at the tails were reduced (half the data points >1SD from the mean; see Table 19). This produced an error effect on LM of 0.1 to 0.7%. Bland-Altman plots, in Figure 26 and Figure 27, show the effect of reducing data at the tails of the distribution. In both scenarios the error increased at the tails of the distribution, particularly at greater weights.

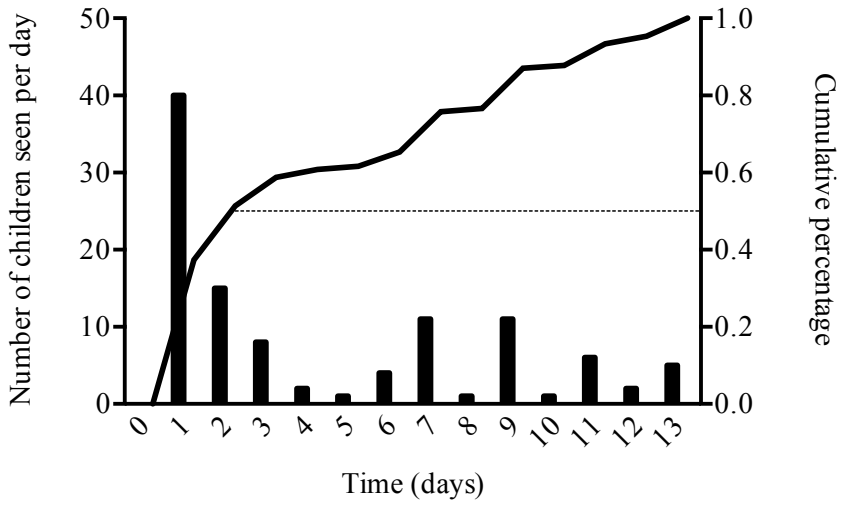

Figure 25: Time taken to complete the study

Table 18: Model fit and error in lean mass when collecting a smaller range of weights

	Model 1 All data	Model 2 <1.5 SD	Model 3 <1.25 SD	Model 4 <1SD	Model 5 <0.75 SD
Number	100	89	76	66	50
ht^2/Z	0.681	0.641	0.639	0.592	0.618
Weight	0.211	0.254	0.264	0.309	0.326
Sex	-0.363	-0.233	-0.311	-0.255	-0.297
Constant	1.946	1.550	1.455	1.221	0.375
R^2	0.93	0.94	0.92	0.89	0.84
Root MSE	0.95	0.79	0.78	0.81	0.87
Residuals	86.53	53.32	43.74	40.56	34.63
Mean (kg)	17.32	17.21	17.24	17.17	17.09
95% confidence intervals	17.13 to 17.51	17.01 to 17.41	17.04 to 17.44	16.97 to 17.37	16.88 to 17.30
Standard deviation	2.46	2.50	2.52	2.54	2.66
Difference (g) from Model 1		113	82	149	231
Percentage difference in mean		0.7	0.5	0.9	1.3

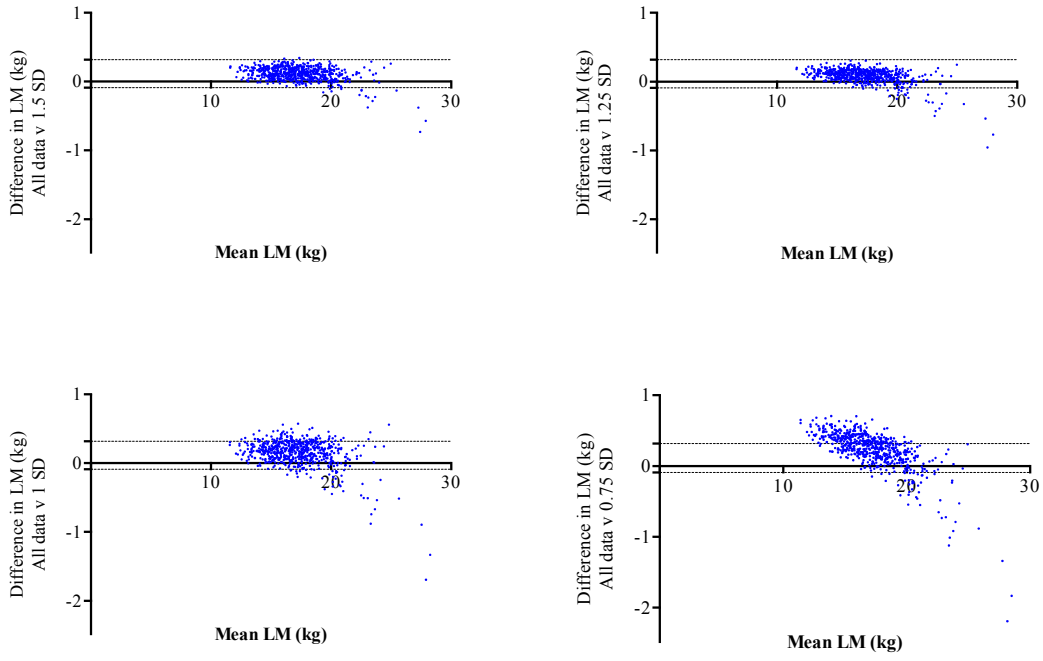

Figure 26: Graphs showing impedance index against lean mass when smaller ranges of weights were tested.

Table 19: Halving the number >1 standard deviation from the mean

	Model 1 All data	Model 2 half data >1SD	Model 3 half data >1SD	Model 4 half data >1SD	Model 5 half data >1SD
Number	100	83	83	83	83
ht^2/Z	0.681	0.636	0.731	0.656	0.681
Weight	0.211	0.250	0.150	0.220	0.229
Sex	-0.363	-0.196	-0.397	-0.503	-0.341
Constant	1.946	1.691	2.416	2.235	1.514
R^2	0.93	0.93	0.91	0.93	0.93
Root MSE	0.95	0.85	0.93	0.87	0.93
Residuals	86.53	57.48	68.53	59.33	58.38
Mean (kg)	17.32	17.21	17.39	17.33	17.28
95% confidence intervals	17.13 to 17.51	17.02 to 17.40	17.20 to 17.58	17.14 to 17.52	17.08 to 17.48
Standard deviation	2.46	2.48	2.41	2.41	2.52
Difference (g) from Model 1		112	66	10	40
Percentage difference in mean		0.7	0.4	0.1	0.2

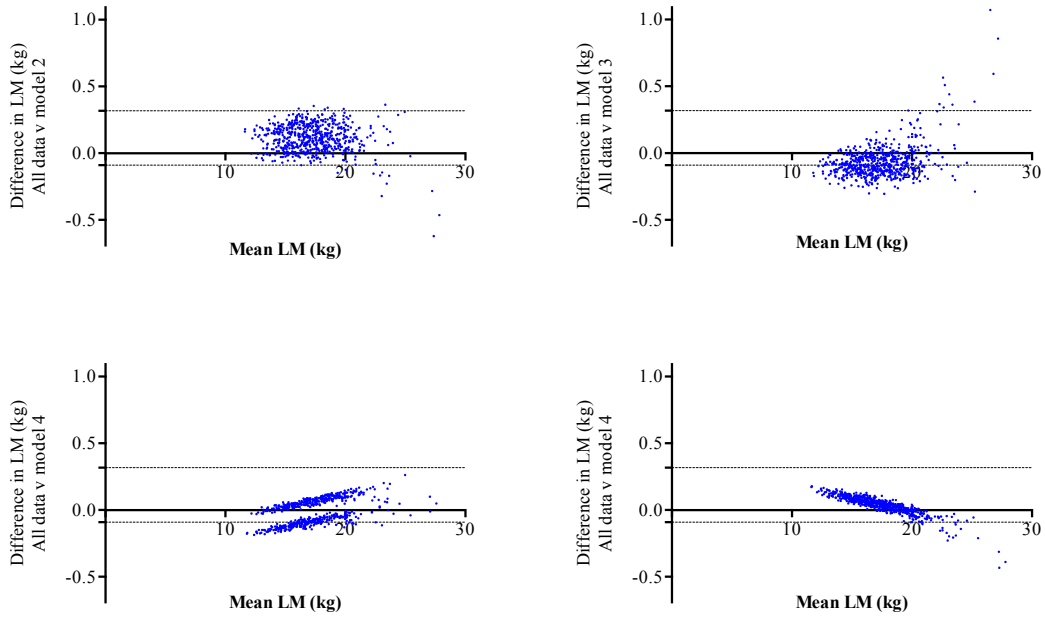

Figure 27: Graphs showing impedance index against lean mass when smaller ranges of weights were tested.

4.8.3 Discussion

The use of BIA is becoming increasingly popular, but body composition estimation is still under-researched in South Asia.[317] I believe this study is the first to produce prediction equations for BIA for a Nepalese population. It is advantageous to be able to separate a person's body weight into fat and lean mass with a simple test as knowledge of body composition is important, both as a marker of health in children [135] and a predictor of chronic disease later in life. [130] An extension of BIA, bioelectrical impedance spectroscopy can also be used to estimate the intra- and extra-cellular compartments using tracers such as bromide and potassium isotopes.[350] Using this method, BIA can estimate hydration in clinical settings, for example in dengue fever. [351,352]

In children, obesity is generally defined using BMI-for-age or weight-for-age, ideally using international growth charts. BMI is not a good predictor of adiposity in that it includes both FM and LM. Definitive confirmation of body composition is not possible without cadaveric examination, but it can be estimated via a number of methods. The more techniques that are used, the more components the body can be divided into. The three-component model also estimates fat-free dry tissue, while the four-component model divides this into protein and mineral. This would be the gold standard method for estimating body composition.

It is rarely possible to use the most accurate methods, such as those using water or air displacement, for large-scale epidemiological studies as they are impractical or expensive. There are a number of alternatives to BIA, such as dual energy X-ray absorptiometry, densitometry and MRI, but few are as easy to use. Even skinfold thickness relies on a level of expertise to be able to get accurate results. Other simple methods such as waist circumference or waist:hip ratio are also advocated by some as predictors of disease.[130,136,353]

4.8.3.1 Bioelectrical impedance

BIA is a relatively simple, inexpensive and accurate method to assess body composition in healthy subjects and to assess fluid distribution and changes in both healthy subjects and those with disease. [308] Isotope calibration studies of BIA in children in different settings have produced different prediction equations. [316-319] The most likely reason for this is a difference in the phenotypes of different groups. Liu et at investigated the relationship between BIA and TBW in pre-pubertal children aged 8-10 years in five Asian countries. They noted the variability between the populations and recommended that ethnicity be included in all predictive equations. [354,355] Deurenberg et al also showed that body composition in the three main ethnic groups in Singapore- Chinese, Malays and Indians- varied by group. The

variation depended mostly on the distribution of body water and arm length relative to height.[356]

Body composition also varies by age[357] and the relative proportions of intracellular and extracellular water, as current does not completely penetrate the cell membrane. Extracellular fluid normally accounts for around 25% of body weight in the adult man and has a lower specific resistivity than intracellular water.[356,358] This is corrected for by using age-specific hydration factors.

The built-in equations of the Tanita BC-418 instrument were designed for Western European or North American phenotypes. This study has shown that they are inappropriate for children in Nepal, producing an error of approximately 0.3 kg, but up to 4.5 kg, with the instrument tending to underestimate LM and potentially overestimate FM. The Tanita system's equations are copyrighted and are not freely available for comparison.

4.8.3.2 Methodological changes

If care is taken in the selection of participants and in carrying out the procedures, a prediction equation with a low standard error can be produced, giving greater confidence in body composition estimates. We made some improvements to the standard method to improve feasibility and accuracy. First, we removed weight error due to clothing by standardising it. Second, we recorded and standardised the intake of fluids throughout the study. Third, we centrifuged saliva samples immediately so that we could get a further sample if necessary.

I then examined the difference that arm position makes to lean mass estimates. The theory of segmental analysis divides the body into cylinders: the arms and legs, which are long and thin, with relatively high resistance, and the shorter, thicker trunk. Despite their low mass, the arms make up approximately 45% of whole body resistance.[310] Even small changes to arm position can introduce bias. Achieving a 90° angle accurately is difficult and we found that even small changes to wrist position affected overall impedance values by about 15 Ohms. To enable us to get BIA results from smaller children, we adapted a standard technique of flexing the arms to 90°. This reduced the impedance by 50-60 Ohms and extended the range of the Tanita BC-418 by approximately 5%, an important difference when dealing with undernourished or young children. The different arm position did result in a small difference in lean mass overall, but when limiting this to children who were less than 16 kg, in whom the technique would be most useful, the methods appear to be similar. Specific isotope calibration is required for each arm position.

To improve the feasibility of carrying out isotope calibration, I looked at the effects of a reduction in the number of participants needed. If time and resources permit, sampling across the weight range creates greater certainty in the regression line, highlighted by the narrow confidence intervals in our graphs. If we had sampled according to our population, in a normal distribution, there would have been few smaller and larger children, making the regression line less stable as the few data points at the extremities would have greater influence. I showed that a reduction in numbers did result in greater error. The difference in mean values was small, but the error became large at the extremities of the distribution. Limiting the number of individuals at the extremes of the data range could dramatically reduce time and resources needed to conduct the study, but - as shown in the hypothetical scenarios - does result in substantial error for heavier and lighter children.

In summary, the isotope calibration study produced a population-specific equation for the children in the larger cohort. The importance of taking care of the position of the arms was highlighted, and if conducting the study with a different arm position, a specific calibration of this is required. A smaller sample reduces resource requirements substantially, but results in large errors at the tails of the weight distribution.

Chapter 5 Confounders

Chapter 5 describes the analysis and results for the three main confounders considered in the study: air pollution, socioeconomic status and food security. The relationship of these variables with the outcomes is described in Chapter 6. The air pollution section describes the analysis of the gravimetric and photometric data and the creation of a 24-hour time-weighted average for each child. I have estimated socioeconomic status in two ways: the Multi-dimensional Poverty Index (MPI) and an asset score based on a Principal Components Analysis (PCA) approach. The MPI was chosen to summarize the data and to be able to compare with other regions or countries. The asset score was used to compare within the group, and in further analyses such as in section 6.6. The food security section describes use of the Household Food Insecurity Access Scale, Household Dietary Diversity Score and Months of Adequate Household Food Provisioning tools with the creation of food security and dietary diversity scores.

5.1 Air pollution

We sampled the respirable fraction of particle mass (PM_4) for children aged 7 to 9 years in the microenvironments in which they spent time. Using the children's time-activity patterns from the questionnaire data, I produced a 24-hour time-weighted average (TWA) exposure. The results have been published in Environment International. [359]

5.1.1 Analysis

Concentration was calculated using Equation Q. After reweighing the filters, the average pre-weight was subtracted from the average post-weight. This was then adjusted for the change in mass of the batch of field blanks. The sampling volume was calculated from the duration of sampling and the average of the start and end flow rates recorded.

Equation Q

$$\text{Concentration} = \frac{(\textit{Average post weight - Average pre weight}) \pm \Delta\, \textit{field blank}}{\textit{Volume}}$$

The sampling schedule was based on the first 100 houses. There were no houses with metal roofs in this group, so these were not tested. Homes with metal roofs were given the average value for urban or rural houses. The lower limit of detection (LOD) was calculated using 3 times the standard deviation of the weight change recorded in the field blanks (see Table 1, Appendix 4.2). Filters showing weight change below the limit of detection were assigned a value of one-half the LOD. Arithmetic and geometric means were calculated for each location in each season. These were then combined to produce an average for the year, by calculating a standardized mean, applying a weighting to each sample of 1/(n×3), where n = number of samples in that location in a season. The time-weighted average (TWA) was calculated using Equation R. The arithmetic mean concentration for each location was multiplied by the amount of time the child spent in it to produce the average exposure concentration over a 24 hour period.

Equation R

$$\text{24-hour TWA} = \frac{\sum bt_1 + vt_2 + ot_3 + st_4 + ct_5 + kt_6}{24}$$

where:

TWA = time weighted average; b = bedroom arithmetic mean; v = verandah arithmetic mean; o = outdoor arithmetic mean; s = school arithmetic mean; c = kitchen during cooking,

arithmetic mean; k = kitchen outside cooking hours, arithmetic mean; t = time spent in each location

The average concentration was given on the DustTrak readout. A correction factor was required as the DustTrak is calibrated to "Arizona road dust", and would differ between aerosols and other factors such as relative humidity. The values recorded were converted to $\mu g/m^3$ and a correction factor was applied, obtained from DustTrak-Apex side-by-side calibration: 0.43 for rural and 0.51 for urban samples (see Table 2, Appendix 4.2).

5.1.2 Results

5.1.2.1 Exposure data

In total, 291 microenvironment measurements were collected from 55 households (6.6% of the cohort), 8 representative outdoor locations, and 8 schools. Fifty-eight were discarded: 14 repeated weighing produced differences greater than 0.1 mg, 13 had damage to the filter, in 15 cases there was equipment failure or inaccurate technique and in 16 cases there were mistakes in timing, labeling or location. Fifty-nine samples were below the limit of detection. In total, 110 days worth of air pollution data were gathered from across the various microenvironments, with a cumulative sampling duration of 2649 hours. Table 20 shows the average duration and total time at each location.

Table 21 shows the concentration level by location in each season, and Table 22 average concentrations at each location over the year. In the bedroom, school and outdoors, concentration levels were highest in winter, followed by spring/autumn, and lowest in the monsoon season. The kitchen samples were lower in spring/autumn, possibly because more cooking is done outdoors and ventilation improves. In cold periods fires are useful for heat. Verandah sample concentrations were lowest in the monsoon season. The microenvironment measurements showed seasonal variation, with winter generally having higher concentration levels than spring/autumn, which were higher in turn than the monsoon season.

Seven DustTrak samples were taken in kitchens to look at variation in respirable fraction concentration over time. The two non-biomass samples had adjusted average (peak) concentrations of 53 $\mu g/m^3$ (425 $\mu g/m^3$) and 166 $\mu g/m^3$ (1080 $\mu g/m^3$). The biomass samples tended to have both average and peak concentrations approximately ten times higher. Both the dung and wood samples produced similar concentrations. The highest peak concentration was just below 60 000 $\mu g/m^3$. Both dung samples produced similar concentrations, but the wood samples varied from an average of 167 $\mu g/m^3$ to 831 $\mu g/m^3$. Depending on kitchen structure, levels could rapidly come down to the non-cooking level, even in biomass-using

houses. A summary of the DustTrak data is shown in Table 3, Appendix 4.2, and time-concentration graphs from three kitchens are shown in Figure 28.

Table 23 shows the average exposure levels for children at each location, calculated by extrapolating the particulates in the samples from 55 representative households to the overall sample. The 24-hour TWA was modeled for 429 boys and 405 girls. The mean 24-hour TWA was 168 $\mu g/m^3$ (SD= 25.9), with no difference in exposure level between the sexes. The distribution of 24-hour TWA calculated for all children is shown in Figure 29 and the average contribution of each location to the total concentration in Table 23. The bedroom/living room made the largest contribution because of the large amount of time spent there. The verandah, bedroom and outdoors all made similar contributions. The kitchen contribution was low, due to the short amount of time, although a small increase in time spent in the kitchen made a big difference to a child's exposure.

5.1.2.2 Time activity

Questionnaire data were collected on 851 children out of a possible 1053. Three children had died and the data for 14 children were removed because they lived outside the region where we did not have air pollution measurements. Time activity data are summarized in Table 23. Children spent most of their time indoors, amounting to about half the day on average. The amount of time at each location was similar for boys and girls. Two per cent of children did not go to school.

5.1.2.3 Fuel use

Households used a variety of cooking fuels (Table 24). A different fuel was often used at the start of cooking or to light the fire. For example, straw and kerosene were commonly used at the start of cooking, but not as the main cooking fuel. About one-third of households used wood, one-third animal dung, and one-third gas, as their main fuels. It was common for families to alternate between fuels, usually wood and dung. Animal dung cakes normally contained a small amount of wood or plant products to give them structure. The use of gas as a main fuel was more common in urban than rural settings (60.8% compared with 15.8%). The opposite was true for wood (27.2% compared with 41.7%) and dung (9.4% and 36.2%). Stoves were usually simple open fire designs of mud and clay with no chimney or hood. Cooking was done mainly indoors in the kitchen, and 786 (94%) households cooked indoors every day of the year.

5.1.2.4 House characteristics

Forty-one percent of the sample lived in urban households. Houses had an average 3.6 rooms (range 1-15). Ninety-four households (11%) did not have a separate kitchen and 81 (10%) had only one room. Most house walls were made of cement and brick (63%), with the remainder mostly mud and wattle (branches or sticks woven into the wall to add structure) (34%). Roofs were made from cement (43%), tiles (54%), grass or straw (2%), or metal sheet (1%). Half of floors were cement or brick and the other half dirt.

Table 20: Sampling times and duration

Location	Number of samples	Average start time (range)	Average end time (range)	Average duration (minutes)	Total sampling time (hours)
Bedroom	96	15:49 (14:30 to 17:38)	09:35 (07:52 to 15:25)	1064 (854 to 1397)	1720
Verandah	31	16:53 (15:43 to 17:05)	19:53 (18:42 to 20:05)	180 (179 to 180)	96
Kitchen cooking	31	07:48 (07:00 to 08:50)	10:38 (09:20 to 11:59)	163 (60 to 202)	85
Kitchen no cooking	29	11:43 (09:41 to 15:04)	14:18 (13:17 to 18:04)	184 (180 to 229)	92
Kitchen 12 hour samples	7	07:24 (07:00 to 08:00)	19:24 (19:00 to 20:00)	720 (720 to 720)	96
School	22	09:26 (06:55 to 11:57)	13:55 (11:15 to 16:30)	275 (120 to 330)	101
Outdoors	38	07:11 (05:07 to 10:30)	19:02 (17:07 to 20:24)	725 (678 to 796)	459

Table 21: Respirable particle mass concentration by season in different locations

Location		Winter			Spring & Autumn			Monsoon		
		Number of samples	Arithmetic mean concentration (SD) ($\mu g/m^3$)	Geometric mean concentration (SD) ($\mu g/m^3$)	Number of samples	Arithmetic mean concentration (SD) ($\mu g/m^3$)	Geometric mean concentration (SD) ($\mu g/m^3$)	Number of samples	Arithmetic mean concentration (SD) ($\mu g/m^3$)	Geometric mean concentration (SD) ($\mu g/m^3$)
Bedroom	Urban cement roof	16	342 (185)	303 (1.64)	10	113 (96.9)	79.6 (2.60)	4	60.3 (35.4)	49.7 (2.24)
	Urban tiled roof	4	384 (234)	311 (2.33)	4	112 (50.8)	102 (1.73)	4	204 (182)	123 (4.05)
	Urban straw roof	1	256	256	2	62.9 (67.4)	41.1 (4.06)	1	38.5	38.5
	Rural cement roof	8	322 (109)	307 (1.38)	7	153 (95.3)	115 (2.69)	4	115 (68.5)	85.1 (2.97)
	Rural tiled roof	16	398 (216)	352 (1.65)	4	173 (201)	111 (2.78)	2	66.4 (71.3)	43.1 (4.09)
	Rural straw roof	2	217 (1.80)	217 (1.01)	4	374 (304)	285 (2.39)	3	160 (249)	48.8 (6.83)
Verandah	Urban	8	410 (221)	347 (1.98)	2	850 (460)	786 (1.77)	5	58.0 (28.2)	50.3 (1.95)
	Rural	8	771 (1060)	455 (2.72)	2	469 176	452 (1.47)	6	95.0 (63.2)	78.5 (1.99)
School	Urban	5	167 (86.8)	146 (1.85)	4	115 (74.3)	98.4 (1.89)	5	82.8 (45.1)	75.8 (1.54)
	Rural	2	161 (143)	126 (2.83)	4	63.1 (8.93)	62.6 (1.16)	2	71.8 (3.30)	71.8 (1.05)

Outdoor	Urban	7	305 (216)	259 (1.79)	7	105 (67.1)	85.0 (2.15)	5	24.3 (0.32)	24.4 (1.01)
	Rural	5	369 (207)	319 (1.90)	8	112 (66.5)	99.6 (1.65)	6	67.6 (27.6)	61.6 (1.66)
Kitchen cooking	Biomass	7	1550 (1050)	1311 (1.84)	5	433 (368)	318 (2.46)	9	933 (499)	835 (1.63)
	Non-biomass	2	204 (170)	165 (2.60)	5	97.8 (2.33)	97.8 (1.02)	3	177 (147)	143 (2.15)
	Dung	4	1410 (757)	1270 (1.70)	2	557 (649)	315 (5.23)	4	1140 (686)	982 (1.89)
	Wood	2	2270 (1740)	1910 (2.36)	2	244 (47.7)	242 (1.22)	4	775 (304)	730 (1.49)
Kitchen no cooking	Biomass	10	250 (128)	216 (1.85)	4	707 (1220)	216 (5.17)	7	96.0 (2.63)	95.9 (1.03)
	Non-biomass	4	123 (45.7)	117 (1.45)	4	303 (416)	168 (3.12)			
	Dung	4	260 (68.1)	251 (1.36)	3	95.2 (2.92)	95.2 (1.03)	2	92.9 (3.44)	92.8 (1.04)
	Wood	6	234 (168)	183 (2.30)	1	2550	2550	4	97.1 (1.05)	97.1 (1.01)
Total		106			76			66		

NB: Biomass samples are shown together and separated into those that used wood and dung only (the others used a mixture of the two)

SD = standard deviation.

Table 22: Weighted average respirable particle mass over the year

Location		Number of samples	Standardized mean (µg/m^3) (95% CI)*
Bedroom	Urban cement roof	30	116 (88, 144)
	Urban tiled roof	12	233 (133, 333)
	Urban straw roof	4	134 (109, 159)
	Rural cement roof	19	175 (134, 217)
	Rural tiled roof	22	125 (51, 199)
	Rural straw roof	9	236 (120, 351)
	Combined roof urban	47	166 (117, 215)
	Combined roof rural	50	192 (135, 249)
Verandah	Urban	15	592 (281, 902)
	Rural	16	445 (264, 627)
School	Urban	14	121 (82, 160)
	Rural	8	123 (38, 209)
Outdoors	Urban	19	131 (81, 181)
	Rural	19	202 (128, 276)
Kitchen cooking	Biomass	21	908 (614, 1203)
	Non-biomass	10	175 (63, 286)
Kitchen no cooking	Biomass	21	438 (-138, 1010)
	Non-biomass	8	213 (-16, 442)

*Calculated by applying a weighting to each sample of 1/(n×3), where n = number of samples in that location in a season.

Table 23: Time activity and exposure level to respirable particulates by location, for girls and boys

Location		Time activity				Exposure*				
		Mean (hours)	Standard deviation	Minimum (hours)	Maximum (hours)	Mean ($\mu g/m^3$)	Standard deviation	Minimum ($\mu g/m^3$)	Maximum ($\mu g/m^3$)	Proportion of total contribution (%)
Bedroom/living room	Boys	12.2	1.65	7.5	17	1750	530	936	3500	44
	Girls	12.3	1.77	8	18	1800	506	998	3730	44
Verandah	Boys	1.24	1.10	0	5.5	600	540	0	3250	15
	Girls	1.33	1.23	0	5.5	640	603	0	3250	16
Kitchen during cooking	Boys	0.04	0.23	0	2	20.2	147	0	1820	1
	Girls	0.03	0.19	0	2	19.7	136	0	1820	1
Kitchen when there is no cooking	Boys	0.21	0.53	0	4	66.7	180	0	1310	2
	Girls	0.21	0.49	0	3	71.0	170	0	875	2
Outdoors	Boys	5.07	1.64	1	12.5	915	369	196	2420	23
	Girls	4.83	1.56	0	12	884	355	196	2420	22
School	Boys	5.29	1.01	0	10	647	123	0	1210	16
	Girls	5.26	1.04	0	11	644	128	0	1330	16
Total concentration in 24 hours	Boys					4000	607	2850	6120	
	Girls					4060	636	2830	6440	
24 hour time-weighted average	Boys					167	25.3	119	255	
	Girls					169	26.5	118	268	

* Exposure levels were calculated by multiplying average concentrations in each location by the time each child was in it.

Table 24: Fuel usage. The number of households using a fuel to light the fire and during cooking

Fuel type	Fuel used to light the fire*	Fuel used continuously*	Main fuel used by a household (%)
Wood	7	606	309 (37.1)
Dung	0	392	231 (27.7)
Straw	138	10	6 (0.7)
Charcoal	2	3	4 (0.5)
Other plant products	14	34	21 (2.5)
Kerosene	230	6	3 (0.4)
Gas	241	324	251 (30.0)
Biogas	7	8	8 (1.0)
Electricity	3	4	1 (0.1)
Plastic	10	0	0
Paper	4	NA	NA
Matches	9	NA	NA

* Multiple fuels may be used by the household. If more than one fuel was used, the main fuel used for cooking was also recorded.

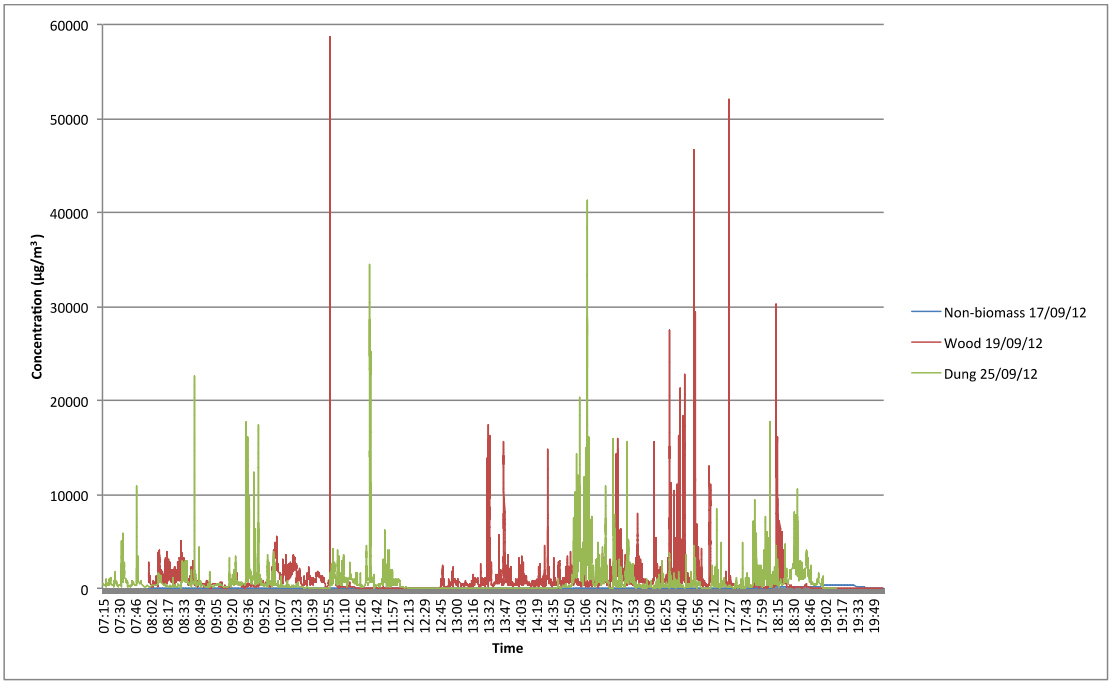

Figure 28: 12-hour kitchen samples taken using the DustTrak II.

The graph shows the concentration levels in a kitchen using non-biomass fuel, wood or dung as the main cooking fuel.

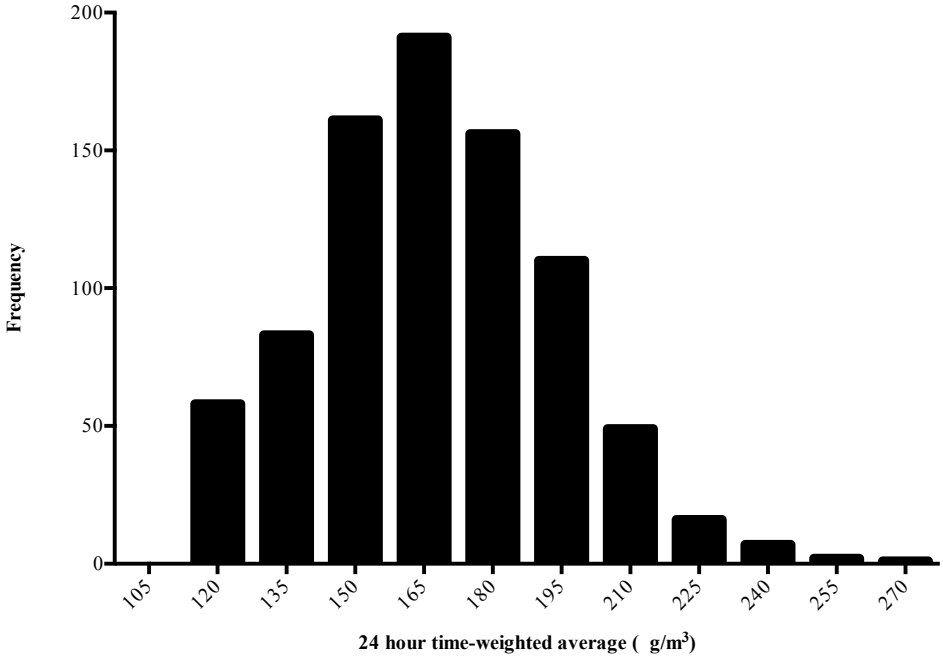

Figure 29: 24-hour time-weighted average histogram, for all children

5.1.3 Discussion

5.1.3.1 Exposure data

This study is one of few to have examined children's exposure levels with measurements of air pollution concentrations in low resource rural settings, and adds valuable information on their time activity. Most estimates of household air pollution come from binary or categorical data on exposure to biomass fuels or the type of fuel used. This type of data is limited in its ability to differentiate exposure among members of the family. We found high exposure levels with substantial variability from season to season and by fuel type. The mean 24-hour TWA of 168 $\mu g/m^3$, and mean exposure levels in all locations, were very high compared with the World Health Organisation recommendation of a maximum 24-hour mean of 25 $\mu g/m^3$ for $PM_{2.5}$ [360] and the United States Environmental Protection Agency 'National Ambient Air Quality Standards' recommendation of 35 $\mu g/m^3$. [361] The levels were also much higher than the national ambient air quality standards for Nepal recommendation of 120 $\mu g/m^3$ PM_{10}. [362]

While exposure levels were high, as shown by other research using comparable methods of exposure estimation, they were not unusual. Balakrishnan et al found higher 24-hour TWA mean respirable fraction levels of 227 $\mu g/m^3$ for boys and 237 $\mu g/m^3$ for girls aged 6 to 15 in solid fuel using households in southern India. [363] In studies of other particle fractions, Dasgupta et al found mean levels of 156-196 $\mu g/m^3$ of PM_{10} in children in Bangladesh [364] and Dionisio et al found levels of 131-157 $\mu g/m^3$ of $PM_{2.5}$ exposure in children from the Gambia. [365] Baumgartner et al found mean levels of 46-70 $\mu g/m^3$ of $PM_{2.5}$ in China, in which children carried the monitor with them where possible. [366] These samples were taken only in the summer- in adults exposure was approximately double in the winter. If this were to be maintained for children, these results would be comparable to ours. A similar study of microenvironment exposure of total suspended particles in the Himalaya region of northern India found similar levels. Saksena et al showed a geometric mean level of 4500 $\mu g/m^3$ in the kitchen during cooking, 640 $\mu g/m^3$ in the kitchen during non-cooking, 470 $\mu g/m^3$ in the living room and 190 $\mu g/m^3$ outdoors during non-cooking times. [367]

There is limited information on indoor air pollution levels in Nepal. Kurmi et al found much higher 24-hour $PM_{2.5}$ concentrations of 455 $\mu g/m^3$ (95% CI 426, 485 $\mu g/m^3$) in biomass-using homes, and 101 $\mu g/m^3$ (95% CI 96,106 $\mu g/m^3$) in gas-using homes in Nepal. [52] The study was carried out in the Hill region where the climate is colder and ventilation is minimal - our study area had high temperatures in summer and well ventilated rooms - and households used hard wood rather than the mixed biomass in our sample. [368] Our DustTrak:Apex calibration factors (Table 2, Appendix 4.2) were similar to those produced by Kurmi et al. [368]

Kurmi et al showed an approximately ten-fold higher concentration in rural households, attributed to biomass fuel burning, whereas in our study the levels were similar. [368] These differences could be due to housing construction materials used in different climatic conditions and to the types of fuel used. In a study from Malawi, Fullerton et al also showed little difference between urban and rural samples (a significant difference was, however, seen when looking at the total inhalable dust levels). [369]

Most indoor exposure arises from burning of biomass fuels, as indicated by the very high levels during cooking. The high peak levels recorded during cooking are also in keeping with other studies that have shown very high air pollution levels during cooking in the South Asian context. [370] Children were only in the kitchen for short periods but, as the extended 12-hour photometric sampling showed, they were exposed to extremely high peak levels with a great deal of variability from minute to minute during cooking: particulate concentrations at times reached almost 60 000 $\mu g/m^3$.

We used microenvironment sampling, which I feel is superior to sentinel sampling in a few specific locations. We simplified the house to the kitchen, verandah and bedroom (or main room in the house). This does not capture more complex houses or the brief amount of time in other locations such as the bathroom or corridors. We assumed that the bedroom had the same exposure level as any other room except the kitchen. I chose to focus on the bedroom because, as the time activity data show, children spent most of their time in the bedroom and it accounted for two-fifths of total exposure. In most Nepalese households, rooms used for sleeping are also used for sitting in to do homework or watch television. Contrary to our initial assumption, there did not seem to be a large difference between different roof types.

Percentage contribution was somewhat different to that of Balakrishnan et al, who showed about two-thirds of the exposure occurring in the living area. Their kitchen contribution was a little higher at 7-8% and outdoors was similar. This may be due to a difference in how the time activity was classified, in that we also included the verandah as a separate microenvironment. Together, the bedroom/living room and verandah amount to 60% of exposure in our study. [363] School, verandah and outdoor locations contributed similar proportions. To our surprise, concentration levels on the verandah were high in winter, spring and autumn seasons. The high exposure levels were likely to be from fires inside the house, outdoor fires and from other houses. The samples were taken in the early evening, which is the main cooking time. Dust may also be displaced from outdoors where levels were also fairly high, particularly in winter. Some of it may have been from fires, but most was probably displaced from fields and unsealed roads. Exhaust emissions would have made up a small proportion as mechanized traffic was relatively uncommon. Household air pollution is

thought to contribute to about 16% of ambient air pollution worldwide. [32] To a certain extent any source of particle mass is detrimental to health,[371] and we are unable to quantify the source of particle mass without further work. The effects of air pollution on the respiratory system also depend on a number of individual factors aside from the level of exposure, such as the age of the person, respiratory rate and exercise level (particularly for outdoor air pollution) and predisposing disease. Marked seasonal variation was seen in non-kitchen samples, with winter concentrations being higher than summer, which were in turn higher than in the monsoon. As would be expected, rain and moisture in the air reduce airborne particle mass in the monsoon season. This pattern was described by Saksena et al [367] and Baumgartner et al, who showed an approximate doubling of the concentration in winter compared to summer.[366] The kitchen samples were fewer and did not show an obvious pattern.

Women are known to experience higher exposure levels due to their role in cooking and female children have been thought to spend more time in the kitchen helping with cooking [364,367,372,373]. This view was not supported by our data. We did not see a difference between boys and girls in time activity or exposure levels. It may be that the children in our study were a little too young to be helping with the cooking. The experience of our staff was that boys are more exposed when they in their first two years, while girls start to help their mothers with cooking at around 10-12 years and will be more exposed during their early teens.

5.1.3.1 Time activity

While pollution is likely to emanate from the kitchen where concentrations are high, its contribution to overall exposure was low because children spent little time there. This highlights the importance of time activity data. Ezzati and Kammen recommend using "time budgets" for this.[373] We did not think self-completed diaries would be possible as our participants were children and levels of literacy were potentially low. Our questionnaire approach may have been prone to recall bias, but it is unclear in which direction it might occur. It may also be that the activity of children changes from one season to another, but we did not think that the recall method would be able to distinguish this accurately enough. Other methods include direct observation, often considered the gold standard, and the use of Global Positioning System (GPS) monitoring. Neither of these methods was considered appropriate. Apart from being logistically infeasible, following a child is intrusive and could affect her behaviour, and GPS was not accurate enough to distinguish which room she was in.

5.1.3.2 Fuel usage

The data on fuel usage emphasize the importance of biomass fuels in the region. Of those commonly in use, animal dung is regarded as the least efficient and most polluting and is the bottom rung of the WHO "energy ladder". [196] Nationally, about two thirds of households use wood as their source of fuel, while a fifth and a tenth use gas and dung, respectively. In Dhanusha the proportions are different, with 43% using wood, 44% using dung and 10% using gas. [40] The results from our study were quite different from national data, but were comparable with data from Dhanusha. The main difference was the approximately three times higher proportion of households using gas. [40] This may be due in part to our semi-urban sample proportion, but the figures are an oversimplification as most households used a mixture of fuels. Some used a more combustible and often more expensive fuel to start cooking, and then continued with a cheaper fuel. More expensive fuels like gas may be used for short cooking occasions like making tea and snacks, while biomass fuels are used for cooking main meals. Many households used two fuels together or alternately, particularly dung combined with firewood or agricultural residues. This is related to supply issues such as availability and demand issues such as family income. In a study of the association between biomass usage and respiratory infection in Nepal, Bates et al found an increased odds of infection related to not only biomass fuels, but also kerosene and gas usage, in comparison with electricity. This has important implications for our entire sample [53].

5.1.3.3 House characteristics

The make-up of the house is important. The location of the kitchen and ventilation can change air pollution levels greatly. Compared to the national figures in the census report, our data are very different for the percentage of houses with mud brick or stone walls (2.9% versus 62.8% nationally), but similar to the Dhanusha figure of 5%. The figure of 62.8% for cement and brick walls was higher in our sample than the national figure (28.7%) or the Dhanusha figure (30.5%). For roof type, the percentage with cement roofs was also higher at 43.2%, than the national (22.5%) or regional (19.9%) figures. [40] Cement roofs and brick walls, combined with the proportion using gas, point to a more affluent group in our sample. These tend to be the people who live in the town of Janakpur, but with increasing remittances from family members who migrate overseas, brick and cement houses are becoming more common in rural areas too. While those living in cement and brick houses are more likely to have a cleaner source of fuel, ventilation tends to be reduced, leading to greater containment of any smoke produced.

5.1.3.4 Limitations

Although a large study of particle mass exposure in a low resource setting in children, our study has limitations. We assumed that the samples were representative of the true average concentration level. Sample variability was wide within one location and from one season to another. In a study with limited resources and time, we cannot be certain that we captured all the variability. Gravimetric sampling over short periods in microenvironments where particulate levels are low is problematic. Filter weight-gains will often be small and results below the limit of detection are of questionable utility. [374] While still advocating the use of gravimetric sampling in general, for future sampling I would also recommend greater use of photometric devices where concentration levels are thought to be low or for short samples. Given the exposure levels found from the other samples, I feel that the non-detect values are likely to be lower, leading to an under-estimate of mean concentration. I would, however, reinforce the need for calibration with gravimetric sampling when photometric methods are used.

Another limitation was that we were unable to quantify the amount of time a child was next to a fire, and how far away from the fire she was during cooking. The photometric data suggest that peak levels can be very high. Barnes et al compared different methods of locating individuals and found that, while they were similar in terms of the time spent in a room, they differed in distance from a fire. [375] More detailed descriptions of housing construction materials and fuel use, combined with measurements and recordings, would be needed to take this further.

In summary, the study found high concentrations of air pollution in all locations, with wide variation from season to season. The highest concentration was in the kitchen but the greatest contribution to a child's exposure was the bedroom.

5.2 Socioeconomic status

5.2.1 Analysis

5.2.1.1 Multi-dimensional Poverty Index

The MPI is a measure of poverty reported by the United Nations Development Programme. Developed by the Oxford Poverty and Human Development Initiative, it aggregates individual level data from surveys such as the Demographic and Health Surveys and can be applied at country and regional level. The score is a combination of the "incidence of poverty" (more strictly, this is the prevalence as it is the proportion of people who are deprived in at least one-third of the weighted indicators) and the "average intensity of poverty" (the average number of deprivations people experience at the same time). The MPI is a composite of ten indicators, grouped into three domains, as shown in Figure 30: Education- years of schooling and school attendance; Health- child mortality and nutrition; and Living standards- cooking fuel, water, sanitation, electricity, floor and assets.[376] [377]

The questionnaire was designed in order to calculate the MPI, with the appropriate questions included (Table 25). I followed the instructions documented by Santos and Alkire for calculating the MPI.[378] The questions were coded using Stata software to create the indicator variables. These were then weighted accordingly with the health and nutrition variables given a score of 1.667, and the standard of living variables a score of 0.556 if deprived. The proportion who were deprived was calculated and each dimension stratified into urban and rural and by allocation group. The variables were then added together to give a score for each dimension and a total MPI intensity score. If data were missing, as was the case for ten children in whom we did not have anthropometry results, we assumed they were not malnourished. The multidimensional headcount ratio, intensity of poverty and MPI score were calculated according to Equation S, Equation T and Equation U respectively.

Equation S

$$\text{Multidimensional Headcount ratio} = \frac{\text{Number of people in households that are MPI poor}}{\text{Total number of people}}$$

Equation T

$$\text{Intensity of poverty} = \frac{\sum(\text{In MPI poor households, Deprivation score} \times \text{Number of people in household})}{\text{Number of people in households that are MPI poor}}$$

Equation U

$$\text{MPI} = \text{multidimensional headcount ratio} \times \text{average intensity across the poor}$$

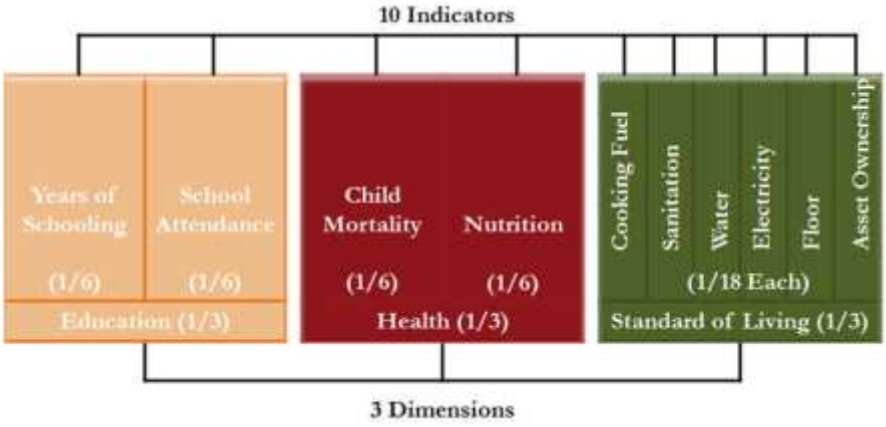

Figure 30: MPI indicators and dimensions [376]

5.2.1.2 Asset score

The asset score was constructed from the questionnaire data using the methods described by Vyas and Kumaranayake.[379] Variables for consideration in the PCA are shown in Table 26. To choose variables with a range of values over the population, they were included if the mean prevalence was >0.10 and <0.90. This left radio, TV, telephone, fridge, bicycle, motorcycle, biomass fuel, toilet, wall type and floor. Including these variables produced a bimodal kernel density plot. After removal of floor and toilet, the scree plot and kernel density graphs in Figure 31 were produced. This graph shows little truncation and has a fairly normal distribution. The first component was retained for the final PCA score. The second component also had an eigenvalue greater than one, but by convention the first factor is usually used. [379,380]

Table 25: MPI dimensions and sources of information [381]

Dimension	Indicators	Definition of deprivation	Source of information
Education	Years of schooling	Deprived if no household member has completed five years of schooling.	Answer "no" to: *Has any member of the household completed 5 years of schooling?*
	School attendance	Deprived if any school-aged child is not attending school in years 1-8.	Answer "no" to: *Are all school-age children currently enrolled in school (Years 4-16)?*
Health	Child mortality	Deprived if any child in the family have died.	Answer "yes" to: *Have any of the child's siblings died?* OR Answer "dead" to *State of the child*.
	Nutrition	Deprived if any adult or child for whom there is nutritional information is malnourished.	WHO BMI-for-age >2 SD below median
Standard of living	Electricity	Deprived if household has no electricity	Answer "no" to electricity: *What things do you have?*
	Drinking water	Deprived if household does not have access to clean drinking water or clean water is >30 minutes walk from home.	Answer "public well", "traditional well", "river, stream, canal, pond" to: *What is the main drinking water source for your household?* OR Answer ">30 minutes" to: *How far do you have to walk to get drinking water?*
	Sanitation	Deprived if household does not have an improved toilet or if toilet is shared.	Answer "yes" to anything except a "flush toilet": *What kind of toilet do your family members use?* OR Answer "no" to: *Do you share your toilet with other households?*
	Floor	Deprived if household has dirt, sand or dung floor.	Answer "dirt", "sand" or "dung" to: *What is the floor mainly made of?*
	Cooking fuel	Deprived if household cooks with wood, charcoal or dung.	Answer "wood", "charcoal", "dung", "straw", "other plant products" or "plastic" as a main fuel to: *Which fuels do you usually use for cooking?*
	Asset ownership	Deprived if household does not own more than one of: radio, TV, telephone, fridge, bike or motorbike; and do not own a car or tractor.	Excluding "electricity", answer "yes" to less than two items and does not answer "yes" to "Bus/Truck/jeep/car/tempo" or "tractor" to: *What things do you have?*

Table 26: Potential variables for inclusion in Principal Components Analysis

	Variable	Comment	Mean (SD)	Keep or Drop*
Assets	Electricity	1 if present, 0 if not	0.98 (0.15)	Drop
	Radio		0.47 (0.50)	**Keep**
	TV		0.77 (0.42)	**Keep**
	Telephone		0.11 (0.31)	**Keep**
	Mobile phone		0.93 (0.25)	Drop
	Fridge		0.12 (0.33)	**Keep**
	Bicycle		0.67 (0.47)	**Keep**
	Rickshaw		0.01 (0.08)	Drop
	Motorcycle		0.25 (0.43)	**Keep**
	Tractor		0.02 (0.14)	Drop
	Other asset		0.02 (0.12)	Drop
Fuel	Biomass	Use biomass as their main fuel	0.54 (0.50)	**Keep**
Water		1 if tap, own/neighbour's well, deep bore hole, hand-pump 0 if they use a public well, traditional well, river, pond	0.98 (0.14)	Drop
Toilet		1 if flush or pan toilet. 0 if pit, open area or other	0.65 (0.48)	**Keep**
House	Walls	1 if brick. 0 if stones or plant materials or wood	0.66 (0.47)	**Keep**
	Roof	1 if cement or tiles. 0 if metal or grass	0.97 (0.17)	Drop
	Floor	1 if cement or brick. 0 if dirt or sand	0.50 (0.50)	**Keep**

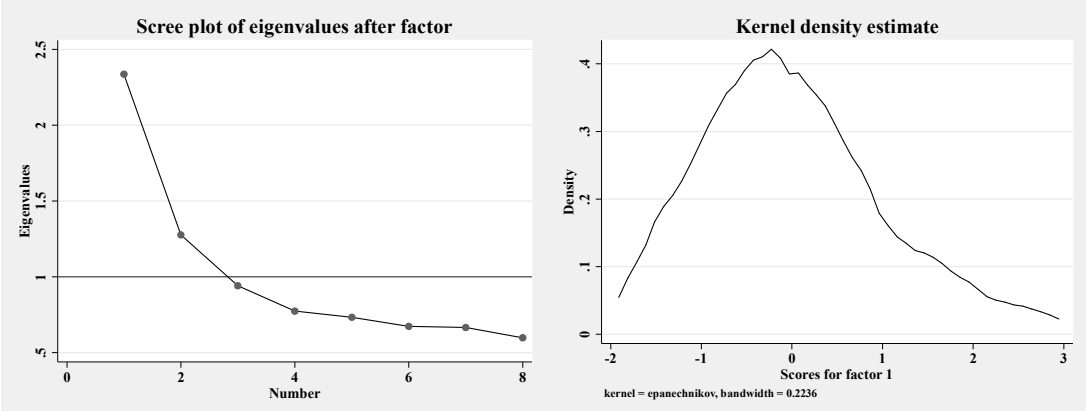

Figure 31: Scree plot and kernel density graph from final factors chosen for asset score

5.2.2 Results

5.2.2.1 MPI

Table 27 shows the number of people who were deprived in each indicator and dimension with the results shown graphically in Figure 32. Table 28 stratifies the dimensions by place of residence and allocation group. The majority of households were considered poor for sanitation and cooking fuels used, while for school attendance, electricity and water the percentages were very low. 308 households (36.2%) had a MPI score of $\geq$3.3 and were regarded as being poor. Of these, 100 (11.8%) were considered to have severe poverty with a score >5. A further 184 (21.6%) were vulnerable to poverty.

Figure 33 shows the distribution of MPI deprivation scores in the sample, with the distributions for each dimension shown in Appendix 5.1. The mean score used was 2.59 (SD 1.60) and median score was 2.22 (IQR 1.11, 3.33). The mean score is the "average intensity" for the whole sample when conducting the MPI, but as the data were skewed (and, strictly speaking, MPI is an ordered categorical variable), I have reported the median as well. Stratification by allocation group gave the same median deprivation score of 2.22 (IQR 1.11, 3.33) in both groups.

There were 5389 people in 851 households and the number of people in households that were MPI poor was 1980. This gave a Multidimensional Headcount ratio of 0.367. Intensity of poverty was 0.422 and MPI for the sample was 0.155.

5.2.2.2 Asset score

An asset score with a mean of 0 and a standard deviation of 1 was produced and divided into quintiles for use in further analyses. A comparison of the asset and MPI scores can be seen in Figure 34. This shows an inverse association and a correlation coefficient of 0.48. The dotted horizontal line represents the cut-off above which a household is considered MPI poor.

Table 27: Number and percentage of the sample who were considered deprived in each indicator

Dimension	Indicator	Number of people who were deprived (Total number = 851)	Percentage (%)
Education	Years of schooling	140	16.5
	School attendance	36	4.2
Health	Child mortality	172	20.2
	Nutrition	295	34.7
Standard of living	Electricity	20	2.4
	Drinking water	19	2.2
	Sanitation	840	98.7
	Floor	423	49.7
	Cooking fuel	643	75.6
	Asset ownership	95	11.2

Table 28: Number and percentage of the sample considered deprived in each dimension

	Whole sample	Urban	Rural	Control	Intervention
Dimension*	Number who are deprived (%)	Number who are deprived (%)	Number who are deprived (%)	Number who are deprived (%)	Number who are deprived (%)
Education	157 (18.4)	56 (16.0)	101 (20.1)	74 (17.3)	83 (19.6)
Health	406 (47.7)	158 (45.3)	248 (49.4)	210 (49.2)	196 (46.2)
Standard of living	841 (98.8)	340 (97.4)	501 (99.8)	425 (99.5)	416 (98.1)
Total number	851 (100.0)	349 (100.0)	502 (100.0)	427 (100.0)	424 (100.0)

*A household may be deprived in more than one dimension.

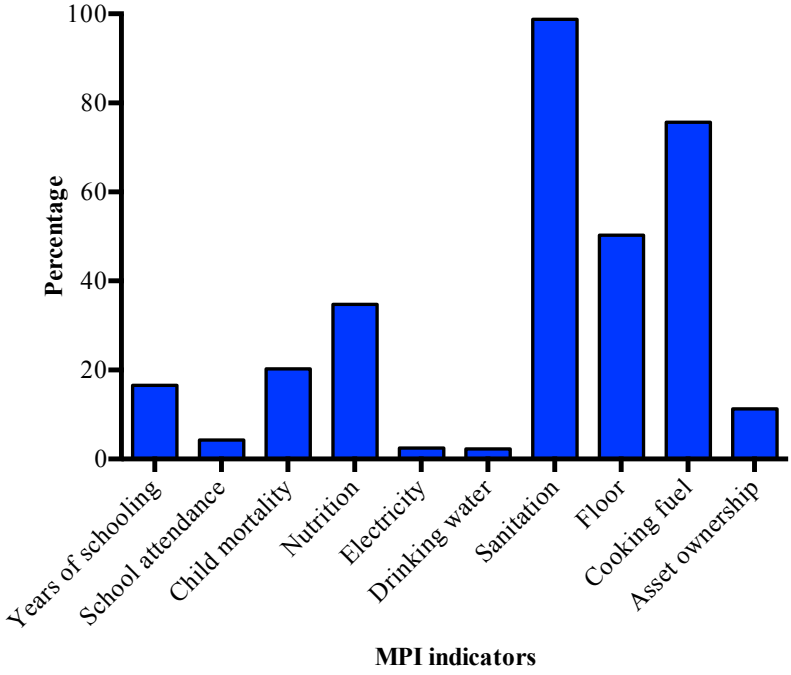

Figure 32: Distribution of proportion of deprived households in MPI indicators

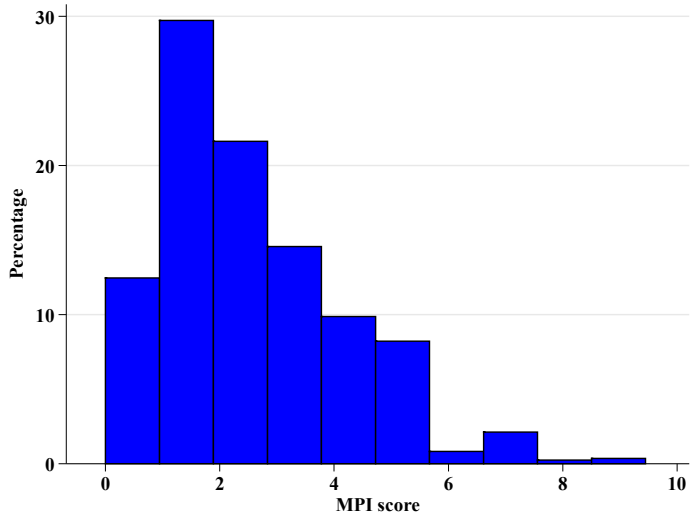

Figure 33: MPI deprivation score distribution across the sample

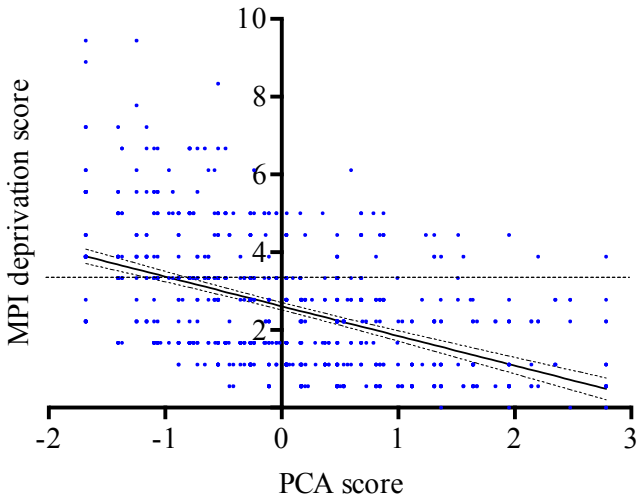

Figure 34: Scatterplot with line of best fit (95% confidence intervals) of asset score and MPI deprivation scores. Horizontal dotted line represents MPI score of 3.33.

5.2.3 Discussion

Lower socioeconomic status is associated with poorer health outcomes in childhood,[382] decreased birth weight[124,383] and child mortality.[384,385] Socioeconomic status in early life is also associated with shorter adult height,[27] lower educational achievement and reduced income,[386] all of which help to perpetuate the cycle of intergenerational malnutrition and poverty. [5] [387] People in less well-off groups tend to have a greater number of risk factors for illness combined with reduced access to ameliorative care.

Socioeconomic status is a complex concept that incorporates social status (including social position, power and capital) as well as wealth.[387] Poverty can be measured in a number of ways using either single or multiple indicators combined. As described by Howe et al, the approaches to measuring socioeconomic status fall into the following categories: asset based, consumption, income/wealth, education, occupation and subjective measures for which people rank themselves.[380] Each has its strengths and limitations and none is perfect. Asset based indices are commonly used, for example in demographic and health surveys. A systematic review was performed to compare wealth indices. Twenty-two of the 33 datasets included showed weak agreement. Those with a higher number of participants and broader range of indicators tended to have better agreement, as did the datasets from richer countries. [388] I did not think income would provide an accurate measure of socioeconomic status. Many people in the study region are subsistence farmers who do not have a regular income, but may have land and durable assets. We did not have the necessary information to be able to construct a measure of consumption and there is some evidence to say that asset scores perform as well as consumption indicators.[389]

5.2.3.1 Multidimensional Poverty Index

The MPI was chosen as a summary as it is an absolute score that can be used to compare our sample with other populations and can be broken down to show in which components people are poor. According to the most recent United Nations Development Programme report, Nepal has an MPI score of 0.217. This corresponds with an incidence of poverty of 44% for the year 2011, and with 25% living under $1.25 per day.[390] Parts of Nepal have the highest MPI scores in the sub-continent: the Central Terai area that includes Dhanusha has an MPI of 0.233. This is made up of an incidence of poverty of 46.2% and average intensity of 50.4%.[376]

As with any summary tool, the MPI has limitations. For example, in our sample the majority of households had electricity and most children went to school. This does not express the reality of life in the winter as “load shedding” leaves a house without electricity for up to 18 hours a day and the quality of schooling varies greatly. While this is a criticism of the MPI,

tools designed for comparison are bound to be simplifications of reality. There is inherently a trade-off between the complexity of a tool and its ease of use. The MPI can also be criticized for what it does not include; for example, security or empowerment indicators.

There is also debate over whether a single composite score can ever truly represent "poverty". Ravallion argues that a composite score loses the valuable information of the individual indices and may be misleading. He goes further to question the validity of a scoring system in which child mortality can be equated with the "combined deprivation of having a dirt floor, cooking with wood, and not having a radio, TV, telephone, bike or car".[391] Intuitively, this would seem wrong, and I would give child mortality a higher weighting. The mortality indicator is a little different though from the others (possibly with the exception of asset ownership) in that it is an outcome in itself. The other indicators could be argued to be mediators or surrogate markers of a construct. For example, poor sanitation may be a mediator for childhood infection. In this respect, the other factors may contribute to child mortality.

5.2.3.1 Asset score

In contrast to the MPI, the asset score approach was used to provide a relative comparison within the group. PCA is a data reduction technique that is used to select variables highly predictive of a larger group of variables. This can be sub-divided to create categories with similar numbers of people, for example quintiles, that can be used in analyses such as in section 6.6. It is useful because it can stratify a population, allowing comparisons to be made, and is becoming an increasingly common way to combine assets.

PCA-derived scores have been used in Nepal, for example in the Demographic and Health Survey. [49] They cannot be used directly to compare one group with another, unless the specific components are known. It is not clear, for example, how the asset score in the DHS was constructed. In addition, PCA does not allow for a predefined weighting of the importance of the indicators unless a greater number of variables on a particular topic are included.

5.2.3.2 Comparison of PCA and MPI

As shown in Figure 34, there was an inverse association between PCA and MPI deprivation scores meaning both tend to identify poorer and richer households the same. This shows some correlation between the scores, but there is quite a wide degree of variation, as indicated by

the correlation coefficient. For example, 62 (7.3%) of households were in the poorest quintile for asset score, but not considered poor for MPI. The assets used in constructing both scores were similar, but differed in the incorporation of health and education indicators in the MPI. If accurate, the sample could be said to be asset poor, but not multi-dimensionally poor. The MPI therefore has a broader range of indicators as it aims to document different types of poverty. Being deprived in assets alone will not classify a household as poor under the MPI. The method of construction is also different. The MPI uses predefined questions and weightings in a particular way. The PCA creates a score from the available data and is prone to biases in data collection. Neither technique provides information at a macro level, nor does it differentiate how resources are used within a household.

5.2.3.3 Generalisability of results

The MPI score found in our sample was lower than that found previously in Dhanusha, and on par with more affluent parts of the country. [376] The difference may be due to data quality issues (the MPI is often calculated from large routine datasets), but may also be due to biases in the selection of our sample. The sample was self-selected in that the women who participated in the original trial chose to enter the study and intended to have their delivery in the regional hospital. This was likely to lead to a lower MPI as it would select women who had the resources to go to the hospital. In addition, we selectively followed up the children who had survived past the age of two (the time of the previous follow-up). This would exclude the 40 women who had a miscarriage or stillbirth. It is possible that an indicator of poverty may have been underlying this death, for example under-nutrition in the mother leading to an inability to sustain the pregnancy. Another 42 children died before the second follow-up at two and a half years of age. By selecting those who did not die in early life, when the mortality rate is the highest in childhood, the "child mortality" indicator would be lower. Another source of bias is loss to follow-up, which may change the MPI score in either direction. Those who were unable to attend the hospital or take the micronutrients may have been more deprived. Alternatively, those who migrated from the area were probably less deprived.

In summary, both the MPI and asset score can be used to measure SES. Neither method is a perfect measure and the correlation between them is not ideal. However, both can provide important information about the population in question, if interpreted in relation to the data used to create them. SES is a complex and often poorly defined concept, and multiple measures are helpful.

5.3 Food security

Food security describes the availability to a household, region or country of enough food both currently and in the future. Food insecurity increases the risk of malnutrition and changes in anthropometry. It was therefore considered important to investigate levels of food security in the sample. The questionnaire included questions on access, availability of food over the year and dietary diversity.

5.3.1 Analysis

We assessed food security with the Household Food Insecurity Access Scale (HFIAS),[302] Household Dietary Diversity Score (HDDS)[303] and Months of Adequate Household Food Provisioning (MAHFP)[304]. The analysis was carried out according to the instructions for questionnaire tools.

HFIAS is made up of nine questions (Table 29). The questions can be divided into three "access-related domains": anxiety, quality and physical consequences. Anxiety is represented by answering "yes" to question 1; low quality food by answering "yes" to questions 2, 3 or 4; and physical consequences by answering "yes" to questions 5, 6, 7, 8 or 9. The HFIAS questions can then be categorized into levels of food security: food secure and mildly, moderately and severely food insecure, as shown in Appendix 5.2.

The HDDS was used to examine the breadth of a child's diet in the preceding week. The HDDS questions asked what food groups children had eaten in the last week. We modified the usual question about the last 24 hours to get a broader idea of what children were eating. The maximum score was 12, relating to a score of one for each of the following foods: cereal, roots and tubers, vegetables, fruits, meat, fish or shellfish, eggs, dairy, pulses, oils or fats, sugar or honey and other (spices, tea, coffee, etc.).

The MAHFP indicates seasonality and duration of food insufficiency over the previous 12 months. Months without adequate food supplies were recorded and summarized for each household. The food security indicators were then divided into the trial groups for comparison.

5.3.2 Results

5.3.2.1 HFIAS

The median HFIAS score was 0, with a range from zero to a maximum of 27. The prevalence of food insecurity was 8.6% (n=73). The proportion of households considered anxious was 6.8% (n=58), as having low quality food was 16.0% (n=136) and as having physical consequences from food insecurity was 11.3% (n=96). The levels of food security are shown in Table 30, with the results from the Nepal DHS as a comparison. This shows that less than one in ten households were food insecure. There was no difference between rural and urban locations.

5.3.2.2 HDDS

Median HDDS was 9 (interquartile range 8, 10). Eating fruits and vegetables was very common and no different between trial groups. Meat or fish was only consumed by about half the children in the last week (see Table 31).

5.3.2.3 MAHFP

The median MAHFP score was the maximum score of 12, indicating that the household did not lack food for any months in the previous year. 7.6% of households had some difficulty obtaining food in the past 12 months, with 3% reporting difficulty obtaining food for ≥3 months. Though the numbers were small, in the post-monsoon season, rural households were more likely to have food insecurity (5.2% for rural, 1.7% for urban)- Table 32.

5.3.2.4 Trial group

The difference between the trial groups was less than 2% for all three indicators (Table 33).

Table 29: Household Food Insecurity Access Scale Score questionnaire results

	Question	Total number answering yes (%)	Of those who answered yes, what is the frequency of occurrence		
			Rarely	Sometimes	Often
1	Did you worry that your household would not have enough food?	58 (6.8)	11 (19.0)	30 (51.7)	17 (29.3)
2	Were you or any household member not able to eat the kinds of food you preferred because of lack of resources?	58 (6.8)	12 (20.7)	36 (62.1)	10 (17.2)
3	Did you or any household member just eat a few kinds of food day after day because of lack of resources?	35 (4.1)	4 (11.4)	22 (62.9)	9 (25.7)
4	Did you or any household member eat food that you did not want to eat because of a lack of resources to obtain other types of food?	43 (5.1)	11 (25.6)	23 (53.5)	9 (20.9)
5	Did you or any household member eat a smaller meal then you felt you needed because there was not enough food?	27 (3.1)	7 (25.9)	14 (51.9)	6 (22.2)
6	Did you or any other household member eat fewer meals because there was not enough food?	26 (3.1)	3 (11.5)	18 (69.2)	5 (19.2)
7	Was there ever no food at all in your household because there were no resources to get more?	18 (2.1)	5 (27.8)	10 (55.6)	3 (16.7)
8	Did you or any household member go to sleep hungry because there was not enough food?	16 (1.9)	3 (18.8)	12 (75.0)	1 (6.2)
9	Did you or any household member go a whole day without eating because there was not enough food?	9 (1.1)	2 (22.2)	6 (66.7)	1 (11.1)

Table 30: Household Food Insecurity Access Prevalence

	Number	Percentage	Food insecurity percentage for Nepal[37]
1. Food Secure	778	91.4	49
2. Mildly Food Insecure Access	21	2.5	12
3. Moderately Food Insecure Access	29	3.4	23
4. Severely Food Insecure Access	23	2.7	16
Total	851	100	100

Table 31: Household Dietary Diversity Score (HDDS)

	Number	Percentage
Cereal	848	100
Roots and tubers	823	97.1
Vegetables	729	86.0
Fruits	604	71.2
Meat	463	54.6
Fish or shellfish	366	43.2
Eggs	232	27.4
Dairy	683	80.5
Pulses	837	98.7
Oils or fats	835	98.5
Sugar or honey	800	94.3
Other (spices, tea, coffee, etc.)	660	77.8

NB. Three children died, so the total number is 848.

Table 32: Months of Adequate Household Food Provisioning (MAHFP) by Nepali month and season

Season	Winter (Push-Phagun)	Spring/Summer (Chait-Jyesth)	Monsoon (Ashadh-Bhadau)	Post-monsoon (Asoj-Mangsir)
Number households without enough food	2	36	40	32

Month

Push (Dec-Jan)	Magh (Jan–Feb)	Phagun (Feb–Mar)	Chait (May–Apr)	Baisakh (Apr–May)	Jyesth (May - Jun)	Ashadh (Jun–Jul)	Shrawan (Jul–Aug)	Bhadau (Aug – Sep)	Asoj (Sep-Oct)	Kartik (Oct–Nov)	Mangsir (Nov–Dec)
1	2	0	7	9	30	26	28	24	25	24	4

Table 33: Food security prevalence by allocation group

	Control	Intervention
	Number* (%)	Number* (%)
Households that are food insecure	39 (9.2)	35 (8.4)
Households with not enough food for the whole year	29 (6.9)	35 (8.4)
Median HDDS	9	9

* Limited to children who were able to come for anthropometry measurements.

5.3.3 Discussion

Food security was defined by the World Food Summit 1996 as existing "when all people at all times have access to sufficient, safe, nutritious food to maintain a healthy and active life". Other aspects of food security include the affordability of food, its quality and safety. The WHO categorizes "three pillars" of food security:

- "Food availability: sufficient quantities of food available on a consistent basis.
- Food access: having sufficient resources to obtain appropriate foods for a nutritious diet.
- Food use: appropriate use based on knowledge of basic nutrition and care, as well as adequate water and sanitation."

In the Declaration of Rome, countries pledged to halve the number of undernourished people by 2015. The Nepal government reiterated this pledge in 2006 when the interim constitution stated that food security was a fundamental human right for all its citizens. The government

announced a Three Year Interim Plan (2010-2013) to reduce food insecurity. It plans to identify and target groups who are vulnerable to food insecurity, especially in remote areas, with specific programmes (although it does not specify what these will be). [37] A country or region's food security can be affected by a number of factors. In Dhanusha district most food is grown locally. Many people live off their own land as subsistence farmers. Food availability therefore varies by the time of the year and the region is particularly dependent on the weather.

The tools used in the study were designed to capture as much of this information as possible and to be similar with those used by the Nepal DHS. They were developed in conjunction with a review of 11 studies of food security by Coates et al, which confirmed the importance of food access and dietary diversity. The review also showed a number of common concerns across different settings, such as worry about adequate quantity of food. [392]

The results from all three food security questionnaires indicated that food insecurity was rare in the sample and that children had diverse diets. Food insecurity was also uncommon at all points in the year and, although the numbers were low, rural residents were a little more vulnerable than urban residents in the post-monsoon period.

Comparing our data with the Nepal DHS (2011) for the Central Terai region, we found a greater proportion of food secure households (50.1 vs 92.2%, $p<0.001$). The DHS would have sampled the population to be representative of the region and should be a more accurate descriptor of the level of food security. However, the number of households sampled (n=1975) was not much greater than our sample. The DHS also used the same questionnaire tools, but modified from a one-month period to 12 months to "allow for seasonal variations". [37] This could account for the difference, as families are more likely to experience aspects of food insecurity over a longer period. The use of a 12-month period does make it difficult to compare the Nepal DHS to others that have used the same questionnaires.

Chapter 6 Main analysis and results

In this section I describe the main findings from the study. I start by considering the mortality with reference to the previous trial and follow-up. I then consider the growth of all the children together, before dividing them by allocation. I examine anthropometry, blood pressure and respiratory results. I perform univariable and multivariable regressions based on a pre-specified conceptual model and explore potential effect modification. This is followed by a summary of the health outcomes and two further analyses investigating the effect of air pollution on asthma symptoms and socioeconomic status on growth.

6.1 Mortality

A discussion about mortality at birth has been presented in detail elsewhere. [7,251] There was a non-significant excess neonatal mortality in the original Janakpur trial in the MMN group. The meta-analysis of this and similar trials by Ronsmans et al did not find a statistically significant difference in neonatal mortality, but as described in section 2.9.2, when the large trial from Lombok, Indonesia was removed, the OR rose to 1.40 (95% CI 1.08, 1.82). [252]

After the high mortality in early life, rates drop in mid-childhood, so mortality was unlikely to be an important factor to consider in this follow-up. Nevertheless, considering the previous debates on this issue, I updated the profile to show the number of children who died. Mortality rates were calculated as in Appendix 6.1.

Overall, a similar number of children had died in both allocation groups. At the two-year follow-up (including stillbirths but not miscarriages), one more child had died in the intervention group. Three further children died between the two-year and eight-year follow-ups. Two of these were from the control group and the third from the intervention group. The number of miscarriages, stillbirths and postnatal deaths is shown in Figure 35. With the caveat that no time period showed a statistically significant difference at the 95% level between intervention and control groups, my impression is that the MMN supplement might change the time at which deaths occur, but does not contribute to a difference overall. If this is true, it is not clear to me whether it is good or bad. It does seem safe to say that MMN supplementation does not reduce mortality as was initially hypothesised.

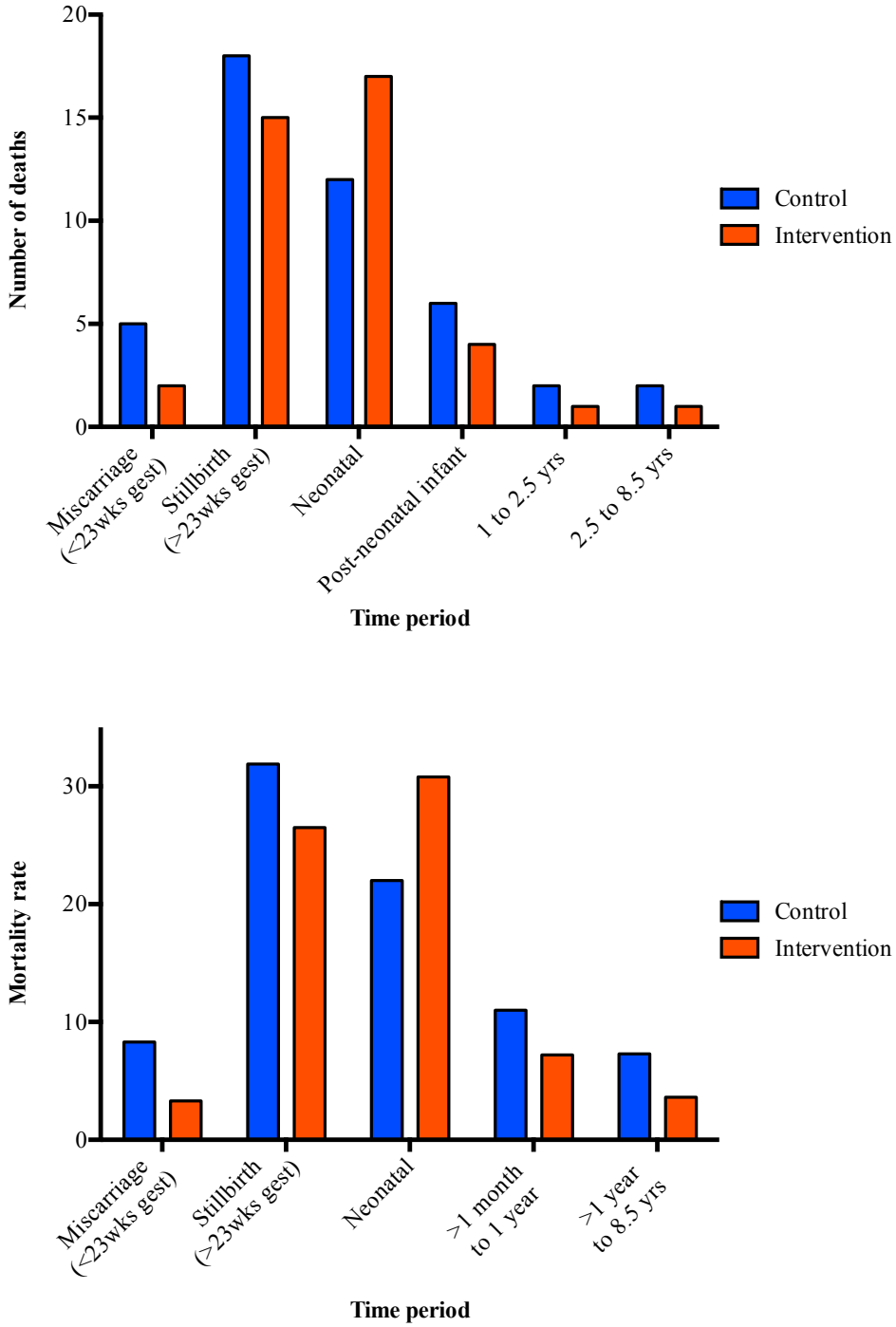

Figure 35: Mortality in control (blue) and intervention (intervention) groups

6.2 Growth of the whole cohort

6.2.1 Analysis

6.2.1.1 Application of WHO reference ranges

The anthropometry data were normalised using the WHO Child Growth Standard reference ranges to create z scores for weight-for-age (WAZ), height-for-age (HAZ) and BMI-for-age (BAZ) for children aged 5-19. [25] Most reference ranges have been created for children under five, but the WHO recently developed ranges for school children and adolescents: the 2007 WHO Reference Curves. To create these reference ranges, they initially examined 34 datasets from 22 countries, but found a great deal of heterogeneity. They then re-examined the National Center for Health Statistics/WHO international growth reference data and created new reference ranges from them using the Box-Cox power exponential method. This uses curve smoothing by cubic splines and can cope with skewed and kurtotic data. [25]

6.2.1.2 Regression to the mean

The statistical phenomenon of regression to the mean may occur when categorisations are applied to the data. An example would be when children who are stunted at one time point move closer to the mean value over time, i.e. show greater catch-up growth. As described by Cameron et al, regression to the mean can be accounted for by calculating the difference between the second height and the product of first height and the correlation between the heights. [393]

6.2.1.3 Exclusion of children with illness

As illness may adversely affect growth, it was decided that we would also restrict the analysis to children who were well. Major and chronic illnesses were coded as described in section 3.6.

6.2.1.4 Body composition

Fat mass (FM) was calculated as the difference between lean mass (LM) and total mass and BMI as body weight (kg) divided by height (m) squared. Both LM and FM can be divided by height squared, to give the lean mass index (LMI) and the fat mass index (FMI). Such a model allows independent evaluation of both LM and FM relative to body size. [394 395]

6.2.2 Results

6.2.2.1 Anthropometric status of the children

The anthropometry results for the whole cohort are shown in Table 1, Appendix 6.2. and a summary of the main results at 8.5 years, adjusted for age and sex, is presented in Table 34. Girls were a little shorter and lighter than boys.

The distributions of WAZ, HAZ and BAZ for age are shown in Figure 36. The graphs show normal distributions at all time points, but shifted to the left: the children in our sample were both lighter and shorter than the global standard applied. Figure 37 shows scatter plots for HAZ, WAZ and BAZ from birth to eight years. For height, there was a reduction of 1.15 z scores over the period. For weight, the reduction was a little less at 0.87 z scores, but the infants were relatively lighter to begin with. For HAZ, there appeared to be some recovery from 2.5 to 8.5 years, whereas WAZ showed a steady decline. BAZ showed recovery at 2.5 years, but this was due to greater reduction in HAZ, leaving children with an appropriate weight for height. The standard deviation for WAZ was similar over the three time points, but for HAZ reduced from 1.26 to 0.94.

Table 35 shows the proportion of children underweight, stunted and with low BMI, at the three time points. At 8.5 years of age, just over half the children had low weight-for-age, and approximately one-third had stunting and low BMI. For children who were stunted (limited to complete data), WAZ scores reduced progressively: the changes from birth-to-2.5 years and 2.5-to-8.5 years were -0.53 and -0.33, respectively. However, there appeared to be some catch-up growth in HAZ in childhood: the changes from birth-to-2.5 years and 2.5-to-8.5 years were –1.91 and 0.80, respectively. The proportion stunted at 8.5 years fell by 30% from that at 2.5 years. However, accounting for regression to the mean caused this positive change in mean score to disappear (-0.56 z scores).

Only 1.4% of the children were overweight (defined as BAZ >1 SD). For girls the figure was 0.5% and for boys 2.3%.

Table 34: Main anthropometry results for the whole cohort at 8.5 years, stratified by sex

		Number	Mean	Standard deviation	Range
Weight-for-age	Girls	406	-2.09	0.97	-4.6 to 1.4
	Boys	435	-2.03	1.09	-4.8 to 2.7
Height-for-age	Girls	406	-1.54	0.92	-5.7 to 1.1
	Boys	435	-1.45	0.95	-4.0 to 1.4
BMI-for-age	Girls	406	-1.66	0.92	-4.2 to 1.7
	Boys	435	-1.64	1.01	-4.5 to 3.3

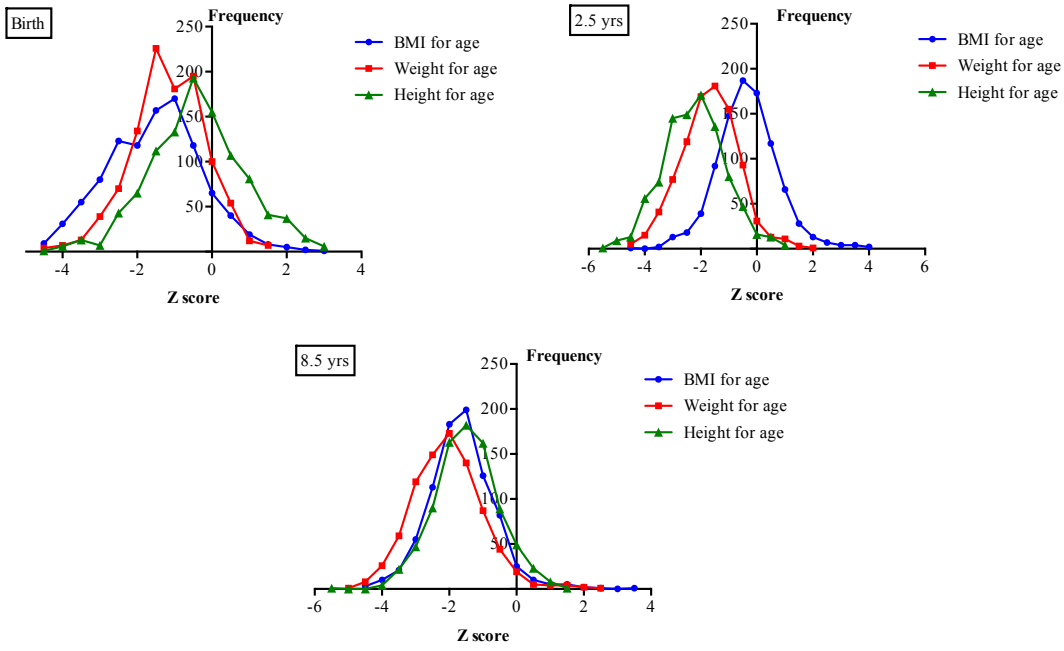

Figure 36: Frequency distributions of weight-for-age, height-for-age, and body mass index for age, at birth, 2.5-years, and 8.5 years

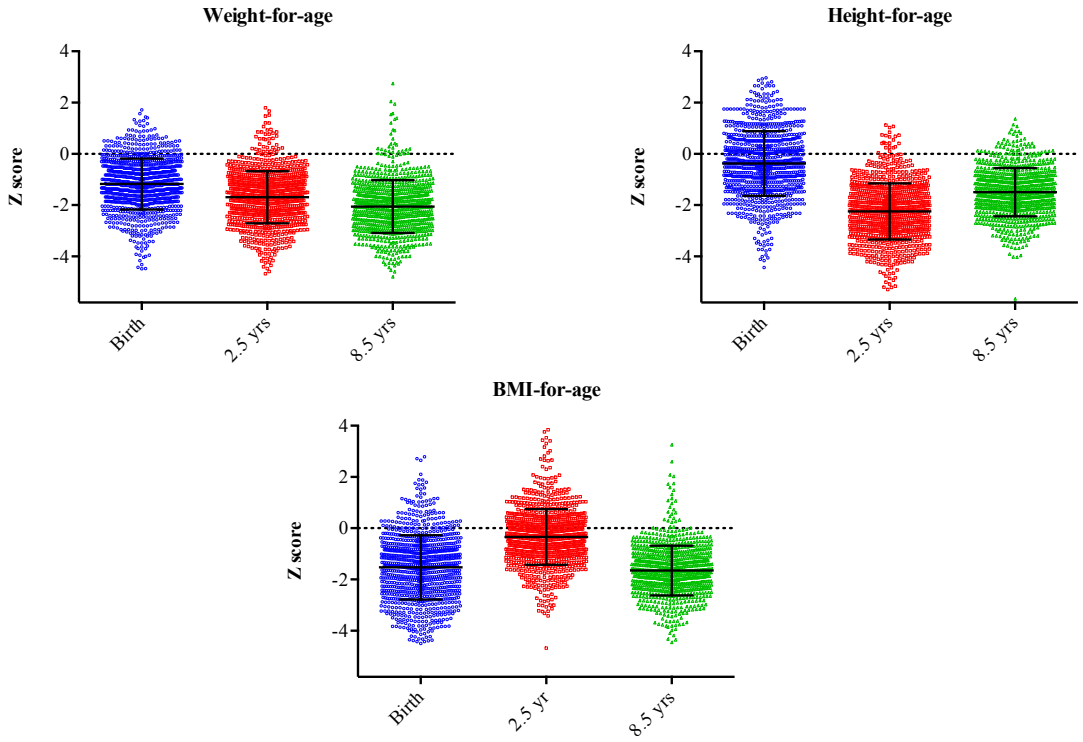

Figure 37: Change in z scores. Black bars denote mean ± 1 standard deviation

Table 35: Proportion of infants/children underweight, stunted and wasted

	Underweight (weight-for-age <-2 z)		Stunted (height-for-age <-2 z)		Low BMI (BMI-for-age <-2 z)	
	Number (%)	Mean for children underweight	Number (%)	Mean for children stunted	Number (%)	Mean for children wasted
Birth	186 (17.9)	-2.7	91 (9.0)	-2.7	348 (34.8)	-2.9
2.5 years	340 (37.2)	-2.7	537 (58.8)	-3.0	54 (5.9)	-2.5
8.5 years	444 (52.8)	-2.8	242 (28.8)	-2.6	293 (34.8)	-2.6

6.2.2.2 Exclusion of children with illness

The anthropometry results for the whole cohort, stratified into well children and children with a history of major or chronic illness (n=34) are shown in Table 2, Appendix 6.2. The list of illnesses included achondroplasia, congenital heart disease, developmental delay of unknown cause, leishmaniasis, prolonged diarrhoea, renal conditions (hydronephrosis, nephritic syndrome), rheumatic fever, and tuberculosis. Children with a history of illness tended to be a little shorter and lighter, but as the numbers were small we cannot draw definitive conclusions.

6.2.2.3 Body composition

The mean fat mass proportion was 14.5% (girls 15.2%, boys 13.7%). The distribution of fat mass was normal, but with a few much heavier children (Figure 38). Figure 39 shows scatterplots of lean and fat mass. There was a positive association between lean and fat mass (β coefficient 0.28; 95% CI 0.24, 0.33). This association was no longer present when controlling for height (squared) using LMI and FMI, i.e. their association was explained by each correlating with height.

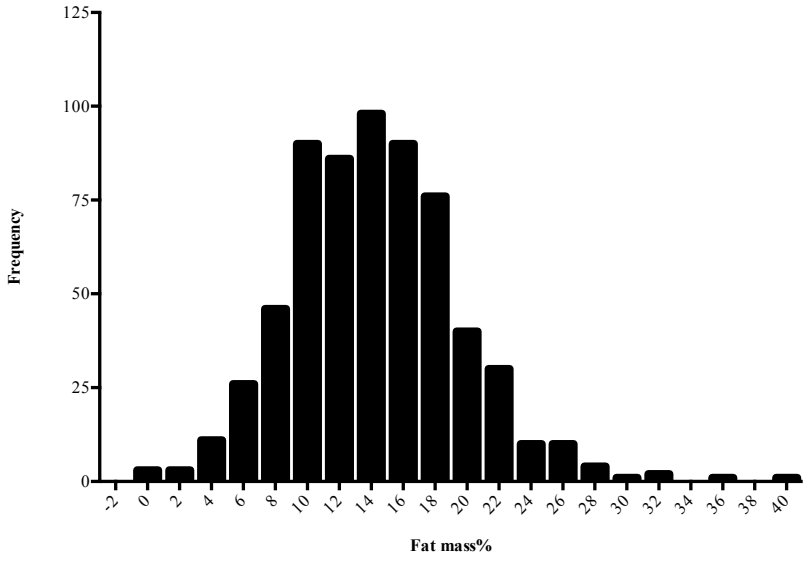

Figure 38: Distribution of fat percentage in the whole cohort

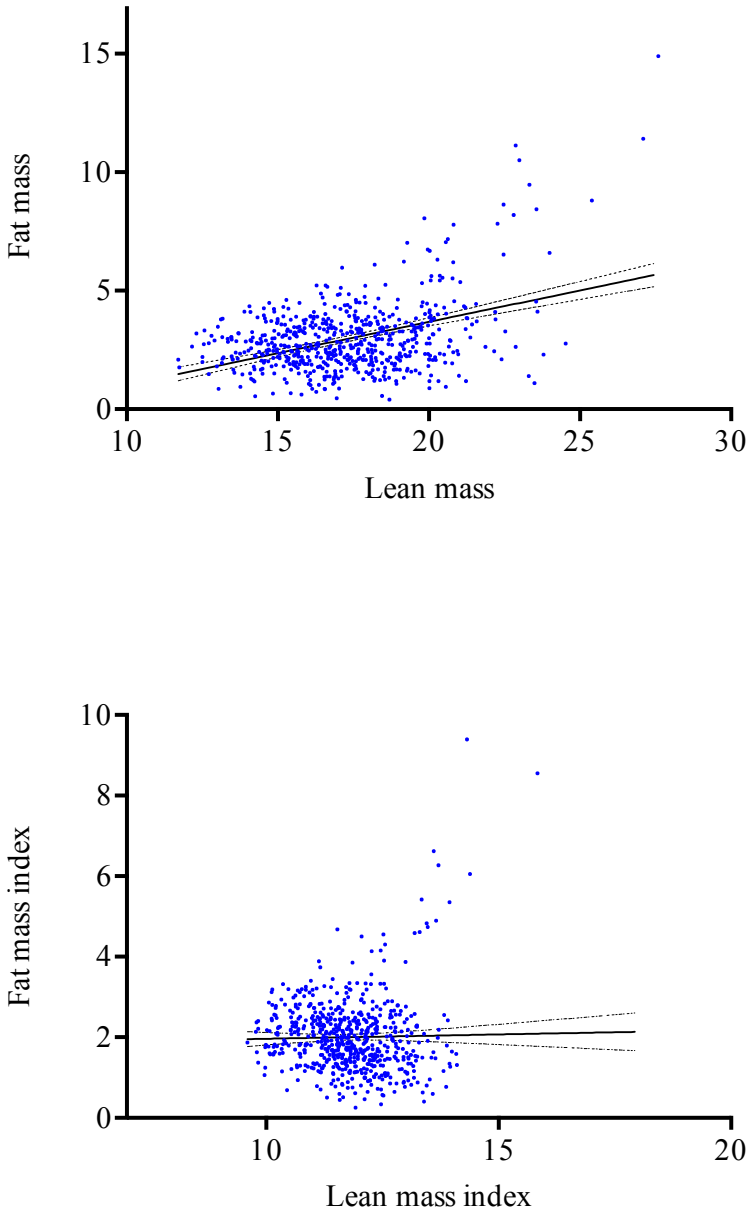

Figure 39: Scatterplots of fat mass against lean mass and fat mass index against lean mass index

6.2.3 Discussion

Levels of malnutrition were high. Estimates for the *Terai* from the 2011 Nepal Demographic and Health Survey suggested that 37% of children under five were stunted, 11% were wasted, and 30% underweight. Boys fared slightly worse than girls in all categories, and estimates for rural populations were higher than urban figures.[49] We found a similar proportion of stunting, but a 23% higher estimate of low BMI as an index of underweight. The fact that our children were older raises concerns about further deterioration in nutritional status beyond the age of five years.

The results show that the children started off life small and then continued to get smaller. Most research tends to focus on the first year or five years of life, but we have shown that even after two years of age there was a reduction in weight z score. While growth failure was greatest in early life, there appeared to be some scope for catch-up. Contrary to the idea that height trajectory is set early,[396] the children in our study did show some catch-up growth. The proportion who were stunted fell by 30% from 2.5 to 8.5 years, but this catch-up in the smallest children is potentially explained by regression to the mean. As recommended by Cameron et al, analysis of groups that have been categorised should include correction for regression to the mean. [393]

The levels of obesity found in our study, as defined by BMI, were very low, but the percentage of fat was relatively high. This compares to an estimate of mean fat percentage (using bioelectrical impedance) in UK 8 year olds of 21% in girls and 17% in boys. [397] The relatively high fat percentage is in keeping with the idea of the "thin-fat" phenotype that is common in the Indian subcontinent, in which there is a relatively greater amount of fat for the size of the person. It may be due to inadequate nutrition at critical periods and may possibly confer a survival advantage.[398,399]

As described in section 4.4, we can be confident of the findings as TEM and TEM% values were below consensual norms of 3 mm for height, 2 mm for body circumferences, and 1% or 5% for skinfold thicknesses. [336] [337] The standard deviation of HAZ reduced over the three time points, which may be a true phenomenon or potentially due to a selection bias. For example, we may have found less children in the tails of the distribution. This is possible, but unlikely, as there was little indication of it when considering children lost to follow-up. The difference may also be due to measurement error. Length/height measurement in newborns and at two years is much more difficult to perform than at eight years. Similar data on measurement error are not available for comparison. While potentially leading to a wider standard deviation, it would not necessarily lead to a change in the mean value as the error could occur in both directions.

A potential limitation was that the references used to calculate anthropometric z scores differed from those for children under five years used in the trial and previous follow-up.[25] This might have led to inconsistencies, but the references for older children are based on statistical extrapolation from the younger ranges and were designed for compatibility. In addition the ranking of children should stay the same.

6.3 MN Anthropometry and blood pressure

6.3.1 Analysis

6.3.1.1 Primary analysis: unadjusted comparison of allocation groups

The primary analysis compared allocation groups with t tests and univariable regression models by intention-to-treat.

6.3.1.2 Secondary analysis

A. Multivariable regression

Although allocation was balanced, I adjusted for potential confounders to increase precision. To choose possible confounders, I built a causal diagram and used the rules set out in section 2.10 to identify variables to include in multivariable models. In the main analysis, controlling for confounding was not essential as the antenatal MMN were randomised (with balanced allocation) and appropriately blinded. The intervention and control groups appeared to be similar for most of the information we collected, but adding in covariates using the causal diagram can help to improve precision in linear models (and potentially efficiency in non-linear models). [400] Adjustment would also act to increase the statistical power.[401]

The causal diagram is only an approximation and at times a gross simplification of reality. It can, however, play a useful role in determining the relationship between variables and suggestions for which factors should be controlled for. Importantly, it also helps to prevent over-adjustment by controlling for things that are unnecessary or are mediating factors. The structure of the causal diagram was based on *a priori* knowledge. I created it using the data from the previous trial and follow-up and my reading of the subject matter. The diagram was based on the hierarchical models produced by Victora et al[402] and, more recently, Fenske et al[403]. My model is similar to these, but goes further in showing the actual relationships between the factors, albeit mostly limited to the data available. Fenske et al undertook reviews of the literature to identify the factors to include in their conceptual model of stunting. They divided the factors into underlying, intermediate and immediate determinants, broadly similar to the hierarchical levels described by Victora et al.

- Underlying - maternal, household and regional characteristics
- Intermediate – household food competition, water, sanitation, hygiene, indoor air pollution, healthcare and infections (both chronic and recurrent), nutrition (breastfeeding, complementary feeding, micronutrient deficiency)
- Immediate - IUGR, inadequate caloric nutrient intake and uptake

They then estimated the effects of each of the components through a complex statistical approach involving quantile regression and boosting (a statistical inference method for complex models), using data from the Indian National Family Health Survey 2005/2006. They confirmed sex and age as being important non-modifiable factors. Both were incorporated in the analyses by conversion of the data to z-scores. They also identified wealth, maternal education and maternal BMI.[403] Wealth was included as an asset score. Maternal BMI was considered a possible effect modifier in the meta-analyses by Fall[17] and Haider[21]. Other factors such as religion, partner's occupation, sex of household head, urban/rural location, number of household members, drinking water, meal frequency by age, and iron supplementation were not found to be important determinants of stunting. [403]

The diagram was passed to all involved in the design and analysis of the study and to independent researchers at the Institute for Global Health for corrections, refinements and comments. The final diagram is shown in Figure 40. A number of directed acyclic graphs were produced from it for specific outcomes, as described in the individual analyses. I discussed each of the diagrams with Dr Rhian Daniel, the statistician who advised on the choice of confounders. The diagrams were not used for choosing effect modifiers.

This conceptual model was used as the basis for the multivariable regression analyses in this research. It was designed primarily around the information collected in the study, but also included a few other important variables. Multivariable linear regression models based on the diagram included covariates describing air pollution (24 hr-TWA), dietary diversity (HDDS score), food security (HFIAS score), maternal education (no education, primary or secondary and above), maternal height, household asset score and residence (urban or rural). Adjustment for maternal height was not considered essential, but was included in the model to augment information on diet and socioeconomic status. Maternal BMI was not included as it was not measured at an optimal time (rather than pre-pregnancy, BMI was measured during pregnancy and at the two-year follow-up) and would go into the same box as parental height. I included covariates for maternal education and residence to offset the potential effects of differential loss to follow-up.[340]

Figure 40 was designed specifically for this study. It should be used with caution in other research as the *a priori* assumptions on which the model is based may not be universally applicable. Some of the arrows are likely to be appropriate in any setting - for example, anthropometry of boys and girls would be different everywhere - but other arrows are context-specific. An example is the arrow from SES to maternal education. In some settings maternal education would lead to a change in SES and is sometimes considered part of the same construct. I assume here that, primarily due to the lack of social mobility, SES of the

family or household is a more important factor in determining the level of education the female members of the family receive. The model would be the same or similar in other parts of the Terai, but would be different if the population in question were from the capital city, Kathmandu. While not directly generarisable to other contexts, the diagram in Figure 40, could be used as a starting point upon which similar models are constructed.

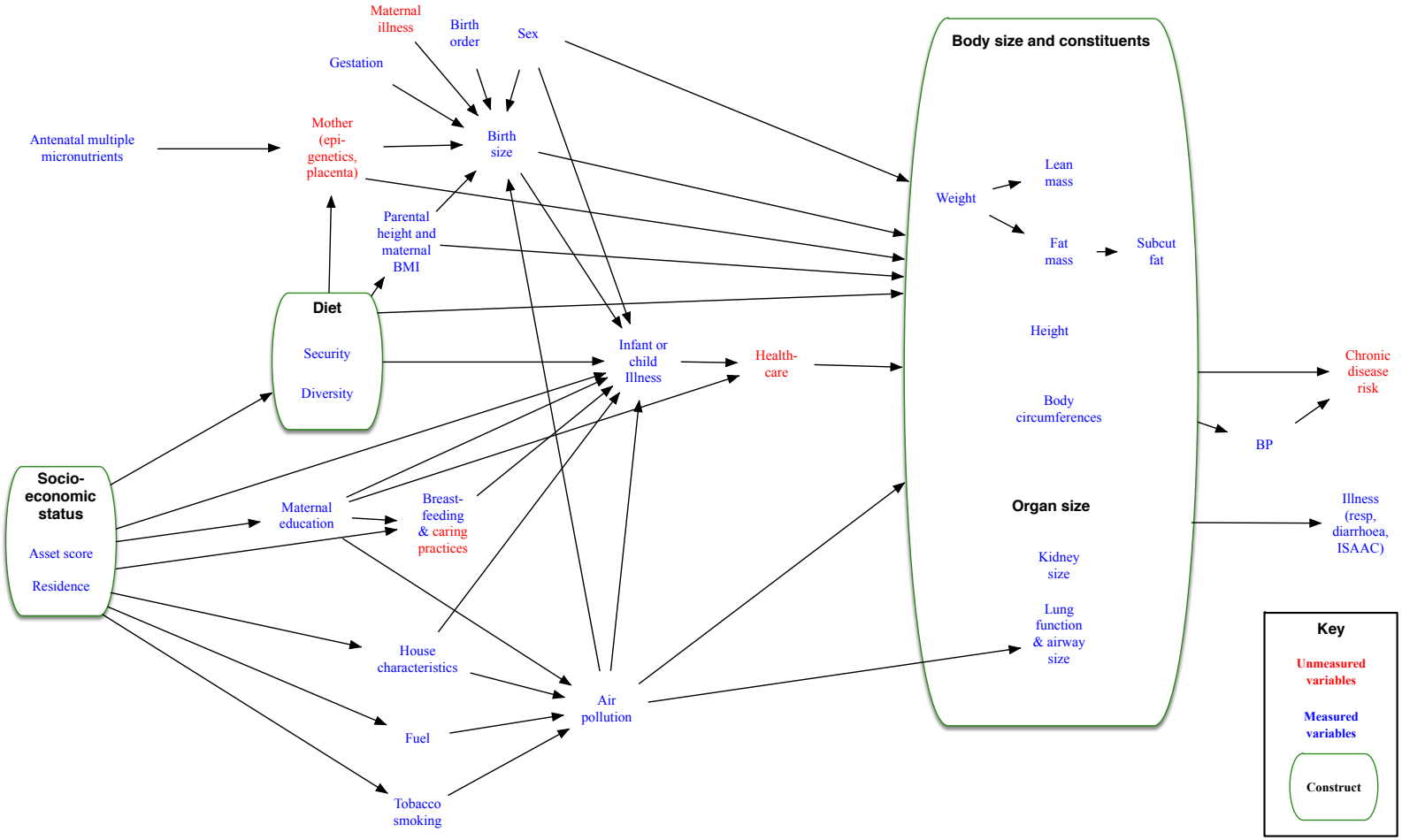

Figure 40: Conceptual model

Assumptions in linear regression

Multivariable linear regression models were created for the outcome variables and the inherent assumptions were tested for linearity, normality, homoscedasticity, leverage and multi-collinearity.[404]

Linearity

The association between predictor variables and outcome variables should be linear when using linear prediction models. This was tested by plotting the residuals against each covariate separately, which should create a random scatter around the zero value. An example of graphs from the multivariable regression for weight-for-age z scores is shown in Figure 41.

Normality

A normal distribution of the variables is required for hypothesis testing, but not for regression analysis. Unbiased coefficients can still be obtained with non-normal data. Rather, the residuals, or errors, should be identically and independently distributed. This was checked by looking at a kernel density plot of residuals, an example of which is shown in Figure 42. As linear regression is quite robust to non-normality, the decision was made to not transform the data.

Homoscedasticity

Linear regression assumes homogeneity of the variance of residuals, i.e. a constant error of variance. If a pattern exists, it is said to be heteroscedastic. This can be shown graphically using the rvfplot command in Stata, which plots residuals against predicted values. An example from the multivariable regression for WAZ is shown in Figure 43. Homoscedasticity can also be tested with the Breusch-Pagan test of the null hypothesis that there is homogeneity of residuals. Heteroscedasticity was thought to exist for most outcomes, so robust standard errors were used.[405] Robust standard errors produce wider confidence intervals and are therefore more conservative.

Leverage

Data points that differ greatly from the mean value may have a large influence on a regression. The influence of a data point is also a product of the degree to which it is an outlier, i.e. how large the residual values are. Other than removing children with chronic diseases as discussed previously, even if a data point had high leverage, it did not seem justified to remove it from the analyses.

Multi-collinearity

Collinearity occurs when predictor variables have a near-perfect linear association. This makes the regression model unstable, increasing the standard errors. Multi-collinearity of the multiple regression models was checked by calculating the variance inflation factor (vif). Vif scores of greater than ten may indicate multi-collinearity. Tests of all models showed scores of <2, indicating that multi-collinearity was unlikely to exist.

Independence

We assumed that individuals were independent.

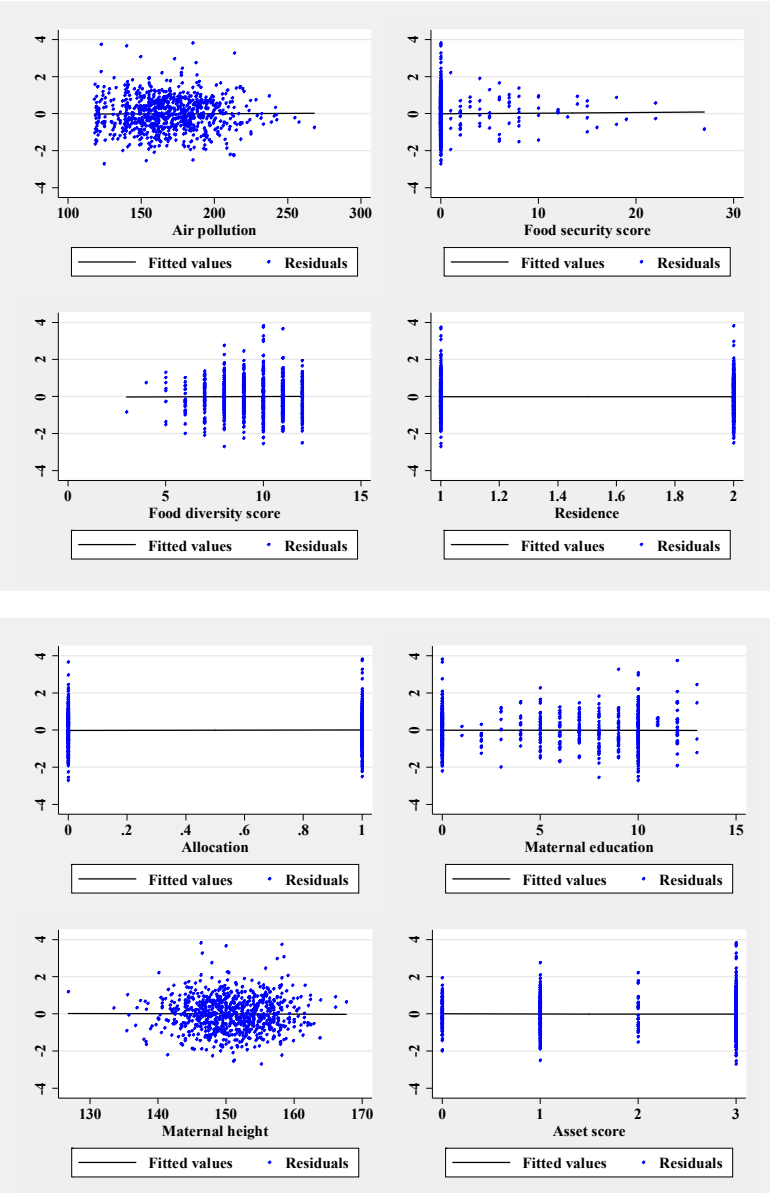

Figure 41: Tests for linearity: plots of each covariate against residuals from the multi-variable regression of weight-for-age

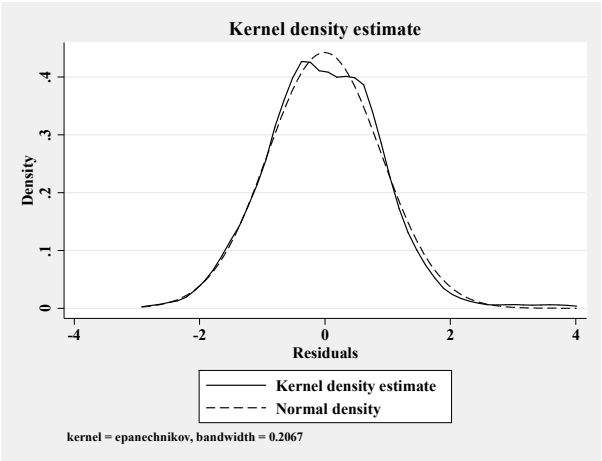

Figure 42: Kernel density plot of residuals for multivariable regression for weight-for age z scores

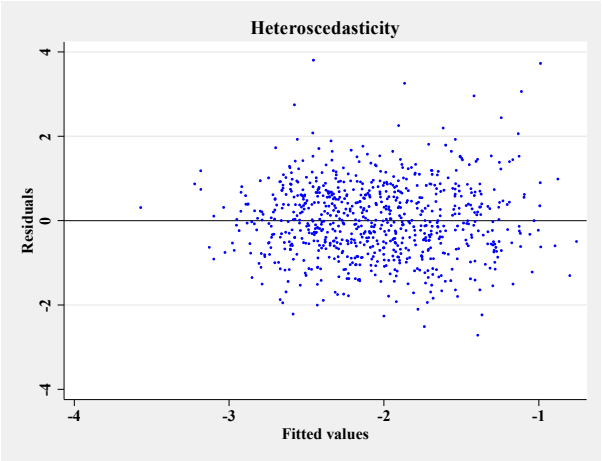

Figure 43: Scatterplot of residuals against fitted values for multivariable regression for weight-for-age z scores

B. Secondary analysis: Exclusion of children with illness

Prior illness may affect childhood growth, and children with major or chronic illness were excluded as described in section 6.2.

C. Secondary analysis: adjusted for birth size

The direct effects of antenatal multiple micronutrient supplementation, not mediated by birth size, were investigated for the main outcomes. By removing the effect of birth size, the independent effect of MMN on anthropometry at 8.5 years can be seen. Based on the causal diagram in Figure 40, this was done by adding birth anthropometry indices to the multi-variable regression models (including maternal height, air pollution, food security and dietary diversity) and comparing them with models that did not include birth indices.

D. Secondary analysis: conditional growth

Conditional growth was calculated for two time points - 2.5 years and 8.5 years - to examine the difference between actual and expected growth based on previous measurements. A positive value represented growth faster than expected. I regressed current size on previous measures using the method described by Adair et al.[406] Conditional relative height takes into account previous height and weight measures, while conditional weight accounts for previous height and weight, and also current height. Standardised residuals were used as the outcomes in univariable and multivariable linear regression models.

E. Effect modification

Sex

I stratified the results by sex to look for a different magnitude of effect in girls and boys as a difference was seen at birth.

Maternal BMI

Maternal BMI was considered a possible effect modifier on birth outcomes in the meta-analyses by Fall et al[17] and Haider and Bhutta[21].

6.3.2 Results

6.3.2.1 Primary analyses

The main trial profile is shown in Figure 16. Figure 44 shows the number of children included in each aspect of the analysis. Not shown are the missing data for particular outcomes, as described in Section 4.3: 213 BIA measurements (104 control and 109 intervention), one kidney ultrasound (intervention) and one skinfold thickness (intervention).

The primary unadjusted analyses showed no difference between children whose mothers received antenatal MMN or the control of iron and folate. Unadjusted differences (intervention minus control) with p-values from independent t tests were 0.047 z scores (95% CI -0.093, 0.186; p 0.51) for WAZ, 0.023 z scores (95% CI -0.104, 0.150; p 0.72) for HAZ and 0.043 z scores (95% CI -0.088, 0.175; p 0.52) for BAZ. There was no difference in systolic (0.017 mmHg; -1.016, 1.051; p 0.97) or diastolic blood pressure (0.128 mmHg; -0.932, 1.187; p 0.81). Table 36 shows a comparison with the birth and 2.5 year data.

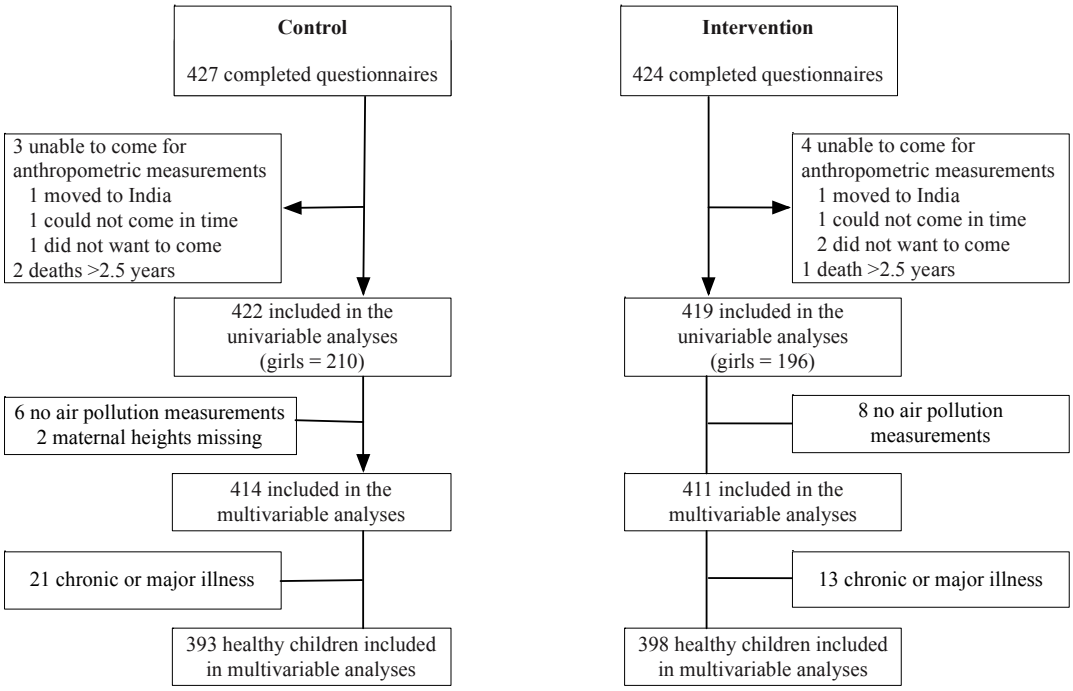

Figure 44: Flow chart for anthropometry analysis

Table 36: Anthropometric indices at birth, 2.5 and 8.5 years, by allocation

				Control	Intervention
		Sample	Frequency <-2 SD (%)	Mean (95% CI)	Mean (95% CI)
Birth*	WAZ	1044	186 (17.9)	-1.28 (-1.35, -1.19)	-1.08 (-1.17, -1.00)
	HAZ	1016	91 (9.0)	-0.41 (-0.52, -0.30)	-0.34 (-0.45, -0.24)
	BAZ	1002	348 (34.8)	-1.63 (-1.74, -1.52)	-1.42 (-1.53, -1.31)
2.5 years	WAZ	915	340 (37.2)	-1.76 (-1.85, -1.67)	-1.62 (-1.72, -1.53)
	HAZ	915	537 (58.8)	-2.29 (-2.39, -2.19)	-2.20 (-2.31, -2.10)
	BAZ	915	54 (5.9)	-0.40 (-0.50, -0.30)	-0.29 (-0.39,-0.18)
8.5 years	WAZ	841	444 (52.8)	-2.08 (-2.18, -1.98)	-2.03 (-2.13, -1.93)
	HAZ	841	242 (28.8)	-1.51 (-1.59, -1.42)	-1.48 (-1.57, -1.39)
	BAZ	841	293 (34.8)	-1.67 (-1.76, -1.58)	-1.63 (-1.72, -1.53)

BMI: body mass index; WAZ: weight-for–age; HAZ: height-for-age; BAZ: BMI-for-age

*Excluding birth data <-4.5 and >3 z scores

6.3.2.2 Secondary analyses

A. Multivariable regression

Multivariable regression models tended to increase the effect size, but the results did not reach statistical significance. The only outcome that did was head circumference, at 0.19 cm difference (95% CI 0.02, 0.37). The results are shown in Table 37. Adjusting for current size (in the form of WAZ, HAZ, BAZ, lean mass or fat mass) for blood pressure did not make an important difference to the results.

B. Exclusion of children with illness

Contrary to our initial assumption, excluding children with prior history of major or chronic illness tended to reduce the effect size slightly. The difference was small and the number of children excluded was only 34.

C. Adjustment for birth size

There was no difference between allocation groups due to the direct effects of antenatal multiple micronutrient supplementation, not mediated by birth-size, on z score: 0.044 (95% CI -0.083, 0.171) for WAZ, 0.056 (95% CI -0.060, 0.171) for HAZ, and 0.054 (95% CI -0.075, 0.183) on BAZ.

Table 37: Child anthropometric indices by allocation, showing mean values, unadjusted, and adjusted differences

		Control Mean (SD)	Intervention Mean (SD)	Unadjusted difference (95% CI)	Adjusted difference * (95% CI)	Adjusted difference restricted to children without major or chronic illness (95% CI)
Weight (kg)	Weight	20.04 (3.31)	20.14 (3.35)	0.10 (-0.35, 0.55)	0.30 (-0.08, 0.67)	0.25 (-0.12, 0.63)
	Lean mass	17.34 (2.44)	17.30 (2.49)	-0.05 (-0.43, 0.34)	0.10 (-0.23, 0.43)	0.08 (-0.25, 0.42)
	Fat mass	3.01 (1.6)	2.94 (1.58)	-0.07 (-0.32, 0.18)	0.02 (-0.21, 0.25)	0.05 (-0.18, 0.27)
Height (cm)	Standing	120.72 (5.91)	120.73 (6.06)	0.00 (-0.81, 0.81)	0.35 (-0.31, 1.01)	0.27 (-0.38, 0.93)
	Trunk length	64.14 (2.94)	64.16 (2.96)	0.02 (-0.38, 0.42)	0.14 (-0.20, 0.48)	0.07 (-0.28, 0.41)
z score	Weight-for-age	-2.08 (1.01)	-2.03 (1.06)	0.05 (-0.09, 0.19)	0.09 (-0.03, 0.22)	0.07 (-0.06, 0.19)
	Height-for-age	-1.51 (0.92)	-1.48 (0.96)	0.02 (-0.10, 0.15)	0.06 (-0.05, 0.17)	0.05 (-0.06, 0.16)
	BMI-for-age	-1.67 (0.96)	-1.63 (0.98)	0.04 (-0.09, 0.18)	0.07 (-0.06, 0.20)	0.05 (-0.08, 0.17)
Skinfold thickness (mm)	Triceps	7.39 (2.56)	7.36 (2.41)	-0.03 (-0.37, 0.31)	0.07 (-0.24, 0.38)	0.10 (-0.20, 0.37)
	Biceps	3.95 (1.34)	3.95 (1.40)	-0.01 (-0.19, 0.12)	0.06 (-0.11, 0.23)	0.08 (-0.10, 0.25)
	Subscapular	4.91 (1.29)	4.93 (1.51)	0.01 (-0.18, 0.20)	0.06 (-0.12, 0.24)	0.07 (-0.11, 0.26)
	Supra-iliac	5.76 (2.54)	5.57 (2.35)	-0.19 (-0.52, 0.14)	-0.11 (-0.42, 0.21)	-0.06 (-0.36, 0.25)
Body circumference (cm)	Head	49.19 (1.48)	49.37 (1.47)	0.18 (-0.02, 0.38)	0.19 (0.02, 0.37)	0.15 (-0.02, 0.32)
	Chest	55.59 (3.39)	55.74 (3.64)	0.15 (-0.33, 0.63)	0.28 (-0.14, 0.71)	0.27 (-0.16, 0.70)
	Waist	49.01 (3.76)	49.20 (3.96)	0.19 (-0.33, 0.71)	0.29 (-0.19, 0.77)	0.23 (-0.25, 0.71)
	Hip	57.30 (4.00)	57.36 (4.11)	0.07 (-0.48, 0.61)	0.33 (-0.14, 0.81)	0.26 (-0.22, 0.73)

	Upper leg	31.11 (2.91)	31.21 (2.91)	0.10 (-0.30, 0.49)	0.26 (-0.09, 0.61)	0.21 (-0.14, 0.56)
	Mid-upper arm	15.94 (1.40)	15.99 (1.38)	0.04 (-0.15, 0.23)	0.11 (-0.06, 0.27)	0.10 (-0.07, 0.26)
Renal dimension (cm)	Right length	7.90 (0.55)	7.89 (0.57)	-0.01 (-0.08, 0.07)	0.00 (-0.07, 0.07)	-0.01 (-0.08, 0.06)
	Right antero–posterior distance	2.98 (0.26)	3.00 (0.28)	0.02 (-0.01, 0.06)	0.02 (-0.01, 0.06)	0.02 (-0.02, 0.06)
	Left length	8.25 (0.57)	8.22 (0.58)	-0.03 (-0.11, 0.05)	-0.03 (-0.10, 0.05)	-0.05 (-0.12, 0.03)
	Left antero–posterior distance	3.29 (0.32)	3.30 (0.32)	0.02 (-0.03, 0.06)	0.02 (-0.03, 0.06)	0.01 (-0.03, 0.06)
Blood pressure (mmHg)	Systolic	98.06 (7.14)	98.08 (8.10)	0.02 (-1.02, 1.05)	-0.06 (-1.10, 0.98)	-0.20 (-1.23, 0.83)
	Diastolic	61.16 (7.36)	61.29 (8.27)	0.13 (-0.93, 1.19)	0.19 (-0.87, 1.25)	0.15 (-0.92, 1.22)

*Multivariable regression models included variables describing air pollution, dietary diversity, food security, maternal education and height, household asset score, and residence, using robust standard errors. Age and sex were included if not intrinsic to z score.

D. Conditional growth results

In general, there was a tendency for positive effect sizes up to 2.5 years and negative effect sizes up to 8.5 years on both WAZ and HAZ, but these did not reach statistical significance.

Table 38: Conditional growth: effect of antenatal multiple micronutrient supplements on growth at different time points

	2.5 years		8.5 years	
	Unadjusted difference (95% CI)	Multivariable regression * (95% CI)	Unadjusted difference (95% CI)	Multivariable regression * (95% CI)
WAZ	0.08 (-0.06, 0.22)	0.09 (-0.05, 0.24)	-0.06 (-0.20, 0.08)	-0.04 (-0.18, 0.09)
HAZ	0.05 (-0.10, 0.18)	0.08 (-0.04, 0.21)	-0.10 (-0.24, 0.04)	-0·09 (-0.12, 0.04)

* Multivariable regression models included variables describing air pollution, dietary diversity, food security, maternal education and height, household asset score and residence, using robust standard errors.

E. Effect modification

Sex

Table 39 shows stratification by sex, with birth and 2.5 year data for weight shown for comparison in Appendix 6.3. In boys, there was no difference in anthropometry outcomes. While there were no statistically significant differences, in girls there was a tendency towards increased weight. There was a 0.17 (95% CI -0.01, 0.35) z score difference in WAZ, equivalent to approximately 500 g. When formally tested, there was no evidence of interaction (p=0.24).

Maternal BMI

No difference was seen due to effect modification by maternal BMI (categorised as <18.5 and >18.5 kg/m^2). For mothers with BMI<18.5, WAZ was 0.15 (95% CI -0.06, 0.37), while for mothers with BMI>18.5, WAZ was 0.06 (95% CI -0.09, 0.21).

Table 39: Child anthropometry by allocation group, by child sex

		Control Mean (SD)	Intervention Mean (SD)	Unadjusted difference (95% CI)	Multivariable regression * (95% CI)	Multivariable regression restricted to children without major or chronic illness (95%CI)
Weight	Girls	19.55 (2.96)	19.71 (3.15)	0.16 (-0.44, 0.75)	0.51 (-0.03, 1.04)	0.51 (-0.03, 1.06)
	Boys	20.53 (3.57)	20.53 (3.49)	-0.00 (-0.67, 0.66)	0.101 (-0.43, 0.63)	0.01 (-0.51, 0.53)
Height	Girls	120.40 (5.92)	119.98 (5.93)	-0.42 (-1.57, 0.74)	0.33 (-0.65, 1.32)	0.44 (-0.51, 1.39)
	Boys	121.05 (5.89)	121.38 (6.12)	0.33 (-0.80, 1.47)	0.36 (-0.55, 1.27)	0.14 (-0.78, 1.06)
WAZ	Girls	-2.14 (0.92)	-2.02 (1.02)	0.12 (-0.07, 0.31)	0.17 (-0.01, 0.35)	0.17 (-0.01, 0.35)
	Boys	-2.02 (1.09)	-2.04 (1.09)	-0.02 (-0.23, 0.18)	0.03 (-0.15, 0.21)	-0.02 (-0.19, 0.16)
HAZ	Girls	-1.55 (0.91)	-1.53 (0.93)	0.02 (-0.16, 0.20)	0.06 (-0.11, 0.23)	0.08 (-0.08, 0.24)
	Boys	-1.47 (0.93)	-1.44 (0.98)	0.03 (-0.15, 0.21)	0.06 (-0.10, 0.22)	0.02 (-0.14, 0.18)
BAZ	Girls	-1.73 (0.87)	-1.58 (0.97)	0.15 (-0.03, 0.33)	0.18 (0.00, 0.36)	0.16 (-0.02, 0.34)
	Boys	-1.61 (1.04)	-1.67 (0.99)	-0.06 (-0.25, 0.13)	-0.02 (-0.20, 0.16)	-0.05 (-0.23, 0.13)

6.3.3 Discussion

6.3.3.1 Main results

At follow-up at 8.5 years, we saw no differences in anthropometric outcomes (with or without adjustment for birth size), conditional relative growth, or blood pressure between groups whose mothers were allocated antenatally to either multiple micronutrient or iron and folic acid supplements. In addition, we found no difference in body composition measured by BIA and skinfold thickness, suggesting neither a global difference in lean or fat mass nor a difference in their distribution.

Because of balanced allocation, the primary analysis was unadjusted. We developed adjusted models from a conceptual diagram whose assumptions are uncertain in view of the complexity of childhood growth as an outcome. We used causal diagram analysis to make them explicit, and attempted to adjust for important variables, without over-adjustment.

Overall, the similar follow-up studies have not shown a lasting difference in anthropometry in children born to mothers taking antenatal multiple micronutrient supplements. In Burkina Faso, Roberfroid et al showed increased length-for-age and weight-for-age z scores after one year, but the difference had disappeared by 2.5 years.[259] In China, Wang et al found no difference in wasting, stunting and underweight at 30 months of age.[262] In Bangladesh, Khan et al found no difference in weight or body composition at 54 months of age, but reported lower linear growth than in the iron and folic acid control group.[260,261]

The only outcome that showed a difference between allocation groups was head circumference in multivariable analysis. Is the difference in head circumference a true result? One could argue not: I would be cautious in over-interpreting one outcome amongst the many tested, the difference was only 2 mm (very close to our detection limit), the univariable regression results did not show a statistical difference and other body circumferences showed no difference. On the other hand, one might say that it is important in that we are confident in anthropometry measurements as highlighted by the low error values, multivariable regression may be more likely to produce an accurate answer by reducing the effect of confounding and the difference was very similar in absolute terms to that found at 2.5 years (potentially indicating canalisation of this trait). If we were to apply a correction for multiple testing, the result would become insignificant. Whether or not to do this is a point of debate amongst statisticians. Researchers, deliberately or not, search for interesting (usually positive) results that are more likely to be published, and multiple testing could lead to false positive results. Contrary to this, having an *a priori* hypothesis, to which we attach importance by making it our primary outcome, is somewhat arbitrary and to not use the data to their full extent seems wasteful. In this case I consider most of the outcomes to be measuring the same thing - body

size and composition (the only one that may be different is blood pressure as this also comes under hormonal and nervous control) - and in this analysis it is a moot point as the results were null: applying a Bonferroni correction simply increased the p value. My personal view is that correction for multiple testing is over-cautious, but I would recommend that the results of each analysis conducted should be shown so that the reader can put them into context.

We assume that the previous follow-up showed a true difference between intervention and control groups in early life: the trial was of high quality and the results in keeping with similar trials. There are therefore two interpretations of the current results: (1) the difference had disappeared by mid-childhood, or (2) any differences were not manifest in our indicators (we were unable to find them). If (1) is correct and there were no lasting effects of antenatal MMN, it may be that their effects were transient, for example through reversible epigenetic changes. Methylation early in life tends to be irreversible, but it may be that any changes happened too late to modify growth trajectory or are, in fact, reversible. Any beneficial biological consequences of the supplement may have been vitiated by the detrimental effects of the environment. The second possibility is that long-term physiological effects were present but undetected. Our follow-up rates were high and measurement error was low so, other than by chance, it would seem unlikely that there was a large effect that we were unable to find. It is conceivable that the difference in anthropometry was still present, but had diminished over time.

Anthropometric differences may emerge at subsequent follow-up as the current ages of cohorts across all the trials may be insufficient to see effect. At seven-to-nine years of age, children are in their lowest growth rate period and the groups may diverge again in adolescence. There is some evidence for this from a study in baboons, in which an effect of changing weaning diet was manifest in females only in adolescence.[407]

6.3.3.2 Mediation and conditional relative growth

The results seem to show no direct effects of antenatal MMN after removing the effect on birth size. Looking at the conditional growth changes, the weight- and height-for-age outcomes did not show a statistical difference, but the 2.5 year data tended to show positive effect measures, while at 8.5 year findings were negative. Presumably the MMN had a positive effect on growth in-utero (to produce a difference in weight at birth), which diminished over the first two years and then became negative in later childhood, allowing the control group to catch-up in size.

6.3.3.3 Blood pressure

At 2.5 years, there was a 2.5 mmHg (95% CI: 0.5, 4.6) lower blood pressure in the MMN group, but in this follow-up we did not find a difference in systolic or diastolic blood pressure or in kidney dimensions. The mechanism by which birthweight relates to later blood pressure may be via its relationship with kidney size or, more specifically, nephron number, which is hypothesized to be related to essential hypertension.[225] Presumably, as we found no difference in kidney size and body composition, blood pressure should also be the same. However, the relationship between nephron number and hypertension is not straightforward as compensatory hyperfiltration can occur, maintaining a lower pressure when there is a lower nephron number. [231]

We cannot say whether the two-year results were a true effect or one that occurred by chance. The practicalities of measuring blood pressure in two-year-olds in a field setting make it likely that there was a higher degree of error. The mean blood pressure was higher than the 8.5 year measurements and the standard deviation was approximately two-and-a-half times as large. This should, however, have been the same for both trial groups.

6.3.3.4 Effect modification

Though not statistically significant, when stratifying by sex, there was a tendency for a larger effect of MMN in girls. This was seen at birth in the secondary analysis: girls +108 g (95% CI 36, 179 g) and boys +49 g (95% CI -33, 122 g). A different effect might be biologically plausible, for example through sex-specific DNA methylation from alterations in maternal diet,[408-410] smoking,[411] or through differential fetal mortality. However, the test for interaction was negative and the study was not powered to find a difference in sex, so I would be cautious in the interpreting it. Other trials have been inconclusive. Friis et al showed a "marginally significant" interaction with length,[273] Stewart et al showed a non-significant difference in height-for-age z scores, with boys being taller,[268] and Roberfroid et al showed no interaction.[253]

Maternal BMI also showed no difference, but the effect size was a little larger for mothers with BMI <18.5. This analysis was exploratory in that it was not powered to find an interaction and the maternal BMI variable may not have been accurate. An interaction cannot, therefore, be ruled out. Stratification reduces the sample size in each group, leading to wider confidence intervals and to find an interaction the sample size needs to be approximately four times larger. A further limitation was that we did not have pre-pregnancy maternal BMI, but

used maternal BMI from the 2-year follow-up, assuming that maternal BMI would not have changed dramatically within the time frame.

6.3.3.5 Generalisability

Generalisation of the findings may be limited by non-representative recruitment to the original trial or differential loss to follow-up. It is possible that women who chose to participate differed systematically from those who did not. Although we adjusted internally for socioeconomic status, rural participants may have been from more affluent groups who could afford to travel to the urban hospital for antenatal care.

In summary, the anthropometric findings showed that the difference in weight, present at birth and at two years, was not evident at eight years of age. These results do not rule out a future difference in anthropometry emerging, nor do they rule out health benefits, but they do suggest no lasting effects of antenatal multiple micronutrient supplementation on weight in mid-childhood.

6.4 Respiratory results

On the basis of a similar trial of antenatal vitamin A supplementation in humans,[177] and of animal studies,[176,184] I hypothesised that children who had received antenatal MMN supplementation would have better spirometric lung function at 8 years of age than control children.

6.4.1 Analysis

Where possible, we did not perform spirometry if the child was unwell. Six children were unable to perform spirometry: five had developmental delay and one poor co-ordination. Spirometry data from a further 42 (5.0 %) children were excluded because of poor technique, representing an overall failure rate of 5.7%. I examined all spirometry curves and chose FEV_1, FVC and FEF_{25-75} values as described in section 3.12. To confirm that any low results were not due to poor technique, I re-examined all 119 results where the values were <1 litre. The trial profile for the respiratory analysis including exclusions is shown in Figure 45.

For the restricted analysis, children were excluded due to illness:

1. Acute illness or symptoms during spirometry: n=8.
2. Chronic or major illness (asthma, congenital heart disease, hydronephrosis, nephritic syndrome, recurrent pneumonia, rheumatic fever, single kidney and tuberculosis), including an ISAAC asthma diagnosis or wheezing/whistling in the past year: n=51.
3. History of pneumonia requiring hospital treatment (2 possible bronchiolitis, 2 neonatal pneumonia and 47 pneumonia classified as major illness): n=51.

In total, 95 children were excluded (some children had multiple illnesses).

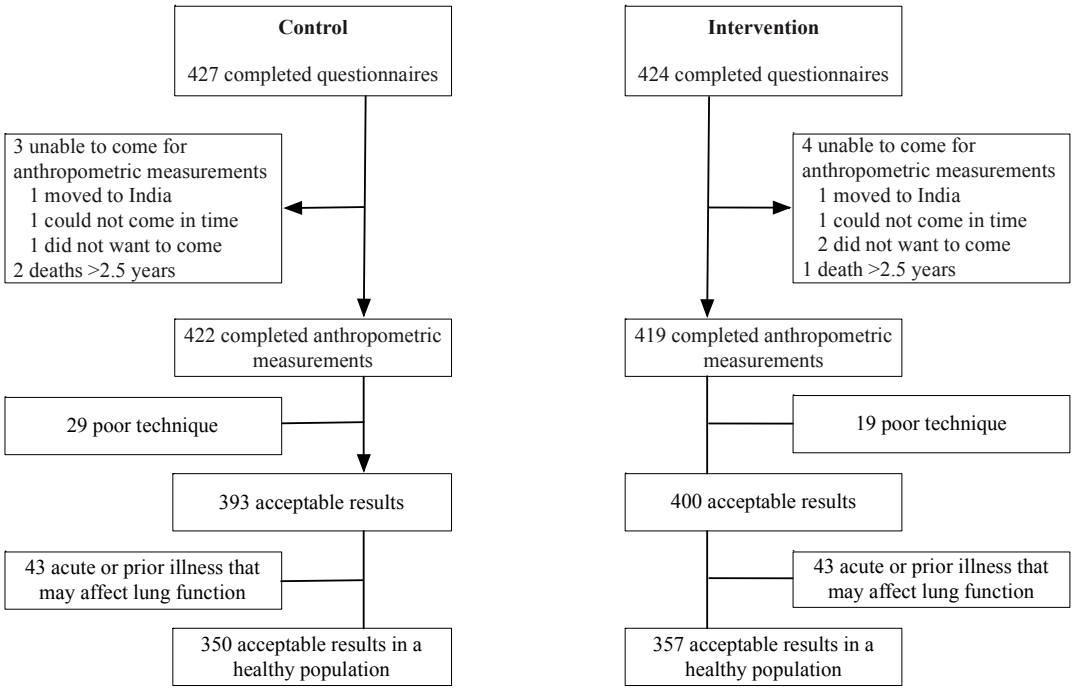

Figure 45: Flow chart for respiratory analysis

6.4.1.1 Primary analysis

Lung function varies with age, sex, height and ethnic group. Lung function data were therefore adjusted for sex, age and height at time of testing using the Global Lung Function Initiative (GLI-2012) multi-ethnic, 'all-age' reference ranges, based on equations produced recently because lung function is known to differ by ethnic group. [26] These cover Caucasians, African-Americans, East-Asians ('North' and 'South East Asia'). An 'Other/Mixed' category was also created from a composite of the four equations. As specific reference ranges do not yet exist for South Asia, the data were applied to each of the reference range equations using data from 'healthy' children to ascertain best fit, based on the fact that mean (standard deviation) spirometry z-scores in a healthy population should approximate 0 (1). Figure 46 shows the results graphically and the data can be seen in Table 1, Appendix 6.4. A poster describing this work was presented at the European Respiratory Society annual conference 2013.

None of the reference ranges were perfect. The African-American GLI equation provided the best fit for FEV_1 (mean (SD) 0.09 (0.86) z scores) and the SE Asia equation was slightly better for FVC (Figure 1). The 'NE Asia' and 'Other' equations were found to be inappropriate. FEV_1/FVC was interpreted appropriately using all the equations except the equation for SE Asia. After consultation with Professor Janet Stocks, who advised on the

respiratory aspect of the project, it was decided that, in the absence of South Asian equations, the Caucasian ones should be used for group comparisons as these are based on the largest amount of data and may be considered a normative standard.

Primary analysis was based on all children with technically acceptable spirometry results. The association between birthweight and child lung function was first investigated. Data were examined by allocation group, the distribution of the variables was assessed for normality, and allocation groups were compared with two-tailed t tests.

6.4.4.2 Secondary analysis

The secondary analysis excluded children with acute or chronic illness or prior pneumonia requiring hospitalisation, adjusted for potential confounders and looked at effect modification by sex. A directed acyclic graph was created showing our *a priori* assumptions about the causal relationship between the variables in the study (Figure 47). It was used to decide which variables to control for in the multivariable regression to increase the precision of the result. I developed multivariable linear regression models adjusting for air pollution, food access and diversity, maternal education and height, household asset score, residence and maternal height (the latter to augment information on diet and socioeconomic status). I also included covariates for maternal education and residence to offset the potential effects of differential loss to follow-up[340], and a term identifying the spirometer used.

The model assumptions were tested as in section 6.2. Since distribution of FEV_1/FVC was heteroscedastic, robust standard errors were applied to the regression models for this outcome. All analyses were conducted as intention to treat.

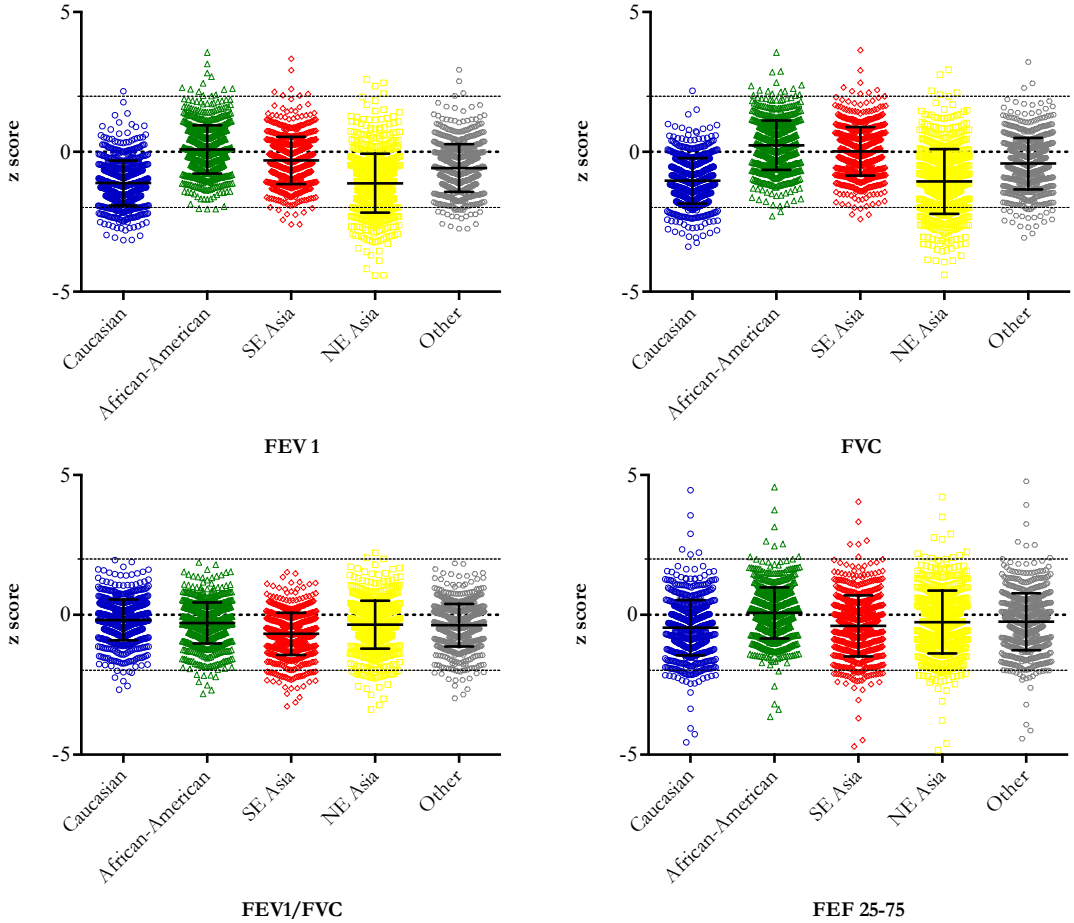

Figure 46: Comparison of data from Nepalese children according to ethnic-specific GLI equations. Solid horizontal lines = mean (SD)

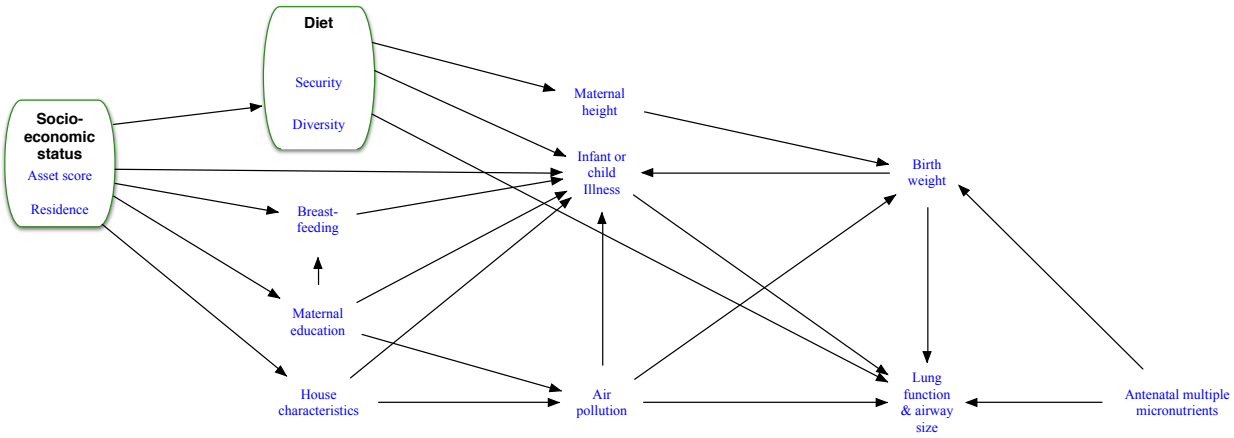

Figure 47: Directed acyclic graph showing association between variables measured.

6.4.2 Results

841 children attempted spirometry. There were no differences between observers for FEV_1 or FVC, nor within the biological control over time. A small difference in FVC was observed between the two spirometers (mean 0.15; 95% CI 0.03, 0.26 z-scores), and this was therefore included in the multivariable model.

6.4.2.1 Exclusions

Although the prevalence of asthma was extremely low (< 2%, see section 6.6), 95 (11.3%) children had evidence of acute or chronic illness that might have affected lung function, some of whom had multiple diagnoses; nine out of 95 (9.5%) also had poor technique. Although lung function was somewhat lower in children who were excluded on health grounds, excluding them had relatively little impact on summary results from either trial group (Table 40). There were no anthropometric or sex differences between children excluded on health grounds and the remaining children.

6.4.2.2 Lung function of entire cohort

When expressed in relation to predicted values for Caucasian children, mean (SD) z-scores for the entire group of 'healthy' Nepalese children with acceptable spirometry were -1.15 (0.8) for FEV_1 and -1.05 (0.8) for FVC, corresponding to reductions of 13.7% and 12.4%, respectively, when compared with healthy Caucasian children (Figure 48). [26] The proportional reductions in both these outcomes meant that the FEV_1/FVC z-score was, however, close to that predicted for Caucasian children (within 1.5%). Data for the whole cohort stratified by sex are shown in Table 2, Appendix 6.4.

6.4.2.3 Birthweight and lung function

Though a great deal of variability existed, there was a positive association between birthweight and childhood lung function. A one z-score increase in birthweight was associated with an increase of 0.10 z scores (95 % CI 0.04, 0.15) in FEV_1 and 0.11 z scores (95% CI 0.06, 0.18) in FVC. Multivariable regression for the effect of birthweight on lung function, controlling for air pollution, dietary diversity, food security, maternal education and height, household asset score and residence, produced similar results. Scatterplots of birthweight against lung function can be seen in Figure 49.

6.4.2.4 Effect of antenatal micronutrients

Anthropometry and lung function results stratified by trial group in those with technically acceptable spirometry, along with those excluded due to illness, are shown in Table 40. The full spirometry results showing exclusion categories by allocation group are shown in Table 3, Appendix 6.4.

Table 41 shows the difference in lung function values between the allocation groups in univariable and multivariable regression analyses. There were no significant differences in lung function between the control and intervention groups. The multivariable regression results in z scores (intervention – control) were -0.08 (95% CI -0.19 to 0.04) for FEV_1, -0.05 (95% CI -0.17 to 0.06) for FVC, -0.04 (95% CI -0.15 to 0.07) for FEV_1/FVC and -0.06 (95% CI -0.20 to 0.09) for $FEF_{25-75\%}$. Adjustment for confounders and exclusion of children on health grounds made little difference to the results. When analysed according to sex, girls in the intervention group had slightly worse FEV_1 than those in the control group, on univariable analysis (-0.18 95% CI -0.34, -0.02. Table 42), but not in the multivariable model. The effect on lung function independent of birthweight in girls was -0.21 (95% CI -0.37, -0.05) in univariable regression. In boys there was no difference (-0.05; 95% CI -0.22, 0.12).

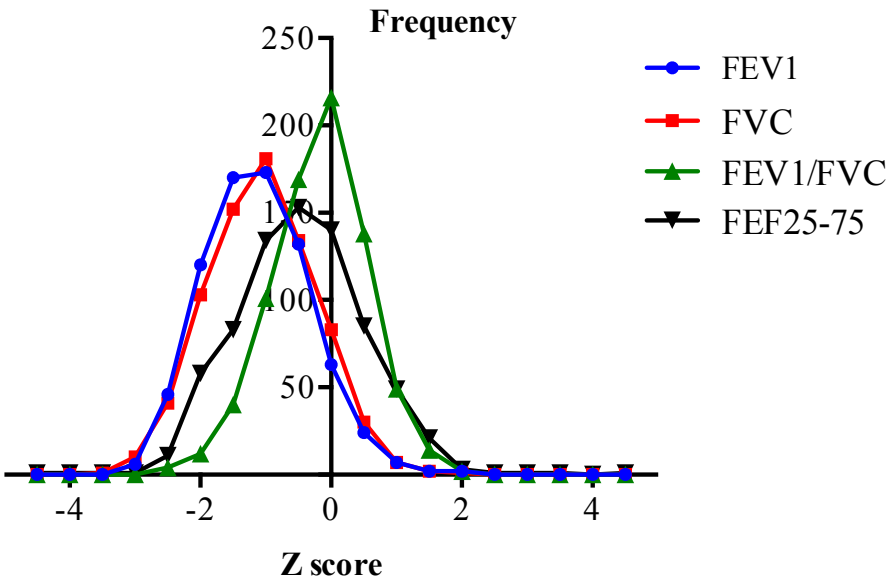

Figure 48: Frequency distributions for spirometry indices.

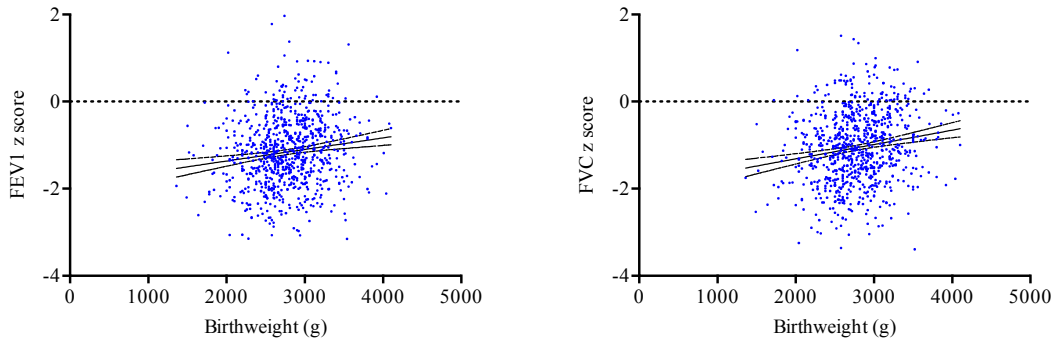

Figure 49: Scatterplots and linear fit for birthweight against FEV_1 and FVC z scores

Table 40: Lung function and anthropometry by allocation group in those with acceptable spirometry

Entire cohort	**Control n=393 Mean (SD)**	**Intervention n=400 Mean (SD)**
Age (years)	8.5 (0.4)	8.4 (0.4)
Weight z-score [a]	-2.1 (1.0)	-2.0 (1.0)
Height z-score [a]	-1.5 (0.9)	-1.5 (0.9)
BMI z-score [a]	-1.7 (0.9)	-1.6 (1.0)
FEV_1 [b]	-1.11 (0.8)	-1.18 (0.8)
FVC [b]	-1.02 (0.8)	-1.07 (0.8)
FEV_1/FVC [b]	-0.20 (0.8)	-0.24 (0.8)
$FEF_{25\%-75\%}$ [b]	-0.48 (1.0)	-0.53 (1.0)
Children without evidence of prior significant disease	**Control n=350**	**Intervention n=357**
Age (years)	8.5 (0.4)	8.5 (0.4)
Weight z-score [a]	-2.1 (0.9)	-2.0 (1.1)
Height z-score [a]	-1.5 (0.9)	-1.5 (0.9)
BMI z-score [a]	-1.7 (0.9)	-1.6 (1.0)
FEV_1 [b]	-1.07 (0.8)	-1.16 (0.8)
FVC [b]	-1.01 (0.8)	-1.05 (0.8)
FEV_1/FVC [b]	-0.15 (0.7)	-0.22 (0.7)
$FEF_{25\%-75\%}$ [b]	-0.42 (1.0)	-0.50 (1.0)
Lung function results in children with technically acceptable data but who had evidence of acute or chronic illness or had been hospitalised for pneumonia	**Control n=43**	**Intervention n=43**
Age (years)	8.5	8.4
Weight z-score [a]	-2.1 (1.3)	-2.0 (0.9)
Height z-score [a]	-1.5 (1.1)	-1.5 (1.2)
BMI z-score [a]	-1.8 (1.2)	-1.6 (0.8)
FEV_1 [b]	-1.44 (1.0)	-1.41 (0.9)
FVC [b]	-1.16 (0.9)	-1.22 (0.8)
FEV_1/FVC [b]	-0.57 (0.9)	-0.34 (1.0)
$FEF_{25\%-75\%}$ [b]	-0.92 (1.3)	-0.77 (1.2)

[a] Anthropometry z-scores calculated according to WHO reference ranges.[25]

[b] Spirometry z-scores calculated according to the Quanjer GLI-2012 spirometry equations based on Caucasian subjects.[26]

Table 41: Comparison of lung function (expressed as z-scores) in children, by allocation group

	Control n=393 Mean (SD)	Intervention n=400 Mean (SD)	t test p for difference	Unadjusted difference (95% CI)	Multivariable regression * (95% CI)	Multivariable regression restricted to children without illness (95% CI) n= 707
FEV_1	-1.11 (0.84)	-1.18 (0.79)	0.41	-0.08 (-0.19, 0.04)	-0.06 (-0.18, 0.05)	-0.07 (-0.19, 0.05)
FVC	-1.02 (0.83)	-1.07 (0.81)	0.63	-0.05 (-0.17, 0.06)	-0.04 (-0.15, 0.08)	-0.03 (-0.15, 0.09)
FEV_1/ FVC	-0.20 (0.75)	-0.24 (0.77)	0.60	-0.04 (-0.15, 0.07)	-0.04 (-0.15, 0.07)	-0.08 (-0.19, 0.03)
$FEF_{25-75\%}$	-0.48 (1.02)	-0.53 (1.02)	0.56	-0.06 (-0.20, 0.09)	-0.05 (-0.20, 0.10)	-0.08 (-0.22, 0.07)

*Multivariable regression includes variables describing air pollution, dietary diversity, food security, maternal education and height, household asset score and residence, using robust standard errors. Analysis restricted to those with technically satisfactory results.

Table 42: Lung function by allocation group, stratified by sex

		Control Mean (SD)	Intervention Mean (SD)	Unadjusted difference (95% CI)	Multivariable regression * (95% CI)	Multivariable regression restricted to children without illness (95%CI)
FEV_1	Girls	-1.18 (0.81)	-1.36 (0.72)	-0.18 (-0.34, -0.02)	-0.15 (-0.31, 0.02)	-0.15 (-0.32, 0.02)
	Boys	-1.04 (0.86)	-1.04 (0.82)	0.00 (-0.16, 0.16)	-0.02 (-0.19, 0.14)	-0.05 (-0.22, 0.12)
FVC	Girls	-1.13 (0.84)	-1.26 (0.82)	-0.14 (-0.30, 0.03)	-0.10 (-0.28, 0.07)	-0.10 (-0.29, 0.08)
	Boys	-0.92 (0.80)	-0.91 (0.76)	0.01 (-0.14, 0.16)	-0.00 (-0.16, 0.15)	-0.00 (-0.17, 0.16)
FEV_1/ FVC	Girls	-0.15 (0.74)	-0.23 (0.80)	-0.08 (-0.23, 0.08)	-0.06 (-0.23, 0.10)	-0.08 (-0.25, 0.09)
	Boys	-0.24 (0.76)	-0.24 (0.76)	-0.00 (-0.15, 0.14)	-0.03 (-0.18, 0.12)	-0.08 (-0.24, 0.07)
$FEF_{25\%-75\%}$	Girls	-0.57 (0.96)	-0.69 (0.95)	-0.12 (-0.32, 0.07)	-0.07 (-0.27, 0.14)	-0.08 (-0.29, 0.12)
	Boys	-0.39 (1.07)	-0.40 (1.07)	-0.01 (-0.22, 0.19)	-0.06 (-0.27, 0.15)	-0.10 (-0.31, 0.11)

*Multivariable regression includes variables describing air pollution, dietary diversity, food security, maternal education and height, household asset score and residence, using robust standard errors. Analysis limited to those with acceptable spirometry data.

6.4.3 Discussion

6.4.3.1 Main results

Contrary to our initial hypothesis, there was no evidence of an effect of antenatal multiple micronutrient supplementation on lung function in Nepalese children at 8 years of age. Despite undertaking the study under somewhat challenging field conditions, we performed spirometry on 80% of the children available for follow-up between the ages of 7-9 years, with technically satisfactory results achieved in 94.3% of subjects. After excluding children with a significant prior medical history, and adjusting for height, age and sex, FEV_1 and FVC in 'healthy' Nepalese children were approximately 13% lower than expected for Caucasian children.

As described in section 2.6, previous animal and human evidence has suggested potential long-term effects of antenatal micronutrient exposure. The best evidence exists for vitamin A.[178,412] Supplementation in both animals[176] and humans[177] has shown positive effects on lung function. In a follow-up at 9-13 years of age of an antenatal vitamin A supplementation trial from the adjoining district of Sarlahi, the intervention group had greater mean adjusted FEV_1 and FVC, of 46 ml in both cases. The differences in trial design (cluster versus individually randomised trials), comparator group (placebo) and dosage (longer period of supplementation with a 20% higher dose), as described in section 2.6, may account for the differing results of our study and that of Checkley et al. [177,182] The difference in the control group may be important. Though not a clear association, antenatal folate, a potent DNA methylator, has been implicated in respiratory disease in children. [243-245]

Since no specific reference range currently exists for the South Asian population, we chose to use the GLI Caucasian equations [26] to adjust for age, sex and height before investigating the potential effect of antenatal MMN supplementation. As expected, FEV_1 and FVC values were lower than would be expected for Caucasian children, and were slightly lower than those observed in children from South Asia whether living in the UK [413] or in India.[414] Ethnic differences may be due to differences in anthropometry or body composition. While the Caucasian equations were not a perfect fit, they were chosen for comparison as they have been produced with the greatest amount of evidence and could be considered a normative standard. The larger population on which the Caucasian equations are based would give greater precision, but may be less accurate. [415]

6.4.3.2 Birthweight

The difference in FEV_1 of 0.02 z scores was equivalent to 43 ml per 500 g increase in birthweight. This is comparable to the studies looking at the association between birthweight and adult lung function, which showed an approximately 50 ml FEV_1 increase per 500 g birthweight. [168,416]

6.4.3.3 Effect modification

In a secondary analysis I looked at the effect in boys and girls separately. While generally not reaching statistical significance, there was a tendency for antenatal MMN to have a detrimental effect on FEV_1 and FVC in girls, but not in boys. The effect was similar for both indices, so there was no difference in the ratio and also no difference in FEF 25-75. While not statistically significant, there was also a tendency for girls to be a little heavier than boys, but not taller. Overall, the difference was small and may well have occurred by chance.

6.4.3.4 Strengths and limitations

The sample size for the study was large, with an excellent follow-up rate, no evidence of bias in those lost to follow-up and few exclusions due to poor spirometry technique. The trial groups were well-balanced and potential confounding factors were documented and controlled for. It is possible that any effect of MMN supplementation could have been masked by unadjusted confounding. While we controlled for air pollution in the regression models, an antenatal nutritional intervention may have no effect at such high levels. Our air pollution estimates were directly measured, but were only based on a subsample. It is, however, unlikely that there would have been marked differences in results had it been possible to undertake personal exposure estimates in all individuals.

In summary, we found lower average spirometry values compared to Caucasian children, but no long-term difference in lung function or respiratory disease resulting from antenatal multiple micronutrient supplementation.

6.5 Health questions

6.5.1 Results

The questionnaire asked about illness in the last seven days and in the last year. The results for the whole cohort are shown in Table 43. Fever was very common, experienced by approximately 5% in the last week and 80% in the last year. Other illnesses were rare. The results by allocation group are shown in Table 44. No difference in the occurrence of illness was apparent between the groups, so no statistical analyses were done.

Table 43: Health results for the whole cohort

	In the last 7 days	In the last 12 months		
	Number of children (%)	Number of children (%)	Median number of episodes	Range
Fever	46 (5.4)	687 (81.0)	2	1 to 12
Diarrhoea	4 (0.5)	6* (0.7)	1	1 to 5
Blood in stools	3 (0.4)	30 (3.5)	2	1 to 12
Pneumonia	2 (0.2)	19 (2.2)	1.5	1 to 12
Fast breathing	4 (0.5)	16 (1.9)	2	1 to 12
Chest indrawing	3 (0.4)	15 (1.8)	2	1 to 12

Table 44: Self-reported illness over the last week and last year and food security by allocation group. Data presented as number (%) unless otherwise specified.

	Control (n = 422)	Intervention (n= 419)
Illness in the last 7 days		
• Fever	23 (5.5)	22 (5.3)
• Pneumonia	0 (0.0)	2 (0.5)
• Fast breathing	1 (0.2)	3 (0.7)
• Chest indrawing	0 (0.0)	3 (0.7)
Illness in the last 12 months		
• Fever	346 (82.0)	336 (80.2)
• Persistent diarrhoea*	2 (0.5)	4 (1.0)
• Blood in the stools	11 (2.6)	19 (4.5)
• Pneumonia	9 (2.1)	10 (2.4)
• Fast breathing	7 (1.7)	9 (2.2)
• Chest indrawing	8 (1.9)	7 (1.7)
• Doctor diagnosis of asthma	4 (0.9)	9 (2.1)
• Wheeze	7 (1.7)	9 (2.1)
• Dry cough	91 (21.6)	107 (25.5)

*Persistent diarrhoea lasting for >13 days.

6.5.2 Discussion

Reported morbidity was high, as indicated by how common fever was, but the occurrence of specific symptoms was rare. The questions were chosen to represent conditions that would affect growth and respiratory outcomes, particularly pneumonia and persistent diarrhoea. As discussed in section 6.6, the prevalence of asthma, based on questionnaire or clinical, diagnosis, was low.

It is difficult to corroborate illness reports without reliable medical records. Hospital or clinic records are often not kept and only represent a proportion of children seen in hospital. Children from poorer households may not be taken to see health providers when unwell, particularly those who have to travel large distances. Anecdotally, it seemed that it was common for parents to take their children to a medical practitioner of some sort - a medical

assistant or pharmacist, for example - but records of these interactions are not kept. We relied on parental recall, which is potentially prone to bias. This may act to increase the recall of major illness, but to forget less common ones. We would, however, expect recall bias to be similar in both trial groups.

In summary, while fever was common, recall of specific illness was rare. There was no obvious difference between the trial groups.

6.6 Asthma and air pollution

Using the ISAAC questions, in this analysis I sought to estimate the prevalence of asthma in children aged between seven and nine years, and then to investigate the association between air pollution and the risk of wheeze and rhinitis. Exposure to indoor air pollution at an individual level is generally high due to the use of biomass fuels for cooking by about 75% of households,[40] and has been considered before for children in Nepal,[417] but this study went further by using personal exposure estimates of air pollution both indoors and outdoors for each child.

6.6.1 Analysis

A directed acyclic graph based on *a priori* knowledge was constructed, from which potential confounding factors were selected for inclusion in multivariable logistic regression models for boys and girls separately (Figure 50). I controlled for socioeconomic status, maternal education and urban or rural residence. In a similar method to that described in section 5.2, a socioeconomic score variable was created using principal components analysis, including household assets and house structure, divided into quintiles. Biomass fuel use was not included as a potential component as it was the exposure variable in this analysis.[379] To explore the association between air pollution and dry cough, the exposure variable was divided into tertiles (the numbers were too small to investigate wheeze and sneezing in this way).

6.6.2 Results

Questionnaire data were obtained from 848 children. Fourteen were excluded from the analysis as they were from outside the region (where we did not have air pollution estimates). While 14.7% of children had ever had wheezing or whistling, there was a very low reported prevalence of wheeze of 1.9% in the last 12 months (see Table 45). A further six children not detected by the ISAAC questionnaire had a clinical diagnosis of asthma from their doctor. In contrast, dry cough at night was very common, reported by approximately a quarter in the last year. Approximately one-in-ten children had nasal symptoms and these were slightly more common in the winter season.

The air pollution exposure results are described in section 5.1. The results for association of air pollution with wheeze, dry cough and sneezing, runny or blocked nose are shown in Table 46. In girls, there was no association between air pollution and any of the outcomes investigated in univariable or multivariable regression models. In boys, there was an

association with dry cough at night: odds ratio (OR) 1.15 (95% CI 1.05, 1.26) for a 10 μg/m^3 increase in air pollution. There was no association with sneezing, runny or blocked nose. In comparison with the lowest tertile of air pollution, the OR in boys for dry cough at night was 1.66 (95% CI 0.94, 2.91) for the middle tertile and 2.37 (95% CI 1.35, 4.17) for the upper.

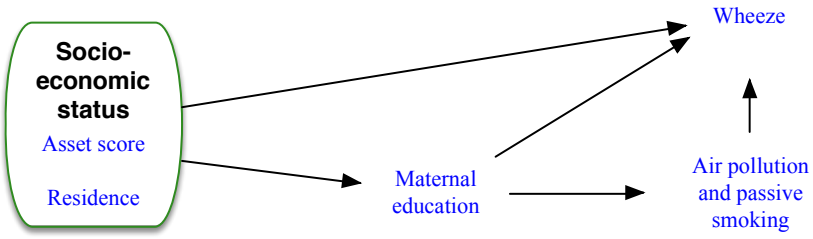

Figure 50: Directed acyclic graph showing the relationship between air pollution and wheeze

Table 45: Responses to the ISAAC questions, for 834 children in southern Nepal, 2012

		Number who answered yes (%)	
Asthma		**Girls**	**Boys**
Has your child ever had wheezing or whistling in the chest at any time in the past?		52 (12.8)	70 (16.3)
Has your child had wheezing or whistling in the chest in the past 12 months?		9 (2.2)	7 (1.6)
How many attacks of wheezing has your child had in the past 12 months?	1-3	6	6
	4-12	3	1
In the past 12 months, how often, on average, has your child's sleep been disturbed due to wheezing?	Never woken with wheezing	3	2
	Less than one night/week	3	4
	One or more nights/week	3	1
In the past 12 months, has wheezing ever been severe enough to limit your child's speech to only one or two words at a time between breaths?		4	2
Has your child ever had asthma?		3 (0.7)	6 (1.4)
In the past 12 months, has your child had a dry cough at night, apart from a cough associated with a cold or chest infection?		90 (22.2)	108 (25.2)
Rhinitis			
Has your child ever had a problem with sneezing, or a runny, or blocked nose when he/she DID NOT have a cold or the flu?		49 (12.1)	55 (12.8)
In the past 12 months, has your child had a problem with sneezing, or a runny, or blocked nose when he/she DID NOT have a cold or the flu?		42 (10.4)	44 (10.3)
In the past 12 months, has this nose problem been accompanied by itchy-watery eyes?		5 (1.2)	5 (1.2)
In which of the past 12 months did this nose problem occur?	Monsoon	10	8
	Post-monsoon	23	13
	Winter	21	31
	Spring/summer	13	13
In the past 12 months, how much did this nose problem interfere with your child's daily activities?	Not at all	37	38
	A little	2	4
	A moderate amount	3	0
	A lot	0	2
Has your child ever had hay fever?		8 (2.0)	12 (2.8)

Table 46: Associations between air pollution and ISAAC outcomes, for 834 children in southern Nepal, 2012

		Univariable regression			Multivariable regression*		
		Odds ratio**	95% CI	p	Odds ratio**	95% CI	p
Wheeze in the last 12 months	Girls	0.93	0.69, 1.25	0.620	1.10	0.80, 1.51	0.574
	Boys	1.41	0.96, 2.08	0.078	1.47	0.96, 2.25	0.074
Dry cough at night in the last 12 months	Girls	0.99	0.91, 1.08	0.864	1.01	0.92, 1.10	0.906
	Boys	1.16	1.06,1.27	0.001	1.15	1.05, 1.26	0.002
Sneezing/ runny, or blocked nose in last 12 months	Girls	0.96	0.69, 1.35	0.830	1.04	0.71, 1.53	0.847
	Boys	1.02	0.99, 1.05	0.235	1.02	0.99, 1.05	0.291

*Adjusted for socioeconomic circumstances, maternal education, presence of a smoker within the household and urban or rural residence.

** Odds ratios are for a 10 $\mu g/m^3$ increase in PM_4 (24-hour time-weighted average)

6.6.3 Discussion

The findings showed a low prevalence of wheeze overall and severe symptoms were rare. Asthma is common in children worldwide, but its prevalence varies greatly by location. Estimates of "wheeze in the last 12 months" can range from around one-in-fifty to one-in-three children. There is a positive association with gross national income and it is generally a little less common in girls than boys.[418-420] While the prevalence was low compared with worldwide figures, it was in keeping with a review of studies from India that found a median prevalence of 4.75%, with some studies reporting <1%.[421] A study from Nepal's Kathmandu Valley that used a definition of wheeze *or* cough showed a prevalence of 6%. [417]

Dry cough was very common, reported in the last year by approximately a quarter of the sample. This may be a true phenomenon as the region is very dusty outside of the rainy season and air pollution levels are high, but it may also in part be a function of how the question was interpreted. A previous study from the Terai found slightly higher figures for wheeze in children aged 9-13 years, but the prevalence of cough was much lower at 3-4%.[422] As discussed previously, the main limitation of our study for assessing prevalence was that the sample may not have been fully representative of children from the region. The initial trial recruited women who chose to have antenatal care at a public hospital, and may have excluded both the poorest and the wealthiest.

Exposure to air pollution is known to be associated with exacerbations of asthma on a day-to-day basis, but the evidence for it playing a role in primary causation of asthma is mixed and is based mostly on data from high-income countries.[423] There is evidence of increased prevalence of asthma in biomass-using households,[424] and a case-control study from the Kathmandu Valley showed an increased odds of asthma associated with secondhand tobacco smoke (OR 1.9; 95% CI 1.0, 3.9) and biomass fuels (2.2; 95% CI 1, 4.5). [417] A non-ISAAC study by Shrestha and Shrestha looked at respiratory symptoms in adults and found an increased odds of both wheeze and cough of 4.75 (95% CI 1.4, 16.7) and 3.37 (95% CI 1.3, 8.7), respectively, for people who used unprocessed fuels.[425] Similarly, a trial of chimney-stoves in Guatemala showed a reduction in wheeze in adult women of 58% (95% CI 0.25, 0.70), but there was no difference in lung function. [426] Worldwide estimates have shown no association between PM_{10} and current wheeze,[427] and a large multi-centre comparison of asthma prevalence showed an inverse association overall, but the results were inconsistent. [423]

Overall, we did not find an association between respirable particle mass and symptoms of asthma or rhinitis, except for dry cough in the last 12 months among boys. At this age, exposure to air pollution was similar in boys and girls as the children were generally not involved in cooking. It is possible that the gender difference in association with cough was

related to boys having a narrower airway calibre before puberty. [428] The lack of association with most outcomes may be a true phenomenon or potentially due to a plateau effect that has been described for respiratory illness.[207] All the children were exposed to a high average concentration of air pollution and we had no individuals with lower exposure to compare with. It is possible that air pollution is associated with asthma prevalence at the much lower levels common in high-income countries. In keeping with our results, a review of the evidence showed an association of air pollution with cough alone. [429]

This study could not rule out alternative explanations due to its cross-sectional design. We can only make assumptions about causal associations. Air pollution, for example was measured at the same time as the outcome. We assume that the exposure to air pollution was similar to that experienced by the child previously. Other confounding factors may not have been included in our model: we limited our measurements to particle mass, but specific toxic components in the air may also be important.

In summary, participants described a low prevalence of asthma and rhinitis symptoms consistent with other studies from South Asia. Air pollution levels were high in general, but a specific association was seen only with cough at night in boys.

6.7 The effect of socioeconomic status on growth

Socioeconomic status (SES) is associated with nutritional status in children in low- and middle-income countries,[386,430-432] but how it affects the growth of different tissues in different periods is less well understood. Evidence is emerging of the importance of growth in different periods during childhood, not only on immediate health, but also on longer-term health and education. [406] A greater understanding of how and when SES may affect growth is important in countries in which levels of poverty are high and under-nutrition is common. I looked at the effect of SES on growth in two different periods in childhood: early in life at 0-2.5 years, and in mid-childhood at 2.5-8 years.

6.7.1 Analysis

I defined SES on the basis of owning a set of predefined household assets, using questionnaire data collected at birth. Asset score at birth was included as an ordered categorical variable as described previously. The questions included an asset rank used by the World Health Organization that stratified households into four categories, with more expensive items like a motor vehicle or a refrigerator given the highest ranking and having none of the items as the lowest rank. The two middle categories were collapsed due to low numbers.[433]

I was not able to use the measures of SES as described in Section 5.2, because they were taken at 8 years, i.e. after the outcome. Only individuals for whom we had complete data at all three time points were included in the analysis.

HAZ and WAZ[25] at the three time points (birth, 2.5 and 8.5 years) were summarized. Variables representing stunting (HAZ <-2 z scores) and underweight (WAZ <-2 z scores) were created and used as dependent variables in logistic regression models. Linear regression was used to look at the relationship between asset score and lean and fat mass at 8.5 years.

I then examined the association between SES and conditional height (CH) and conditional relative weight (CRW) in the two periods. Conditional relative growth at 0-2.5 years and 2.5-8.5 years was estimated using the method described by Adair et al.[406] Conditional height takes into account previous weight and height, while conditional relative weight accounts for previous weight and height, and also current height. Height can be considered a marker of skeletal growth and weight a marker of soft tissue growth. The inclusion of birth data in the 8.5 year relative growth analysis had no effect on the model. Standardized residuals from linear regression models, using asset score as an ordered categorical variable were used as the

outcome. I constructed a causal diagram based on *a priori* assumptions (Figure 1, Appendix 6.5). No confounding variables were thought to be present.

6.7.2 Results

Complete anthropometry data were available for 793 children (48.3% girls). Approximately half the families were in the wealthiest asset group. Our data showed a high prevalence of under-nutrition. At 8.5 years of age, just over half the children were underweight and approximately one third were stunted.

HAZ and WAZ scores by SES are shown in Table 47. They suggest an inverse relationship for both indices, as confirmed by the reduction in odds of being stunted and underweight for each increase in rank of asset score. From level 1 to 2, there tended to be a non-significant reduction in odds, while a significant effect was seen when moving to level 3 in all cases except HAZ at birth. Similarly there was a no association between moving from level 1 to 2 on lean mass (0.46 kg; -0.15, 1.07 kg) and fat mass (0.05 kg; -0.35, 0.46 kg). A significant association was seen when moving to level 3 on lean mass (1.34 kg; 0.77, 1.92 kg) and fat mass (0.59 kg; 0.22, 0.97 kg).

The regression analysis showed a positive association between asset score and conditional height, with the exception of weight from 0-2.5 years (Table 48). Moving from asset level 1 to 2 showed non-significant changes, while moving to asset level 3 had significant associations. The largest effect was on CH in the younger period. At 2.5-8.5 years, there was a similar effect for both CH and CRW.

Table 47: Mean weight- and height-for age z score at 0, 2.5 and 8.5 years in three asset score categories.

		Asset score			Odds of stunting and underweight (95% CI)*	
		1 (poorest)	2	3 (least poor)	OR asset level 2	OR asset level 3
Birth	HAZ	-0.42	-0.37	-0.30	0.97 (0.53, 1.78)	0.78 (0.44, 1.40)
	WAZ	-1.46	-1.15	-1.11	0.63 (0.41, 0.97)	0.54 (0.36, 0.82)
2.5 years	HAZ	-2.63	-2.42	-2.03	0.75 (0.53, 1.07)	0.49 (0.35, 0.68)
	WAZ	-2.00	-1.83	-1.52	0.91 (0.63, 1.31)	0.60 (0.42, 0.85)
8.5 years	HAZ	-1.84	-1.66	-1.28	0.88 (0.60, 1.32)	0.49 (0.33, 0.72)
	WAZ	-2.45	-2.25	-1.82	0.88 (0.62, 1.25)	0.52 (0.37, 0.73)

*Comparator group is asset level 1. The odds ratios for stunting (<-2 z scores) and underweight (<-2 z scores) are shown in the right hand columns.

Table 48: Conditional relative growth, showing standardised residuals for height and weight in the two periods considered

	Height		Weight	
	0-2.5 years Standardised residuals (95% CI)	2.5-8.5 years Standardised residuals (95% CI)	0-2.5 years Standardised residuals (95% CI)	2.5-8.5 years Standardised residuals (95% CI)
Asset level 2	0.117 (-0.083, 0.318)	0.166 (-0.048, 0.380)	-0.009 (-0.214, 0.195)	0.060 (-0.154, 0.275)
Asset level 3	0.473 (0.285, 0.661)	0.320 (0.119, 0.522)	0.106 (-0.086, 0.297)	0.221 (0.190, 0.423)

*Comparator group is asset level 1.

6.7.3 Discussion

The results confirm the finding of other research that SES is an important factor determining the anthropometric status of children. I have gone on to show that it may affect the growth of skeletal tissue, represented by conditional growth in height in both early and later childhood, and soft tissue, represented by conditional relative growth in weight in mid-childhood only.

There was a consistent inverse relationship between SES and both HAZ and WAZ. In addition, the higher the household SES, the lower the risk of stunting or underweight in childhood and underweight at birth. A significant effect was seen when moving from level 1 to level 3 in the asset score, i.e. the more expensive assets. SES was not associated with being short at birth,

We defined growth as the residual of the regression model, conditional on previous anthropometry measures. It was therefore not just a change in height or weight, but a deviation from expected values. Similar to the results for stunting and wasting, moving from asset level 1 to 2 tended to have positive, but non-significant associations, while moving to asset level 3 had significant associations. The effect of SES was stronger on growth in height than weight, particularly in early life. This was supported by the greater effect on lean mass than fat mass at 8.5 years. Of interest was the lack of effect on soft tissue growth in early childhood. One possible contributing factor may be the high prevalence of breastfeeding across all socioeconomic levels in the region, such that - at least in early life - infants were all likely to have access to similar nutrition and this might mitigate some of the detrimental effects of low SES. At 2.5-8.5 years, there was a similar effect for growth in both height and weight.

SES is a proximal factor that does not directly affect a child's ability to grow, but acts through a number of complex pathways. An example is its association with illness. Low SES may increase the risk of contracting an illness, perhaps related to poor sanitation and inappropriate health behaviours, and also the ability to treat it through the family's capacity to access and afford healthcare. These factors act to influence the risk and severity of illness, which in turn increases the metabolic demands on a growing child and reduces their ability to absorb nutrients and, if severe, halts growth. Cross-sectional studies in low- and middle-income countries show that SES is positively associated with anthropometry and growth in children. Four longitudinal studies, all from Brazil, have confirmed these findings.[430,434-436] Lourenço et al considered children of a similar age to ours and showed a positive association between wealth and height up to 10 years of age.[434] The only study to look at a change in anthropometry was by Gigante et al,[435] who showed that, while income was associated with height at 4 and 18 years, it was not associated with a change in height between the two time

points. They concluded that SES is important for early, but not for later, growth. They also suggest that in the lower social classes, social mobility is unable to significantly change the trajectory of height attainment.[435 430]

A potential limitation of the study was the use of an asset score as an index of SES, as is commonly done with DHS data and was recommended by WHO at the time at the initiation of the study. SES is a complex concept to define and that there are many alternative methods and ideally other measures would have been used in addition to this, but they were not available.[380] Accurate measures of consumption were not present and I felt that income would be unreliable as in this setting it is often seasonal and may be non-monetary.

In summary, SES affects the growth of children when the family own more expensive assets. The greatest influence is on skeletal growth in early life.

7 Public engagement and dissemination

"We don't see things as they are, we see them as we are."

Anais Nin

As part of the research process we felt it was important to report the results to the participants and community. The public engagement aspect of this project involved dissemination of the main research findings to the participants and local community and an art project focusing on air pollution. In addition to this some of the results have been published and presented at scientific conferences. When conducting the air pollution measurements, it became evident that our participants did not fully understand the results and their implications. To help with this and also to develop further understanding ourselves, we ran a project with local artists exploring the use of biomass fuels for cooking.

7.1 Public engagement – main project

There are a number of definitions of public engagement, the one I find most useful being from the National Co-ordinating Centre for Public Engagement:

"Public engagement describes the many ways in which higher education institutions and their staff and students can connect and share their work with the public. Done well, it generates mutual benefit, with all parties learning from each other through sharing knowledge, expertise and skills. In the process, it can build trust, understanding and collaboration, and increase the sector's relevance to, and impact on, civil society." http://www.publicengagement.ac.uk/what

7.1.1 Participants and local community

We held two identical public engagement events over consecutive days in Janakpur, to which we invited the participants, the head teachers of the schools in which we did air pollution measurements and local government and NGO leaders. Approximately 150 people attended these events. During the events, senior MIRA staff summarised the results prior to the study, described the current project and mentioned the main results. Attendees were given written information about the project to take with them.

We then presented the public engagement art project and showed replicas of the pictures the artists produced. Finally, we showed the main project and Smokescreen films that were created. The main project film was shot and produced by Sebastian Roberts, a medical student who did his elective with me. This film can be seen through the following link: http://youtu.be/nFkLOI9UHyw

In addition, I spoke to a journalist from a local newspaper about the results of the project.

7.1.2 Scientific community

Summary of the results to the scientific community took the form of posters and presentations at scientific conferences and meetings and the publication of papers.

Posters

- Kular D, Devakumar D, Manandhar DS, Shrestha BP, Saville N, Osrin D. Food security status in southern Nepal: application of the Household Food Insecurity Access Scale questions". Nutrition Society Sustainable Diet and Food Security conference, Lille, France 28-29th May 2013
- Devakumar D, Kirkby J, Manandhar DS, Osrin D, Shrestha B, Chaube SS, Stocks J. Applicability of the Global Lung Function Initiative (GLI) reference ranges to

spirometry data from children in Nepal. European Respiratory Society Annual Congress 2013. 7-11th September 2013

- D Devakumar, JCK Wells, SS Chaube, NM Saville, DS Manandhar, A Costello, JA Ayres, J Stocks, D Osrin. The long-term effects of antenatal multiple micronutrient supplementation in Nepal. 8th World Congress on Developmental Origins of Health and Disease, Singapore. 17th Nov 2013
- Bartington SE, Bakolis I, Gulliver J, Devakumar D, Kurmi O, Osrin D, Ayres JG, Saville N, Costello A, Manandhar DS, Hansell AL. Domestic exposure to Carbon Monoxide and $PM_{2.5}$ in biomass fuel households in Janakpur, Nepal. UK and Ireland Exposure Science Meeting. Manchester, UK. 4th March 2014

Presentations

- Devakumar D. "Antenatal multiple micronutrient supplementation in Nepal". School of Public Health, University of Saõ Paulo.
- Devakumar D. "The long-term effects of antenatal multiple micronutrient supplementation in Nepal". Wellcome Trust Bloomsbury group conference, Kings College London, 1-3rd July 2013.
- Devakumar D. "Antenatal multiple micronutrient supplementation in Nepal". Body composition group, Institute of Child Health, UCL, 8th July 2013.
- Presented by my MIRA colleague Mr Sushil Yadav at the Perinatal Society of Nepal conference:
  Devakumar D, JCK Wells, SS Chaube, Yadav SK, NM Saville, DS Manandhar, A Costello, JA Ayres, J Stocks, D Osrin. "Follow-up of antenatal multiple micronutrient trial in Dhanusha district, Nepal", PESON conference, Kathmandu, Nepal 28th November
- Devakumar D, JCK Wells, SS Chaube, NM Saville, DS Manandhar, A Costello, JA Ayres, J Stocks, D Osrin. "The long-term effects of antenatal multiple micronutrient supplementation in Nepal". Commonwealth Association of Gastroenterology and Nutrition 13th scientific congress, Colombo, Sri Lanka, 6th Dec 2013
- Devakumar D. "Longitudinal follow-ups. An example from antenatal multiple micronutrients in Nepal". Faculty of medicine, University of Colombo, 9th Dec 2013.

Scientific papers published

- Devakumar D, Semple S, Osrin D, Yadav SK, Kurmi OP, Saville NM, Shrestha BP, Manandhar DS, Costello A, Ayres JG. Biomass fuel use and the exposure of children to particulate air pollution in southern Nepal. Environment International. 2014 Feb 14; 66C: 79-87. http://www.sciencedirect.com/science/article/pii/S0160412014000166

Papers prepared

- Devakumar D, Grijvala C, Roberts S, Chaube SS, Saville NM, Manandhar DS, Costello A, Osrin D, Wells JCK. Isotope calibration of the Tanita BC-418 bioelectrical impedance machine in children in Nepal.

- Devakumar D, Ayres JG, Bartington S, Stocks J, Chaube SS, Saville NM, Manandhar DS, Costello A, Osrin D. Cross sectional study of asthma and rhinitis in the context of exposure to air pollution in Nepal.
- Devakumar D, Chaube SS, Wells JCK, Saville NM, Ayres JG, Manandhar DS, Costello A, Osrin D. The effects of antenatal multiple micronutrient supplementation on anthropometry and blood pressure in mid-childhood in Nepal: follow-up of a double-blind randomized controlled trial
- Devakumar D, Stocks J, Ayres JG, Kirkby J, Yadav SK, Saville NM, Devereux G, Wells JCK, Manandhar DS, Costello A, Osrin D. Effects of antenatal multiple micronutrient supplementation on lung function in mid-childhood: follow-up of a double-blind randomised controlled trial in Nepal
- Devakumar D, Kular D, Daniel R, Osrin D, Shrestha BP, Wells JCK, Manandhar DS, Costello A, Saville NM. The effect of socioeconomic status on the growth of children in Nepal

Abstract published

- Kular D, Devakumar D, Manandhar DS, Shrestha BP, Saville N, Osrin D. Food security status in southern Nepal: application of the Household Food Insecurity Access Scale questions. Proc Nutr Soc. 2013, 72 (OCE5), E314

http://journals.cambridge.org/download.php?file=%2FPNS%2FPNS72_OCE5%2FS0029665113003480a.pdf&code=377e2f0b8e813fa7e051a6bcc1b17fc3

7.2 Smokescreen

Following the main data collection, we ran a public engagement project focusing on air pollution. The project used art to explore the issues around indoor air pollution from cooking. Air pollution in the region is mostly caused by the indoor burning of biomass fuels and is disproportionately experienced by the women of the household who do the cooking. When performing the air pollution testing, a number of the participants asked us for more information about what we were doing and the problems with air pollution. While quantification of the level of air pollution is important, I felt that more could be done to identify the problems they face and come up with potential solutions. Our target audience was the women within our current micronutrient follow-up project in whose houses we obtained air pollution measurements. To help both them and us gain further understanding of the research, we designed a public engagement project to follow on, but the direction of the project was determined by the participants involved. I sought and obtained additional funding through a UCL Beacon public engagement award.

The main aims of the project were:

1. Through the use of art, to get the people affected by indoor air pollution to help us understand the issues they face and the obstacles to change.
2. To find potential solutions to the problem of indoor air pollution to inform future research.
3. To involve some of the participants in our current follow-up, providing something tangible.
4. To provide junior members of UCL and MIRA experience in public engagement.

7.2.1 Art project summary

The project ran over a period of four months: a month of set-up, two months of primary activities, and a month for the evaluation and final event. We brought together local women who had participated in previous projects and artists from the Janakpur Women's Development Centre (JWDC), to look at problems and think about potential solutions in a creative environment. The JWDC is a co-operative of female artists from poor local communities who produce Maithili art. As well as helping to preserve their artistic heritage, the centre works as a functioning business that helps to empower women. In addition to having experience of painting on walls, they have also worked on other health promotion pictures and have created a picture for our micronutrient follow-up project.

In the initial phase, 50 women were identified and asked to take part. Of these, 34 attended focus group discussions. After starting the project one group ceased due to illness and time commitments, leaving a total of 29 participants who completed the project. Initially we had hoped to involve some men from the households as well, possibly in a separate group. This would have been important as the head of the household (and decision maker) is often a man. Unfortunately, it was not possible because of a combination of a lack of male volunteers and, on our part, not searching hard enough to find men.

We held four focus groups at the start of the project, in which the women came together with an artist and a facilitator. They discussed the issues around air pollution such as the fuels they use and why they use them, the health effects of air pollution and possible ways to mitigate the effects.

Women chose to meet regularly to design pictures that would describe the health effects of air pollution and ways to reduce it. The groups met approximately weekly with an artist and the MIRA facilitator. The original intention of the project was that small pictures would be produced in the kitchens of the participants. However, the participants chose to produce large

pictures that would be painted directly on walls and metal boards around the city. In total, 11 pictures were made. A community meeting with local officials was held at which decisions were taken about where to display the pictures, after which negotiations took place with local officials. As well as in locations close to the participant's home, the pictures were placed around the city; for example, in the main public hospital, the railway station and in a school. The pictures were also shown at our public engagement events. Examples of some the pictures produced are shown in Figure 51.

To evaluate the project six semi-structured interviews (four with participants and two with artists) and a final focus group discussion with representatives of all the groups were performed. The main findings from these were:

1. *Issues and obstacles related to air pollution.* While individual understanding of the problems was limited, collectively, and with the help of the facilitator, the women mentioned most of the detrimental effects of air pollution. One aim of the project was for the participants to teach us about the problems they face. Through the focus group discussions, interviews and artwork they helped to deepen our understanding. Choice of fuels, for example, is about more than just availability and cost. Issues such as the taste of the food and cooking traditions are also important.

2. *Solutions*. The participants came up with some solutions that were portrayed in the artwork, such as improving kitchen ventilation and keeping children away when cooking. The project also drew attention to the subject of indoor smoke in the community. The women could see that air pollution is an important issue and were able to highlight the problem to others through their large pictures. In terms of change, they described changes they had made to their cooking practices, such as cooking in better ventilated areas. One woman made a structural change to her house. She knocked down a wall and had a new one built to allow the smoke to escape.

3. *Involvement of participants*. The most pleasing aspect of the project was the enthusiasm of the participants, who all reported back positively. Twenty-nine of the 50 people asked completed the project, committing their time for four months. Even though the women could meet as often as they liked, or not at all, they chose to do so weekly. They had long, detailed discussions about the subject and really seemed to enjoy the art work. As a testament to this, the facilitator said that normally when she runs women's groups once a month she has to contact the women to persuade them to come. In this project, the participants were calling her weekly to ask her when she was coming.

This was the first experience of public engagement for myself and the MIRA staff member who ran the project. There were also two interns who helped with the project, both of whom were contributing to a public engagement project for the first time. As shown in the staff evaluation, all junior members enjoyed the project and learnt a great deal from it, both in terms of the public engagement process and about the subject matter. One member commented that it was a "thrilling, eye-opening experience". Another said how happy she was to have this opportunity: she learnt how to "think for myself" and "computer skills and English". On the negative side, language limited the degree to which the two British interns could contribute.

7.2.2 Film

A film documenting the project process was made by Dinesh Deokota, a Nepalese filmmaker I hired. He visited the site a number of times, filming the groups and the artists to tell the story of the project. The full version of the film in English can be seen at http://youtu.be/dHvlzrVBLOY. The original version in Nepali shown at the event can be seen at http://youtu.be/Xa-5eEY57MI.

Figure 51: Photos from the Smokescreen project showing the creation and some of the final pictures

8 General discussion

"When my information changes, I alter my conclusions. What do you do, sir?"

John Maynard Keynes

The PhD set out to investigate the lasting effects of multiple micronutrient supplementation in pregnancy, with a focus on growth and lung function. In addition to this, I considered a number of other factors: air pollution exposure, measures of food security and socioeconomic status, and public engagement. The findings of each have been discussed up to this point. In this chapter, I begin by discussing the main objectives of the study in terms of the long-term effects of antenatal multiple micronutrients on growth and lung function of children. I describe possible explanations for the results and questions arising from them. In the second half of the chapter I consider whether multiple micronutrients should be given to pregnant women in low- and middle-income countries and the policy options related to nutrition supplementation.

8.1 Multiple micronutrient supplementation in pregnancy

In this section I consider whether the findings from the study are correct and possible explanations for why an effect was not seen. I then discuss whether a health advantage might emerge in the future and whether the results could be generalizable.

The results showed that MMN supplementation did not lead to apparent differences in phenotype at eight years of age. Our results were similar to those of the other follow-up studies described in Section 2.9, which found no difference in long-term outcomes. The only outcome that may have been different was head circumference, but this could have been a chance finding. Efficacy trials also tend to show more positive findings than in a real-life setting, so any effects are likely to be further attenuated. It is important to remember, though, that the comparator group was not placebo. The results suggest that MMN – at least at one recommended daily allowance (RDA) and over the second and third trimesters - had no *additional* benefits over iron and folic acid, rather than having no effect at all.

Explanations for the results are either that there was truly no difference at eight years or that we were unable to detect a difference. If there were no lasting effects of antenatal MMN, it may be that their effects were transient, perhaps through reversible epigenetic changes. Methylation early in life tends to be irreversible, but it may be that any changes happened too late to modify growth trajectory or are, in fact, reversible. Neither transgenerational nor current environmental influences are likely to be redressed over the course of two trimesters of micronutrient supplementation. Any beneficial effect may be dwarfed by the detrimental effects of the environment.

The second possibility is that long-term physiological effects were present but undetected. We can have confidence in our results. Our follow-up rates were high, with little evidence of differential loss to follow-up, and measurement error was low so, other than by chance, it would seem unlikely that there was a large effect that we were unable to find. In addition, we assumed that, if not measured and included in the multivariable regression, alternative causes of growth were balanced across the intervention and control groups as a result of randomisation. It is therefore unlikely that a type II error occurred. It is conceivable that the difference in anthropometry was still present, but had diminished over time. Multivariable models showed a difference in mean weight of 295 g, similar to the difference at 2.5 years of age, but a much smaller proportion of the weight of an 8-year-old. It may be that the intervention only had an effect in early childhood, and this difference was maintained in

absolute terms. To be able to detect a difference of this magnitude would require a sample approximately ten times as large.[9]

If nutritional interventions in-utero do lead to phenotypic differences in childhood, as predicted by the DOHaD theory, potential reasons for a lack of effect are that the dose or timing of the micronutrients were incorrect or that a threshold effect exists.

Dose - despite being given at one RDA, the dose was potentially suboptimal. Calls have been made for an increased dose of micronutrients.[22] Levels were tested in a subsample and vitamin A and iron were about 10% lower than expected. It may also be that a "one-size fits all" approach is not appropriate and more tailored supplements are needed relating to the likely micronutrient, and possibly also macronutrient status, of pregnant women.

Timing - At 12 weeks' gestation, MMN given in pregnancy may have been too late to effect changes in the woman's phenotype and energy stores. It is also probably too late to improve micronutrient stores sufficiently. In-utero weight gain happens mostly in the third trimester, but DNA methylation occurs mainly around the time of implantation. By 12 weeks, the fetus is mostly formed and the scope for change is limited.

Threshold - While diets were likely to be balanced, it may be that a threshold rather than a dose-response effect exists. Potentially, a deficiency leads to a deficit in lung function, but once adequate levels are reached there is no further benefit. An important micronutrient to consider here is vitamin A because supplementation programmes for children under five years of age have existed in Nepal since 1993. The 2011 Nepal Demographic Health Survey showed that 79% of children in the Terai aged 6-59 months received vitamin A supplements in the last six months. [43] Lung development continues into childhood[161] and supplementation may correct any deficit in both trial groups, resulting in similar lung function in childhood. The degree to which the childhood supplementation programme had an effect would presumably depend on vitamin A sufficiency in a child, when exactly they receive the supplement and the level of maturity the lungs have reached.

In addition to the main findings, the effect of antenatal MMN may have been a little different in boys and girls. Girls were slightly heavier and had a lower FEV_1 (approximately 0.2 z scores for each), while there was no effect in boys. Head circumference was also a little larger

[9] A difference of 0.2 z scores is approximately 740 g (700 g in boys and 780 g in girls) in an eight-year-old. Based on the number of children found, the power to detect a 200 g (approximately 0.06 z scores) difference would be 14%. To find a difference of 200 g would require a sample of 8700 children.

in girls (0.27 cm; 95% CI -0.00, 0.54), compared to boys (0.09 cm; 95% CI -0.14, 0.32) and the data from birth showed that girls were heavier. [7] The differences were not statistically significant and no interaction was found, so we should be cautious in the interpretation, but the findings are potentially consistent with biological mechanisms described previously and do raise an interesting question of whether a trade-off in tissues has occurred. The expensive-tissue hypothesis describes how the body reduces the size of one organ to preserve energy for another, as implicated in the thrifty phenotype hypothesis. [437] Such trade-offs have been seen in studies comparing brain mass and gut mass in fish[438] and pectoral muscle mass (used for flight) in birds.[439] Research in mammals has generally found evidence against this hypothesis, but the results did indicate that lung tissue mass may be negatively correlated with adipose tissue. [10] [440]

Later effects

While the findings at 8 years were null, we cannot rule out the re-emergence of a difference between the trial groups later and it is still possible that the birthweight advantage may have longer-term health effects. While they seemingly lose the advantage they had, the slower relative growth of children of greater birthweights may be linked with physiologic differences, as it is believed that being born small and having catch-up growth is detrimental to health in terms of later non-communicable disease.[406] As seen in animal studies where rats were malnourished over many consecutive generations, it may also take multiple generations to return to a "normal" phenotype. [441,442] The difference in birthweight in girls, if real, could have important implications for the next generation. Research from the USA showed that the strongest predictor of offspring birthweight is maternal birthweight. [443] Evidence from cohort studies in Brazil also shows that maternal weight at birth and 20 months is associated with offspring birthweight. [126] It can also affect outcomes later in life. Barker et al showed that the birthweight of a woman was associated with the birthweight of her children and their blood pressure as adults. There was a 2.4 mmHg (95% CI 0.1, 4.7 mmHg) reduction in systolic blood pressure per one pound (454 g) increase in birthweight. [444]

[10] The authors did not specifically test the correlation between lung mass and adipose tissue, but did see a positive correlation between lung and digestive tract tissue and a negative correlation between digestive tract and adipose tissue.

Generalisability of findings

The main findings should be generalisable to other women and children in similar settings in low- and middle-income countries (LMICs). A potential bias is that the women recruited to the trial were not from the richest or poorest demographics, as they were from a group who chose to give birth at Janakpur Zonal hospital. Recent data show that 32% of women in the Terai deliver in an institution, [43] this despite deliveries in government hospitals being free of charge. The figure is likely to be higher for people living in Janakpur as transport to the hospital is easier. I am uncertain as to whether this would have made a difference to the outcomes. The effect might have been greater in poorer, undernourished mothers, but no difference was seen when stratifying by maternal BMI. While not the poorest, compared to other countries, the women within the study were not well-off.

Further questions arising from this study

Follow-up of current cohort

- Does an effect of antenatal MMN or of changing growth trajectories alter disease risk later in life, for example cardiovascular disease rates in middle-age?
- Is there a difference in other organ systems between the groups, such as the nervous system?
- Will MMN supplementation lead to a difference in the offspring of the children born in the study?

Alternative studies

- Would the outcomes be different if supplementation was started at a different time: in the first trimester or pre-conceptually?
- Would a different combination, either of micronutrients or dosages, lead to a different outcome?
- Other than sampling variation, what accounts for the differences between populations or the same population in different locations that have been shown in systematic reviews and follow-up studies? In studies that have examined antenatal MNN is there a sex-specific difference in outcomes?

8.1.2 Anthropometry and lung function of Nepalese children

A striking finding was the low anthropometry (mean WAZ = -2.1, HAZ = -1.5) and spirometry (mean FEV_1 = -1.2, FVC = -1.1) values for the children in our sample in relation to international standards. Approximately half the children were underweight, a third stunted and they had spirometry values >10% lower than Caucasian children. [26] The relatively lower percentage of lean mass (and higher fat percentage) is in keeping with the idea of the "thin-fat" phenotype described in India, [445] and has implications for studies of anthropometry in children as reporting BMI may not be reliable.

The children started off life small and continued to get relatively smaller. This is supported by analysis of the 2011 Nepal Demographic and Health Survey, which showed that children <12 months of age were significantly less likely to be underweight than those between 12 and 60 months. [43] Victora et al, who looked at growth up to the age of five in 54 LMICs, showed a reduction in anthropometry scores up to two years of age, but normal growth thereafter.[446] Contrary to this, we saw a reduction in weight-for-age (but not height-for-age) from two years onward, as well as earlier.

The growth patterns seen may be a consequence of using worldwide growth reference ranges that do not account for different growth patterns in a particular population of children. The WHO growth curves were designed to provide normative standards for child growth, i.e. "how children should grow". [447] While population-specific reference ranges could be more appropriate, they may not be able to predict full growth potential, for example if all children are exposed to a particular risk factor.

Aside from issues of classification, the reasons for growth deficiencies in children in our sample involve a complex web of genetic and environmental factors. Of interest was that our findings were in a context of low disease burden in mid-childhood, in a sample who were a little better off than some and in whom food security and dietary diversity were generally good. Alternative explanations include environmental stressors such as air-pollution and environmental enteropathy. As discussed in detail in Section 5.1, all children in our sample were exposed to much higher levels of air pollution than both national and internationally recommended upper limits, mostly due to the indoor burning of biomass fuels, but also to high outdoor levels most likely from displaced dust. Environmental enteropathy occurs when there is breakdown of the gut intestinal barrier resulting in flattened gut villi and inflammatory infiltrates. This leads to microbial translocation across the gut wall, resulting in a chronic inflammatory process and poor weight gain. Despite being very common, the causes are poorly understood, but are related to unhygienic conditions. [448] We were unable to test for this, but it does provide one explanation for the continued poor growth of all children.

Further questions arising from this study

- Are the findings of continued poor growth of children in mid-childhood mirrored in other populations in LMICs?
- Does growth in mid-childhood (and differences in lean and fat mass) have long-term implications in terms of adult anthropometry and health?

Air pollution

- Would repeat measures of air pollution taken over longer time periods produce similar results?
- Would behaviour change interventions at the household level work to reduce air pollution exposure?

8.3 Policy implications

Policies pertaining to nutrition are important for improving mortality and morbidity of women and children in LMICs. The concept of developmental origins of health and disease is gaining evidence and could have an important role to play in public health nutrition policy, by highlighting windows in which interventions can have maximal effect. Pregnancy is believed to be a time in which a woman can be motivated to change her behaviour for lasting benefit to herself and her offspring. The short-term goals within pregnancy can make health promotion activities more achievable; for example, smoking cessation to improve the growth of the fetus, rather than to reduce risk of cancer decades later. In addition to the immediate effects and the reduction in the burden of long-term chronic disease, such policies also touch on ideas of intergenerational equity or justice. The underlying factor for most, if not all, the outcomes investigated in this project is the socioeconomic status of the child and their family. Restructuring the social and economic hierarchy of a society is complicated and will take decades, possibly generations, but is likely to provide the most sustainable improvements.

Several policies exist regarding food production and other factors relating to child growth and health. Shrimpton describes two categories of policy: normative or rights-based and needs-based. MMN are primarily needs-based in terms of correcting maternal micronutrient deficiency, but also applies the normative standards of growth charts. [449] Designing policies that may be effective is a complicated process that only in part relies on the evidence. Potentially more important is the political will of the government and other international bodies. Walt and Gilson for example, describe the importance of context, process and actors, as well as the content of a policy in their description of the Health Policy Triangle. [450] The post-2015 “sustainable development goals”, may provide an opportunity in which the context is appropriate and relevant actors are willing to listen.

The policy choices that pertain to the field of nutrition, at a user level, are supplementation programmes, food fortification or monetary policies. Fortification, recommended by WHO,[451] can occur at a universal level or be targeted at a specific group. Fortification of all foods has the advantage that everyone gets the micronutrient. It may be appropriate for certain micronutrients that would not cause toxicity in high doses, but not for many micronutrients combined. Targeted supplementation with MMN can be used if certain foods can be identified for the relevant group. This would be difficult to do in this setting. [452] The extent to which fiscal policies would work in low resource, particularly subsistence farming, settings is questionable. They may help to make undesirable foods prohibitively expensive and a tax credit scheme could work in some populations, but I do not think it would be appropriate for

rural Nepal. The rest of this chapter will consider supplementation, specifically in relation to the UNIMMAP supplement in pregnancy.

8.3.1 Should multiple micronutrient supplements be given to pregnant women from low and middle-income countries?

Despite the relatively good evidence base for antenatal MMN, it is true that more research is needed and clear answers may only be available in the next generation. While we do not have a definitive answer to whether antenatal micronutrients are beneficial, the question remains and policy recommendations need to be made in the light of imperfect evidence.

Supplementation has the advantage of being able to deliver the appropriate dose in a reliable form. In our study, the tablets were well accepted with very high compliance rates in both trial groups. As described by Shrimpton, delivery, rather than compliance, is the most important aspect.[449] Delivering the micronutrients to pregnant women would be complicated but can be achieved, as shown by the example of immunisations. For energy and protein, community based programmes are probably best, but for micronutrients it is difficult to provide the right dosage. Supplementation has a clear causal mechanism and a relatively good evidence base. The argument against supplementation is that these programmes are not sustainable. It is a top-down approach decided by governments and often international bodies and may not have the support of the people affected. Taking a pill would create reliance on others and would not be as appealing as food. Supplementation does not solve the specific problems that individuals or populations may have.

The main benefit of antenatal UNIMMAP supplementation is a small increase in birthweight and the corresponding reduction in the percentage of babies who are born with low birthweight or SGA. Birthweight has been shown in many instances to be positively associated with long-term health (excluding large babies). In this respect, MMN supplementation has been recommended by many. [22,452] Bhutta and colleagues (from The Lancet Nutrition Interventions Review Group and the Maternal and Child Nutrition Study Group) recommend the use of MMN supplementation instead of iron and folate. They calculate that 43 715 deaths would be averted by giving MMN in pregnancy at 90% coverage in the 34 countries most in need. Along with nine other nutrition interventions, their models suggest it would reduce deaths by 15% in children under 5 years of age and reduce stunting by 20%. [452]

Cost

Changing from the recommended alternative of iron and folic acid would be more expensive, but most of the costs are in the delivery of the supplement to pregnant women. The supplement itself would be <20% more than iron and folic acid in terms of production costs, though the price would vary with economies of scale. [453] Analyses by Bhutta et al suggest that the additional cost of MMN in pregnancy (at 90% coverage) in the 34 LMICs in which it would be most useful would be (International)$472 million ($244 million for consumables, $70 million for personnel and $157 million for other costs). The package of all ten nutrition interventions they recommend would cost (International)$9.6 billion. The other interventions they consider in pregnancy are calcium supplementation to mothers at risk of low intake and energy protein supplements as needed. [452] Using these figures and assuming a 20% higher cost of MMN than iron and folic acid and the same delivery costs, MMN would cost an additional (International)$50 million. In global terms this is small.

An additional "cost" to consider is the opportunity cost. This is the next best alternative forgone. If (International)$472 is spent on MMN, what interventions would not be spent on? This is more difficult to gauge, but it is likely that supplementation beginning earlier or with macronutrients, or interventions to tackle upstream factors would be more effective. Opportunity cost would be context specific depending on a country's budget and needs.

8.3.2 Supplementation policy options

Within supplementation programmes, there are four main options for policies: single micronutrients, multiple micronutrients, food or a combination of these.

8.3.2.1 Single

Single micronutrient supplementation is common worldwide. An evidence base, including mechanism of action and dosage, can be established relatively easily and the supplements can be targeted to the most useful time period (for example, folate periconceptually) or can be population specific (for example, iodine). It is also easier to convey why they are necessary: folate, for example, will reduce neural tube defects. The disadvantage, especially in populations with multiple nutritional deficits, is that women rarely require supplementation with only one micronutrient. Taking many separate tablets is complicated, confusing and likely to lead to reduced adherence.

8.3.2.1 Food

Dietary changes are thought to be the best options as they can be incorporated into a person's lifestyle. Meta-analyses have shown that balanced protein/energy supplements probably lead to an increase in birthweight. Imdad and Bhutta found an increase in birthweight of 59.9 g (95% CI 33, 87 g) and a reduction of SGA of 0.69 (95% CI 0.56 to 0.85) due to the supplements.[454] Ota et al found a similar increase in birthweight of 41.0 g (95% CI 4.6, 77.3 g), corresponding to a reduction in SGA of 0.79 (95% CI 0.69 to 0.90) and increase in birth length of 0.16 cm (95% CI 0.01, 0.31 cm). The effect was a little larger in undernourished women (67.0 g; 95% CI 13.1, 120.8 g), while no effect was seen in adequately nourished women. The effects did not appear to last into childhood. [455]

The foods used need to be culturally appropriate and adaptable enough to be prepared in a variety of doses. When giving energy supplements, care should be taken not to increase the caloric intake to a level that would lead to obesity and gestational diabetes, as this can be detrimental to both fetus and mother. Food supplements are the most likely to be consumed and sustainable, but are more difficult to deliver safely with the right constituents and variety.

8.3.2.1 Multiple micronutrients with other constituents

The composition of the micronutrient supplements can be debated. The UNIMMAP supplement contains most micronutrients that would be useful, but some are missing. An example of one that might be useful is calcium, which has been shown to increase birthweight (by 85 g; 95% CI 38, 134 g) and may also reduce preterm birth (0.76; 95% CI 0.60, 0.97).[154] Due to the potential interaction with iron absorption, the addition of calcium may require a change in the iron dose, or it may need to be given as a separate supplement. [456]

8.3.2.1 Food and multiple micronutrients

Intuition would suggest that the most useful supplement would be a combination of micronutrients and macronutrients to provide all the requirements for pregnant women. Huybregts et al looked specifically at whether food supplements with MMN are beneficial. They showed no effect on birthweight, but did find a difference in birth length of 4.6 mm (95% CI 1.8, 7.3) in the adjusted analysis. [457] The Bangladesh MiniMat trial compared MMN to iron and folic acid in two groups that either received protein-energy supplementation early (as soon as pregnancy was identified: usually first trimester) or at the usual time (their own choice: usually second trimester). MMN plus early food supplementation (2696 g; 95% CI 2663, 2729g) had a slightly lower birthweight than later food supplementation (2710 g; 95%

CI 2657, 2745g), but this was not a significant difference. They found no difference in birth length. [11,271]

An example of this approach is described in Box 7 with two new "ready to use" products for pregnant women by Nutriset: Enov'Mum and Plumpy'Mum. These products provide an appropriate combination of nutrition, but have all the problems of sustainability. In my opinion, this would seem most useful for countries or regions suffering from an acute problem, for example a natural disaster, rather than being a long-term solution.

Enov'Mum

A lipid-based supplement designed for non-emergency or chronic emergency contexts, though based on the guidance the WHO, World Food Program and UNICEF for populations affected by emergency.[458] The supplement is very similar to UNIMMAP (with slightly different doses) but also contains calcium, phosphorus, potassium and magnesium, protein and lipids. The cost is 2.90 €/kg.

Plumpy'Mum

This is for women with BMI <18.5. The micronutrient content is similar, but it contains approximately three times the lipid and five times the protein content. The cost is approximately three times higher than Enov'Mum.

Box 7: Examples of nutrition supplements containing both macronutrients and micronutrients

8.4 My recommendation

The wider goal of this project and other similar research is to provide evidence for public health policy. Health policies for low-income countries such as Nepal need to be a combination of factors that lead to short-term improvements in health and remediation of suffering, and longer-term prevention of the underlying causes of illness. Supplementation was designed to be an example of the former, but this research set out to investigate whether it could also relate to longer-term benefits. The meta-analyses showed an increase in birthweight which, as described in Chapter 2, is associated with improvements in health. The study by Bhutta et al, based predictions of mortality and morbidity on this difference in birthweight. The authors argue that this difference is sufficient to warrant nutritional policies with a resulting predicted fall in child mortality. [452]

Based on the evidence in my research, I would not advise a shift in nutrition policy from the current recommendation of antenatal iron and folic acid supplements. As described in Section 2.8, the evidence base for these micronutrients can also be questioned, but they have been used for many decades and are considered safe.

Both the positive and negative aspects of antenatal MMN supplementation need to be balanced when deciding whether to recommend the UNIMMAP supplement for pregnant women. While others may arise in the future, the main advantage of MMN supplementation at the moment is the small increase in birthweight. The main negative effects are the potential increase in neonatal mortality and the cost. I do not think antenatal MMN supplementation does increase mortality (at least, overall mortality) and the cost, while large, is relatively small for a global supplementation programme.

I think supplementation can be a useful policy. In itself it does not make a difference to the root causes of nutrition problems, but can help to make a population healthier while other upstream policies are implemented. My reservation in recommending the UNIMMAP supplement is that the health benefits are based on the difference in birthweight and the actual evidence for improvements in mortality and long-term health is absent or limited. Birthweight is a composite of many processes that occur in-utero and the role of antenatal MNN in this is unclear. On balance, therefore, I would not recommend MMN.

The null findings from the study do not definitively rule out future benefits later in life or in the next generation. If a difference in weight does truly exist in girls, as described by Prentice and Moore in Figure 4,[86] and by Wells,[459] improvements in maternal size may lead to improvements in birthweight in future generations. We therefore may not be able to assess the importance of MMN for many years to come, and the policy implications should be reassessed as new evidence accrues.

If not a change to antenatal multiple micronutrient supplementation, what should be done? Bhutta et al recommend calcium supplements, protein-energy supplements and iodine where required.[452] Though not possible to assume from my research, it is likely that policies designed to improve the macronutrient intake of girls and women, in conjunction with micronutrients, would produce the best results in a population in southern Nepal. The results of previous meta-analyses indicate the need for pre-existing macronutrient status, [17] although the trials in Burkina Faso[457] and Bangladesh[11] did not find a difference.

Understanding of causation can help us advise on the most productive policies and interventions. The conceptual diagram in Figure 40 shows the complex nature of child growth, with many interrelated factors. MMN supplementation makes up only a small part of this. Focusing on factors that have multiple connections and that are central to the causal pathways is likely to have the best chance of improving health. An example is maternal education. Based on our assumptions, improving maternal education will result in changes to child illness, breastfeeding and caring practices, air pollution exposure and healthcare seeking behaviour. In addition to improvements in the education system, improvements in gender equality would help to achieve this. The diagram does not, however, show the relative importances of these factors and further work would be required to elucidate it. The model also highlights the limitations of potential interventions in that a number of factors cannot be controlled for; for example, birth order and gestation. Understanding causation cannot influence the many other aspects involved in policy formation, such as the financial and political implications.

In addition to nutrition policy, this research has provided evidence that can help to shape policy on air pollution exposure mitigation. Air pollution concentrations we found were very high. Policy has tended to encourage improved stoves and chimney ventilation based on the only trial in this subject area, the results of which were mixed.[426,460] This work shows that exposure levels can potentially be altered by behaviour change (for example, avoiding areas of high air pollution concentration during certain times of the day). Further research into this topic area would be required as this study only points to a potential intervention and is not strong enough to warrant policy change.

8.5 Conclusions and summary

Overall, my research has shown that MMN supplementation in pregnancy does not have lasting effects at 7-9 years of age. In addition to the main outcomes, I have documented the growth and respiratory status of children in southern Nepal and the degree of air pollution that they are exposed to. The study has shown that children in Nepal are both underweight and stunted and have lower lung function than international standards, and are exposed to very high levels of air pollution.

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Appendices

Appendix 1

Appendix 1.1 Funding letter

wellcome trust

Private and Confidential

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Your Ref:
Our Ref: 092121/Z/10/Z

20th August 2010

Dear Professor Costello

I am writing to let you know that the Trust has agreed to award University College London a grant of up to **£277,204** to provide a Research Training fellowship for Dr Delanjathan Devakumar over 36 months for his study entitled "**The effects of antenatal micronutrient supplementation and current air pollution on growth and lung function in 8 year old children**", under your sponsorship and the supervision of Dr David Osrin, Professor Jon Ayres and Dr Jonathan Wells.

The grant has been given a notional start date of 1st December 2010 and is intended to provide support as follows:

RING-FENCED FUNDS:	
	Total
CLINICAL FELLOW	
Dr Delanjathan Devakumar	172,516
OVERSEAS ALLOWANCES	
Airfare	2,515
Housing contribution	5,300
Personal freight	3,760
Health and travel insurance	5,448
Vaccinations	300
Visa costs	2,500
Sub Total	**£192,339**

TRANSFERABLE FUNDS:	
	Total
FLEXIBLE FUNDING ALLOWANCE	2050
INFLATION ALLOWANCE	820
TRAVEL TO MEETINGS	4,500
MATERIALS & CONSUMABLES	5148
EQUIPMENT	
See attached list	15,534

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TRAVEL-COLLABORATIVE	
Supervisor visits	3,555
OTHER	
Salaries for Nepal staff	21,676
Field staff local travel	1,920
Questionnaire printing	209
Compensation (travel, refreshments)	3,522
Doctor for consulations	5,478
Office rent	3,200
Office furniture	278
Nepal communication	696
Transport of equipment to/from Nepal	916
Ethical Approval Nepal	713
Testing of mineral levels in hair samples	6,000
Mess spec of hair samples (ICH)	6,600
External advisory meetings	400
Workshops	300
Training courses	1,350
Sub Total	**£84,865**
GRAND TOTAL:	**£277,204**

Ring-fenced funds may only be used for the purpose stated above. The budgets for transferable funds are indicative only and movement of funds between these budget headings is allowed without prior permission from the Trust.

The ring-fenced funds provided for Dr Devakumar include a basic starting salary of £39,693 per annum, as set by the Host Organisation, plus inflation.

The grant is cash-limited; supplementary funding will only be provided in specific circumstances (see Information to Note).

The Trust anticipates that Research Training Fellows will be able to focus on their research throughout the duration of their fellowship. Clinical duties should be restricted to a maximum of two programmed activities per week, where appropriate, and only if agreed by the supervisor.

The Trust's Grant Conditions (COND/01/10), referred to in the attached Information to Note, detail the conditions under which the grant is awarded.

We would remind you that with regard to clause 6 (iii) of the Grant Conditions, all research papers that have been accepted for publication in a peer-reviewed journal, and are supported in whole or in part by Wellcome Trust funding, must be made available from PubMed Central and UK PMC as soon as possible, and in any event within six months of publication, in line with our Open Access policy (www.wellcome.ac.uk/About-us/Policy/Spotlights/Open-access/index.htm).

It is a condition that the Head of your Host Organisation will administer the grant in accordance with the purposes for which it has been awarded. I should be grateful if your Organisation would confirm, in writing, the acceptance of this grant on the conditions detailed in this letter and the notes. A Grant Start Certificate for this purpose can be downloaded from the Trust's website (see Information to Note). The grant cannot be activated until this confirmation has been received. The Trust will not accept liability for any expenses incurred on the grant until a signed Grant Start Certificate has been returned.

When accepting this Award of Grant, the Organisation recognises that the UNDERTAKINGS given by the Organisation and others at the time of signing the Application Form are "conditions precedent" and the Organisation will ensure that they, their agents, servants, employees and students will continue to abide by the undertakings given throughout the lifetime of the grant.

Copies of this letter and the notes should be forwarded to the Head of your Host Organisation, your Head of Procurement, your Research Grants Office and your Finance Officer. For payment of grant funds see Information to Note.

If you would like to discuss any administrative issues regarding the grant, please contact me at this office.

Yours sincerely,

Angharad Jones
Grants Adviser
Populations & Public Health
Grants Management Department

Att: Equipment list

Cc: Dr Delanjathan Devakumar
Dr David Osrin
Professor Jon Ayres
Dr Jonathan Wells

Appendix 2

Appendix 2.1 PRISMA guidelines

Structured summary	Objectives: to summarise the birthweight and SGA outcomes from trials using the UNIMMAP supplement in pregnancy and then to look at the long-term outcomes of these trials in childhood. Data sources: Medline, Embase and Psychinfo databases Study eligibility criteria: Intervention group uses UNIMMAP supplement in pregnancy Control group uses iron 30 mg and folic acid Participants: Pregnant women from low- and middle-income countries Interventions: UNIMMAP supplement Results: Birthweight and proportion of SGA Long-term outcomes: anthropometry, cognitive and cardiovascular
Rationale	The review was designed to look for any new trials of the UNIMMAP supplement, look at the combined effect of the UNIMMAP supplements in isolation and to look for long-term effects of these supplements.
Objectives	To summarise the birthweight and SGA outcomes from trials using the UNIMMAP supplement in pregnancy to look at the long-term outcomes of these trials in childhood.
Protocol and registration	NA
Eligibility criteria	All trials that have tested the UNIMMAP supplement in pregnancy in low- and middle-income countries. All studies that have followed up children from these trials. Trials reported in English
Information source	Databases: Medline No date restrictions.
Search	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.
Study selection	Trials that use the UNIMMAP combination in pregnancy
Data collection process	Data were not confirmed with the authors. Though, the data from the original trials were compared with previous meta-analyses. Where there was a discrepancy with one trial, the authors of the meta-analysis were contacted.
Data items	Birthweight and SGA proportion Any follow-up outcomes
Risk of bias in individual studies	No specific methods were used to assess bias in the original trials. In the follow-up studies, differential loss to follow-up was recorded.
Summary measures	Principal summary measures birthweight and proportion of SGA. Follow-up outcomes depended on the outcome.
Synthesis of results	Random effects meta-analysis was used to combine results of birthweight. This was done using adjusted data from the original trials and data from the meta-analysis by Fall et al (*Food Nutr Bull* 2009; 30(4): S533-46), who had access to the original data. I^2 were reported for each meta- analysis.
Risk of bias across studies	The trials have a low risk of bias. All were double blind randomized controlled trials.
Additional	An additional meta-analysis was performed with the addition of five of similar

analyses	multiple micronutrient supplements.
Study selection	See section 2.9.3
Study characteristics	See section 2.9.3
Risk of bias within studies	
Results of individual studies	
Synthesis of results	Meta-analysis of UNIMMAP trials only using the data from Fall et al (*Food Nutr Bull* 2009; 30(4): S533-46): birthweight +24.01 g (95% CI 8.60, 39.41 g), $I^2 = 0\%$
Risk of bias across studies	Meta-analysis of UNIMMAP trials only using original the data from the trials: birthweight +44.26 g (95% CI 32.41, 56.10 g), $I^2 = 6.5\%$
Additional analyses	Meta-analysis of UNIMMAP and similar trials: birthweight +35.90 g (95% CI 21.54, 50.25 g), $I^2 = 32\%$
Summary of evidence	Using either the original data or that from the Fall et al meta-analysis showed a small increase in birthweight (24 to 44 g), with corresponding reduction in SGA. These differences were in keeping with the previous meta-analyses conducted. Adding the six similar micronutrient trials produced a result between these two estimates. With the exception of the Janakpur trial, the follow-up studies generally did not show long-term changes in anthropometry or cardiovascular symptoms. Some differences were seen in cognitive tests, but may tests were conducted in these studies.

Table 1: Profile for Systematic review of UNIMMAP trials describing birthweight (with meta-analysis) and SGA and long-term outcomes using the PRISMA guidelines **(Liberati A, et al. *PLoS Med* 2009; 6(7): e1000100)**

Appendix 2.2 Example of when the traditional theory of confounding and the counterfactual theory differ

As described by Hernan and Robins (Causal Inference, Chapman & Hall/CRC; 2012), there can be examples of when the traditional theory of confounding introduces bias by creating confounding where it did not exist. Take this example, where C is associated with exposure E and outcome O. C is not on the causal path from E to O, so would be adjusted for using the traditional method. Using the counterfactual definition however, you would not condition on C because C is a collider. By conditioning on the variable C, it opens up the backdoor path from E to O, introducing confounding.

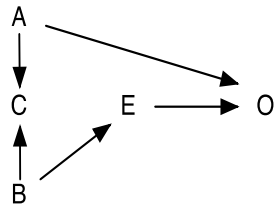

Figure 1: Model showing no confounding, but where it can be induced through inappropriate adjustment

Appendix 3

Appendix 3.1 Power calculation

Assuming the same sample size in each group, the statistical power for a difference in means can be calculated as follows:

$$n = \frac{\sigma^2 (Power \times \alpha)^2}{difference^2}$$

$$400 = \frac{1^2 (Power \times 0.05)^2}{0.2^2}$$

$$Power = \frac{\sqrt{\frac{400 \times 0.04}{1}}}{0.05}$$

Where

n = sample size

α= significance level

σ = standard deviation

difference = expected difference in mean

Appendix 3.2 Gantt Chart

Year 1, 2011	1	2	3	4	5	6	7	8	9	10	11	12
Training: body composition & spirometry												
Formulation of questionnaire												
Advertising and employing staff												
Training of data collection staff												
Identifying and locating children												
Piloting questionnaire and data collection												
Data collection												
Advisory group meeting												

Year 2, 2012	1	2	3	4	5	6	7	8	9	10	11	12
Data collection												
Data analysis												
Advisory group meeting												

Year 3, 2013	1	2	3	4	5	6	7	8	9	10	11	12
Writing up of thesis												
Dissemination of results												
Public engagement art project												

Appendix 3.3 Consent form (piloting)

Informed Consent Form for Parents or Guardians

Piloting of body measurements

This project will involve measuring your child's body size, blood pressure and respiration. None of these measurements will harm your child. This is being done to help train our staff in data collection. The information we collect will be anonymised and used only to look at the data collector's technique. A report will be provided for you and a small gift for your child.

If you are happy for your child to be involved please sign and return the form. Thank you for your help.

Signed:

Date:

Appendix 3.4 Calliper calibration

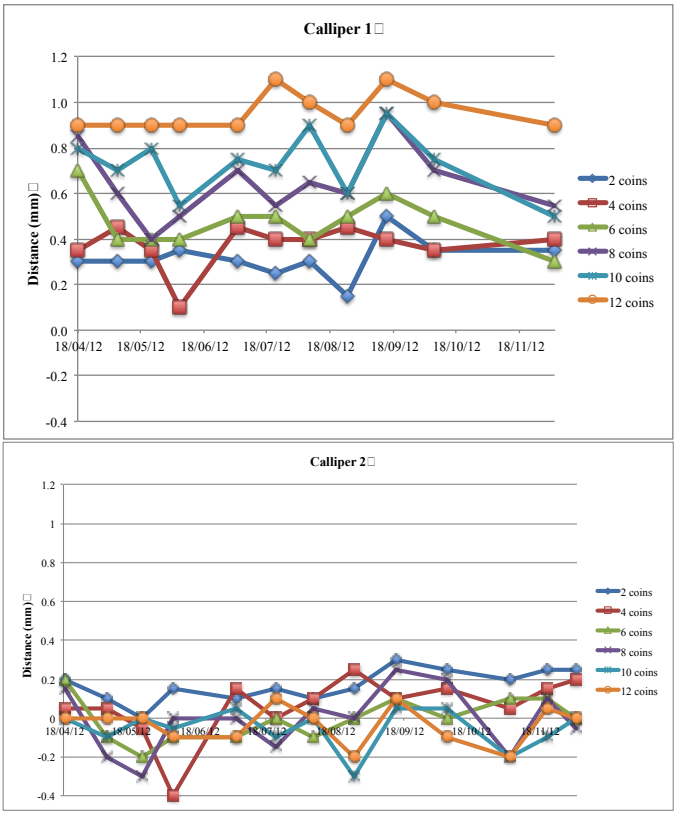

Figure 1: Harpenden calliper calibration repeated measures over time.

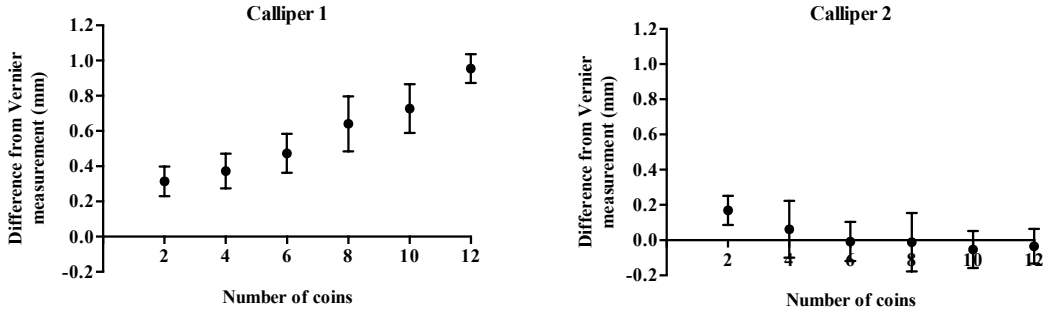

Figure 2: Graphs to show the change in difference (mean and standard deviation) from the Vernier calliper over increasing distance

Appendix 3.5 Questionnaire

MIRA Dhanusha
Micronutrient follow up programme
9 Year Follow up form

Nepali date अन्तरवार्ता लिएको मिति (साल महिना र गते) 20☐☐ ☐☐ ☐☐	Time अन्तरवार्ताशुरू गरेको समय ☐☐:☐☐ am/pm	Data collector's initials or ID number अन्तरवार्ताकारको आई डी नं ☐☐
GPS waypoint number: ☐☐☐	Telephone number of parent:	

बच्चाको अवस्था जिवित Alive ① मृत Dead ②

State of the child बच्चाके अवस्था

यदी बच्चाको मृत्यु भएको भए बच्चाको मृत्यु भएको साल महिना र गते लेख्नुहोस।

20☐☐ ☐☐ ☐☐

If dead, what was the date of death? यदी बच्चाके मृत्यु भईल अछि त बच्चाके मृत्यु भईल साल, महिना आ गते लिखु .

बच्चाको मृत्यु हुनुको कारण बारेमा स्पष्ट रुपमा लेख्नुहोस ।

What was the cause of death? बच्चाके मृत्यु होईके कारणबारेमे प्रष्ट रुपस लिखु ।

……

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यदि बच्चाको मृत्यु भएमा A,C,D सोध्नुहोस।

Section A – Demographics

A1: आमाको नाम..... ID number आई डी नं. ☐☐☐☐

Mother's name माईके नाम

यदि आमा उत्तरदाता नभएमा उत्तर दिने व्यक्तिको सम्बन्ध लेख्नुहोस।.....

Name & relationship to child if **not** the mother

यदि माई उत्तरदाता नई अछि त उत्तर देवेवाला व्यक्तिके सम्बन्ध लिखु ।

A2: आमा दिदी, दाई, बहिनी, भाई मध्ये कुन हो लेख्नुहोस् ☐☐ जम्मा भाई, बहिनी संख्या लेख्नुहोस् ☐☐ - मृत्यु भएको भाई, बहिनी, दाईको बारेमा पनि सोध्नुहोस _

Birth order of mother? (please ask if mother's siblings died)

भाई दिदी भाई मध्ये कोन छि लिखु . - मृत्यु भेल भाई दिदी भाई के बारे मे सेहो पुछु _

बासस्थान Location

A3: जिल्ला: धनुषा Dhanusha ① महोतरी Mahottari ② सर्लाही Sarlahi ③ सिराहा Siraha ④ अन्य Other⑤ .....

नगरपालिका Municipality ① गा बि स VDC ②

नगरपालिका / गा बि स को नाम Municipality /VDC name.....

वडा नं Ward..... टोल Tole.....

A4: घर कहाँ छ?

Where is the house? घर कत अईछ ?

Janakpur City **जनकपुर सहर** ① Town with large market (e.g. Mahendranagar) **बजार** ② Village **गाँउ** ③

A5: घर सग संगै ब्यस्त सडक छ?

Is the house beside a busy road? घरके आसपास बेसी गाडीघोडा चलवाला रोड अइछ ?

Yes - Tarmac main highway कालो पत्रे मुख्य सडक ① Tarmac other road कालो पत्रे सानो सडक ②

Dirt road धुले सडक ③ No छैन ④

A6: कति बर्ष देखि तपाईको बच्चा स्कुलमा छ? ☐☐
Number of years child has completed at school?
कते वर्ष सँ अहाँके बच्चा स्कुलमे छई ।

A7: जम्मा परिवार सदस्य संख्या ☐☐
Number of people in the household जम्मा परिवार संख्या

बच्चा संग परिवारका सदस्यहरुको सम्वन्ध Relationship to child	बर्ष Age (years)	धुम्रपान गर्ने गर्नुहुन्छ? Smoker	के पिउनु हुन्छ? Smokes what? Cigarettes सिगरेट = 1 Beedi बिडी = 2 Chillum चिलिम = 3 Hooka हुका = 4	दिनमा कति वटा सम्म पिउने गर्नु हुन्छ? How many per day?	प्राय गरेर कुन ठाउँमा धुम्रपान गर्ने गर्नु हुन्छ? Where do you mostly smoke?
	☐☐	Yes छ ① No छैन ②		☐☐	Indoors घर भित्र ① Outdoors घर बाहिर ②
	☐☐	Yes छ ① No छैन ②		☐☐	Indoors घर भित्र ① Outdoors घर बाहिर ②
	☐☐	Yes छ ① No छैन ②		☐☐	Indoors घर भित्र ① Outdoors घर बाहिर ②
	☐☐	Yes छ ① No छैन ②		☐☐	Indoors घर भित्र ① Outdoors घर बाहिर ②
	☐☐	Yes छ ① No छैन ②		☐☐	Indoors घर भित्र ① Outdoors घर बाहिर ②
	☐☐	Yes छ ① No छैन ②		☐☐	Indoors घर भित्र ① Outdoors घर बाहिर ②
	☐☐	Yes छ ① No छैन ②		☐☐	Indoors घर भित्र ① Outdoors घर बाहिर ②
	☐☐	Yes छ ① No छैन ②		☐☐	Indoors घर भित्र ① Outdoors घर बाहिर ②

Section B- Child's health

B1: गएको दुई हप्ता भित्र बच्चालाई कुनै किसिमको बिमारी भएको छ? छ Yes ① छैन No ②
Has the child been unwell in the last 2 weeks?
बितल दुई हप्ता भितरमे बच्चाके कोनो किसिमके बिमारी भेल छल?

B1.1: यदी बच्चा बिरामी भएको भए कुन प्रकारको बिमारी हो स्पष्ट रुपमा लेख्नुहोस?
If yes, what illness? यदी बच्चा बिमारी भेल छल त कोन बिमारी भेल छल प्रष्ट रुपसँ लिखु?

……………………………………………………………………….

……………………………………………………………………………………

(यदी बच्चा अहिले बिरामी अवस्थामा छ भने बिरामी निको भएपछि मात्र स्पाईरोमिटरको लागी ल्याउन सल्लाह दिनुहोस)
(If currently unwell, please delay the spirometer until better)
(यदी बच्चा अखन बिमारी छै त बिमारी निक भेलाके बाद मात्रे स्पाईरोमिटरके लेल लैबाक सल्लाह दियौं)

B2: तपाईको बच्चालाई कुनै किसिमको दिर्घ रोग छ ।? Yes छ ① No छैन ②
Does your child have a chronic illness? अहाँके बच्चाके कोनो किसिमके दीर्घ रोग अैछ ?

B2.1: यदी छ भने कस्तो खालको दिर्घ रोग छ स्पष्ट रुपमा लेख्नुहोस

If yes, what illness? अगर छई त केहन किसिमके दिर्घ बिमारी छई स्पष्ट रुपमे लिखु ।

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B3: बिगतमा बच्चालाई कुनै किसिमको ठूलो खालको बिमारी भएको थियो? Yes छ ① No छैन ②

Has the child had any major illnesses in the past? बितल दिनमे बच्चाके कोनो किसिमके बढका बिमारी भेल छल ?

B3.1: यदी छ भने कस्तो खालको समस्या भएको हो स्पष्ट रुपमा लेख्नुहोस्

If yes, what illness? अगर भेल छल त केहन किसिमके समस्या भेल छल, प्रष्ट रुपसँ लिखु ।

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B4: गएको **१२ महिनामा** बच्चालाई तलका मध्ये कुनै लक्ष्ण देखिएको थियो?

Has your child had any of the following symptoms in the last **12 months**? बितल **१२ महिनामे** बच्चाके निच्चके कोनो लक्ष्ण देख्ने छेलि ?

		If yes, how many episodes?
ज्वरो Fever	Yes छ ① No छैन ②	☐☐
१३ दिन भन्दा बढी लगातार पखालालागेको Persistent diarrhoea (episodes lasting for >13 days)?	Yes छ ① No छैन ②	☐☐
दिसामा रगत देखिने Blood in stool?	Yes छ ① No छैन ②	☐☐
निमोनिया Pneumonia?	Yes छ ① No छैन ②	☐☐
छीटो छिटो सास फेर्ने Fast Breathing?	Yes छ ① No छैन ②	☐☐
कोखा हान्ने Chest Indrawing?	Yes छ ① No छैन ②	☐☐

B5: गएको **७ दिनमा** बच्चालाई तलका मध्ये कुनै लक्ष्ण देखिएको थियो?

Has your child had any of the following symptoms in the last **7 days**? बितल **७ दिनमे** बच्चाके निच्चके कोनो लक्ष्ण देख्ने छेलि ?

ज्वरो Fever	Yes छ ① No छैन ②
पखाला लागेको diarrhoea?	Yes छ ① No छैन ②
दिसामा रगत देखिने Blood in stool?	Yes छ ① No छैन ②
निमोनिया Pneumonia?	Yes छ ① No छैन ②
छीटो छिटो सास फेर्ने Fast Breathing?	Yes छ ① No छैन ②
कोखा हान्ने Chest Indrawing?	Yes छ ① No छैन ②

B6: उमेर अनुसार बच्चाको शरिरमा आउने परिवर्तन संगै बच्चाको वृद्धि र विकास छिटो छिटो हुन्छ । त्यसकारण हामी तपाईको बच्चामा शुरु भएको परिवर्तन बारेमा जान्न चाहन्छौ । केटामा प्रायः गरेर अण्डकोषको वृद्धी हुन थाल्छ, र केटीमा स्तनको विकास हुन थाल्छ ।

When puberty starts children grow quickly, so we want to know whether your child started puberty? In boys usually starts with an increase in the size of the testes and in girls it is breast development.

बच्चामे होईवाला शारीरिक परिवर्तनसँ बच्चाके वृद्धि विकास जल्दी होईछई । तईसँ हमसब आँहाके बच्चामे सुरु भइल परिवर्तनके बारेमे जाने चाहइछि । बेटामे प्रायः अण्डकोषके वृद्धि होइछई आ बेटीमे छाती के विकास होइछइ ।

तपाईको छोरामा गुप्ताङ्गमा रौ आएको महसुस गर्नु भएको छ ? Yes छ① No छैन ② Don't know थाहा छैन③

Boys: have you noticed any pubic hair growth? आहाके बेटामे गुप्ताडमे केश आइल से महसुस कइनेछि ।

तपाईको छोरीमा स्तनको बिकास भएको पाउनु भएको छ ? Yes छ ① No छैन ② Don't know थाहा छैन ③

Girls: have you noticed any breast development in your daughter? आहाके बेटीमे छातीके विकास भेल छई से महसुस करैछि ।

B7: ISAAC questions for asthma

1. विगतमा कुनै पनि समयमा तपाईको बच्चालाई घ्यार–घ्यार (छातिमा सुसेले जस्तो आवाज आउने) भएको थियो ? Yes छ ① No छैन ②

Has your child ever had wheezing or whistling in the chest at any time in the past?
बितलाहा कोनो समयमे आहाके बच्चाके घ्यार–घ्यार (छातिमे सुसकारी जेहन आवाज आइल छल) भेल छल ?

यदी छैन भन्ने उत्तर आएमा प्रश्न नं. ६ मा जानुहोस । IF YOU HAVE ANSWERED "NO" PLEASE SKIP TO QUESTION 6

2. गएको १२ महिनामा तपाईको बच्चालाई घ्यार घ्यार (छातीमा सुसेले जस्तो आवाज आउने) भएको थियो?

Yes छ ① No छैन ②

Has your child had wheezing or whistling in the chest in the past 12 months?
बितलाहा १२ महिनामे आहाके बच्चाके घ्यार घ्यार (छातिमे सुसकारी जेहन आवाज आइल छल) भेल छल ?

यदी छैन भन्ने उत्तर आएमा प्रश्न नं. ६ मा जानुहोस् । IF YOU HAVE ANSWERED "NO" PLEASE SKIP TO QUESTION 6

3. गएको १२ महिनामा कति पटक तपाईको बच्चालाई सुसेले जस्तो आवाज आउने भएको थियो?

How many attacks of wheezing has your child had in the past 12 months?
बितलाहा १२ महिनामे कतेवेर आहाके बच्चाके घ्यार घ्यार (छातिमे सुसकारी जेहन आवाज आइल छल) भेल छल?

None भएन ① 1 to 3 एक पटकदेखि ३ पटकसम्म ② 4 to 12 ४ पटकदेखि १२ पटकसम्म ③

More than 12 १२ पटक भन्दा बढी ④

4. गएको १२ महिनामा कतिको मात्रामा (सरदर) तपाईको बच्चालाई घ्यार घ्यार भएर सुत्न गाह्रो भएको थियो?

In the past 12 months, how often, on average, has your child's sleep been disturbed due to wheezing?
बितलाहा १२ महिनामे कतेदेरतक आहाके बच्चाके घ्यार घ्यार भक सुतमे दिक्कत भेल छल ?

Never woken with wheezing कहिल्यै पनि नबिउझिएको ①

Less than one night per week एक हप्तामा एक रात भन्दा कम ②

One or more nights per week हप्तामा एक वा एक भन्दा बढी रात सम्म ③

5. गएको १२ महिनामा कडा घ्यार घ्यारको कारण बच्चाले एउटा वा दूईवटा मात्र शब्द एक पटक सास फेर्दा बोल्न सक्ने भएको थियो? Yes छ ① No छैन ②

In the past 12 months, has wheezing ever been severe enough to limit your child's speech to only one or two words at a time between breaths?
बितलाहा १२ महिनामे बहुत घ्यार घ्यारके कारण सँ एकटा आ दुटा मात्रे शब्द एकवेर सास फेरकाल बोलै छलै ?

6. तपाईको बच्चालाई कहिल्यै दम भएको थियो? Yes छ ① No छैन ②

Has your child ever had asthma? आहाके बच्चाको कहियो दमा भेल छल ?

7. गएको १२ महिनामा तपाईको बच्चालाई रातीमा सुख्खा कफ आउने भएको थियो? Yes छ ① No छैन ②

In the past 12 months, has your child had a dry cough at night, apart from a cough associated with a cold or chest infection?
गेलहा १२ महिनामे आहाके बच्चाके राईतमे सुख्खल कफ अवै छलै?

B8: ISAAC questions for rhinitis

1. तपाईको बच्चालाई कहिल्यै रुघा नलागेको बेलामा पनि छिक आउने वा सिगान बग्ने वा नाक बन्द हुने भएको छ?

Has your child ever had a problem with sneezing, or a runny, or blocked nose when he/she DID NOT have a cold or the flu?
आहाके बच्चाके कहियो सर्दि नई भेल समयमे छिकै छलै या नाक सँ पानी या नाक बन्द होइछलै?

Yes **छ** ① No **छैन** ②

यदी छैन भन्ने उत्तर आएमा प्रश्न नं. ६ मा जानुहोस । IF YOU HAVE ANSWERED "NO" PLEASE SKIP TO QUESTION 6

2. गएको १२ महिनामा तपाईको बच्चालाई रुघा नलागेको बेलामा पनि छिक आउने वा नाक बन्द हुने भएको छ?

In the past 12 months, has your child had a problem with sneezing, or a runny, or blocked nose when he/she DID NOT have a cold or the flu?

गेलहा १२ महिनामे आहाके बच्चाके सर्दि नई भेल समयमे छिकै छलै या नाक सँ पानी या नाक बन्द होइछलै ?

Yes **छ** ① No **छैन** ②

यदी छैन भन्ने उत्तर आएमा प्रश्न नं. ६ मा जानुहोस । IF YOU HAVE ANSWERED "NO" PLEASE SKIP TO QUESTION 6

3. गएको १२ महिनामा नाक सम्वन्धी समस्याको कारणले आखा चिलाउने र आसु झर्ने भएको छ?
In the past 12 months, has this nose problem been accompanied by itchy-watery eyes?
गेलहा १२ महिनामे नाकके समस्या सँ आँख हउवाई छेलै या आँख से पानी खसैछेलै?

Yes छ ① No छैन ②

4. गएको १२ महिनामा कुन महिनामा नाक सम्वन्धी समस्या देखियो? कुन महिनामा भयो चिन्ह लगाउनुहोस ।
In which of the past 12 months did this nose problem occur? (Please circle any which apply)
गेलहा १२ महिनामे कोन महिनामे नाकके समस्या भेल छल ? कोन महिनामे भेल छल चिन्ह लगाउ ।

[A] बैशाख Baisakh (Apr – May)	[B] जेष्ठ Jyesth (May - Jun)	[C] असाढ Ashadh (Jun – Jul)	[D] श्रावण Shrawan (Jul – Aug)	[E] भाद्र Bhadau (Aug – Sep)	[F] असोज Asoj (Sep – Oct)
[G] कार्तिक Kartik (Oct – Nov)	[H] मंसिर Mangsir (Nov – Dec)	[I] पौष Push (Dec – Jan)	[J] माघ Magh (Jan – Feb)	[K] फाल्गुण Phagun (Feb – Mar)	[L] चैत्र Chait (Mar – Apr)

5. गएको १२ महिनामा नाक सम्वन्धी समस्याले बच्चाले गर्ने दैनिक कृयाकलापमा कतिको मात्रामा असर पार्यो?
In the past 12 months, how much did this nose problem interfere with your child's daily activities?
गेलहा १२ महिनामे नाकके समस्या सँ बच्चाके दिनभरके कामकाजमे कते दिक्कत भेल छल ?

Not at all ① असर गरेन
A little ② थोरै मात्रामा असर गर्यो]
A moderate amount ③ केहिमात्रामा असर गर्यो
A lot ④ धेरै मात्रामा असर गर्यो

6. तपाईको बच्चालाई कहिल्यै बिरुवाको एलर्जीको कारणले आँखा र नाकबाट पानी आउनाको साथै आँखा चिलाउने भएको छ? Yes छ ① No छैन ②
Has your child ever had hay fever? आहाके बच्चाके कहियो एलर्जीके कारण आँइख या नाकसँ पानी आबैके साथे आँईख हउवाई छेलै?

Section C: Socioeconomic status

शिक्षा **Education**

C1: तपाईको परिवारको कुनै सदस्यले ५ वर्ष सम्म स्कुलमा पढ्नुभएको छ? Yes छ ① No छैन ②
Has any member of the household completed 5 years of schooling?
आहाके परिवारके कोनो सदस्य ५ वर्ष स्कुलमे पढने छई ?

C2: स्कुल जाने उमेरका सबै बच्चाहरु हालसालै स्कुलमा भर्ना भएका छन्?(४ वर्ष देखि १६ वर्ष सम्मका) Yes छ ① No छैन ②
Are all school-age children currently enrolled in school (Years 4-16)?
स्कुल जाईवाला उमेरके सब बच्चासब भरखर स्कुलमे भर्ना भेलछईर (४ वर्ष से १६ वर्ष तकके)

स्वास्थ्य **Health**

C3: बच्चाको कुनै भाई बहिनी वा दाई दिदी को मृत्यु भएको छ? Yes छ ① No छैन ②
Have any of the child's siblings died? बच्चाके कोनो भाई बहिन या भैया दिदीके मृत्यु भेल छई ?

Standard of Living

C4: तपाईको घरको लागि खानेपानीको मुख्य श्रोत के हो?
What is the main drinking water source for your household? आहाके घरके लेल पियवाला पानीके मुख श्रोत कि छई ?

(1) घरैमा ल्याइएको निजी धारा Piped drinking water in residence	(7) बोरिङग Deep bore hole
(2) सार्वजनिक धारा Public water tap	(8) आफ्नो चापाकल Own (private) handpump in courtyard
(3) निजी इनार Own (private) well	(9) छिमेकीको चापाकल Neighbour's handpump
(4) सार्वजनिक इनार Public well	(10) सार्वजनिक चापाकल Public handpump
(5) छिमेकीको इनार Neighbour's well	(11) नदी, खोला, नहर, पोखरी River, stream, canal, pond
(6) पारम्परिक सार्वजनिक इनार Traditional public well	

C5: खाने पानी ल्याउनको लागी तपाईलाई कति टाढा सम्म हिड्नु पर्छ? ३० मिनेट भन्दा कम <30 minutes ①
३० मिनेट भन्दा बढी >30 minutes ②

How far do you have to walk to get drinking water?
पियवाला पानी लावके लेल आहा सबके कते दुर चल परइय ।

C6: तपाईको परिवारका सदस्यले कस्तो प्रकारको शौचालयको प्रयोग गर्नु हुन्छ?
What kind of toilet do your family members use?
आहाके घरके सदस्य केहन प्रकारके पैखाना टटी प्रयोग करैइय ?

[A] Flush toilet फ्लस [B] Pan toilet प्यान [C] Pit toilet खाल्डे चर्पी
[D] Bushes / stream / open areas खुल्ला ठाँउ [E] Other's toilet अरूको चर्पी

C7: तपाईको चर्पी अर्को घरका सदस्यले पनि प्रयोग गर्नु हुन्छ? Yes छ ① No छैन ②
Do you share your toilet with other households?
आहाके पैखाना टटी दोसरा घरके आदमी सेहो प्रयोग करैइय ?

C8: तपाई बस्ने घरमा कुन कुन बस्तुहरु छन्?
What things do you have?
आहा रहेवाला घरमे कोन कोन समानसब अइछ ?

[A] बिजुली Electricity	[B] रेडियो Radio	[C] टेलिभिजन TV	[D] टेलिफोन Telephone
[E] मोबाइल Mobilephone	[F] फ्रीज Fridge	[G] साइकल Bicycle	[H] रिक्सा Rickshaw
[I] मोटरसाइकल Motorcycle	[J] ट्याक्टर Tractor	[K] बस,ट्क, जिप, कार, टेम्पो Bus/Truck/jeep/car/tempo	

C9: घर के ले बनेको छ? (पर्खाल)
What are the walls of the house mainly made of? घर कथि सँ बनल अैछ ? (भित्ता)

① Cement and bricks सिमेन्ट र इँटा	② Mud and bricks माटो र इट्टा	③ Mud and Stone माटो र ढुङ्गा
④ Planks of wood काठको पटाहा	⑤ Grass / Straw Thatch खर पराल	⑥ Metal sheets क्रकट पाता
⑦ Mud and woven stems or bamboo (tat) टाट		⑧ Other अन्य .....

C10: घरको छानो के ले बनेको छ?
What is the roof mainly made of? घरके चार कथि सँ बनल अइछ ?

① Cement सिमेन्ट	② Traditional tiles (Khapadaa) खपडा	③ Tiles टायल
④ Metal sheets जस्ताको छाना	⑤ Grass / straw thatch फुसको छाना	⑥ Other अन्य .....

C11: भुई के ले बनेको छ?
What is the floor mainly made of? भुईया कथि के अइछ ?

Dirt **माटो** ①	Dung **गोबर** ②	Sand **बालुवा** ③	Cement सिमेन्ट④	Other ⑤ .....

C12: तपाई प्रायः गरेर बस्ने घरमा जम्मा कति वटा कोठा छन्? ☐☐
How many rooms are there in total in the house you usually live in?
आहा प्रायः रहेवाला घरमे जम्मा काई हन्ना अइछ ?

C13: कुन कोठामा तपाईको बच्चा सुत्ने गर्छ?

In which room does your child sleep? आहा के बच्चा कोन हन्नामे सुतै अइछ ?

Room where food is cooked खाना पकाउने कोठा ①

Bedroom छुटै सुत्ने कोठा ②

Room where people sit मान्छे आएर बस्ने कोठा ③

C14: तपाईको बच्चा सुत्ने कोठामा :

In the room where your child sleeps: आहाके बच्चा सुतवाला कोठलिमे :

तपाईको बच्चा सुत्ने कोठामा सिसा भएको झ्याल कति वटा छन् ? How many windows are there with glass? आहाके बच्चा सुतवाला हन्नामे सिसावाला काइटा खिडकी अइछ ?	☐☐
तपाईको बच्चा सुत्ने कोठामा सिसा नभएको झ्याल कति वटा छन् ? How many windows are there without glass? आहाके बच्चा सुतवाला हन्नामे सिसा नइभेल कइटा खिडकी अइछ ?	☐☐
तपाईको बच्चा सुत्ने कोठामा ढोका कति वटा छन् ? How many doors are there? आहाके बच्चा सुतवाला हन्नामे काइटा केवारी अइछ ?	☐☐
तपाईको बच्चा सुत्ने कोठामा कति वटा बाहिर निस्कने ठाउँहरु छन ढोका नभएको] ? How many seperate doorways without door are there? आहाके बच्चा सुतवाला हन्नामे काइटा बाहर निकलवाला जगह अइछ ?	☐☐

C15: खाना पकाउनकोलागी तपाईको घरमा धेरैजसो कुन इन्धन प्रयोग गर्नु हुन्छ?

Which fuels do you usually use for cooking? खाना पकावकेलेल आहाके घरमे सबसे बेसी कुन चिज सँ पकवई छि

	आगो बाल्नको लागी प्रयोग गर्ने ईन्धन Fuel used for lighting the fire (please tick **all** that apply)	खना पकाउनको लागी निरन्तर प्रयोग गर्ने इन्धन Fuel used continuously for cooking (please tick **all** that apply)	एक भन्दा बढी इन्धन प्रयोग गरेको भए मूख्य रुपमा प्रयोग गरेको ईन्धनमा टिक लगाउनुहोस If more than 1 fuel is used, which is the main fuel (please tick **one** fuel)
Firewood दाउरा	[A]	[A]	[1]
Dried cow dung (goitha) गुइठा	[B]	[B]	[2]
Straw पराल	[C]	[C]	[3]
Charcoal कोईला	[D]	[D]	[4]
Other plant products झारपात	[E]	[E]	[5]
Kerosene मट्टितेल	[F]	[F]	[6]
Gas ग्यास	[G]	[G]	[7]
Biogas (gober gas) गेवर ग्यास	[H]	[H]	[8]
Electricity बिधुत	[I]	[I]	[9]
Plastic प्लाष्टीक	[J]	[J]	[10]
Other (please state) अन्य भए स्पष्ट रुपमा लेख्नुहोस	[K]	[K]	[11] .....

C16: के तपाईसग छुट्टै भान्सा कोठा छ? Yes छ ① No छैन ②

Do you have a separate kitchen? आहा लग छुटे भन्सा कोठा छइ ?

C17: घर भित्र खाना पकाउने चलन कतिको छ? months/yr बर्षमा कति महिना ☐☐ days/wk हप्तामा कति

दिन □□

What is the custom of cooking indoors? घरभितरमे खाना बनाबके चलन केतेके छई ?

C18: तपाईको घर परिवार भित्रबाट कोही सदस्य बैदेशिक रोजगारमा जानुभएको छ अथवा गइरहनु हुन्छ? Yes छ ① No छैन ②

Has any member of your family (household) migrated overseas or outside the district for work? Or does anyone go regularly?
आहाके घरपरिवार भितरसे कोनो सदस्य वैदेशिक रोजगार मे गेलछई या जाइवाला छई ?

यदि छ भने कहाँ?

[A] India भारत	[B] Arab countries अरब मुलुकहरु	[C] Malaysia मलेसिया	[D] Other countries specify अन्य देश (उल्लेख गर्नुहोस्).....

Section D Food security and vulnerability

अब म तपाईलाई केहि प्रश्न तपाई बसेको घरको खाना वा खाधान्नको पहुंचको बारेमा सोध्न लागेको छु । कृपया गएको ३० दिनको बारेमा मात्र जवाफ दिनुहोला र तपाई वा तपाई बसेको घरको परिवार सदस्यको बारेमा भन्नु होला। श्रोत भन्नाले पैसा वा खाधान्न जनाउने छ ।

I am now going to ask you some questions about food and food access in the house where you stay. Please answer about the last 30 days only and please answer for yourself and your household. By 'resources' we mean money or food.
आब हम आहाके किछ बात आहा रहैछि से घरके खाना आ खाइवाला चिजके पुगेके सम्बन्धमे पुछब । कृपया बितलाहा ३० दिनके बारेमे मात्रे जवाफ देवेपरत आ आहा और आहा रहैछि से घरके परिवार सभके बारेमे मात्र कहब । श्रोत के मतबल पैसा या खाइवाला चिज बुझपरत ।

D1 तपाईको घरमा खाना नपुग्ला भनेर चिन्ता भएको थियो? Yes I worried चिन्ता थियो ① No I did not worry चिन्ता थिएन ②

Did you worry that your household would not have enough food?
आहाके घरमे खाना नई पुगत तईके लागि चिन्ता भेल छल *?*

(यदि थिएन भने प्रश्न नं. *D2* मा जानुहोस्) If no go to question D2

D1.1 यदि खान नपुग्ने चिन्ता थियो भने कहिले कहिले?

If you worried that you would not have enough food how often?
अगर खाना नपुगत तईके चिन्ता भेलछल त कहिया कहिया ?

Rarely (very little) आक्कल झुक्कल (एक्दम कम) ① Sometimes कहिलेकाँही ② Often प्रायः जसो ③

D2 श्रोत को अभावले गर्दा कुनै मनपर्ने खानेकुरा खान नसकेको थियो?

Were you or any household member not able to eat the kinds of food you preferred because of lack of resources?
पैसाके अभावके कारण कहियो मन परेवाला खाईवाला खाना नई खाइसकलि ?

Yes we were not able to eat खान नसकेको थियो ① No थिएन ②

(यदि थिएन भने प्रश्न नं. *D3* मा जानुहोस्) *If no go to question D3.*

D2.1 मनपर्ने खानेकुरा खान नसकेको थियो भने कहिले कहिले?

If you were not able to eat foods you preferred how often?
मनपरेवाला खाना नपुगल त कहिया कहिया ?

Rarely (very little) आक्कल झुक्कल (एक्दम कम) ① Sometimes कहिलेकाँही ② Often प्रायः जसो ③

D3 श्रोतको अभावले गर्दा दिन दिनै एकै प्रकारको खानेकुरा खानु परेको थियो?

Did you or any household member just eat a few kinds of food day after day because of lack of resources?
पैसाके अभावसँ दिन दिने एके रंगके खाना खाई परल छल ?

Yes थियो ① No थिएन ②

(यदि थिएन भने प्रश्न नं. *D4* मा जानुहोस्) *If no go to question D4*

D3.1 यदि थियो भने कहिले कहिले?

If yes how often? अगर छेल त कहिया कहिया?

Rarely (very little) आक्कल झुक्कल (एक्दम कम) ① Sometimes कहिलेकाँही ② Often प्रायः जसो ③

D4 श्रोतको अभावले गर्दा अन्य खानेकुरा पाउन नसकेको कारणले मन नपर्ने खानेकुराहरु खानु परेको थियो?

Did you or any household member eat food that you did not want to eat because of a lack of resources to obtain other types of food?
श्रोतके अभावसँ कोनो दोसर खाईवाला चिज नई भेटल त मन नई परल खाईवाला चिज खाई परल *?*

Yes थियो ① No थिएन ②

(यदि थिएन भने प्रश्न नं. *D5* मा जानुहोस्) *If no go to question D5*

D4.1 यदि थियो भने कहिले कहिले?

If yes how often? अगर छल त कहिया कहिया ?

Rarely (very little) आक्कल झुक्कल (एक्दम कम) ① Sometimes कहिलेकाँही ② Often प्रायः जसो ③

D5. खानेकुराको अभावले छाकमा चाहना भन्दा कम खाना खानु भएको थियो**?** **Yes** थियो ① **No** थिएन ②

Did you or any household member eat a smaller meal then you felt you needed because there was not enough food?
खाईवाला चिजके अभावसँ छाकमे चाहैसे कम खाना खाइ परल छल **?**

(यदि थिएन भने प्रश्न नं. ***D6*** मा जानुहोस्) ***If no go to question D6***

D5.1 यदि थियो भने कहिले कहिले?

If yes how often? अगर छल त कहिया कहिया?

Rarely (very little) आक्कल झुक्कल (एक्दम कम) ① Sometimes कहिलेकाँही ② Often प्रायः जसो ③

D6 *खानाको अभावले गर्दा खानाको छाक घटाउनु परेको थियो?* **Yes** थियो ① **No** थिएन ②

Did you or any other household member eat fewer meals because there was not enough food?
खाईके अभाव सँ खानाके छाक घटावे परल छल **?**

(यदि थिएन भने प्रश्न नं. ***D7*** मा जानुहोस्) ***If no go to question D7***

D6.1 यदि थियो भने कहिले कहिले?

If yes how often? अगर छल त कहिया कहिया ?

Rarely (very little) आक्कल झुक्कल (एक्दम कम) ① Sometimes कहिलेकाँही ② Often प्रायः जसो ③

D7 श्रोतको अभावले खाना पटक्कै वा कत्ती पनि नभएको अवस्था थियो?

Was there ever no food at all in your household because there were no resources to get more?
पैसाके अभाव सँ खाना साफे या कमो नई भेल अवस्था छल ?

Yes थियो ① No थिएन ②

(यदि थिएन भने प्रश्न नं. *D8* मा जानुहोस्) *If no go to question D8*

D7.1 यदि थियो भने कहिले कहिले?

If yes how often?
अगर छल त कहिया कहिया ?

Rarely (very little) आक्कल झुक्कल (एक्दम कम) ① Sometimes कहिलेकाँही ② Often प्रायः जसो ③

D8 खानाको अभावले गर्दा भोकै सुत्नु परेको थियो *?*

Did you or any household member go to sleep hungry because there was not enough food?
खानाके अभावसँ भुखले सुते परल छल *?*

Yes थियो ① No थिएन ②

(यदि थिएन भने प्रश्न नं. *D9* मा जानुहोस्) *If no go to question D9*

D8.1 यदि थियो भने कहिले कहिले?

If yes how often? अगर छल त कहिया कहिया ?

Rarely (very little) आक्कल झुक्कल (एक्दम कम) ① Sometimes कहिलेकाँही ② Often प्रायः जसो ③

D9 खानाको अभावले गर्दा दिनभरी भोकै बस्नु परेको थियो?

Did you or any household member go a whole day without eating because there was not enough food?
खानाके अभाव सँ दिनभैर भुखले रहल परल छल ?

Yes थियो ① No थिएन ②

(यदि थिएन भने प्रश्न नं. *D10* मा जानुहोस्) *If no go to question D10*

D9.1 यदि थियो भने कहिले कहिले?

If yes how often? अगर छल त कहिया कहिया?

Rarely (very little) आक्कल झुक्कल (एक्दम कम) ① Sometimes कहिलेकाँही ② Often प्रायः जसो ③

D10 गएको १२ महिनामा कुनै महिना तपाईको परिवारको आवश्यकता अनुसार खाना नपुग्ने अवस्था थियो***?***

Over the last 12 months, were there any months in which you did not have enough food to meet your family's needs?
बितलाहा १२ महिनामे कोनो महिना आहाके परिवारमे चाहल जते खाना नई पुगल अवस्था भेल छल ***?***

Yes थियो ① No थिएन ②

(यदि थिएन भने प्रश्न नं. *D11* मा जानुहोस्) *If no go to question D11*

D10.1 यदि थियो भने कुन महिनामा?

If yes which months? अगर छल त कहिया कहिया ?

[A] बैशाख Baisakh (Apr – May)	[B] जेष्ठ Jyesth (May - Jun)	[C] असाढ Ashadh (Jun – Jul)	[D] श्रावण Shrawan (Jul – Aug)
[E] भाद्र Bhadau (Aug – Sep)	[F] असोज Asoj (Sep – Oct)	[G] कार्तिक Kartik (Oct – Nov)	[H] मंसिर Mangsir (Nov – Dec)
[I] पौष Push (Dec – Jan)	[J] माघ Magh (Jan – Feb)	[K] फाल्गुण Phagun (Feb – Mar)	[L] चैत्र Chait (Mar – Apr)

D11 गएको ७ दिन भित्रमा तपाइको बच्चाले तलका मध्ये कुन कुन (घरमै बनाएको) खानेकुरा खानुभयो?

In the last **7 days** which of the following foods did **your child** eat (i.e. foods that were made at home)?
बितलाहा ७ दिन भितरमे आहाके बच्चासभ यि निचका सबमे से कथि कथि (घरमे बनाउँल) खाईवाला खैलक ?

[A] Cereals e.g. rice, wheat, maize, millet, अन्न जस्तै चामल, गहुँ, मकै, कोदो
[B] Root tubers e.g. potatoes, yams, sweet potatoes, taro, elephant's foot etc जरामा हुने जस्तै :आलु, तरुल, सखरखण्ड, पिड ओल आदि
Vegetablesतरकारीहरु: [C] *yellow colour like pumpkin & carrots* पहेंलो तरकारीहरु जस्तै फर्सी, गाँजर आदि [D] *green leaves*हरियो सागपातहरु
Fruits फलफुलहरु [E] *yellow like mango and papaya* पहेंलो जस्तै आँप तथा मेवा [F] *other non-yellow fruit* *अन्य फलफुल*
Meatमासु: [G] *goat* खसी [H] *chicken* कुखुरा [I] *duck* हाँस [J] *buffalo* रागां [K] *pig* ब [L] *mouse/rat* मुसा [M] *cow/bull* गाई/गोरु
Fish/shell fish माछा/घोंगी: [N] *small fish* सानो माछा [O] *large fish* ठुलो माछा [P] *snails* घोंगी [Q] *crabs* गँग
[R] Eggs अण्डा [S] Dairy दूध पदार्थहरु
[T] Pulses & nuts दाल :गेडागुडी /बदाम [U] Oil / fats तेल :चिल्लो पदार्थहरु [V] Sugar / honey चिनी :मह/मि
[W] Other e.g. spices, tea, coffee अन्य जस्तै: मसला, चिया, कफी

Where is your child during an average day?

01:00	02:00	03:00	04:00	05:00	06:00
07:00	08:00	09:00	10:00	11:00	12:00
13:00	14:00	15:00	16:00	17:00	18:00
19:00	20:00	21:00	22:00	23:00	24:00

IB=Indoor bedroom IK= Indoor kitchen IKC= Indoor kitchen when cooking

O=Outdoors V=Veranda S=School Please state if other

Appendix 3.6 Anthropometry: Childhood nutrition research centre, standard operating procedure

Weight (digital scales)

1. Operator checks that scales are on level floor, away from any objects and tared to zero.
2. Subject wears underwear or swimsuit
3. Subject stands on centre of scales, keeping still, facing forwards and hands at sides.
4. Operator records measurement once read-out is stable.
5. The measurement is recorded to the nearest 0.01 kg

The measurement is repeated and recorded twice after excluding any erroneous values.

Height (wall-mounted stadiometer)

1. Remove shoes and any hair ornaments that will interfere with the measurement
2. Subject stands straight with
   (a) feet flat on floor,
   (b) back, shoulders, head, buttocks (and calves if backboard reaches) against back-board of stadiometer,
   (c) heels against heel plate and
   (d) head in horizontal Frankfurt (orbito-meatal) plane passing through upper margins of the external acoustic meatuses and the lower margin of the left orbit.
3. Subject takes a breath in and on expiration the headboard is moved down gently onto the head compressing the hair and the measurement recorded.
4. The measurement is recorded to the nearest 0.1 cm

The measurement is repeated and recorded twice after excluding any erroneous values.
NB If it is not possible to have all the back, buttocks and calves in contact with the backboard make sure the subject is standing with a upright spine.

Circumferences

Use appropriate insertion tape depending on size of circumference to be measured.
Measurement of circumferences should not be over clothing unless it is light and close fitting such as a swimming costume.

Head circumference

1. Remove hair ornaments so that hair can be compressed close to the skull.
2. Subject stands straight with head in Frankfurt plane.
3. Operator places insertion tape horizontally around widest point of occipital bones and forehead perpendicular to the long axis of the face.
4. Operator records measurement at point on tape indicated after compressing hair by pulling tape tight.
5. The measurement is recorded to the nearest 0.1 cm

The measurement is repeated and recorded twice after excluding any erroneous values.

Mid-upper arm circumference (MUAC, left side)

1. Subject stands straight with left arm at side and elbow bent at an angle of 90^{o}.
2. Operator finds the depression at the end of the acromion process and locates the lateral tip of the acromion process.
3. Operator finds the point of the olecranon process (elbow).
4. Operator measures the distance between acromion tip and point of elbow.
5. Operator marks a point halfway between these anatomical landmarks.

6. Subject lets arm hang loosely at the side. If also measuring bicep and tricep skinfolds, extend this line horizontally to the anterior and posterior of the upper arm.
7. The operator measures, and records, mid-upper arm circumference at this point, with the tape perpendicular to the long axis of the arm and pulled tight so that it is in contact with the skin without compression.
8. The measurement is recorded to the nearest 0.1 cm

N.B Ensure that the arm is relaxed and hanging at the side whist taking the measurement.
The measurement is repeated and recorded twice after excluding any erroneous values.

Waist circumference (WHO/ Lohman method)

1. Subject stands straight with abdomen relaxed and arms hanging at the sides and feet together.
2. Operator faces the subject and finds the narrowest girth as seen from the front. If this proves to be difficult then the subject may be asked to bend to the side and the operator then identifies the point at which the trunk folds, and uses this as the landmark. For obese subjects the smallest horizontal circumference at a point between the lowest rib and top of iliac crest is measured.
3. At the end of normal expiration, measure and record the waist circumference at this point with the tape horizontal and in contact with the skin without compressing the waist.
4. The measurement is recorded to the nearest 0.1 cm

The measurement is repeated and recorded twice after excluding any erroneous values

Hip circumference

1. Subject stands straight with arms at the sides and feet together.
2. Whilst squatting at the side of the subject the operator finds the widest girth of the hips, usually around the greater trochanters of the femur, but may vary according to the shape of the subject.
3. The tape is passed around the widest point and the operator checks this is horizontal and in close contact without compression.
4. The measurement is recorded to the nearest 0.1 cm

The measurement is repeated and recorded twice after excluding any erroneous values.

Skinfold thickness measurements

General technique:

1. Always check the dial of the calipers start at zero and adjust if necessary.
2. Use an eyeliner pencil to mark the points to measure.
3. Assuming the operator is right handed, then once the site has been located and marked the thumb and forefinger of the left hand are used to elevate a fold of skin and subcutaneous fat about 1cm away from the measurement point. If necessary the skin can be lifted using both hands and then held by the left hand whilst measuring with the right.
4. The thumb and finger must be far enough away from the point of measurement so that the fingers are not compressing the point of measurement and the skinfold is pulled away from the musculature in order to form a fold with almost parallel skin surfaces. It may not be possible to achieve parallel sides when the skinfold is large. Care must be taken to only grasp skin and subcutaneous fat.
5. The right hand is used to open the calipers and place them over the skinfold perpendicular to the long axis of the fold approximately half way between the crest of the fold and the body surface.
6. The calipers are released whist continuing to hold the skinfold with the left hand.
7. A reading is taken once the dial first slows to almost a stop. This should be around 3 secs but never more than 4 secs because if the calipers are left in position too long, particularly

in obese subjects, they will start to compress the skinfold and give an inaccurate measurement.

8. Once a reading is taken release the calipers FIRST before releasing the fold held by the left hand. In young children there is a danger that they may suddenly pull away and there is a risk of damage to the skin. The operator should be aware and release the calipers quickly.
9. Whilst taking a reading the operator's head should be positioned so as to avoid errors due to parallax.
10. A measurement is recorded to the nearest 0.2 cm.
11. The measurement is repeated and recorded three times after excluding any erroneous values

Biceps (on left)

1. Subject stands straight with arm held loosely at the side.
2. Operator finds the level of the mid-upper arm circumference measurement (see MUAC above) and locates the point on the anterior aspect of the arm. Using the previous mark for MUAC, a line is drawn perpendicular to that line and directly over the humerus.
3.The skinfold measurement is taken by the operator gently pinching the skin and adipose tissue immediately above this site and pulling it away from the underlying muscle.
4. The caliper is then used for the measurement at the exact level on the mid-upper arm plane.

The measurement is repeated and recorded three times after excluding any erroneous values

Triceps (on left):

1. Subject stands straight with arm held loosely at the side.
2. The operator finds the level of the mid-upper arm circumference measurement (see MUAC above) and locates the point on the posterior aspect of the arm. Using the previous mark for MUAC, a line is drawn perpendicular to that line and directly over the humerus.
3. The skinfold measurement is taken by the operator gently pinching the skin and adipose tissue immediately above this site and pulling it away from the underlying muscle.
4. The caliper is then placed at the exact level on the mid-upper arm plane.

The measurement is repeated and recorded three times after excluding any erroneous values

Subscapular (on left)

1. Subject stands straight with arms held loosely at the side.
2. The operator finds the inferior angle at the lower margin of the scapula. If this is difficult to locate then gently take the left forearm and place behind the back, mark the scapular angle and then release the arm.
3. The skinfold is grasped diagonally so that the point of measurement is just inferior to this point and the fold is inclined infero-laterally at approximately 45 ^{0} to the horizontal and in the natural cleavage line of the skin.
4. The caliper is placed approximately 1 cm from the fingers of the left hand, perpendicular to the long axis of the skinfold.

The measurement is repeated and recorded three times after excluding any erroneous values

Suprailiac (on left)

1. Subject stands straight with arms held slightly abducted to aid access to the measurement site.
2. The operator palpates the top of the iliac crest and marks a cross aligned with the mid-axillar.
3. An oblique skinfold is grasped just above the centre of the marked cross (see 2 above) and following the natural cleavage line of the skin so that the fold is approximately 1cm above the iliac crest at the mid-axillary point. It should be aligned inferomedially at approximately 45 ^{0} to the horizontal. Two hands may be used to grasp the skinfold initially and then the right hand removed to use the caliper.
4. The caliper is placed on the fold at the mid-axillar point.

The measurement is repeated and recorded three times after excluding any erroneous values

Appendix 3.7 Anthropometry data collection form

ID

English date

Data collector initials for anthropometry

Time

:

Weight Tanita 418 = Kg Tanita Solar = Kg

Height

Standing height = cm Standing height = cm

Sitting height = cm Sitting height = cm

Skinfold thicknesses: Caliper 1 ☐ 2 ☐

	First (mm)	Second (mm)	Third (mm)
Triceps			
Bicep			
Subscapular			
Suprailiac			

Circumferences Tape 1 ☐ 2 ☐ 3 ☐

	First (cm)	Second (cm)
Head		
Chest		
Waist		
Hip		
Upper leg		
Mid-upper arm		

Blood pressure: **Monitor 1** ☐ **Monitor 2** ☐

1. / mmHg **2. / mmHg**

Ultrasound kidney size: **Right length cm, A/P cm**

Left length cm, A/P cm

Spirometry: 1 ☐ or **2** ☐

Data collector's initials:

FEV1 =

FVC =

MEF 25-75 =

Hair sample Yes ☐ No ☐

Nail sample Yes ☐ No ☐

Bioelectrical impedance (Please staple)

Add 0.2kg for clothes

Whole body = Ω

Right leg = Ω

Left leg = Ω

Right arm = Ω

Left arm = Ω

Appendix 3.8 Janakpur Deuterium Calibration Protocol

Summary of proceedings

1. Gain Consent
2. Check that child hasn't drunk water in last 30mins
3. Explain saliva sample technique and deal with questions
4. Collect pre-dose samples
5. Administer dose, recording time & planning the post-dose interval
6. Perform bioelectrical impedance (BIA), weight, standing height and sitting height measurements
7. Monitor child during 4 hour waiting period & give standard meal & drinks
8. Collect post-dose saliva sample at the 4 hour mark Thank participants and give participation gifts.
9. Spin down all samples and freeze ready for analysis.
10. Enter all participant data.

Explanation For Consent

Read the following information to participants

We would like to do a test to measure the total amount of water in your body. This can give us information of about your body composition, which means information about the basic constituents of your body (e.g. muscle, bone, fat and water). This gives us lots of information about your general health. We will use this data to help us in a larger study, which we are also conducting in Dhanusha. None of the tests we are performing today are harmful or painful. The testing will take between about 5 hours, 4 hours of which is waiting time.

The testing involves 2 stages.

PART 1: Before the test begins we need to make sure that you have not eaten or drunk for 30 minutes. The first stage involves you giving one saliva sample and then drinking a "special" water drink. The saliva test involves wetting a cotton wool bud in your mouth. The "special drink" is drunk through a straw from a small plastic bottle. At this stage we will also do other measurements of your height and weight and will ask you to stand on a machine called a "bioelectrical impedance machine".

The special water drink is a substance called deuterium - also known as heavy water. Deuterium is entirely harmless - it is simply a different form of water. It is normally present in low quantities in the human body. We give you a concentrated amount of the substance enabling us to measure it in your body from the saliva samples that you give us during the test. From this we can measure the amount of water in your body. The Bioelectrical impedance test involves you standing on this machine *(SHOW THEM THE MACHINE)* and holding the handles. The machine sends a tiny electrical current that you cannot feel through your body and measures the resistance through different parts of your body. From this we can calculate percentages of water, fat, muscle and bone in your body.

We will then measure your height and weight using scales and measures. Combing the results from the deuterium, the bioelectrical impedance and other tests enables us to measure your body composition accurately. Some of these tests take time to calculate the result so unfortunately some, but not all, of the information will be available today. After you have done the first saliva sample and the other tests, the first stage is complete.

PART 2: The second part of the test involves waiting before performing the second saliva sample. We need total of four hours for the deuterium drink to distribute evenly through your body. During this waiting time we will give you food and drinks. You must not drink anything other than what we give you as this will affect the test. In the final 30 mintues we will ask you not to eat or drink anything. After this time you will perform the final saliva sample, also using a cotton bud in the mouth.

Once this done, the test is complete.

If you have any questions please free to ask them now, or at any stage during the test.

Protocol for researchers

Preparation for the test - 30minutes

The first priority is to make sure that a valid period of nil by mouth is obtained. On arrival participants and their guardians should be told that the study cannot start until we have waited 30 mins without the children eating or drinking (including chewing gum). During the period of the study, the children must not eat or drink anything without instruction from the researchers. Once this has been explained and the time recorded the consent can be gained.

Gaining Consent

Explain about 30 min nil-by-mouth period before starting the test

Deliver explanation/watch film

Discuss project with parent/guardian and answer any questions

Obtain signature of parent/guardian

This period is unlikely to take the full 30 mins so this time should be spent with resolving any questions and demonstrating the technique of saliva sample collection.

Saliva Collection

In order to obtain an adequate sample, the following technique should be explained clearly to the child. Approximately 2ml of saliva is needed to run the total body water test. The cotton wool buds need to be made VERY wet to give an adequate sample. Below 1ml of saliva the test risks becoming unusable. Observing expansion of the cotton bud to 150-200% of its "dry" size is a means of assessing adequacy of the sample. Inadequately wet sample will show minimal expansion of the cotton bud. This is more reliable that assessing adequacy on how "wet" the sample looks.

Demonstrate the following to the child:

1. The various components of the test: the salivette, the drink and the sample container. Focusing on the salivette show the cotton wool bud and the container itself, where the saliva collects after centrifugation.

2. The technique for getting a good sample.

Build up lots of saliva in mouth.

Put cotton wool in mouth.

Roll it around mouth with your tongue. DO NOT chew it.

Do this for 1-2 mins and make the cotton wool bud as wet as possible.

Remove with fingers and replace into receptacle. Do NOT drop on the floor!

If there is time prior to performing the pre-dose sample, check the child's understanding and repeat explanation as required.

Steps for Collecting Pre-dose sample

Explain clearly how to get an adequate sample, checking understanding

Encourage the child to build up saliva in the mouth

Don disposable gloves and snap the cap off the salivette

Remove the cotton bud from the container and place the cotton bud in child's open mouth

Encourage the child to move the cotton bud round the mouth. Remind them not to chew the bud. Wait at least 1.5 minutes.

Ask the child to present the bud at the front of the mouth and check for adequacy

If adequate, reholster in salivette.

Shake the salivette. It should “thud” and stick when shaking.

Store sample safely ready for centrifugation.

Deuterium drink

The accuracy of the total body water test relies on knowing the precise amount of deuterium that has been consumed. Spillage of the prepared drink should be avoided at all costs. Explanation to the child in a clear fashion will increase success of avoiding error. In the event of a spillage, if it is only small droplets these can be approximated to 0.1 ml per beaded droplet. Larger losses which are not possible to quantify accurately will invalidate the test.

Shake the drink before giving to the child.

Say the following: “The drink needs to be drunk very carefully with the straw to avoid spilling any of the drink.”

The drink needs to be kept in the bag, so that if any does spill we can catch the drops.

Try to drink the whole drink & when you are finished give the drink back to me.

Explain that they are about to drink the special "heavy water" drink and discuss the proper technique to avoid spilling.

Steps for administering deuterium dose

Open sealed prepared deuterium drink, undo lid and insert straw.

Give the drink, within the bag, to the child. Ask them to take the drink with both hands.

Instruct to drink all drink using the straw.

When finished take empty container, bend straw into bottle and seal with lid. Next seal bag around container.

Record the time the dose was given.

Calculate the time to perform "post-dose", four hours later.

Waiting Period

As soon as the deuterium drink has been drunk the time must be recorded, correct to the nearest minute. The post-dose sample must be conducted at 4 hours following, making sure that the participant has not drunk or eaten for 30 minutes prior to this.

During this period children will be given standardised drink (fizzy drink or water filled up to demarcation on plastic cups), administered at set times. Additional tests are performed at this stage.

During this period the pre-dose samples should be centrifuged. The samples should be spun for a minimum of 3000 rpm for 3 minutes. In exceptionally low yield samples try running for another couple of minutes - harvest after this point is minimal however.

Weight, Height and BIA

Height: Height was measured with a Leicester stadiometer, accurate to 0.1cm. The stadiometer was calibrated with a 50cm calibration rod.

Position: The child’s feet should be placed together with their heels touching the stadiometer. Their knees were kept extended and their head was placed in the Frankfort position. The child is asked to take a breath in just before the reading is taken. The measurement should be taken with the observer reading at the appropriate height to avoid parallax.

The sitting height measurements are taken with the child seated on a custom-made stool. Their legs should be supported so that the knees were bent at 90°.

Weight: Prior to weighing and BIA the child should empty their bladder. Body weight was measured with a Tanita Solar stand on scale accurate to 0.1 kg. The children are weighed wearing only their underwear, a vest and a sarong that together weigh 200g. The weighing scales are calibrated fortnightly with calibration weights.

BIA: The children stood on the metal plates with their legs apart and their arms not touching their body. Two recordings were taken, one with the child's arms at 90° and one with their arms at 180°. The weight of clothes used was 200g.

Second saliva sample

The second saliva sample must be performed at least 4 hours following consumption of the deuterium dose but only if there has been a clear period of 30 minutes of nil-by-mouth prior to performing the sample. The test saliva sample must not be collected earlier than this. If the child eats or drinks anything during this period of nil-by-mouth, the test must be delayed 30 minutes after the last drink or foodstuff consumed.

Steps for collecting post-dose sample are the same as above (page 339)

Avoid lapses in concentration and technique from both researcher and child

Ensure adequacy of sample.

Once the sample has been successfully collected the test is complete

After the test: Once the final saliva sample has been given this test is complete. To complete the testing the following tasks are required.

Re-weigh dose bottle and straw

Storage

Data Entry

Centrifugation and sample storage

Spin down samples, colour code label and freeze

Pipette into sample freezing tubes - expel air from pipette (squeeze it fully), introduce the bottom of the receptacle and then release allow the pipette to fill.

Discharge pipette into the freezing tube close to the top - avoid forming bubbles

Repeat process until freezing tube is 90% full

Seal tube, check label and then place in freezer box and into freezer.

Making up the dose

A standard dose of 1.2g of deuterium is used in this study representing 0.06g of deuterium x average weight of children in this study (20kg)

Labelling

Colour Label all components before starting using indelible marker pens. Label participant ID and nature of the sample in question on all parts. Thus: lid and bottles for dosing and freezer tubes and body and cap for salivettes.

Colour coding:

Dose and dosing bottles (GREEN)

Pre-dose salivettes and tubes (RED)

Post-dose salivettes and tubes (BLUE)

List of Components per Test:

Salivettes x3, Freezer tubes x3, Dosing bottle, Straw, Re-sealable plastic bags, Fresh Cold Water - 100ml, Deuterium 1.2g, Pipettes x3, Generic components, Marker pens Red, Green, Blue, Bottles still water. Freezer box for receptacles, Bottle of deuterium, Filter (one per day), 5ml Syringes (one per day)

Calibrating scales

To ensure accuracy scales need to be calibrated each day and prior to use.

Method

Turn scales on with held press of "On/zero/Off/Yes" button

Wait 1 minute before performing calibration

Press and hold "Print/Cal" until "CAL" appears on display

Wait for flashing "-C-" to disappear and flashing "100g" to appear

Place 100g calibration weight on scale

Press " On/zero/Off/Yes" Button

Wait for "DONE" to show on display

Steps to make up dose

Wash hands.

Take off lid of dose bottle, place the empty bottle on the scales and wait till weight reading is stable.

Zero scales

Measure out 1.2ml of deuterium using syringe delivered through filter. Go slowly, allowing for measurement lag and observe weight increase using the scales.

Once done - pump one empty (air filled) full syringe slowly through the filter - returning any unused deuterium to the container (normally about 2 drops). Be careful not to push off the filter.

Fill the bottle up 75% with bottled water.

Seal both deuterium container and dosing bottle

Agitate dosing bottle for one minute- (fix elbow and shoulder and rotate wrist prone and supine in full 180 degree motion for this time - rather than simply shaking)

Immediately place down - remove lid and shake off/dry any loose drops externally and those on lid and bottle rims which would otherwise evaporate.

Take clean, fresh pipette and withdraw 2ml of fluid from the centre of the bottle and place in pre-marked (GREEN DOSE) freezer tube.

Seal both bottle and freezer tube. Double check bottle for spilled external drops and dry if found. One violent shake WITH top sealed can dislodge difficult droplets wedged between lid and bottle. Repeat drying process on final time.

Place bottle into plastic bag with straw.

Weigh bottle making sure all aspects of plastic bag are resting on the scale are part of this amount, ensuring no overhang.

Seal bottle bag and leave aside ready for testing.

Sebastian Roberts, Final year medical student, UCL

Dr Delan Devakumar, Wellcome Trust Research Training Fellow, UCL

29/1/12

Appendix 3.9 Air pollution sampling strategy

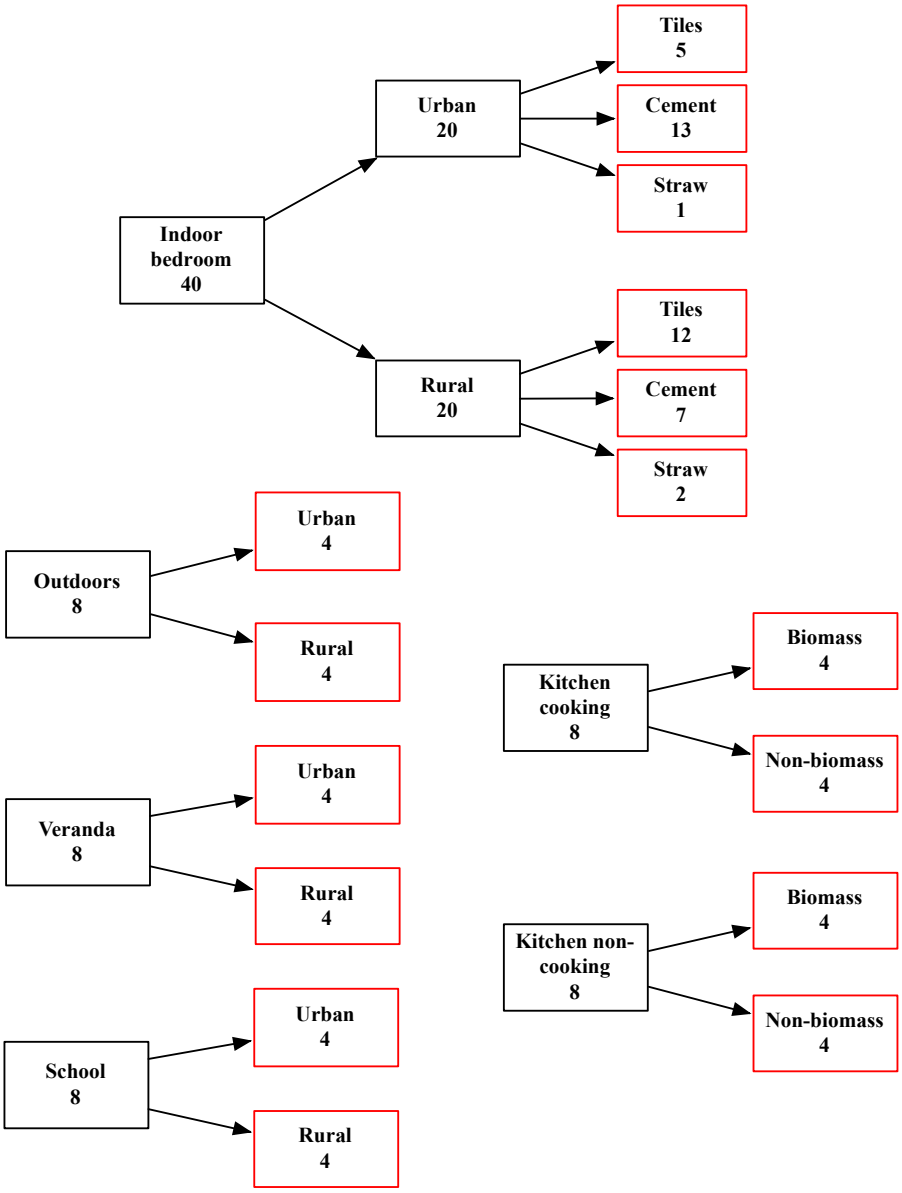

Figure 1: Air pollution sampling profile used for the first season of sampling

Appendix 3.10 Air pollution data collection form

Air Pollution

Child's ID	School	Data collector ID
☐☐☐☐		

Apex number	Nepali date	Filter number	Time start	Volume start (L or m^3)	Temp start (^oC)	Time end	Volume end (L or m^3)	Temp end (^oC)	Calibration end (L)
☐	20☐☐ ☐☐ ☐☐	☐☐☐	_ _:_ _		☐☐	_ _:_ _		☐☐	
☐	20☐☐ ☐☐ ☐☐	☐☐☐	_ _:_ _		☐☐	_ _:_ _		☐☐	
☐	20☐☐ ☐☐ ☐☐	☐☐☐	_ _:_ _		☐☐	_ _:_ _		☐☐	
☐	20☐☐ ☐☐ ☐☐	☐☐☐	_ _:_ _		☐☐	_ _:_ _		☐☐	

Appendix 3.11 Doctor's voucher

Doctor's Voucher

Dr. SN Yadav Date: _ _ _ _ / _ _ / _ _

ID N.....

Child Name: ..... Age..... Sex.....

This voucher can be used to cover the cost of essential, acute medications up to a value of Rs 200. It can be redeemed up to the end of the research project in December 2012.

This voucher can be used for the following medications:

Oral antibiotics: amoxycillin, ciprofloxacillin, norfloxacillin, cefixime, metronidazole

Oral aciclovir

Anti-malarials: chloroquine and sulfadoxine

Appendix 3.12 Ethic approval letters

UCL RESEARCH ETHICS COMMITTEE
GRADUATE SCHOOL OFFICE

Dr David Osrin
Centre for International Health and Development
Institute of Child Health
30 Guilford Street
UCL

16 May 2011

Dear Dr Osrin

Notification of Ethical Approval
Ethics Application: 2744/001: The effects of antenatal micronutrient supplementation and current air pollution on growth and lung function in 8-10 year old children

I am pleased to confirm that in my capacity as Chair of the UCL Research Ethics Committee, I have approved your study for the duration of the project (i.e. until November 2012).

Approval is subject to the following conditions:

1. You must seek Chair's approval for proposed amendments to the research for which this approval has been given. Ethical approval is specific to this project and must not be treated as applicable to research of a similar nature. Each research project is reviewed separately and if there are significant changes to the research protocol you should seek confirmation of continued ethical approval by completing the 'Amendment Approval Request Form'.

The form identified above can be accessed by logging on to the ethics website homepage: http://www.grad.ucl.ac.uk/ethics/ and clicking on the button marked 'Key Responsibilities of the Researcher Following Approval'.

2. It is your responsibility to report to the Committee any unanticipated problems or adverse events involving risks to participants or others. Both non-serious and serious adverse events must be reported.

Reporting Non-Serious Adverse Events
For non-serious adverse events you will need to inform Helen Dougal, Ethics Committee Administrator (ethics@ucl.ac.uk), within ten days of an adverse incident occurring and provide a full written report that should include any amendments to the participant information sheet and study protocol. The Chair or Vice-Chair of the Ethics Committee will confirm that the incident is non-serious and report to the Committee at the next meeting. The final view of the Committee will be communicated to you.

Reporting Serious Adverse Events
The Ethics Committee should be notified of all serious adverse events via the Ethics Committee Administrator immediately the incident occurs. Where the adverse incident is unexpected and serious, the Chair or Vice-Chair will decide whether the study should be terminated pending the opinion of an independent expert. The adverse event will be considered at the next Committee meeting and a decision will be made on the need to change the information leaflet and/or study protocol.

On completion of the research you must submit a brief report (a maximum of two sides of A4) of your findings/concluding comments to the Committee, which includes in particular issues relating to the ethical implications of the research.

With best wishes for the research.

Yours sincerely

Sir John Birch
Chair of the UCL Research Ethics Committee

Cc: Delanjathan Devakumar, CIHD, Institute of Child Health, UCL

UCL Research Ethics Committee
Graduate School
North Cloisters, Wilkins Building
UCL
Gower Street
London WC1E 6BT

Tel: 020 7679 7844
Fax: 020 7679 7043
Email: ethics@ucl.ac.uk

Nepal Health Research Council

Estd. 1991

NHRC

Ref. No. 105

29 September 2011

Dr. David Osrin
Principal Investigator
University College London, UK

Ref: Approval of Research Proposal entitled **The Effects of Antenatal Micronutrient Supplementation and Current Air Pollution on Growth and Lung Function in 8-10 Years Old Children**

Dear Dr. Osrin,

It is my pleasure to inform you that the above-mentioned proposal submitted on 31 May 2011 (**Reg. no. 51/2011** please use this Reg. No. during further correspondence) has been approved by NHRC Ethical Review Board on 25 September 2011 (2068-06-08).

As per NHRC rules and regulations, the investigator has to strictly follow the protocol stipulated in the proposal. Any change in objective(s), problem statement, research question or hypothesis, methodology, implementation procedure, data management and budget that may be necessary in course of the implementation of the research proposal can only be made so and implemented after prior approval from this council. Thus, it is compulsory to submit the detail of such changes intended or desired with justification prior to actual change in the protocol.

If the researcher requires transfer of the bio samples to other countries, the investigator should apply to the NHRC for the permission.

Further, the researchers are directed to strictly abide by the National Ethical Guidelines published by NHRC during the implementation of their research proposal and submit progress report and full or summary report upon completion.

As per your research propnsal, total research amount is US$ 39,305.00 and NHRC processing fee is NRs.85, 370.46.

If you have any questions, please contact the research section of NHRC.

Thanking you.

Sincerely Yours,

Dr. Shanker Pratap Singh
Member Secretary

Tel.+977-1-4254220, 4227460, Fax: +977-1-4262469, RamShah Path, P.O. Box 7626, Kathmandu, Nepal.
Website: http://www.nhrc.org.np, Email : nhrc@nhrc.org.np

Appendix 3.13 Information sheets and consent form

Information Sheet for Parents or Guardians

You will be given a copy of this information sheet. If you decide to take part you will be asked to sign a consent form.

Title of Project: The effects of antenatal micronutrient supplementation and current air pollution on growth and lung function in 8-10 year old children

This study has been approved by the UCL Research Ethics Committee (Project ID Number): **2744/001**

Name	Dr D Devakumar
Work Address	MIRA Dhanusha office, Ramanand Chowk, Janakpur
Contact Details	041523371

We would like to invite to participate in this research project.

- **Details of Study:**

This study is the follow-up of the children born in the previous study in which mothers were given a vitamin and mineral supplement in pregnancy. We want to see all the children (up to 1064 children) to find out the long term effects of this supplement. We would like to know if the supplement makes your child bigger and improves their breathing. This will help to decide whether such supplements should be used in the future.

If you are happy to participate, we would like to ask you some questions about your child's health, your family, where you live and what you eat. We will then ask you to come to the Mother and Infant Research Activities (MIRA) office when convenient, where we will take some measurements of your child: their height and weight, percentage of body fat, body measurements, and skinfolds (by pressing the fat gently on the arm, shoulder and abdomen). They will not be asked to undress completely. We will then check their blood pressure, perform some breathing tests by asking them to blow into a small machine, and look at the size of their kidney with an ultrasound machine. We will take some samples of your child's hair and toenails to find out the level of minerals. A sample of saliva will also be requested to investigate their hereditary make-up (genetic markers related their growth and lung function). As in the previous study, we will use identification numbers and will not mention your child's name on any of the samples.

The whole assessment will take approximately 45 minutes and none of the measurements will harm your child.

We would also like to measure the quality of the air your child is breathing in. This would involve sampling the air with small machines in your home and in your child's school.

We may ask if we can take a photograph of your child during the project. This will only happen with your consent at the time. The photographs may be used to help summarise the work done by MIRA. Your child's name and contact details will not be included with them.

You may decide to personally withdraw or withdraw your data from the research project at any time. This will not affect you in any way.

We will cover the costs of transport to the MIRA office (using public transport). We will also provide a consultation with a doctor in Janakpur to check over your child and cover the costs of essential, acute medications up to a value of NRs 200. We will also provide refreshments and a small gift for your child to say thank you.

All the information we collect will not show your child's name and will be stored securely. Only the researchers will have access to it. The scientific findings may be published in the scientific media or presented at scientific meetings, but your name or identifiable information will not appear anywhere.

Please discuss the information above with others if you wish or ask us if there is anything that is not clear or if you would like more information.

It is up to you to decide whether to take part or not; choosing not to take part will not disadvantage you in any way. If you do decide to take part you are still free to withdraw at any time and without giving a reason.

All data will be collected and stored in accordance with the Data Protection Act 1998.

Information Sheet for Parents or Guardians

You will be given a copy of this information sheet. If you decide to take part you will be given this information sheet to keep and be asked to sign a consent form.

Title of Project: The effects of antenatal micronutrient supplementation and current air pollution on growth and lung function in 8-10 year old children – calibration study

This study has been approved by the UCL Research Ethics Committee (Project ID Number): **2744/001**

Name	Dr D Devakumar
Work Address	MIRA Dhanusha office, Ramanand Chowk, Janakpur
Contact Details	041523371

We would like to invite to participate in this research project.

- **Details of Study:**

This study is designed to measure the body composition of Nepalese children in this region. We will try to measure the total amount of water in your child's body and from this, estimate the amount of body fat and muscle and bone. This information will be useful for other research looking at the size and make-up of children in Nepal.

If you are happy to participate, we would like your child to drink special water drink is a substance called deuterium - more commonly knows as heavy water. This drink is entirely harmless and is simply a different form of water. It occurs naturally in all of us. Before and 4 hours after the drink they will be asked to provide a saliva sample using an absorbent cotton wool swab. The saliva samples will be given a unique identification number, stored in a secure location in the Mother and Infant Research Activities (MIRA) office and transported to London where it will be tested.

The study will be done while your child is at school and it will not disrupt their lessons.

We may ask if we can take a photograph of your child during the project. This will only happen with your consent at the time. The images may be used to help summarise the work done by MIRA. Your child's name and contact details will not be included with the images.

You may decide to personally withdraw or withdraw your data from the research project at any time. You would just need to inform the research assistant if so.

All the information we collect will be anonymised and stored securely. Only the researchers will have access to the data that is collected. The information may be published in the scientific media or presented at scientific meetings, but your name or identifiable information will not appear anywhere.

Please discuss the information above with others if you wish or ask us if there is anything that is not clear or if you would like more information.

It is up to you to decide whether to take part or not; choosing not to take part will not disadvantage you in any way. If you do decide to take part you are still free to withdraw at any time and without giving a reason.

All data will be collected and stored in accordance with the Data Protection Act 1998.

Informed Consent Form for Parents or Guardians

Please complete this form after you have read the Information Sheet and/or listened to an explanation about the research.

Title of Project: **The effects of antenatal micronutrient supplementation and current air pollution on growth and lung function in 8-10 year old children**

This study has been approved by the UCL Research Ethics Committee (Project ID Number): **2744/001**

Thank you for your interest in taking part in this research. Before you agree to take part, the person organising the research must explain the project to you.

If you have any questions arising from the Information Sheet or explanation already given to you, please ask the researcher before you to decide whether to join in. You will be given a copy of this Consent Form to keep and refer to at any time.

Statement of Parent or Guardian

I

- have read the notes written above and the Information Sheet, and understand what the study involves.
- understand that if I decide at any time that I no longer wish to take part in this project, I can notify the researchers involved and withdraw immediately.
- consent to the processing of my personal information for the purposes of this research study.
- understand that such information will be treated as strictly confidential and handled in accordance with the provisions of the Data Protection Act 1998.
- agree that the research project named above has been explained to me to my satisfaction and I agree to take part in this study.

Signed: Date:

Appendix 3.14 Referral form to the nutrition unit

MIRA Dhanusha

Micronutrient follow-up programme

Referral form for children with malnutrition

Date:

Name of child:

Date of birth:

Name of the mother:

Name of the father:

Address:

Weight:	
Height:	
Weight for height Z score:	
BMI Z score:	
Mid-upper Arm Circumference:	

Comments:

Appendix 4

Appendix 4.1 Technical effort of measurement data

Table 1: Results for the technical error of measurement and coefficient of reliability assessments throughout the research project

September 2011 (Piloting):

	Observer 1		Observer 2	
	Intra-observer TEM	Intra-observer TEM %	Intra-observer TEM	Intra-observer TEM %
Height	0.07	0.06	0.07	0.06
Biceps SFT	0.12	3.36	0.06	1.29
Triceps SFT	0.10	1.40	0	0
Subscapular SFT	0.12	2.55	0	0
Suprailiac SFT	0.10	1.92	0.06	0.98
Head circum	0.05	0.10	0.03	0.06
Chest circum	0.05	0.09	0.03	0.05
Waist circum	0.07	0.14	0.03	0.06
MUAC	0	0	0	0

	Inter-observer TEM			Inter-observer coefficient of reliability		
	First	Second	Third	First	Second	Third
Height	0.33			1		
Biceps SFT	0.17	0.89		0.23	0.98	
Triceps SFT	0.32			0.95		
Subscapular SFT	0.25	0.06		0.63	1	
Suprailiac SFT	0.62	0.35		0.90	0.98	
Head circum	0.12			0.97		
Chest circum	0.77			0.96		
Waist circum	2.31	1.07	0.18	0.57	0.85	0.99
MUAC	0.21			0.95		

February 2012:

	Observer 1		Observer 2	
	Intra-observer TEM	Intra-observer TEM %	Intra-observer TEM	Intra-observer TEM %
Height	0.05	0.05	0.04	0.05
Biceps SFT	0.06	1.77	0	0
Triceps SFT	0	0	0	0
Subscapular SFT	0.13	2.80	0.13	2.93
Suprailiac SFT	0.13	2.70	0.06	1.41
Head circum	0.04	0.09	0.04	0.09
Chest circum	0.06	0.12	0.04	0.08
Waist circum	0.04	0.01	0.05	0.11
Hip circum	0.04	0.08	0.04	0.08
Upper leg circum	0.04	0.14	0	0
MUAC	0.03	0.20	0.03	0.20

	Inter-observer TEM		Inter-observer coefficient of reliability	
	First	Second	First	Second
Height	0.33		0.97	
Biceps SFT	0.21		0.94	
Triceps SFT	0.30		0.97	
Subscapular SFT	0.17		0.95	
Suprailiac SFT	0.25		0.95	
Head circum	0.37	0.14	0.82	0.99
Chest circum	1.04	0.77	0.18	0.90
Waist circum	1.27	0.35	0.13	0.97
Mid-upper arm circum	0.11		1	
Hip circum	0.13		0.96	
Upper leg circum	0.46		0.98	

May to July 2012:

	Observer 1		Observer 2	
	Intra-observer TEM	Intra-observer TEM %	Intra-observer TEM	Intra-observer TEM %
Height	0.03	0.03	0.03	0.03
Biceps SFT	0.09	2.60	0.01	2.25
Triceps SFT	0.10	1.54	0.01	1.22
Subscapular SFT	0.09	2.11	0.01	1.87
Suprailiac SFT	0.11	2.46	0.06	1.47
Head circum	0.04	0.09	0.04	0.08
Chest circum	0.04	0.07	0.06	0.11
Waist circum	0	0	0.06	0.12
Hip circum	0	0	0.03	0.05
Upper leg circum	0.03	0.10	0.03	0.10
MUAC	0.04	0.27	0.03	0.20

	Inter-observer TEM		Inter-observer coefficient of reliability	
	First	Second	First	Second
Height	0.16		1.00	
Biceps SFT	0.14		0.95	
Triceps SFT	0.22		0.99	
Subscapular SFT	0.13	0.20	0.94	0.90
Suprailiac SFT	0.20		0.96	
Head circum	0.17		0.97	
Chest circum	0.95	0.63	0.89	0.92
Waist circum	0.67	0.35	0.93	0.98
Mid-upper arm circum	0.07		0.99	
Hip circum	0.31		0.99	
Upper leg circum	0.60	0.64	0.93	0.93

October 2012:

	Observer 1		Observer 2	
	Intra-observer TEM	Intra-observer TEM %	Intra-observer TEM	Intra-observer TEM %
Height	0.05	0.04	0.05	0.04
Sitting height	0.05	0.04	0.02	0.02
Biceps SFT	0.06	1.68	0	0
Triceps SFT	0.06	0.86	0.09	1.20
Subscapular SFT	0.08	1.64	0.06	1.36
Suprailiac SFT	0.09	1.50	0.06	1.05
Head circum	0.03	0.06	0.05	0.10
Chest circum	0.04	0.07	0.06	0.11
Waist circum	0.04	0.08	0.05	0.10
Hip circum	0.04	0.08	0.04	0.08
Upper leg circum	0.04	0.12	0.03	0.10
MUAC	0.04	0.28	0.04	0.24

	Inter-observer TEM	Inter-observer coefficient of reliability
Height	0.26	1.00
Sitting height	0.39	0.96
Biceps SFT	0.15	0.95
Triceps SFT	0.30	0.98
Subscapular SFT	0.16	0.93
Suprailiac SFT	0.28	0.95
Head circum	0.15	0.99
Chest circum	0.53	0.97
Waist circum	0.40	0.99
Mid-upper arm circum	0.22	0.96
Hip circum	0.29	0.99
Upper leg circum	0.17	0.99

Table 2: Results split into intervention and control groups with and without the removal of measurement error. Lt = left, Rt = right

	Control					Intervention				
	Mean	SD	95% CI	SD after adjust-ment for error	95% CI after adjust-ment for error	Mean	SD	95% CI	SD after adjust-ment for error	95% CI after adjust-ment for error
Standing height (cm)	120.72	5.91	120.16, 121.29	5.91	120.16, 121.29	120.73	6.06	120.15, 121.31	6.06	120.15, 121.31
Sitting height (cm)	64.14	2.94	63.86, 64.42	2.94	63.86, 64.42	64.16	2.96	63.87, 64.44	2.96	63.87, 64.44
Skin-fold thickness										
Triceps (mm)	7.39	2.54	7.15, 7.63	2.54	7.15, 7.63	7.36	2.39	7.13, 7.59	2.39	7.13, 7.59
Biceps (mm)	3.95	1.34	3.83, 4.08	1.34	3.83, 4.08	3.95	1.38	3.81, 4.08	1.38	3.81, 4.08
Subscapular (mm)	4.92	1.28	4.80, 5.04	1.28	4.80, 5.04	4.93	1.51	4.79, 5.07	1.51	4.79, 5.07
Suprailiac (mm)	5.76	2.53	5.51, 6.00	2.53	5.51, 6.00	5.57	2.34	5.34, 5.79	2.34	5.34, 5.79
Body circumferences										
Head (cm)	49.19	1.48	49.05, 49.33	1.48	49.05, 49.33	49.37	1.47	49.23, 49.51	1.47	49.23, 49.51
Chest (cm)	55.59	3.39	55.27, 55.91	3.38	55.27, 55.91	55.74	3.64	55.39, 56.09	3.64	55.39, 56.09
Waist (cm)	49.01	3.76	48.65, 49.37	3.76	48.65, 49.37	49.20	3.96	48.82, 49.58	3.96	48.82, 49.58
Hip (cm)	57.30	4.00	56.91, 57.68	4.00	56.91, 57.68	57.36301	4.11	56.97, 57.76	4.11	56.97, 57.76
Upper leg (cm)	31.11	2.91	30.84, 31.40	2.91	30.84, 31.40	31.21456	2.91	30.94, 31.49	2.90	30.94, 31.49
Mid-upper arm (cm)	15.94	1.40	15.81, 16.08	1.40	15.81, 16.08	15.99	1.38	15.85, 16.12	1.38	15.85, 16.12
Kidney										
Rt length (cm)	7.90	0.55	7.84, 7.95	0.49	7.85, 7.94	7.89	0.57	7.84, 7.95	0.54	7.84, 7.94
Rt anterior – posterior (cm)	2.99	0.26	2.95, 3.00	0.21	2.95, 3.00	3.00	0.28	2.97, 3.03	0.24	2.98, 3.02
Lt length (cm)	8.25	0.57	8.19, 8.30	0.55	8.19, 8.30	8.22	0.58	8.16, 8.27	0.56	8.16, 8.27
Lt anterior– posterior (cm)	3.31	0.32	3.28, 3.34	0.26	3.29, 3.34	3.30	0.32	3.27, 3.34	0.27	3.28, 3.33

Appendix 4.2 Additional air pollution results

Table 1: Change in mass of field blanks and Limit of detection value

	Change in field blanks	Limit of detection
Batch 1	-0.000020	0.000069
Batch 2	-0.000017	0.000072
Batch 3	0.000033	0.000077
Batch 4	0.000015	0.000076

Table 2: DustTrak II correction factor. Two urban and two rural samples were taken with both the DustTrak II and the Apex to calculate a site-specific ratio for photometric:gravimetric sample conversion.

	DustTrak II concentration (µg /m^3)	Apex concentration (µg /m^3)	DustTrak:Apex ratio	Average correction factor
Rural	1670	732	0.4387	0.43
	1590	669	0.4211	
Urban	1370	660	0.4897	0.52
	957	509	0.5438	

Table 3: DustTrak II 12-hour kitchen samples, showing the minimum, maximum and average concentrations in each location.

	Length of sample	Fuel used	Minimum concentration (µg/m^3)	Maximum concentration (µg/m^3)	Average concentration (µg/m^3)
1.	12 hours	Non biomass	0	425	53.0
2.	12 hours	Non biomass	53.6	1080	166
3.	12 hours	Wood	26.0	58 800	831
4.	12 hours	Wood	0	1930	167
5.	12 hours	Wood	40.6	10 500	498
6.	12 hours	Dung	58.5	41 400	684
7.	12 hours	Dung	0	52 000	718

Appendix 5

Appendix 5.1 Multi-dimensional poverty index – distribution of dimensions

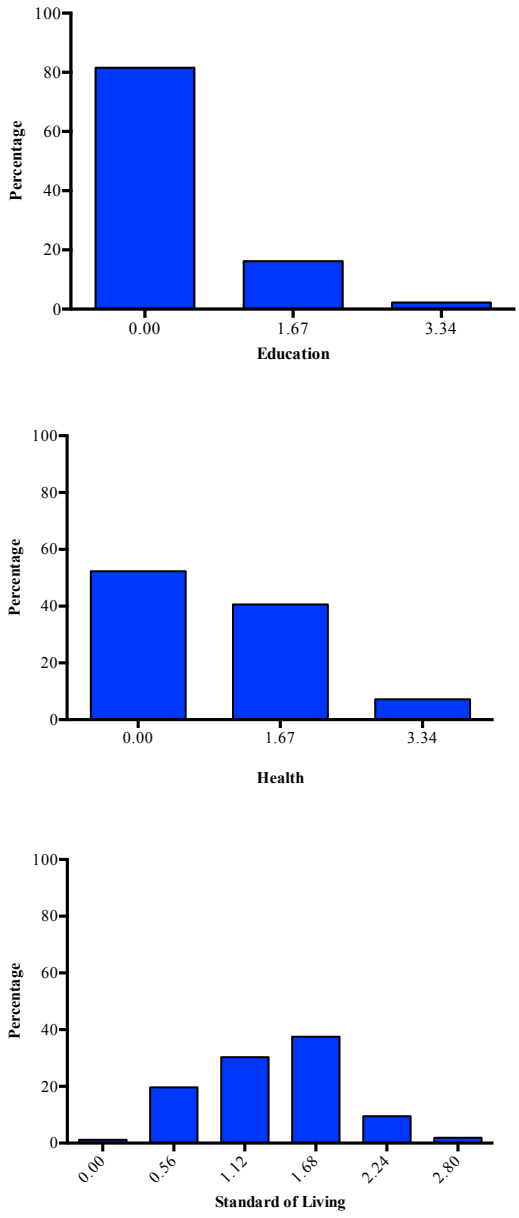

Figure 1: Distribution of the MPI scores by dimension (education, health and standard of living)

Appendix 5.2: Food security criteria for calculation of the HFIAS access scale score

Food Secure HFIA category if [(Q1a=no or Q1a=rare) and Q2=no and Q3=no and Q4=no and Q5=no and Q6=no and Q7=no and Q8=no and Q9=no]

Mildly Food Insecure Access HFIA category if [(Q1a=sometimes or Q1a=often or Q2a=rare or Q2a=sometimes or Q2a=often or Q3a=rare or Q4a=rare) and Q5=no and Q6=no and Q7=no and Q8=no and Q9=no]

Moderately Food Insecure Access HFIA category if [(Q3a=sometimes or Q3a=often or Q4a=sometimes or Q4a=often or Q5a=rare or Q5a=sometimes or Q6a=rare or Q6a=sometimes) and Q7=no and Q8=no and Q9=no]

Severely Food Insecure HFIA category = 4 if [Q5a=often or Q6a=often or Q7a=rare or Q7a=sometimes or Q7a=often or Q8a=rare or Q8a=sometimes or Q8a=often or Q9a=rare

Box 1: Levels of food security definition, using the HFIAS question numbering as shown in Section 5.3 (Coates et al, USAID Food and Nutrition Technical Assistance Project, Academy for Educational Development, 2007)

Appendix 6

Appendix 6.1 Equations for calculating mortality

Equations for calculating mortality rates:

$$\text{Miscarriage rate} = \frac{\text{number of deaths before 23 weeks gestation}}{\text{number enrolled into trial}} \times 1000$$

$$\text{Stillbirth rate} = \frac{\text{number of deaths after 23 weeks gestation}}{\text{number of births}} \times 1000$$

$$\text{Neonatal mortality rate} = \frac{\text{number of deaths from birth to 28 days}}{\text{number of livebirths}} \times 1000$$

$$\text{Post-neonatal infant mortality rate} = \frac{\text{number of deaths from 1 month to 1 year}}{\text{number of livebirths}} \times 1000$$

$$\text{Post-infant child mortality rate} = \frac{\text{number of deaths from 1 year}}{\text{number of livebirths}} \times 1000$$

Appendix 6.2 Additional anthropometry results

Table 1: Anthropometry results in the whole cohort

		Number	Mean	Median	Standard deviation	Range
Age		841	8.45	8.4	0.37	7.2 to 9.9
Weight	Weight (kg)	841	20.09	19.6	3.33	13.5 to 44.8
	Lean mass (arms 90°) (kg)	840	17.07	16.83	2.38	11.68 to 27.47
	Fat mass (arms 90°) (kg)	840	3.03	2.83	1.53	-0.0 to 17.6
	Lean mass (arms 180°) (kg)	628	17.32	17.09	2.46	11.7 to 27.6
	Fat mass (arms 180°) (kg)	628	2.97	2.73	1.61	-0.2 to 17.7
Height	Standing height (cm)	841	120.73	120.4	5.98	95.3 to 140.6
	Sitting height (cm)	841	64.15	64.1	2.95	55.7 to 73.1
BMI		841	13.71	13.57	1.31	10.8 to 24.4
Z scores	Weight-for-age	841	-2.06	-2.07	1.03	-4.8 to 2.7
	Height-for-age	841	-1.49	-1.51	0.94	-5.7 to 1.4
	BMI-for-age	841	-1.65	-1.67	0.97	-4.5 to 3.3
Skin-fold thickness	Triceps (mm)	841	7.38	6.98	2.49	2.7 to 27.5
	Biceps (mm)	841	3.95	3.67	1.37	2.0 to 17.3
	Subscapular (mm)	841	4.92	4.70	1.40	2.8 to 17.5
	Suprailiac (mm)	840	5.66	45.1	2.45	2.9 to 20.7
Body	Head (cm)	841	49.27	49.3	1.48	42.4 to 55.1

circumferences	Chest (cm)	841	55.66	55.4	3.51	46.5 to 79.1
	Waist (cm)	841	49.11	48.7	3.86	39.2 to 77.1
	Hip (cm)	841	57.33	56.9	4.05	47.2 to 85.4
	Upper leg (cm)	841	31.17	30.7	2.91	23.6 to 51.8
	Mid-upper arm (cm)	841	15.96	15.8	1.39	12.7 to 27.2
Kidney dimensions	Right length (cm)	839	7.89	7.9	0.56	5.6 to 10.2
	Right anterior–posterior distance (cm)	839	2.99	3	0.27	2 to 3.9
	Left length (cm)	839	8.23	8.2	0.58	6.5 to 10
	Left anterior–posterior distance (cm)	839	3.30	3.3	0.32	2.3 to 4.6
Blood pressure	Systolic (mmHg)	841	98.07	98	7.63	63 to 143
	Diastolic (mmHg)	841	61.22	61	7.82	36 to 108

Table 2: Anthropometry results applying exclusions that may affect growth

		No illness				Major or chronic illness			
		Number	Mean	Standard deviation	Range	Number	Mean	Standard deviation	Range
Weight	Weight (kg)	807	20.13	3.30	13.5 to 44.8	34	19.31	3.94	14.5 to 34
	Lean mass (arms straight) (kg)	604	17.34	2.45	11.7 to 27.6	24	16.64	2.51	12.2 to 22.9
	Fat mass (arms straight) (kg)	604	2.98	1.59	-0.2 to 17.7	24	2.89	2.11	-0.7 to 11.1
Height	Standing height (cm)	807	120.84	5.87	104.0 to 140.6	34	118.06	7.92	95.3 to 133.3
	Sitting height	807	64.21	2.93	55.7 to 73.1	34	62.80	3.13	57.1 to 70.7

	(cm)								
Z scores	Weight-for-age	807	-2.04	1.02	-4.8 to 2.7	34	-2.35	1.28	-4.4 to 1.6
	Height-for-age	807	-1.48	0.92	-4.0 to 1.4	34	-1.87	1.23	-5.7 to 0.4
	BMI-for-age	807	-1.65	0.95	-4.5 to 3.3	34	-1.69	1.33	-4.3 to 2.1
Skin-fold thickness	Triceps (mm)	807	7.36	2.42	2.7 to 27.5	34	7.70	3.90	4.1 to 21.9
	Biceps (mm)	807	3.96	1.37	2.0 to 17.3	34	3.81	1.30	2.4 to 8.4
	Subscapular (mm)	807	4.92	1.39	2.8 to 17.5	34	4.94	1.79	3.7 to 13.2
	Suprailiac (mm)	806	5.66	2.41	2.9 to 19.9	34	5.76	3.32	3.3 to 20.7
Body circum-ferences	Head (cm)	807	49.29	1.45	45.2 to 55.1	34	48.99	2.00	42.4 to 52.9
	Chest (cm)	807	55.67	3.47	46.5 to 79.1	34	55.65	4.44	49.4 to 72.5
	Waist (cm)	807	49.08	3.81	39.2 to 77.1	34	49.67	4.88	43.1 to 68.1
	Hip (cm)	807	57.38	4.01	48.1 to 85.4	34	56.09	4.88	47.2 to 73.2
	Upper leg (cm)	807	31.12	2.87	23.6 to 51.8	34	30.36	3.59	24.3 to 43.0
	Mid-upper arm (cm)	807	15.98	1.37	12.7 to 27.2	34	15.68	1.79	12.7 to 23.0
Kidney dimensions	Right length (cm)	805	7.90	0.55	5.6 to 10.2	34	7.75	0.69	5.7 to 8.9
	Right anterior–posterior distance (cm)	805	2.99	0.27	2.0 to 3.9	34	2.98	0.30	2.4 to 3.7
	Left length (cm)	805	8.24	0.57	6.5 to 10	34	8.14	0.64	6.9 to 9.4
	Left anterior–posterior distance (cm)	805	3.29	0.32	2.3 to 4.6	34	3.30	0.35	2.6 to 3.9
Blood pressure	Systolic (mmHg)	807	98.00	7.39	63 to 119	34	99.65	7.68	36 to 85
	Diastolic (mmHg)	807	61.08	7.68	36 to 85	34	64.74	10.24	49 to 108

Appendix 6.3 Trial and two-year follow-up results by sex

Table 1: Effect of MMN on weight at birth and 2.5 years

Birth		Control Mean (SD)	Intervention Mean (SD)	Unadjusted difference (95% CI)	Multivariable regression (95% CI)
Weight	Girls	2.67 (0.4)	2.77 (0.4)	0.103 (0.032 to 0.174)	0.119 (0.040 to 0.199)
	Boys	2.79 (0.4)	2.84 (0.5)	0.048 (-0.030 to 0.126)	0.043 (-0.039 to 0.125)
Weight-for-age	Girls	-1.31 (1.0)	-1.09 (1.1)	0.223 (0.052 to 0.394)	0.263 (0.073 to 0.452)
	Boys	-1.26 (1.0)	-1.12 (1.1)	0.137(-0.044 to 0.317)	0.095 (-0.093, 0.282)
2 years		**Control Mean (SD)**	**Intervention Mean (SD)**	**Unadjusted difference (95% CI)**	**Multivariable regression (95% CI)**
Weight	Girls	10.40 (1.3)	10.53 (1.4)	0.127 (-0.127 to 0.381)	0.269 (0.020 to 0.517)
	Boys	11.00 (1.4)	11.24 (1.6)	0.244 (-0.026 to 0.514)	0.276 (0.012 to 0.540)
Weight-for-age	Girls	-1.79 (1.0)	-1.68 (1.0)	0.111 (-0.075 to 0.298)	0.204 (0.021 to 0.386)
	Boys	-1.73 (1.0)	-1.57 (1.1)	0.159 (-0.027 to 0.346)	0.159 (-0.024 to 0.342)

Appendix 6.4 Additional spirometry results

Table 1: Spirometry results for the whole cohort, using the Global Lung initiative (Quanjer et al, ERS 2012) 'Caucasian', 'African-American' and 'South East Asia' reference ranges

GLI Z scores Caucasian		Number	Mean	Standard deviation	Range
Lung function No exclusions	FEV_1	835	-1.16	0.83	-4.94 to 2.17
	FVC	835	-1.07	0.83	-4.11 to 2.19
	FEV_1/FVC	835	-0.21	0.77	-3.62 to 1.99
	$FEF_{25\%-75\%}$	831	-0.52	1.03	-5.33 to 4.45
Excluding: poor technique	FEV_1	793	-1.14	0.82	-4.94 to 2.17
	FVC	793	-1.05	0.82	-4.11 to 2.19
	FEV_1/FVC	793	-0.22	0.76	-3.62 to 1.95
	$FEF_{25\%-75\%}$	793	-0.50	1.02	-5.33 to 4.45
Excluded: poor technique values	FEV_1	42	-1.46	0.96	-3.07 to 1.97
	FVC	42	-1.46	0.97	-3.36 to 1.44
	FEV_1/FVC	42	-0.06	0.90	-1.86 to 1.99
	$FEF_{25\%-75\%}$	38	-0.76	1.19	-4.56 to 1.31
Lung function applying all exclusions	FEV_1	707	-1.11	0.80	-3.15 to 2.17
	FVC	707	-1.03	0.81	-3.39 to 2.19
	FEV_1/FVC	707	-0.19	0.73	-2.68 to 1.95
	$FEF_{25\%-75\%}$	707	-0.46	0.98	-4.27 to 4.45
All excluded values: poor technique, acute & chronic illness and	FEV_1	128*	-1.43	0.93	-4.94 to 1.97
	FVC	128	-1.27	0.90	-4.11 to 1.44

pneumonia	FEV_1/FVC	128	-0.34	0.94	-3.62 to 1.99
	$FEF_{25\%-75\%}$	124	-0.82	1.24	-5.33 to 1.84

GLI Z scores African-American		**Number**	**Mean**	**Standard deviation**	**Range**
Lung function	FEV_1	835	0.04	0.89	-3.94 to 3.56
No exclusions	FVC	835	0.20	0.89	-3.05 to 3.55
	FEV_1/FVC	835	-0.32	0.78	-3.78 to 1.90
	$FEF_{25\%-75\%}$	831	0.02	0.95	-4.35 to 4.56
Excluding: poor technique	FEV_1	793	0.05	0.88	-3.94 to 3.56
	FVC	793	0.23	0.88	-3.05 to 3.55
	FEV_1/FVC	793	-0.33	0.77	-3.78 to 1.87
	$FEF_{25\%-75\%}$	793	0.03	0.94	-4.35 to 4.56
Excluded: poor technique values	FEV_1	42	-0.30	1.05	-2.04 to 3.41
	FVC	42	-0.23	1.05	-2.25 to 2.97
	FEV1/FVC	42	-0.17	0.90	-1.96 to 1.90
	$FEF_{25\%-75\%}$	38	-0.22	1.09	-3.65 to 1.70
Lung function applying all exclusions	FEV_1	707	0.09	0.86	-2.04 to 3.56
	FVC	707	0.24	0.87	-2.30 to 3.55
	FEV_1/FVC	707	-0.29	0.74	-2.83 to 1.87
	$FEF_{25\%-75\%}$	707	0.07	0.90	-3.39 to 4.56
All excluded values: poor technique, acute & chronic illness and pneumonia	FEV_1	128*	-0.25	1.00	-3.94 to 3.42
	FVC	128	-0.01	0.97	-3.04 to 2.97
	FEV_1/FVC	128	-0.45	0.94	-3.78 to 1.90

	$FEF_{25\%-75\%}$	124	-0.26	1.13	-4.35 to 2.18
GLI Z scores SE Asia		**Number**	**Mean**	**Standard deviation**	**Range**
Lung function	FEV_1	835	-0.36	0.87	-4.50 to 3.33
No exclusions	FVC	835	-0.02	0.88	-3.44 to 3.64
	FEV_1/FVC	835	-0.71	0.80	-4.26 to 1.52
	$FEF_{25\%-75\%}$	831	-0.45	1.13	-5.89 to 5.01
Excluding: poor technique	FEV_1	793	-0.34	0.86	-4.50 to 3.33
	FVC	793	0.00	0.87	-3.44 to 3.64
	FEV_1/FVC	793	-0.71	0.79	-4.26 to 1.52
	$FEF_{25\%-75\%}$	793	-0.44	1.12	-5.89 to 5.01
Excluded: poor technique values	FEV_1	42	-0.63	0.97	-2.25 to 2.86
	FVC	42	-0.39	1.00	-2.35 to 2.56
	FEV_1/FVC	42	-0.56	0.93	-2.43 to 1.51
	$FEF_{25\%-75\%}$	38	-0.69	1.29	-5.04 to 1.50
Lung function applying all exclusions	FEV_1	707	-0.30	0.84	-2.60 to 3.33
	FVC	707	0.02	0.86	-2.40 to 3.64
	FEV_1/FVC	707	-0.68	0.76	-3.28 to 1.52
	$FEF_{25\%-75\%}$	707	-0.39	1.07	-4.72 to 5.01
All excluded values: poor technique, acute & chronic illness and pneumonia	FEV_1	128*	-0.65	0.97	-4.50 to 2.86
	FVC	128	-0.24	0.95	-3.44 to 2.56
	FEV_1/FVC	128	-0.84	0.97	-4.26 to 1.51
	$FEF_{25\%-75\%}$	124	-0.79	1.36	-5.89 to 2.09

*6 children attempted spirometry but we could not get any data, therefore the total exclusions are 134.

Table 2: Spirometry data stratifying into boys and girls, using the 'GLI Caucasian' reference range with all data

	Boys			Girls		
	Number	Mean	Standard deviation	Number	Mean	Standard deviation
FEV_1	433	-1.03	0.84	402	-1.30	0.78
FVC	433	-0.91	0.79	402	-1.23	0.84
FEV_1/FVC	433	-0.23	0.76	402	-0.19	0.78
$FEF_{25\%-75\%}$	431	-0.39	1.08	400	-0.65	0.96

Table 3: Child lung function and anthropometry by allocation group

		Control				Intervention			
		Number	**Mean**	**Standard deviation**	**Range**	**Number**	**Mean**	**Standard deviation**	**Range**
Lung function z scores No exclusions	FEV_1	418	-1.14	0.85	-4.9 to 2.2	417	-1.19	0.81	-4.5 to 2.0
	FVC	418	-1.05	0.84	-4.1 to 2.2	417	-1.08	0.82	-3.4 to 1.4
	FEV_1/FVC	418	-0.19	0.76	-2.6 to 2.0	417	-0.22	0.78	-3.6 to 1.9
	$FEF_{25\%-75\%}$	416	-0.49	1.02	-4.8 to 3.6	415	-0.54	1.04	-5.3 to 4.5
Excluding: poor technique	FEV_1	393	-1.11	0.84	-4.9 to 2.2	400	-1.18	0.79	-4.5 to 1.4
	FVC	393	-1.02	0.83	-4.1 to 2.2	400	-1.07	0.81	-3.2 to 1.3
	FEV_1/FVC	393	-0.20	0.75	-2.6 to 2.0	400	-0.24	0.77	-3.6 to 1.9
	$FEF_{25\%-75\%}$	393	-0.48	1.02	-4.8 to 3.6	400	-0.53	1.02	-5.3 to 4.5
Excluded: poor technique values	FEV_1	25	-1.62	0.82	-2.9 to 0.2	17	-1.22	1.13	-3.1 to 2.0
	FVC	25	-1.57	0.88	-2.7 to 0.5	17	-1.29	1.11	-3.4 to 1.4
	FEV_1/FVC	25	-0.17	0.92	-1.9 to 2.0	17	0.09	0.86	-1.5 to 1.6
	$FEF_{25\%-75\%}$	23	-0.83	0.98	-2.7 to 0.6	15	-0.66	1.49	-4.6 to 1.3
Excluding: acute & chronic illness	FEV_1	390	-1.12	0.83	-3.1 to 2.2	393	-1.18	0.79	-3.2 to 2.0
	FVC	390	-1.05	0.83	-3.4 to 2.2	393	-1.08	0.82	-3.4 to 1.4
	FEV_1/FVC	390	-0.16	0.73	-2.5 to 20.	393	-0.21	0.75	-2.7 to 1.9
	$FEF_{25\%-75\%}$	388	-0.45	0.97	-3.4 to 3.6	391	-0.52	1.01	-4.6 to 4.5
Excluded values: acute and chronic illness	FEV_1	28	-1.45	1.07	-4.9 to 1.1	24	-1.36	1.01	-4.5 to 0.0
	FVC	28	-1.08	1.01	-4.1 to 1.2	24	-1.08	0.84	-3.0 to 0.7
	FEV_1/FVC	28	-0.71	1.02	-2.6 to 1.3	24	-0.46	1.19	-3.6 to 1.7
	FEF 25%-75%	28	-1.08	1.43	-4.8 to 1.8	24	-0.87	1.46	-5.3 to 1.8
Excluding: pneumonia	FEV_1	394	-1.12	0.85	-4.9 to 2.2	394	-1.17	0.81	-4.5 to 2.0
	FVC	394	-1.04	0.84	-4.1 to 2.2	394	-1.07	0.83	-3.4 to 1.4

	FEV_1/FVC	394	-0.18	0.76	-2.6 to 2.0	394	-0.23	0.77	-3.6 to 1.9
	$FEF_{25\%-75\%}$	392	-0.47	1.01	-4.8 to 3.6	392	-0.52	1.04	-5.3 to 4.5
Excluded values: pneumonia	FEV_1	24	-1.47	0.79	-3.1 to -0.0	23	-1.41	0.70	-2.7 to 0.0
	FVC	24	-1.22	0.78	-2.7 to 0.5	23	-1.31	0.65	-2.4 to -0.1
	FEV_1/FVC	24	-0.50	0.75	-2.2 to 0.6	23	-0.19	0.89	-2.7 to 1.1
	$FEF_{25\%-75\%}$	24	-0.83	1.15	-3.2 to 1.4	23	-0.75	1.00	-3.3 to 1.4
Excluding: acute & chronic illness and pneumonia	FEV_1	371	-1.10	0.82	-3.1 to 2.2	374	-1.16	0.80	-3.2 to 2.0
	FVC	371	-1.04	0.82	-3.4 to 2.2	374	-1.06	0.83	-3.4 to 1.4
	FEV_1/FVC	371	-0.15	0.73	-2.5 to 2.0	374	-0.21	0.75	-2.7 to 1.9
	$FEF_{25\%-75\%}$	369	-0.44	0.97	-3.4 to 3.6	372	-0.51	1.02	-4.6 to 4.5
Excluded values: acute & chronic illness and pneumonia	FEV_1	47	-1.44	0.96	-4.9 to 1.1	43	-1.41	0.85	-4.5 to 0.0
	FVC	47	-1.16	0.93	-4.1 to 1.2	43	-1.22	0.77	-3.0 to 0.7
	FEV_1/FVC	47	-0.57	0.88	-2.6 to 1.3	43	-0.34	1.01	-3.6 to 1.7
	$FEF_{25\%-75\%}$	47	-0.92	1.30	-4.8 to 1.8	43	-0.77	1.22	-5.3 to 1.8
Excluding: poor technique, acute & chronic illness and pneumonia	FEV_1	350	-1.07	0.81	-3.1 to 2.2	357	-1.16	0.78	-3.2 to 1.4
	FVC	350	-1.01	0.81	-3.4 to 2.2	357	-1.05	0.81	-3.2 to 1.3
	FEV_1/FVC	350	-0.15	0.72	-2.5 to 2.0	357	-0.22	0.74	-2.7 to 1.9
	$FEF_{25\%-75\%}$	350	-0.42	0.97	-3.4 to 3.6	357	-0.50	1.00	-4.3 to 4.5
All excluded values: poor technique, acute & chronic illness and pneumonia	FEV_1	68	-1.50	0.93	-4.9 to 1.1	60	-1.36	0.93	-4.5 to 2.0
	FVC	68	-1.29	0.93	-4.1 to 1.2	60	-1.24	0.87	-3.4 to 1.4
	FEV_1/FVC	68	-0.44	0.89	-2.6 to 2.0	60	-0.22	0.98	-3.6 to 1.7
	$FEF_{25\%-75\%}$	66	-0.89	1.20	-4.8 to 1.8	57	-0.74	1.28	-5.3 to 1.8

Appendix 6.5 Socioeconomic status and growth directed acyclic graph

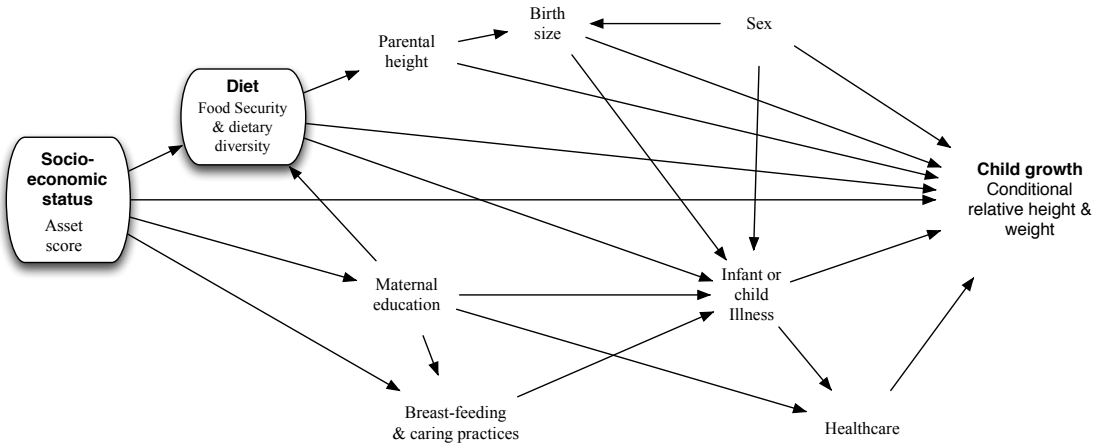

Figure 51: Directed acyclic graph