Impact of maternal nutritional supplementation on offspring blood pressure

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Submitted for degree of Doctor of Philosophy

London School of Hygiene and Tropical Medicine

2010

Candidate statement

I, Sophie Ann Hawkesworth, 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. Certain fieldwork components were conducted prior to the commencement of this PhD and others were conducted in collaboration with other researchers; my direct contribution has therefore been highlighted at the start of each chapter.

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Abstract

Observational studies on the association between birth weight and adult blood pressure provide suggestive evidence that exposures during fetal development can have lasting impacts on health. The effect of maternal nutrition during pregnancy on offspring blood pressure has been demonstrated in animal models, but data from cohort studies in humans have proven inconclusive. The follow-up of randomised controlled trials of nutritional supplementation during pregnancy can add high quality data to this research field; this thesis focuses on the effects in three separate trials.

Protein energy supplementation provided to pregnant women in rural Gambia was unrelated to offspring blood pressure at 11-17 years old (n=1267). Again in The Gambia, maternal calcium supplementation compared to placebo was also unrelated to offspring blood pressure at 5-10 years old (n=350).

In rural Bangladesh there was no effect of maternal food or multiple micronutrient supplementation on offspring systolic blood pressure at 4.5 years old (n=2335). The micronutrient intervention was also unrelated to offspring diastolic blood pressure, but there was evidence that an early invitation to enter a governmental food supplementation programme was associated with marginally lower diastolic blood pressure: 0.58mmHg (95% CI: 0.06, 1.11; P: 0.03). In this setting, randomisation to receive counselling to promote exclusive breast feeding was not associated with offspring blood pressure at 4.5 years of age and none of the interventions were associated with offspring kidney function, assessed as ultrasound-obtained volume and glomerular filtration rate calculated from plasma Cystatin C.

These data suggest that the maternal diet during pregnancy, at least those aspects of intake that can be altered during supplementation trials, may not be directly relevant for the determination of offspring blood pressure. Nutritional exposures during other stages of the life course may prove to be more important.

Acknowledgements

I would like to thank a number of individuals without whom it would not have been possible to conduct this work. I am particularly grateful to my supervisor Dr Sophie Moore for encouraging me to undertake this challenge and for her endless help, enthusiasm and support throughout the process. I would also like to thank Professor Andrew Prentice for his continued help and support of my work. I am grateful also to my other colleagues within the International Nutrition Group, particularly to Dr Tony Fulford for his patience and expert advice with my statistical analysis and to Jenny Howard for her good humoured help with any logistical issue that arose.

I am extremely grateful to the participating children and their families in both of the follow-up studies and for the endless help and guidance provided by field staff in The Gambia and Bangladesh. In particular I would like to express my gratitude for the tireless work undertaken by the field teams, which included Kabiru Sise, Morikebba Sanyang, Saul Jarjou, Kalilou Sanneh and Sherif Kolley in The Gambia and many more in Bangladesh overseen with good humour and skill by Drs Kaniz and Farzina and by Mr Shahidullah.

For The Gambian studies I would also like to thank the more senior staff without whom the research would not have been possible. In particular Dr Landing Jarjou and Yankuba Sanyang at MRC Keneba and Dr Ann Prentice and Dr Gail Goldberg at MRC Human Nutrition Research Cambridge for all of their enthusiastic help and support throughout the project.

I would like to thank my colleagues at the International Centre for Diarroeal Disease Research, Bangladesh who made the collaboration extremely pleasurable with all of their hard work and commitment. In particular Dr Delwer Hossain, Dr Ashraf Khan, Dr Waheed Hoque and Dr Shams Arifeen. I would also like to thank our

collaborators at Uppsala University, Professor Lars-Ake Persson, Dr Lotta Ekstrom and Emma Lindstrom, for welcoming me onto the project and for their help and support from the development of the fieldwork to the statistical analysis. I am also very grateful to Professor Yukiko Wagatsuma for her help in the design of the fieldwork, particularly the assessment of kidney function.

Finally I would like to thank all of my friends and family for their help and support throughout my PhD, in particular my parents and Craig Higgins for their emotional support and encouragement.

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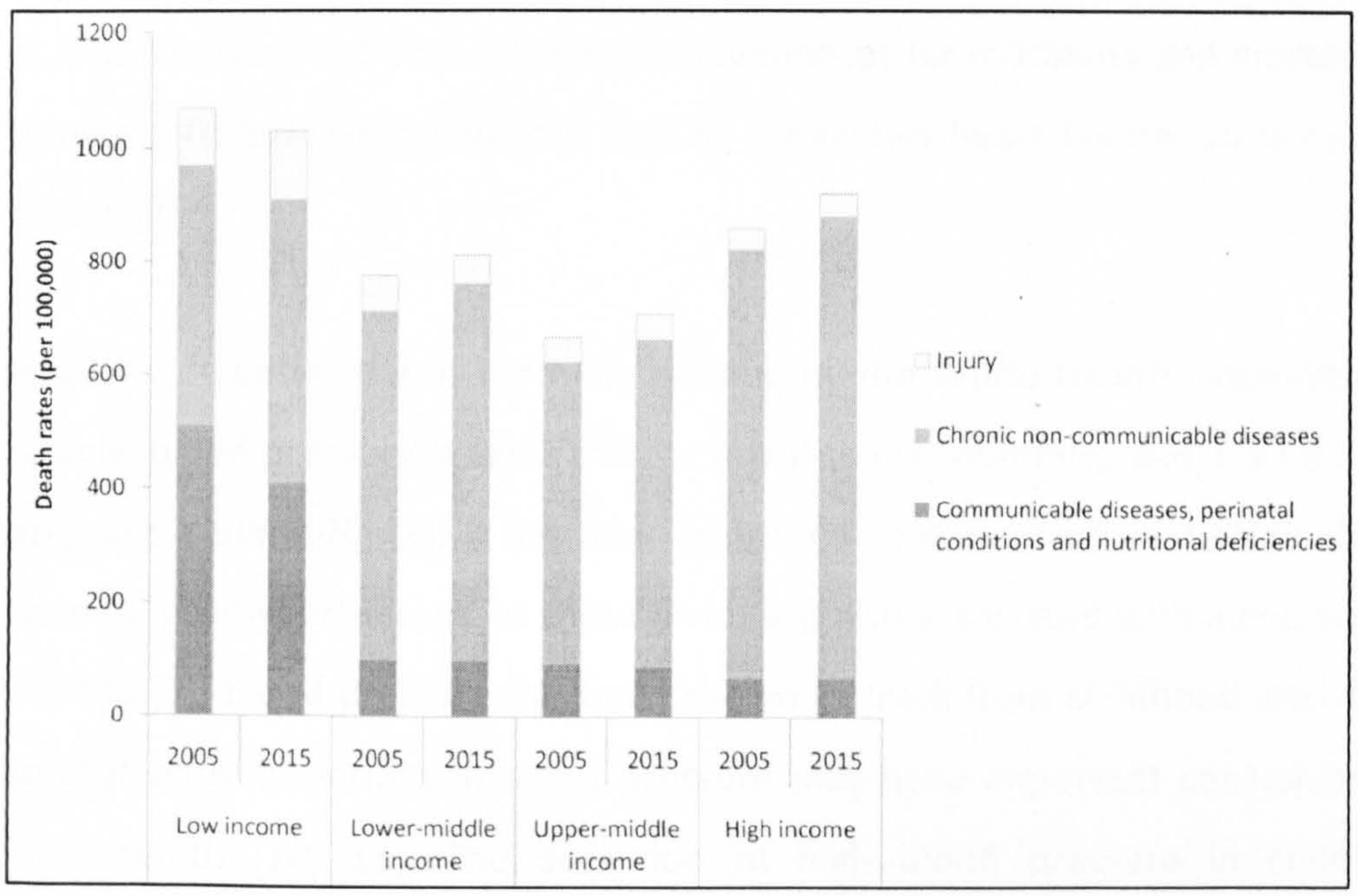
For Mum and Dad

Chapter 1 Introduction

1.1 Cardiovascular disease

Chronic non-communicable diseases, including cardiovascular diseases (CVDs), are responsible for the largest share of death and disability worldwide [1]. CVDs, encompassing coronary heart disease and cerebrovascular disease, contribute the greatest burden of non-communicable diseases and accounted for 17.5 million deaths worldwide in 2005, equating to 30% of global deaths [1]. Traditionally viewed as diseases of the affluent, there is now recognition of the increasing burden of chronic diseases in low and middle income countries as highlighted by two Lancet series [2, 3] and a WHO report [1], each of which called for urgent global action. Indeed, it is currently estimated that 80% of chronic disease deaths occur in low and middle-income countries [4]. The burden of chronic diseases in these countries is projected to increase rapidly, surpassing morbidity and mortality attributed to infectious communicable diseases in even the lowest income countries by 2015 (Figure 1.1) [5].

Figure 1.1 Death rates for World Bank country income groups, 2005 and 2015 (reproduced from Strong *et al* [5])



Intermediate risk factors for cardiovascular disease include overweight or obesity, raised blood pressure (hypertension), raised blood glucose and blood lipids. These are potentially modifiable and thus lend themselves to public health prevention strategies. This thesis focuses on blood pressure, which is responsible for a large burden of death and disability worldwide. In 2000, a WHO analysis attributed 7.1 million deaths and 64.3 million disability-adjusted life years worldwide to non-optimal blood pressure [6]. After a brief discussion of the general determinants of blood pressure this chapter will highlight the role of early-life exposures, particularly prenatal nutrition, which form the basis of this PhD research.

1.2 Definition of blood pressure

Blood pressure refers to the force exerted by the blood against the area of the vessel wall. It is conventionally measured as arterial pressure described in millimetres of mercury (mmHg) [7]. The heart pumps blood into the arteries in a continuous but pulsatile manner; the peak of this pulse is termed systolic pressure, whilst the lowest point is the diastolic pressure [7]. The blood pressure system is highly regulated both in the short-term by the autonomic nervous system and in the long-term by the action of the kidneys. This regulation is vital as even small rises in arterial pressure have important consequences for morbidity and mortality. Over time, raised blood pressure can lead to congestive heart failure, stroke and renal failure [7].

In adults, hypertension is currently defined by the World Health Organisation as a systolic blood pressure greater than or equal to (≥) 140mmHg and a diastolic blood pressure ≥ 90mmHg [8]. It has been proposed that a cut-off of 120/80 should be termed 'pre-hypertension' as these levels are also associated with increased risk of disease [9]. Blood pressure has been shown to track from childhood into adult life so that early elevations in blood pressure may have important consequences for later health [10, 11]. The definition of high blood pressure in childhood is

complicated by the influence of changing body size. One commonly-applied definition utilises population distributions of blood pressure, split by age and height centiles, compiled by the US National High Blood Pressure Education Program (NHBPEP) and last updated in 1996 [12]. Using these distributions, hypertension in childhood is defined as average systolic or diastolic blood pressure above the 95th percentile for age, height and sex [12].

1.3 Concurrent determinants of blood pressure

Blood pressure is related to age, sex, ethnicity, body size and to some extent diet, each of which will be described briefly in this section. Blood pressure is positively associated with age. Data from the Framingham Heart Study for example, demonstrated that systolic blood pressure rose steadily from 30 to 85 years of age, whilst diastolic blood pressure rose initially and then began to fall after 50 years; both changes have been interpreted as a consequence of increasing arterial stiffness [13]. The association between age and blood pressure is less clear for children and adolescents, due to the impact of growth and development, with some studies reporting no association [14, 15]. At a given age, men generally have higher blood pressure than pre-menopausal women and are at higher risk of hypertension [16]. In a Danish study, 24 hour blood pressure was on average 6-10mmHg higher for men than for women between the ages of 20-70 years, after which the differences were no longer apparent [17]. It has been hypothesised that androgens operating on the renin-angiotensin system explain the gender difference in blood pressure, which becomes less apparent after menopause [16]. Sex differences are also apparent in adolescents, with boys demonstrating a higher ambulatory (continually monitored) blood pressure than girls [18, 19].

Body size is an important predictor of blood pressure in children and adolescents [12], with both height and weight independently and positively associated with blood pressure [14, 15, 20, 21]. Few studies have been able to characterise body composition in greater detail than simple anthropometry, but data from the Avon

Longitudinal Study of Pregnancy and Childhood (ALSPAC) demonstrated a positive independent association between systolic blood pressure and both fat and lean mass assessed by dual-energy X-ray absorptiometry (DXA) at nine years of age [21]. In a smaller US study of children and adolescents aged 5-18 years, He *et al* reported an inverse association between fat mass (assessed by DXA and by skinfold thickness measurements) and systolic blood pressure in boys and a positive association between fat mass and blood pressure for girls only, assessed by skinfold thickness measurements [22]. Possible associations with lean mass were not assessed by this study. The importance of fat distribution remains unclear; Brion *et al,* using the ALSPAC cohort, report a potential positive association between trunk fat and systolic blood pressure that was stronger for girls than boys [21], whilst He *et al* report a positive association between trunk fat and both systolic and diastolic blood pressure, but only for boys [22]. At present it is therefore difficult to draw firm conclusions about the association between body composition and blood pressure.

The effect of ethnic background on blood pressure has been suggested by a number of studies, generally reporting higher blood pressure for individuals of African compared to European decent [23-26]. A recent systematic review of UK studies, revealed that the majority reported higher blood pressure for adults of African descent compared to white Europeans [26]. A second review reported similar blood pressure levels for white Europeans and Asian adults living in the UK [27]. However, it should be noted that confounding by socio-economic class and by other factors such as body composition and diet are often not taken into account in these studies, and there is very little data from native African or Asian populations. The existence of ethnic differences in children is less well established; in one US study the observation that blood pressure was higher for black compared to white girls at 10 years of age was entirely explained by the difference in sexual maturation [28]. In a separate study, recruiting children and adolescents aged 1-17 years, no ethnic differences were observed for girls but blood pressure was higher for normal weight black boys compared to normal weight white boys, although no difference was observed for overweight individuals [29]. A systematic review of studies in

children in the UK found that blood pressure levels in the five identified studies were similar across the different ethnic groups studied [30].

The association between dietary factors and blood pressure has been most extensively researched for alcohol and salt intake. High alcohol consumption has been consistently shown to be related to high blood pressure [31-34]. At the lower end of the consumption spectrum however, there has been debate over a possible protective effect of moderate alcohol intake compared to individuals who do not drink [31, 32, 35]. These observational associations have been criticised for confounding by a variety of factors such as diet and smoking status, and a meta-analysis of 15 randomised controlled trials confirmed that a reduction in alcohol consumption resulted in a reduction in blood pressure [36]. In addition, a recent meta-analysis utilising the technique of Mendelian randomisation revealed a dose response effect between low, moderate and high alcohol consumption (defined by genotype) and systolic blood pressure in adulthood [37]. The authors used an individual's genotype for the alcohol dehydrogenase 2 (ALDH2)*2 allele as a randomly allocated lifetime alcohol exposure to investigate the association between alcohol consumption and blood pressure that is not confounded by other factors; individuals homozygous for the ALDH2*2 allele have a reduced ability to process alcohol and therefore drink considerably less [37].

Law *et al* conducted meta-analyses of both observational and randomised controlled trial data on the impact of salt intake on blood pressure [38, 39]. The authors conclude that there is a strong positive association and that a reduction in salt intake of around 50mmols per day would be associated with a reduction in systolic blood pressure of 5mmHg [38, 39]. Data on the impact of other dietary factors on blood pressure is less well established; high fibre intake may be associated with a reduced risk of developing hypertension in men [34] and a vegetarian diet has been associated with lower blood pressure [40], although issues of confounding remain important in these types of dietary association studies.

1.4 Early-life determinants of blood pressure

Exposures throughout the life course are increasingly recognised as playing an important role in the aetiology of chronic diseases [41-43]. The impacts of the prenatal and early postnatal environment in particular, have been highlighted by the Developmental Origins of Health and Disease Hypothesis (DOHaD) [44], which has grown into a multidisciplinary research area. This section of the introduction will outline the current evidence for the impact of the early-life nutritional environment on the development of the blood pressure system. For both pre- and early post-natal stages of the life course, the evidence will be presented first from human observational data, then from animal studies and finally human intervention trials as this follows the historical pattern of how the data has emerged in the literature.

1.4.1 Prenatal nutrition

1.4.1.1 Observational data

Work by David Barker and colleagues from the MRC's Environmental Epidemiology Unit in Southampton in the late 1980s provided some of the first evidence that the fetal environment may be related to blood pressure in later life [45]. Using records that had been routinely collected at birth in various centres around the UK, including a hospital in Preston [46] and midwife records in Hertfordshire [47], individuals were traced to their current residence for recruitment into studies investigating cardiovascular disease risk factors. The analysis revealed an inverse association between birth weight and blood pressure that was apparent in childhood but became stronger with age [48]. Subsequently a number of epidemiological studies have replicated this finding, but the topic has not been without controversy. In a systematic review of 34 studies published up to 1996, Law and Sheill reported that the majority of studies in children and adults showed an inverse association between birth weight and blood pressure, although the association for adolescents was less consistent [49]. However, when Huxley *et al* updated the review in 2002 they highlighted some concern with the literature; only

just over half of the published studies had reported a regression coefficient and the strength of the association between birth weight and blood pressure became weaker as study size increased [50]. The authors concluded that this suggested publication bias in the research topic and that previous claims about the strength of the association had been overstated [50]. Subsequently, an informal review of the literature conducted by Adair and Dahly again provided support for an inverse association between birth weight and adult systolic blood pressure and/or an increased risk of hypertension in adults born smaller at birth [51]. Adair and Dahly reported a range of regression coefficients (-0.55, -6.0mmHg) for the difference in systolic blood pressure associated with a 1kg increase in birth weight and concluded that the often quoted 2mmHg difference was in general supported by the literature [51].

In addition to the issue of publication bias outlined above, past studies have been criticised for the predominantly retrospective design (prone to selection bias) employed in most studies [52], inappropriate adjustment for social economic status [52] and the use of statistical methods that may not be appropriate for the research question, such as adjusting for current size [53]. Large prospective birth cohorts can go some way to addressing these concerns but tend to be relatively young in age at present. The ALSPAC cohort has provided conflicting evidence to date; size at birth was inversely related to blood pressure at three years of age [54] but not at nine years [21]. The impact of birth weight on later blood pressure may arguably be more relevant for less developed countries where there is a greater prevalence of low birth weight [55], but very few data are available from these settings. In one comparison study, birth weight and length were found to be inversely related to blood pressure at 3-6 years of age after adjusting for current size in Guatemala City and Santiago but not for cities in China or Nigeria [56].

Beyond birth weight: maternal diet

The impact of fetal growth restriction on the development of chronic disease in later life has mainly been attributed to the action of nutritional insufficiency [45, 57]. Birth weight is therefore used as a proxy measurement of fetal nutrition, which

is itself multifactorial in origin, comprising maternal body composition and size (determined partly by her own fetal experience), maternal diet and nutrient stores and the placental transport of nutrients [45]. Relatively few studies have investigated the direct effect of the maternal diet during pregnancy on health outcomes in the offspring.

The Dutch Hunger Winter, 1944-45, has been extensively studied as an example of a relatively defined exposure to a period of reduced food intake. Between November 1944 and May 1945 there was a severe shortage of food in the western Netherlands as a result of German occupation, and harsh weather conditions, disrupting supply routes. Rations fell to between 400-800 calories a day during this period but were quickly restored with the Allied liberation in May 1945 [58]. Individuals born or conceived during the famine have been compared to those born before or conceived just after this period of food shortage as 'controls' [58]. Despite an inverse association between birth weight and blood pressure in the Dutch famine birth cohort, there was no relationship between famine exposure and blood pressure at 50 years of age [59]. In a study of a different group of survivors however, Stein *et al* (2006) did find that those exposed to the famine (at any time during gestation) had higher blood pressure and a higher rate of hypertension at 59 years of age compared to 'controls' born before or conceived after the famine [60]. Using record data on the rations available during this time, Roseboom *et al* investigated the association between macronutrient availability and blood pressure in adulthood [61]. Although there was no association with total macronutrient availability, the authors observed an inverse association between the protein/carbohydrate ratio in late gestation and offspring blood pressure in adulthood, both for individuals exposed and unexposed to the famine [61].

Similar studies have been conducted on individuals born during the Leningrad siege revealing no differences in blood pressure (at around 50 years of age) compared to those exposed to the siege during infancy [62]. Interestingly both groups exposed to the Leningrad siege in early life (infancy and *in utero*), demonstrated raised diastolic blood pressure and a non-significant tendency to raised systolic blood

pressure compared to individuals born outside of the city [62]. Whilst these findings are intriguing, one of the main drawbacks with these studies is a lack of individual data on actual intakes.

Some of the observational studies that have directly assessed diet during pregnancy were reviewed by Brion *et al,* concluding that there was no strong evidence that any component of the maternal diet influenced blood pressure in the offspring [63]. This data, and a few additional studies not covered by the review, will be briefly summarised below. The heterogeneity of exposure measures prevents any meaningful tabulation of the results but the methodological designs of the various studies are summarised in Tables 1.1 and 1.2 to give an indication of the variety of studies involved.

The impact of protein and energy intake during pregnancy has been investigated in five observational studies [64-68], mostly reporting no main effect in relation to offspring blood pressure. In Filipino adolescent boys, but not girls, low percentage energy from protein in the maternal diet was related to higher blood pressure at 16 years of age [66]. In contrast, high meat consumption (interpreted as high protein) during pregnancy was associated with higher systolic blood pressure in the offspring of individuals who followed severe dietary advice during pregnancy in a Scottish hospital in the 1960s [67]. In a separate Scottish study in the 1950s, no single nutrient intake during pregnancy was associated with offspring blood pressure 40 years later [65]. However, at intakes of animal protein under 50g a day a high carbohydrate intake was associated with higher offspring blood pressure, whereas the reverse pattern was seen for women with intakes of animal protein over 50g a day [65]. An issue raised by this analysis is the importance of pre-specifying hypotheses to be tested prior to conducting the analysis; it is not clear if this particular analysis would have been conducted for *a priori* reasons. In two contemporary birth cohort studies, one from the US [68] and one from the UK [64], no association was observed between protein intake during pregnancy and offspring blood pressure at six months and seven years respectively.

Table 1.1 Characteristics of observational studies reporting maternal macronutrient intakes during pregnancy and offspring blood pressure

	Campbell *et al*, 1996 [65]	**Shiell *et al*, 2001 [67]**	**Adair *et al*, 2001 [66]**	**Leary *et al*, 2005 [64]**	**Huh *et al*, 2005 [68]**
Study location	Aberdeen, Scotland	Motherwell, Scotland	Cebu, Philippines	Bristol, England	Boston, USA
Participants	Women attending Aberdeen Maternity Hospital, 1948-54	Women attending Motherwell Maternity Hospital, 1967-1968	Pregnant women enrolled into the Cebu Longitudinal Health and Nutrition Survey, 1983-1984	Pregnant women enrolled into the ALSPAC study, 1991-1992	Pregnant women enrolled into Project VIVA, 1999-2002
Exposure	Daily nutrient intakes of calories, protein, fat, carbohydrate, calcium, vitamin A, thiamin, riboflavin, niacin and vitamin C	Meat, fish, eggs, cheese, milk, green vegetable, potatoes, bread, cakes, sweet consumption (portions/wk)	Daily nutrient intakes of energy, % energy from fat and % energy from protein.	Daily nutrient intakes of carbohydrate, protein, fat (total and different types), Ca, K, Mg. Food intake of milk, meat, fish, fruit and vegetables	Daily nutrient intakes of protein, carbohydrate, fat
Exposure measurement	7 day weighed food record in 30th week of pregnancy	Antenatal records of food portions eaten in late pregnancy (>20wk)	24h recall at 30wk gestation	Self-report FFQ (110 items) at 32wk gestation	Self-report FFQ (166 items) towards end of 1st and 2nd trimester
Follow-up	47% (253/544) of singleton children in cohort	44% (626/1432) of eligible individuals recruited	66% (2026/3080) of cohort recruited	51% (6944/13678) of cohort recruited	45% (947/2128) of cohort recruited
Age of children	40.6y	27-30y	14-16y	7.5y	6months
Outcome measurement	Automated device (Dinamap) in duplicate; mean used for analysis	Automated device (Omron HEM 711) in triplicate; mean used for analysis	Mercury sphygmomanometer in triplicate; mean used for analysis	Automated device (Dinamap 9301) in duplicate; mean used for analysis	SBP only. Automated device (Dinamap 8100) up to 5 measurements; mean used for analysis

Table 1.2 Characteristics of observational studies reporting maternal micronutrient intakes during pregnancy and offspring blood pressure

	McGarvey et al, 1991 [69]	Morley *et al*, 2004 [70]	Gillman *et al*, 2004 [71]	Belfort *et al*, 2008[72]	Brion *et al*, 2008 [73]
Study location	Rhode Island, USA	Tasmania, Australia	Boston, USA	Boston, USA	Bristol, England
Participants	Women attending a major obstetric hospital, 1985-1987	Twin children (and mothers) enrolled into the Tasmania Infant Health Study (TIHS), 1988-1995	Pregnant women enrolled into Project VIVA, 1999-2002	Pregnant women enrolled into Project VIVA, 1999-2002	Pregnant women enrolled into the ALSPAC study, 1991-1992
Exposure	Daily nutrient intakes of energy, potassium, calcium and magnesium	Taking calcium supplements during pregnancy	Daily calcium intake (diet and supplement use separately)	Daily iron intake (diet and supplement use separately), Hb and mean cell volume	Daily iron intake (diet and supplement use separately), Hb
Exposure measurement	FFQ (116 item) administered post-partum referencing entire pregnancy period	Post-partum questionnaire on nutritional supplement use	Self-report FFQ towards end of 1st and 2nd trimester.	Self-report FFQ towards end of 1st and 2nd trimester. Iron status from screening laboratory tests in 1st and 2nd trimester	Self-report FFQ at 18 (supplements only) and 32wks. Iron status from routine antenatal records. Anaemia in early (1st and 2nd tri) and late pregnancy defined
Age of children	Newborn, 1, 6 and 12 months	7-11y	6 months	3y	7.5y
Follow-up	Newborn: 100% (212/212) recruited 1mo: 87%, 6mo: 54% 12mo: 33%	50% (230/463) children recruited	44% (936/2128) of offspring recruited	55% (1167/2128) of offspring recruited	Diet: 55% (7484/13678) of offspring recruited Anaemia: 9.2%
Outcome measurement	Automated device (Roche Arteriosonde) in triplicate; mean used for analysis	Automated device (Dinamap Pediatric) in triplicate; mean of last two used for analysis	SBP only. Automated device (Dinamap 8100, Pro 100 or Pro 200), 5 measures; mean used	Automated device (Dinamap Pro100) up to 5 measurements; mean used for analysis	Automated device (Dinamap 9301) in duplicate; mean used for analysis

Maternal intake of calcium during pregnancy has been the focus of a number of studies due to the potential reduction in pre-eclampsia risk associated with increased calcium intake [74]. In turn, the association between calcium intake in pregnancy and offspring blood pressure has also been investigated and reported by three observational studies (Table 1.2), albeit with conflicting results [69-71]. Gilman *et al* reported that calcium intake in the second trimester of pregnancy, although not the first trimester, was inversely related to blood pressure in the offspring at six months of age in a large American birth cohort known as Project Viva [71]. Interestingly this association was only observed with calcium intake from supplements and was not apparent for calcium intake from food sources; mean supplement intake was 264mg/d. After adjusting for confounders, a 500mg increase in calcium intake during pregnancy was associated with a 3mmHg decrease in systolic pressure in infancy [71]. McGarvey *et al* also observed an inverse association between maternal calcium intake (from food and personal supplements) and offspring systolic blood pressure at one month, a borderline association at six months but no association at twelve months of age, although only 33% of the cohort were recruited at this time [69]. No association was observed between maternal calcium supplementation use during pregnancy (no data on dietary intake was recorded) and child blood pressure at nine years of age in Tasmania, Australia [70]. It should be noted that both the latter two studies relied on post-partum questionnaires, which are likely to suffer from recall bias.

The impact of other nutrients during pregnancy has received little attention in relation to offspring blood pressure. In the Philippines, fat intake in pregnancy was associated with reduced blood pressure for girls but not boys [66]. In the ALSPAC cohort, omega-3 fatty acid intake was weakly inversely associated with offspring blood pressure, although this association was no longer apparent after adjustment for confounders [64]. Again in ALSPAC, anaemia during pregnancy (haemoglobin <11g/dl) was associated with lower systolic blood pressure in the offspring at seven years of age, although once again the relationship was lost after adjustment for confounders [73]. No other nutrients in the ALSPAC study were associated with

offspring blood pressure [64]. In the Scottish study reporting on women who had followed a high meat diet during pregnancy, high intake of fruit and vegetables was weakly associated with reduced offspring blood pressure [67]. Belfort *et al* reported a positive association between maternal iron intake from supplements during pregnancy and offspring systolic blood pressure at three years of age in Project Viva [72]. Interestingly there was no association between measures of maternal iron status (either haemoglobin or mean cell volume) and offspring blood pressure in the same study [72]. In addition to an association of calcium intake highlighted above, McGarvey *et al*, reported an inverse association between maternal intake of magnesium and systolic blood pressure at one month of age and an inverse association between potassium intake and offspring diastolic blood pressure at six and twelve months of age [69]. As stated previously, the heterogeneity of study design in terms of nutrients assessed, the method of assessment and the age of the subjects at follow-up make any meaningful combined analysis of these results impossible and the picture that emerges from these various studies is unclear and somewhat contradictory.

1.4.1.2 Animal studies

Methodological issues have plagued the human data in this field from the outset and, as a result, evidence from animal studies has been extremely important for advancing understanding of the impact of the maternal diet during pregnancy on offspring health. There is now a wealth of animal data in this field, which will be briefly summarised below, but which is extensively reviewed elsewhere [75-80].

Hypertensive offspring have been produced by a variety of nutrient restrictions during pregnancy in a variety of species, including rats, sheep and pigs [79, 81]. Global food restriction (15-30% of normal food intake), either during part or all of pregnancy, has been shown to lead to raised blood pressure in both sheep and rat offspring [79]. One of the most commonly studied exposures in the literature is a low protein diet provided to pregnant rats, followed by a normal postnatal diet. Researchers at the University of Southampton have modelled the low protein diet

as 9% casein compared to a control diet containing 18% casein [82]. This experimental model has consistently been shown to produce offspring with raised blood pressure [82]. The balance of amino acids may be an important feature, as low protein diets of different compositions have not induced hypertension in the offspring [80]. One proposed explanation is that rats on the Southampton low-protein diet are provided with additional methionine in their diet, which in turn leads to high levels of homocysteine and may therefore result in changes in DNA methylation effecting organogenesis [80]. Supplementation of the diet with folate reduces plasma homocysteine and results in normotensive offspring [83]. Recently the animal data relating to hypertension in rats has been criticised for certain common methodologies, particularly the measurement of blood pressure using a single tail-cuff reading compared to 24 hour continuous recording by telemetry; the former has been shown to give higher readings due to the stress associated with this technique [84].

There has been some debate about the existence of sex differences in fetal response to nutrient restriction during pregnancy, as recently reviewed by Grigore *et al* [85]. In rat models at least, the existence or absence of sex differences is potentially explained by the nature of the nutritional insult and the method of measuring blood pressure; gender differences are generally reported in studies utilising methods other than the tail cuff [79]. Moderate protein restriction during pregnancy results in male offspring with raised mean arterial pressure but no effect in female offspring, whilst severe restriction leads to raised blood pressure in both males and females [86-88]. Females may therefore be more resistant to the effect of dietary manipulation during *in utero* development, only developing disease outcomes under extreme conditions. However, the existence of sex differences in response to early life nutritional insults in other species is unclear at present [79].

Other dietary exposures that have been studied in animal models include fat, sodium and iron. Rat models of iron deficiency during pregnancy result in pups with higher blood pressure than controls, despite being cross-fostered at birth to control dams [89]. Further experiments utilising the same design have demonstrated

increased expression of placental cytokines in response to iron deficiency, which the authors suggest may partly explain the differences in growth rates in experimental and control pups [90]. A diet deficient in omega-3 fatty acids provided during gestation and early postnatal life has also been shown to promote high blood pressure in rats [91]. High sodium diets provided during pregnancy, lactation and early weaning have been shown to produce lasting hypertension in rat offspring [92], although exposure only during pregnancy does not have the same effect [93].

The relevance of the animal data outlined above, to human populations can be questioned for a number of reasons, particularly the appropriateness of extrapolating between species. Human reproduction requires only incremental increases in nutrients on a daily basis compared to other species (because humans have evolved to have very slow pre- and post-natal growth rates), and the developing fetus is protected by a number of evolved adaptations [94]. In addition, the majority of animal studies involve interventions that are at the extreme end of nutrient restriction, representing situations that are unlikely to occur in human populations.

1.4.1.3 Human intervention trials

Returning to the human situation we are reminded of the paucity of the data and of the issues with those that are available. The majority of evidence is derived from cohort studies which suffer either from a retrospective design and/or from large losses to follow-up, insufficient adjustment for confounding (especially for social economic status) and potential errors associated with the measurement of the maternal diet. It is therefore unsurprising that the evidence base is conflicting and dogged by criticism. One solution to obtain higher quality data in this area is to utilise intervention studies to answer the research question "does the maternal diet during pregnancy affect offspring blood pressure?". Intervention studies, particularly those that have utilised randomised double-blind placebo-controlled designs have the advantage of providing the highest quality data of all

epidemiological designs and, provided the sample size is large and randomisation successful, confer a greater degree of validity on the observed result due to a reduced potential for bias [95]. Although there are no trials that have been published that specifically test this hypothesis, trials of nutritional supplementation during pregnancy for other primary outcomes have been conducted and are available for follow-up. A comprehensive review of the published literature in this area revealed that to date, the only exposures that have been studied are protein-energy, multiple micronutrient and calcium supplementation. These will be sumarised on the following pages.

Protein-energy

Two community-based interventions of protein-energy provision for pregnant women and their children have so far been followed-up to investigate any impact on later blood pressure, amongst other outcomes (Table 1.3). The first was a trial conducted by the Institute of Nutrition of Central America and Panama (INCAP) in rural Guatemala in the 1960s [96]. Two villages received the 'Atole' supplement, a protein and energy dense drink, whilst two control villages received the 'Fresco' supplement, a drink which contained no protein and around one third of the energy [96]. A follow-up study was conducted when the offspring were 20 – 29 years old, recruiting only those who had been exposed to the intervention during gestation and for their first three years of life [97]. There was no difference in systolic or diastolic blood pressure for adults born in the intervention compared to the control villages [97] (Table 1.4). A major limitation of this study in relation to the research question under investigation is that it is not possible to distinguish between pre- and post-natal enhanced nutrition in the intervention analysis. Furthermore, due to the small number of villages, the cluster design of the original trial could not be accounted for in the analysis. Another factor to consider is that although this study is often viewed as a trial of protein supplementation the two drinks also provided different levels of certain micronutrients (calcium, phosphorus, zinc, folic acid and vitamin B12), which may affect interpretation of the results [98].

A comparable follow-up study was recently published from India investigating the impact of a community-based intervention, which provided a cereal meal to pregnant and lactating women and children up to age six, on cardiovascular disease risk factors in the offspring [99]. The intervention was rolled-out in a stepwise manner as part of a national public health programme and the researchers were able to compare individuals born into areas covered by the programme with 'control' areas, which did not yet have that provision. The authors report that there was no difference in systolic or diastolic blood pressure between individuals from intervention compared to control areas at 15 years old [99]. However, there was a difference in augmentation index (viewed as a marker of global arterial stiffness with lower values suggesting a healthier vascular tree [100]) between the two groups; adolescents in the intervention area had a percentage augmentation index that was 3.16% lower than individuals in control areas [99]. Again, it is important to note that this study involved a community-level intervention that provided supplements to pregnant women and to their children up to 6 years of age, hampering conclusions regarding the importance of prenatal compared to postnatal nutrition. In addition, the roll-out of the intervention had not been conducted in a random manner, which should add caution to the interpretation of its results.

Table 1.3 Trials and follow-up studies of the effect of maternal protein-energy supplementation on offspring blood pressure

	Guatemala	India
Original trial	Martorell *et al*, 1995 [96]	Kinra *et al*, 2008 [99]
Design	Community-based randomised trial, 1969-77	Community-based intervention, 1987-90
Randomisation	Village level: 2 intervention villages, 2 'control'	Non-randomised design: 15 intervention villages, 14 control
Participants	All village residents: intake of pregnant women and children up to age 7y recorded	Pregnant and lactating women, children up to age 6y.
Supplement	Drink-based supplement, twice daily in a central location. Intervention villages = 'Atole': 682KJ energy, 11.5g protein/d Control villages = 'Fresco': 247KJ energy, 0g protein/d (consumed twice as frequently as Atole)	Food-based supplement daily from village service centre. Intervention villages = receiving supplement; Control villages = yet to receive roll-out of intervention Pregnant/lactating women: 2090KJ energy, 20-25g protein/d Children: 1250KJ energy, 8-10g protein/d
Follow-up	Webb *et al*, 2005[97]	Kinra *et al*, 2008[99]
Date	1997-98	2003-5
Age of offspring	20-29 y	15 y
Recruitment	450/1308 (34%) of original trial offspring born between March 69 and September 1997. 77% (450/585) of traceable subjects with known birth weight. Loss to follow-up associated with lower birth weight and shorter maternal height.	1165/2601 (45%) of all births in trial area recruited. Loss to follow-up associated with females, fulltime employment and younger individuals.
BP measurement	Three measurements at 3-5min intervals using oscillometric digital sphygmomanometer (UA-767; A&D Medical). Mean of last two measurements used in analysis	Two measurements in supine position using oscillometric device (HEM 705; Omron). Mean of two measurements used in analysis Augmentation index assessed by applanation tonometry (Vx system; Atcor (PWV) Medical) of pressure waveforms.

Table 1.4 Effect of prenatal and early postnatal protein-energy supplementation on blood pressure

Outcome	Study	N	Mean (SD)		Mean difference (95% CI)[a]	P-value
			Intervention area	Control area		
Systolic BP (mmHg)	Webb *et al*, 2005 [97]	M: 225	120.3 (9.9)	119.7 (11.2)	0.6 (-2.17, 3.37)	0.67
		F: 222	103.2 (9.2)	103.7 (11.4)	-0.5 (-3.23, 2.23)	0.72
	Kinra *et al*, 2008 [99]	1118	108.7 (10.3)	109.6 (10.0)	-0.83 (-3.11, 1.44)	0.5
Diastolic BP (mmHg)	Webb *et al*, 2005 [97]	M: 225	73.4 (7.8)	72.4 (8.1)	1.0 (-1.09, 3.09)	0.35
		F: 222	65.6 (7.7)	64.6 (8.0)	1.0 (-1.07, 3.07)	0.34
	Kinra *et al*, 2008 [99]	1118	62.5 (6.5)	62.2 (6.5)	0.23 (-1.25, 1.70)	0.8
Augmentation index (%)	Kinra *et al*, 2008 [99]	862	2.5 (11.4)	5.6 (9.1)	-3.16 (-5.51, -0.80)	0.01

[a]Mean difference in blood pressure between intervention and control areas derived from unadjusted regression analysis, reproduced from publications

Multiple micronutrients

In recent years there has been considerable interest in the potential of maternal micronutrient supplementation during pregnancy to provide important benefits for both the mother and infant. Two trials have to date published findings of follow-up beyond infancy, both of which were based in Nepal [101, 102] (Table 1.5). The trials had different designs: one conducted by Osrin *et al* investigated the impact of a supplement containing 15 micronutrients compared to one containing iron and folate only [101], whilst the trial by Christian *et al* compared five arms of supplementation: vitamin A only; vitamin A plus folic acid; vitamin A, folic acid and iron; vitamin A plus folic acid, iron and zinc; a multiple micronutrient tablet that also contained vitamin A [102]. The follow-up studies of both of these trials have been published recently with conflicting results (Table 1.6 and 1.7). The follow-up of the first trial recruited 2-3 year old children and reported that those born to mothers who received the multiple micronutrients had lower systolic blood pressure (2.5mmHg) than those born to 'control' women receiving only iron and folic acid [103]. In contrast, the follow-up of the second trial recruited offspring who were 6-8 years old and found no difference in blood pressure between the five different trial arms [104].

Table 1.5 Trials and follow-up studies of the effect of maternal multiple micronutrient supplementation on offspring blood pressure

	Nepal 1	**Nepal 2**
Original trial	Osrin *et al*, 2005 [101]	Christian *et al*, 2003 [102]
Design	Double-blind randomised controlled trial, 2002-4	Cluster-randomised, double-blind trial, 1999-2000
Randomisation	Computer generated randomisation; allocation concealment	Randomisation at village sector level (n=426) by pulling numbers from a hat
Participants	Pregnant women attending antenatal clinic in Janakpur Singleton pregnancies. Enrolled up to 20wks gestation	Pregnant women identified from pregnancy test if missed menses reported for previous 30d. Enrolled early pregnancy
Supplement	Tablets taken daily from enrolment until delivery Intervention arm = 15 multiple micronutrients (800µ vitamin A, 10mg vitamin E, 5µg vitamin D, 1.4mg vitamin B1, 1.4mg vitamin B2, 18mg niacin, 1.9mg vitamin B6, 2.6µg vitamin B12, 400µg folic acid, 70mg vitamin C, 30mg iron, 15mg zinc, 2mg copper, 65µg selenium and 150µg iodine)/d Control arm = 60mg iron and 400µg folic acid/d	Tablets taken daily from enrolment until 3 months post partum. Five treatment arms: 1. Multiple micronutrient: 1000µg vitamin A, 400µg folic acid, 60mg iron, 30mg zinc, 10 µg vitamin D, 10mg vitamin E, 1.6mg thiamine, 1.8mg riboflavin, 20mg niacin, 2.2mg vitamin B6, 2.6µg vitamin B12, 100mg vitamin C, 65µg vitamin K, 2mg copper, 100mg magnesium 2. Folic acid, iron, zinc and vitamin A 3. Folic acid, iron and vitamin A 4. Folic acid and vitamin A 5. Vitamin A only
Follow-up	Vaidya *et al,* 2008 [103]	Stewart *et al*, 2009 [104]
Date	2005-06	2006
Age of offspring	2-3y (mean 2.7)	6-8y (mean 7.5)
Recruitment	917/1110 (83%) of original trial offspring recruited Loss to follow-up associated with urban location and husbands who were salaried or owned small business.	3524/4130 (85%) of original trial offspring recruited. Recruitment rates even across trial arms but no data on whether loss to follow-up is associated with characteristics
BP measurement	Single measurement with child on mother's lap using electronic sphygmomanometer (CEO 197; Omron).	Four times at 1min intervals using automated oscillometric device (BPM-300, BPTrue). Mean of last 3 used in analysis.

Table 1.6 Effect of maternal multiple micronutrient supplementation on offspring blood pressure in Nepal, Trial 1 (adapted from Viadya *et al* [103])

		Mean (SD)			
Outcome	N	Multiple micronutrient	Iron and folate	Mean difference (95% CI)[a]	P-value
Systolic BP (mmHg)	915	99.4 (13.7)	101.9 (17.5)	-2.5 (-4.54, -0.46)	0.02
Diastolic BP (mmHg)	915	62.1 (12.8)	63.4 (14.7)	-1.3 (-3.09, 0.49)	0.15

[a]Mean difference in blood pressure between offspring born to women in the multiple micronutrient arm compared to iron and folate, derived from unadjusted regression analysis and reproduced from publication

Table 1.7 Effect of maternal micronutrient supplementation on offspring blood pressure in Nepal, Trial 2 (adapted from Stewart *et al* [104])

		Mean (SD)[a]				
Outcome	N	Multiple micronutrient	Iron, folate, zinc, vitamin A	Iron, folate, vitamin A	Folate and vitamin A	Vitamin A
Systolic BP (mmHg)	3524	95.5 (8.5)	95.0 (8.1)	95.2 (8.3)	95.5 (7.9)	95.8 (8.0)
Diastolic BP (mmHg)	3524	64.4 (8.6)	63.7 (8.7)	63.9 (8.4)	63.7 (8.8)	64.2 (8.3)

[a]Mean offspring blood pressure in each intervention arm of the trial, reproduced from publication

Calcium

A number of maternal calcium supplementation trials have been conducted on pregnant women in recent years, primarily to investigate the potential for reducing the risk of pre-eclampsia [74, 105], and to date three of these trials [106-108] have published follow-up data on offspring blood pressure (Table 1.8 and 1.9). All three trials enrolled only nulliparous women as this has been shown to be a risk factor for developing pre-eclampsia [74], and all were randomised double-blind placebo-controlled trials, which represent the highest epidemiological individual study design [95]. One of the trials was conducted by Levine *et al* in the US providing 2g/d of calcium [109], a second also providing 2g/d was conducted by Belizan *et al* in Argentina [110] and the third conducted by Crowther *et al* in Australia provided 1.8g/d [111].

The follow-up of the US study reported that maternal calcium supplementation during pregnancy was associated with lower systolic blood pressure in the offspring at two years, although only 10% of eligible participants were recruited into the follow-up study and a previous follow-up at three months of age had revealed no difference [107] (Table 1.10). Data from Australia [108] and Argentina [106] showed no association between maternal calcium supplementation and offspring blood pressure at 4-7 years and 5-9 years respectively. Despite no overall association with mean blood pressure, in Argentina the intervention was associated with a reduced risk of having high systolic blood pressure (defined by age and height specific cut-offs) [106]. In this same study, there was also an interaction between childhood BMI and maternal calcium supplementation on offspring blood pressure. For children with BMI above the mean at follow-up, the intervention was associated with lower blood pressure [106].

Table 1.8 Trials of maternal calcium supplementation during pregnancy

	USA	**Argentina**	**Australia**
Original trial	Levine *et al*, 1997 [109]	Belizan *et al*, 1991 [110]	Crowther *et al*, 1999 [111]
Design	Randomised double-blind placebo-controlled trial	Randomised double-blind placebo-controlled trial	Randomised double-blind placebo-controlled trial
Randomisation	Computer generated randomisation: allocation concealment	Computer generated randomisation : allocation concealment	Computer generated randomisation, stratified by centre: allocation concealment
Participants	Nulliparous pregnant women attending 5 medical centres Enrolled between 13 and 20wks gestation Passed a compliance test prior to randomisation	Nulliparous pregnant women attending 3 hospitals Singleton pregnancies Enrolled at 20wks gestation	Nulliparous pregnant women from 5 centres Singleton pregnancy Enrolled less than 24wks gestation
Supplement	4 tablets taken daily from 13-21 weeks gestation until delivery Intervention arm = 2g calcium carbonate (500mg per tablet)/d Control arm = cornstarch placebo	4 tablets taken daily from 20wks gestation until delivery Intervention arm = 2g calcium carbonate (500mg per tablet)/d Control arm = lactose placebo	3 tablets taken daily from 20wks gestation until delivery Intervention arm = 1.8g calcium carbonate (600mg per tablet)/d Control arm = lactose placebo

Table 1.9 Follow-up studies of the effect of maternal calcium supplementation trials during pregnancy on offspring blood pressure

	USA	**Argentina**	**Australia**
Follow-up	Hatton *et al*, 2003[107]	Belizan *et al*, 1997[106]	Hillier *et al*, 2007[108]
Date	Not reported	1995-96	200-02
Age of offspring	Two study periods: 3 months and 2y	5-9 y (mean: 7.1)	4-7 y
Recruitment	Study limited to one site (representing 12% of original trial) 3 mo = 260/559 (47%) from this site recruited 2y = 57/559 (10%) from this site recruited No data on loss to follow-up differences	Study limited to one hospital (representing 50% of original trial) 518/614 (84%) from this site recruited Loss to follow-up associated with younger maternal age and lower maternal blood pressure	179/456 (39%) of original trial offspring recruited Loss to follow-up associated with younger mothers, lower Ca intake from food and lower compliance.
BP measurement	3mo: three measurements at 1 minute intervals using sphygmomanometer with ultrasonic amplification and infant in supine position 2y: three measurements using automated sphygmomanometer with child in supine position	Three measurements at 1 minute intervals following 15 minute rest using mercury sphygmomanometer Mean of three values used for analysis	Three measurements at 1 minute intervals following 10 minute rest using automated recorder (845XT; Dinamap) Median value used for analysis

Table 1.10 Effect of maternal calcium supplementation during pregnancy on offspring blood pressure

Outcome	Study	Supplement		Placebo		Mean difference[a]	P-value
		N	Mean (SD)	N	Mean (SD)	(95% CI)	
Systolic BP (mmHg)	Hatton *et al*, 2003 [107]	3 mo: 130	111.4 (14.3)	3 mo: 130	113.6 (12.6)	-2.2 (-5.5, 1.1)	0.20
		2yr: 35	95.4 (7.6)	2yr: 18	100.2 (7.9)	-4.8 (-9.2, -0.3)	0.04
	Belizan *et al*, 1997 [106]	261	105.3 (11.0)	257	103.9 (10.6)	1.4 (-0.5, 3.3)	0.14
	Hillier *et al*, 2007 [108]	91	95.4 (7.4)	88	95.5 (8.5)	-0.1 (-2.4, 2.3)	0.94
Diastolic BP (mmHg)	Belizan *et al*, 1997 [106]	261	65.8 (9.3)	257	65.4 (9.3)	0.4 (-1.2, 2.0)	0.63
	Hillier *et al*, 2007 [108]	91	57.1 (7.2)	88	56.6 (7.1)	0.5 (-1.6, 2.6)	0.65
		n/N	Percentage	n/N	Percentage	Relative risk (95% CI)[c]	
Risk of high systolic BP[b]	Belizan *et al*, 1997 [106]	29/257	11.4	50/261	19.3	0.59 (0.39, 0.90)	0.01
Risk of high diastolic BP[b]	Belizan *et al*, 1997 [106]	26/257	10.2	33/261	12.7	0.80 (0.49, 1.30)	0.41

[a]Mean difference in blood pressure between offspring born to women in the supplement compared to placebo arm of the trial, derived from unadjusted regression analysis and reproduced from publication

[b]Defined by age, height and sex-specific cut-offs[12]

[c]Relative risk of having high blood pressure for children born to women in the supplement compared to placebo arm of the trial, reproduced from publication

Dietary counselling

One additional study should be mentioned in this section although it is not a trial of nutritional supplementation but of dietary advice. This is a small trial conducted in Finland where women were randomised to three different arms: dietary intervention plus probiotics, dietary intervention plus placebo and a control plus placebo arm. Women in the dietary intervention arms received detailed counselling at 24 and 34 weeks gestation, encouraging them to achieve intake that complied with healthy eating recommendations. Specifically, the counselling highlighted the amount and type of fat in the diet and the importance of fibre intake [112]. Subjects were also provided with conventional food products with favourable fat and fibre content to facilitate adherence to the advice given [112]. There were 256 women recruited into the trial and 216 infants recruited into the follow-up study at six months of age where blood pressure was estimated [113]. The intervention was associated with changes in food habits; participants in the dietary intervention arms consumed higher quality fats as a result of reduced butter and increased margarine and vegetable oil consumption [113]. However, there was no difference in infant blood pressure between the dietary intervention and control groups at six months of age [113].

1.4.2 Early postnatal nutrition

Lucas *et al* propose that the apparent association between size at birth and chronic disease in later life may be explained by the relative change in size experienced postnatally for individuals born small for their gestational age [53]. The majority of birth weight – blood pressure associations published in the literature either are only apparent after adjusting for current size or are substantially stronger after this adjustment, suggesting that change in size may be the important exposure rather than fetal growth restriction *per se* [50]. Whilst more recent data has demonstrated robust associations between restricted fetal growth and blood pressure in later life [51], and the animal data outlined in Section 1.4.1.2 demonstrates the proof of principle that fetal exposures can influence hypertension risk [76, 77, 79], there is a growing interest in the importance of exposures early in postnatal life. The field of

research encompassed by DOHaD has now expanded to include early postnatal influences such as differential patterns of growth, nutrition or infection on the susceptibility to disease in later life [44].

1.4.2.1 Observational data

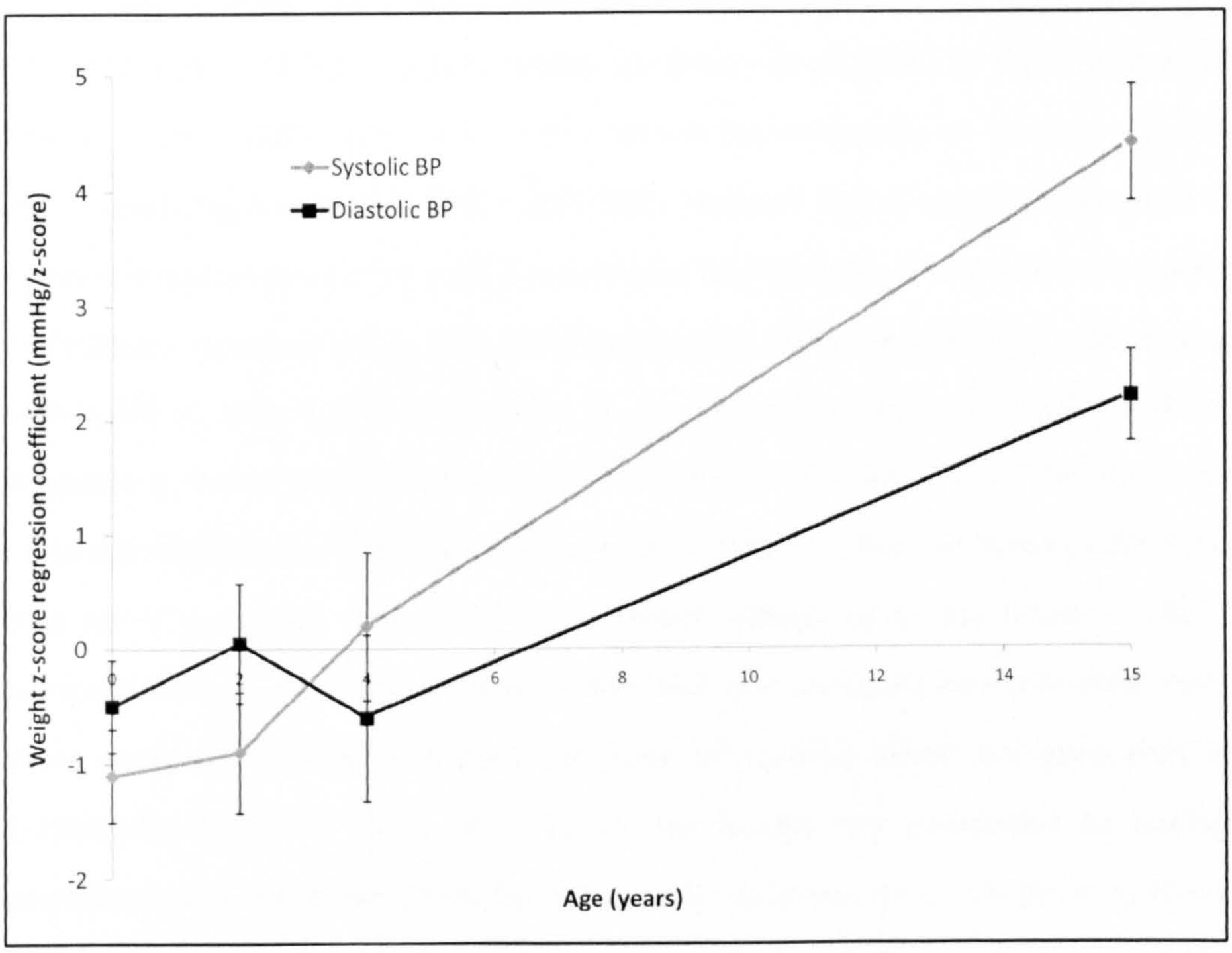
Growth

The importance of growth patterns for the development of disease risk has received the most attention in this field, and underlying this concept are a range of nutritional exposures that in turn influence growth. The highest risk for hypertension is usually observed for individuals who were born small but are relatively large as adults, suggesting that catch-up growth may be aetiologically important and some authors have suggested that coronary heart disease can be conceptualised as a "disorder of growth" [114]. There has however been substantial controversy about the specific patterns of growth which may confer greatest risk, partly stemming from methodological issues relating to characterising an exposure that is fundamentally associated with later size and, by implication, later blood pressure. Data from the Helsinki birth cohort has been influential in this area and appears to implicate catch-up growth between birth and seven years of age; in this cohort hypertensive adults had been born relatively small and by age seven had caught up to average size before surpassing this by age 15 [115]. Many studies have investigated the impact of rapid postnatal growth on blood pressure in later life with the majority reporting the crossing of weight centiles in childhood to be an important exposure [116-120], although there is great heterogeneity in the definition of growth across studies.

Cole has suggested a method for visualising the impact of growth during childhood on blood pressure in later life, termed life course plots [121]. The regression coefficient for the association between weight SD score at different time points and final blood pressure are plotted against age to give an indication of the shape of the relationship. The individual data points represent the strength of the association at a given age and the change from one age to the next represents the effect of

growth [121]. Date from the Pelotas birth cohort in Brazil was used to illustrate this method, showing a negative effect of birth weight on systolic blood pressure and a strong positive effect of weight at 15 years of age. In addition the graphs revealed an essentially linear association between weight centile crossing from birth to 15 years of age and systolic blood pressure at this later age point [121] (Figure 1.2).

Figure 1.2 Life course plot for the association between weight in childhood and blood pressure in adolescence in the Pelotas cohort, Brazil
(reproduced from Cole [121])



Points are linear regression coefficients for the association between weight at each age and blood pressure at 15 years

As the interest in the impact of early life has grown, a number of more sophisticated analysis techniques have emerged. Ben-Shlomo *et al* have suggested modeling individual growth trajectories, which by necessity requires multiple measurements, in order to gain a more complete understanding of the pattern of growth that may be associated with raised blood pressure [122]. Their analysis of

the Barry Caerphilly Growth Study in Wales has demonstrated that rapid postnatal weight and length gains in the first five months of life are positively associated with systolic and diastolic blood pressure at 25 years of age, [122]. In contrast, a recent pooled analysis of cohort data from Brazil, Guatemala, India, the Philippines and South Africa reported that larger size in early childhood was associated with later blood pressure mainly through its association with greater BMI, and that the rate of growth in early life did not affect later blood pressure [123].

Feeding

The impact of infant feeding behavior on the development of high blood pressure in later life was reviewed systematically by Owen *et al* [124] in 2003 and again by Martin *et al* in 2005, with this second review focusing only on studies with follow-up beyond the first year of life [125]. Both reviews found some evidence of lower systolic blood pressure for exclusively breast fed compared to exclusively bottle fed individuals. However, they also both reported a stronger effect in smaller studies, with only a very borderline effect in studies recruiting over 1000 individuals, suggesting some publication bias in the literature [124, 125]. The most recent review and meta-analysis was conducted by Horta *et al* for the WHO in 2007 as part of a wider summary of the long-term health effects of breast feeding [126]. This review included 28 observational studies and one randomised controlled trial. The meta-anlaysis revealed a pooled estimate of systolic blood pressure that was -1.21mmHg (95% CI: -1.72, -0.7) lower for breast fed compared to bottle fed individuals and a smaller effect for diastolic blood pressure of -0.49mmHg (95% CI: -0.87, -0.11) [126]. Again the authors reported evidence for publication bias with smaller sample sizes revealing larger effects, and some evidence that any effect of breast feeding may be attributed to issues of residual confounding [126].

Infections

The impact of infectious diseases in early postnatal life on the risk of chronic diseases in later life could be one interpretation of the observed association between infant mortality rates and death rates from coronary heart disease, which form the foundations of the DOHaD research field [45, 127]. There is little evidence

exploring any link between infectious diseases in early life and blood pressure development. However, some suggestive data were provided by the ALSPAC cohort; hospital admissions for dehydration in infancy were associated with higher diastolic blood pressure at seven years of age, which the authors suggest could reveal a link between diarrhoea in infancy and later blood pressure [128]. Although this finding was replicated in a similar study in Brazil, the more detailed data available in this study failed to show an association between frequency of dehydrating diarrhoea in infancy and blood pressure at a mean age of 5.6 years [129], lending uncertainty to the explanation of the original observation.

1.4.2.2 Animal studies

The majority of animal studies in the DOHaD field have concentrated on the manipulation of the diet during pregnancy and only a few have focussed on the impact of the postnatal diet, recently summarised by Symonds [130]. If a low protein diet is continued from pregnancy throughout lactation in rats, the offspring have blood pressure that is no different from control animals, even if they are given a standard diet after weaning [131]. Interestingly, the offspring exhibit lower blood pressure than controls as adults if they are continued on the low protein diet after weaning [132]. The importance of diet during lactation, at least in rat models, has been highlighted by work by Ozanne and Hales [133]. Male rat pups exposed to reduced nutrition during *in utero* development but cross-fostered at birth to dams on a normal diet show rapid catch-up growth and a reduced lifespan compared to those born to normal dams and cross-fostered to dams on a reduced diet [133].

1.4.2.3 Human intervention trials

Very few studies have investigated the impact of nutritional interventions in early post-partum life on subsequent blood pressure. One of the widely cited examples pertains to an intervention conducted in preterm individuals to investigate the impact of breast *vs* formula milk on health and development. Preterm infants randomly assigned to receive banked breastmilk for the first few weeks of life had

lower diastolic and mean arterial blood pressure at 13-16 years compared to infants given preterm formula [134], although no difference had been observed in an earlier study when the offspring were 7.5-8 years old [135]. In a separate trial of term infants born small for gestational age, individuals randomised to receive protein-enriched formula had increased diastolic and mean arterial pressure compared to those on standard formula at age 6-8 [136]. The authors suggest that the lower risk in these studies relates to slower postnatal growth experienced by breast-fed *vs* formula-fed or standard *vs* enriched formula-fed infants [136]. One additional intervention study provides useful data in this field. A large multicentre cluster-randomised controlled trial promoting breastfeeding in the Republic of Belarus using the steps of the WHO and UNICEF-developed Baby-Friendly Hospital Initiative was conducted between 1996 and 1997 and termed the 'Promotion of Breastfeeding Intervention Trial' (PROBIT) [137]. The trial was successful at increasing the rate of breast feeding; infants in intervention sites were more likely to be breastfed exclusively at three and six months compared to infants in control areas [137]. However, a follow-up study when the children were 6.5 years of age found no difference in blood pressure between the children from intervention and control sites [138].

1.4.3 Other early-life exposures

Stress

The effect of stress during pregnancy on birth weight and subsequent disease risk has been proposed as an alternative explanation for the impact of the fetal environment on the development of the blood pressure system [139]. Animal evidence has supported this hypothesis; administration of the synthetic corticoid stress hormone dexamethasone to pregnant rats results in hypertensive offspring who are born low birth weight [139-141]. Observational studies of human preterm infants have reported that prenatal exposure to corticoids is associated with raised offspring blood pressure [142], although a recent RCT of the corticosteroid betamethasone *vs* placebo provided during pregnancy has shown no adverse effect

on offspring blood pressure [143]. In the US birth cohort Project Viva, a higher ratio of cortisol to cortisone in umbilical venous cord blood (representing increased fetal exposure to cortisol) was found to be associated with increased systolic blood pressure at three years of age [144]. At present, little other data from human studies is available to test this intriguing hypothesis. Moreover, it has been suggested that the mechanism through which stress impacts on the development of offspring blood pressure is through a reduction in maternal dietary intake during pregnancy, a pathway that has been observed in rat models [145].

Seasonality

Season of birth in settings where there is a marked seasonality of farming activities and of food availability has been used by some research groups as a marker of sub-optimal conditions experienced during *in utero* development. In The Gambia, individuals born during the annual hungry season running from July to December have been shown to have a 10-fold increased risk of death in young adulthood, predominantly from infectious causes [146]. This has led researchers to speculate that the development of the immune system may be impaired in individuals born in the hungry season [147]. Interestingly, there is no evidence from the same population that season of birth is related to cardiovascular risk factors in young adulthood, including blood pressure [148]. Data from the Pune Maternal Nutrition study in India has further highlighted the effects of seasonality; marked changes in activity and energy intake correspond with the seasonal changes in farming patterns and in turn are associated with maternal weight fluctuations and with birth weight [149].

1.4.4 Summary of early-life literature

The importance of fetal nutrition for the optimal development of the blood pressure system has been inferred from studies showing a consistently inverse association between birth weight and blood pressure in later life. Direct evidence of

the role of the maternal diet has been demonstrated by a large number of animal experiments, but data on the impact of diet during pregnancy in humans is conflicting, partly due to the heterogeneity of the data collected in observational studies investigating this hypothesis. Follow-up studies of intervention trials of maternal supplementation can provide a useful resource with which to investigate the impact of the maternal diet, which should be free from confounding, but to date very few have been published.

Recent research attention has focused on the postnatal period as another key stage in the life course that may affect later blood pressure. The impact of growth in the first few years of life has been inferred from the inverse associations between birth weight and blood pressure that often are only apparent after adjustment for current size. Again direct evidence of the role of nutrition during the first few years of life on later blood pressure is lacking. One exception is the predominantly observational data on the impact of breast feeding compared to formula feeding, but again the results are conflicting.

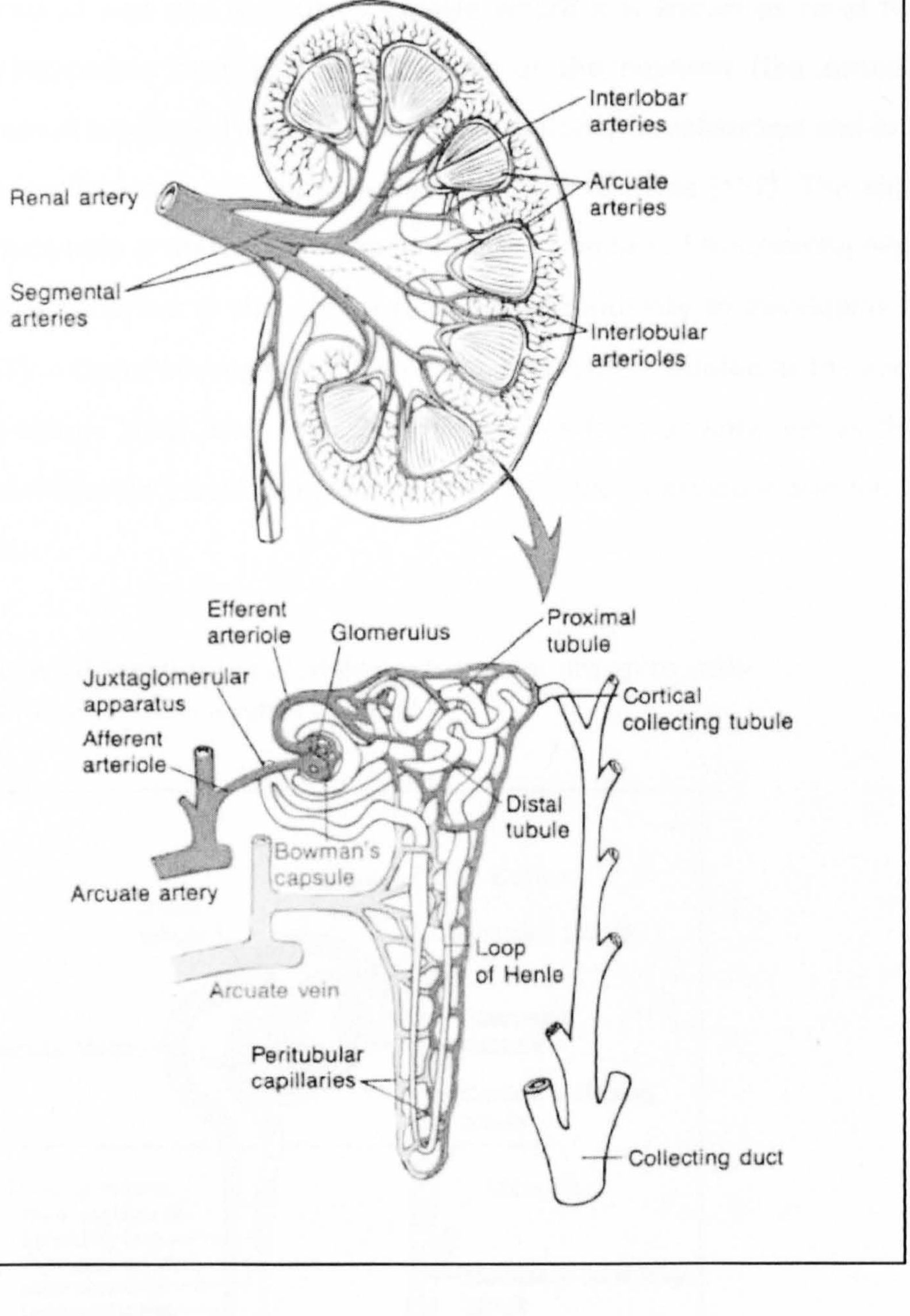
1.5 Potential mechanisms

Despite the paucity and conflicting findings from some of the human data, the evidence does appear to suggest that environmental influences, potentially nutritional, operating in early life can affect an individual's susceptibility to developing chronic disease. It is therefore necessary to consider which mechanisms may be operating during development that impact on the blood pressure system; possibilities are outlined below.

1.5.1 Structural changes

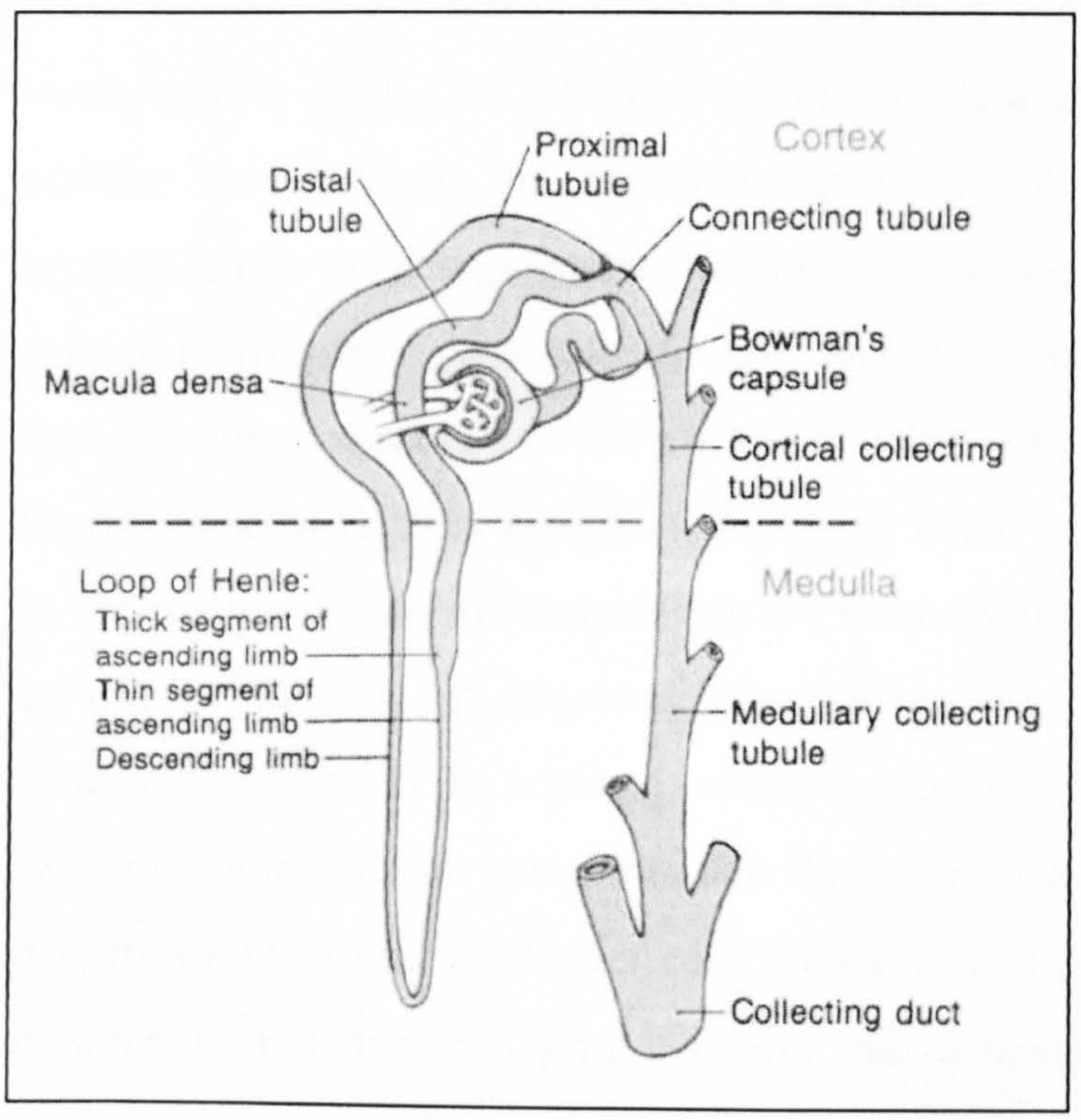
The kidneys (Figure 1.3) are the major organ in the blood pressure system and act as the regulator of long-term arterial pressure [150, 151]. Waste products produced by body cells are excreted in urine formed by the kidneys, a process that maintains the normal volume, electrolyte composition and pH balance of the blood and thus the maintenance of normal blood pressure [152]. Due to the pivotal role the kidneys play in maintaining body fluid composition they are thought to be important in the development of hypertension. Other evidence implicating the kidneys in the development of hypertension is that all known single gene mutations associated with hypertension are characterised by altered expression of kidney proteins [153] and that transplant recipients exhibit blood pressures that reflect those of their donors [154].

Figure 1.3 Cross-sectional image of a kidney with major vessels and the microcirculation surrounding a single nephron
(reproduced from Guyton and Hall [7])



Nephrons are the functional unit of the kidney and the site of urine formation (Figure 1.3 and 1.4). The first sections of the nephron are located in the kidney cortex, these are the glomerulus capillary network, which is surrounded by the expanded end of the renal tubule known as Bowman's capsule (Figure 1.4) [152]. Blood pressure forces plasma, and associated dissolved substances, out of the glomeruli and into Bowman's capsule where it is known as renal filtrate. As the filtrate passes through the remainder of the nephron (the connecting tubule, proximal tubule and loops of Henle), the majority is reabsorbed and only around 1% eventually enters the renal pelvis to be lost as urine [152]. The kidney filtration surface area is therefore dependent on the number of functioning nephrons, and a reduced number is thought to confer a susceptibility to developing hypertension [155]. A figure of roughly 1 million nephrons is often quoted as the average number per kidney [152]. Hoy *et al* describe studies from as long ago as the 1930s that report inverse associations between systolic blood pressure and nephron number [156].

Figure 1.4 Structure of a single nephron, not drawn to scale (reproduced from Guyton and Hall [7])



The rate of filtration into the Bowman's capsule is known as the glomerular filtration rate (GFR). Estimation of the GFR is often used by clinicians to measure kidney function and in the detection of chronic kidney disease [157]. GFR declines with age from an average of around 130 ml/min/1.73m^2 in young men and 120 ml/min/1.73m^2 in young women; a cut-off of 60 ml/min/1.73m^2 is used to detect chronic kidney disease in both men and women [157]. The gold standard measurement of GFR is the plasma clearance rate of injected substances that are exclusively excreted by the kidneys, such as chromium-EDTA, but these techniques are expensive and difficult to perform routinely [157]. As an alternative, serum creatinine has been widely used for the estimation of GFR but the marker has come under recent criticism for its variation in response to other factors than renal function, particularly its association with muscle mass [158]. Recently, Cystatin C (CysC) has been proposed as an alternative estimator of GFR that may be more accurate than creatinine, particularly amongst children [158, 159].

It has been suggested that a reduction in nephron number as a result of inadequate fetal nutrition may explain the association between low birth weight and hypertension [150]. In humans, nephrogenesis occurs between weeks 8 and 36 of gestation with the majority forming in the third trimester and no potential for increase after birth [160]. Brenner *et al* postulate that a reduction in nephron number would cause the remaining glomeruli to undergo hypertrophy and hyperfiltration in compensation, ultimately leading to a loss of functioning glomeruli through glomerulosclerosis and the subsequent development of hypertension in response to this decline in kidney function [155]. There is support for this hypothesis from animal experiments; a low protein diet during pregnancy has consistently been shown to result in a reduced number of nephrons (of around 30%) in rat models [79, 161-164], whilst in the sheep the same effect has been induced by exposure to a global undernutrition diet [165, 166]. However, there remains some debate about the link between reduced nephron number and hypertension; in one rat model combined pre- and postnatal protein restriction resulted in a deficit in nephron number but a lower rather than higher mean arterial pressure [132].

There are few studies in humans investigating the hypothesis that inadequate nutrition *in utero* is associated with structural changes to the kidney, and particularly a reduction in nephron number. In a very small study of stillborn infants, those who were severely growth retarded had a reduced nephron number [167]. Autopsy data have also provided supportive evidence, reporting positive associations between nephron number and birth weight [168, 169]. *In vivo* assessments of kidney function range from structural parameters such as ultrasound assessed volume to estimates of glomerular filtration rate and tubular function via surrogate markers. At autopsy, kidney volume has been shown to correlate well with the number of functioning nephrons, suggesting that this parameter may be used as a proxy indicator of nephron number [170].

Konje *et al* report that small for gestational age fetuses, assessed by ultrasound, have smaller kidneys after 26 weeks of gestation than those appropriate for their gestational age [171]. Australian Aborigines have a high prevalence of both hypertension and low birth weight and a study of children and adolescents demonstrated that low birth weight individuals had a lower kidney volume, assessed by ultrasound, than those of normal birth weight [172]. A study of US adults also reported a strong correlation between glomerular number and birth weight [173]. A larger comparison study involving over 300 people from Australia and the USA reported that Aborigines and US whites with hypertension had fewer nephrons than those groups without hypertension, but the same relationship was not significant for US blacks [156]. Japanese individuals appear to have smaller kidneys than Caucasians, which has been postulated to partly explain the greater prevalence of hypertension in this population [150]. White *et al* recently reviewed the observational data linking low birth weight to chronic kidney disease in later life and their meta-analysis provided some evidence for an association between low birth weight and albuminuria, end-stage renal disease and low estimated glomerular filtration rate, although it should be noted that half of the studies reported a null association [174].

Postnatal growth maybe an important factor to consider in the association between reduced nephron number and raised blood pressure in later life. Bagby postulates that at birth nephron number and body size should be aligned for individuals born small for their gestational age [160]. If however, accelerated growth is experienced postnatally the excretory demand (body mass) exceededs the excretory capacity (nephron number) and it is this imbalance that confers susceptibility to hypertension in later life [160].

Microalbuminuria is one of the first symptoms of renal disease, even before a decrease in glomerular filtration rate (GFR) [175]. In Australian Aborigines, low birth weight was associated with high albumin-creatinine ratio (ACR) (≥34g/mol) in adulthood, which is indicative of renal disease although there was no difference in blood pressure [176]. A study in the Netherlands reported that in individuals born before 32 weeks gestation (and therefore before nephrogenesis is completed) high ACR was related to low birth weight [175]. Evidence for a raised ACR was also observed for individuals exposed to the Dutch Hunger Winter during mid-gestation compared to those who were un-exposed, when individuals were measured at around 50 years of age [177]. However, there was no corresponding difference in blood pressure at this age [59].

It has also been proposed that fetal growth restriction is associated with structural changes of the vasculature. Some authors have suggested that the laying down of elastin *in utero* is impaired in low birth weight individuals and the resulting blood vessels have reduced compliance, thus resulting in raised blood pressure [45]. Small studies of low birth weight individuals have found some evidence of altered function of the microvasculature [178], whilst there is also suggestive evidence from rat and sheep models [80]. However, further evidence on the role of vascular structure in the programming of blood pressure in humans is limited at present.

1.5.2 Hormonal changes

Renin (mainly released by the kidneys) stimulates the conversion of angiotensin I (ANG I) to angiotensin II (ANG II) by angiotensin-converting enzyme (ACE), which acts through a variety of mechanisms to increase blood pressure [179]. In addition, the renin-angiotensin system (RAS) is important for nephron development during gestation [180]. Therefore, the programming of the RAS may represent a mechanism whereby nephrogenesis is affected or an alternative mechanism linking fetal nutrition to later blood pressure. In rats, nutrient restriction has been associated with increased activity of ACE [78]. Human data is very limited, although higher ANG II concentrations have been reported amongst growth restricted infants in Glasgow, compared to those who were appropriate weight for their gestational age [181]. In contrast, lower plasma renin levels were reported for adults in the Sheffield birth cohort who had been born small for gestational age [182].

Insulin-like growth factor (IGF) also plays an important role in kidney development; IGF is expressed at high levels in the developing kidney and knock-out animals without IGF-I have impaired renal development [180]. IGF has been shown to be altered in many of the diseases associated with low birth weight and growth retarded humans have elevated IGF-I [45]. The role of IGF in the programming of hypertension is unclear. Sheep exposed to a 50% reduction in energy requirements during the last trimester produced offspring with upregulated IGF-I receptors but no difference in kidney size [180]. Interestingly, sheep exposed to reduced energy requirements throughout pregnancy showed no effect on IGF-I receptors [180].

1.5.3 Epigenetics

If, as is suggested, there are critical periods of development that respond to cues or stresses in the environment and in turn influence the blood pressure system (amongst others), how are these cues translated into the proposed mechanistic changes outlined above? The role of epigenetics has been highlighted as a likely linking mechanism and the data relating to DOHaD has been recently summarised by Waterland and Michels [183]. Epigenetics refers to heritable changes in gene

expression potential, of which changes in DNA methylation patterns is one of the best characterised examples [183]. DNA methylation patterns are established during fetal and early postnatal development and are thus susceptible to environmental stimuli, such as dietary supply, at this time [183]. One line of evidence that epigenetic mechanisms can be influenced by the maternal diet has been derived from studies in the agouti mouse. In this model, diets rich in methyl donors provided periconceptually and throughout pregnancy were associated with changes in offspring coat colour that operated via altered epigenetic expression [184]. Altered methylation patterns have also been described in the angiotensin receptor gene of rats born to dams fed a low protein diet during pregnancy [185], again implicating epigenetics in the explanation of the effect of nutritional programming of hypertension. There is little data yet published that investigates epigenetic changes in response to fetal nutrition in humans. However, a recent small follow-up study of the Dutch Hunger Winter revealed less DNA methylation of the insulin-like growth factor 2 (IGF2) gene for individuals conceived during the famine compared to unexposed same-sex siblings, providing suggestive evidence of genetic changes in response to environmental exposures during fetal development [186].

1.6 DOHaD theories

Since its inception, the field of DOHaD has stimulated a lively discussion on the possible explanations for the observed inverse association between birth weight and later disease. These have often focused on whether the postulated mechanistic changes, as a consequence of sub-optimal early nutrition, reflect a purely pathogenetic process or whether there could be adaptive advantages. This section will briefly summarise three of the influential theories in this field, in chronological order of their appearance in the literature.

1.6.1 Thrifty genes

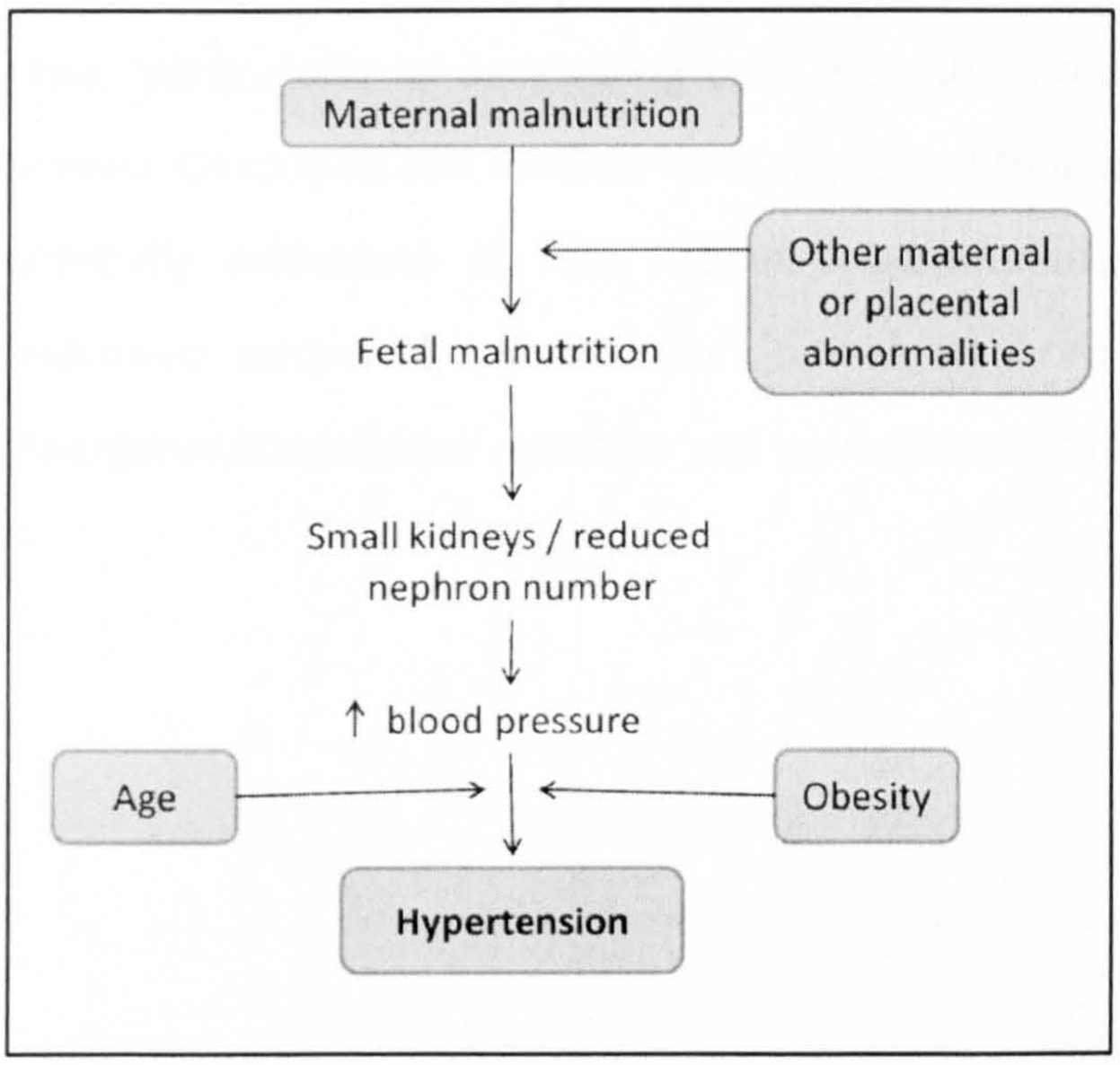
The term the 'thrifty genotype' was first used by Neel in 1962 to describe the potential for genes that allow metabolically frugal processes, which would confer advantages in times of food shortages but which in today's environment result in an increased susceptibility to metabolic diseases [187]. Prentice has argued in support of this theory that much of human evolution has been characterised by periods of extreme famine, providing an evolutionary pressure to select genes that allow increased survival in these conditions, in other words a pressure for genes that are 'thrifty' [188]. In modern society, where there is predominantly an abundance of food, these genes are no longer advantageous and may instead render individuals susceptible to disease [189]. The majority of discussions around the concept of the thrifty genotype have focused on genes involved with metabolic functions or with behavioural adaptations that conserve fuel [189], the applicability of this theory to the blood pressure system is thus debatable.

1.6.2 Thrifty phenotypes

Hales and Barker proposed the 'thrifty phenotype' hypothesis in the early 1990s in an effort to explain the observed inverse association between birth weight and impaired glucose tolerance and the metabolic syndrome [190]. They postulated that fetal malnutrition led to changes in body systems, such as poor pancreatic β-cell development and insulin resistance, which in turn reduced growth and preserved resources for brain development [191]. The resulting reduced capacity for insulin secretion may become detrimental in later life leading to a greater risk of developing glucose intolerance, particularly if an individual were to become overweight or obese [191]. Metabolic adaptations lend themselves to the thrifty phenotype hypothesis as intuitive mechanisms for preserving fuel; the extrapolation of the theory to the development of hypertension is less obvious. However, the potential structural changes in the kidneys outlined in Section 1.5 could also be viewed as 'brain sparing' mechanisms in as far as resources are diverted away from peripheral organs, resulting in a smaller size and functional capacity. Although a necessary adaptation for short term survival, these changes

may confer increased susceptibility to hypertension in later life, particularly in environments of nutritional excess (Figure 1.5).

Figure 1.5 Development of hypertension explained by the thrifty phenotype hypothesis
(adapted from Hales and Barker [191])



1.6.3 Mismatch hypothesis

In essence the thrifty phenotype hypothesis outlined above is a form of 'developmental plasticity', which is an observed phenomenon throughout the animal kingdom. There is a clear survival advantage to an organism that is able to adjust its development optimally in response to the prevailing environmental exposures, a phenomenon that has been observed for many species [192]. Gluckman and Hanson have defined the concept of developmental plasticity within the framework of DOHaD in what they term the 'Predictive Adaptive Response' or 'Mismatch' hypothesis, by which they propose that the developing fetus utilises cues (potentially nutritional) from the uterine environment to predict the

environment that will be experienced postnatally, and thus takes the appropriate developmental pathway [193]. Provided that these cues do accurately predict the future environment there is no increase in disease risk, but if there is a substantial 'mismatch' between the early and later environments a greater risk of disease is incurred [193]. Currently many populations could be experiencing a mismatch between predicted and experienced environments due to the rapidity by which food preferences, consumption patterns and energy expenditure are changing over time, particularly in developing countries undergoing rapid nutrition transitions. Indeed, Gluckman and Hanson have suggested that the epidemic of type II diabetes currently witnessed on the Indian sub-continent may be a consequence of improved postnatal nutrition for individuals born small as a consequence of intergenerational poor nutrition and corresponding maternal constraint [193].

1.7 Study aim and design

The primary research aim of this thesis is to investigate the impact of the maternal diet during pregnancy on offspring blood pressure. The adopted approach is the follow-up of randomised controlled trials (RCTs) of maternal nutritional supplementation. These trials represent a valuable resource with which to investigate this research question, providing two main advantages over observational studies in this field:

1) The RCT design allows us to be less concerned about issues of confounding and therefore more confident that observed effects are due to the treatment regime
2) The diet has been changed by a known amount and therefore, provided compliance data is available, it is not necessary to rely on observational dietary intake data, which has well-documented measurement errors.

To investigate this research question, three intervention studies were chosen (Table 1.11): one of protein-energy supplementation, one of calcium supplementation and one multifaceted design including food and micronutrient supplementation as well as an intervention to promote exclusive breastfeeding.

As a secondary research aim, observational analysis was also conducted for each of the trial 'cohorts' to investigate the impact of other early life factors in the development of blood pressure including, birth weight, season of birth and infant growth.

Table 1.11 Characteristics of the original supplementation trials that form the basis of this PhD research

	Protein-energy	Calcium	Food, MMN, breastfeeding
Location	Rural Gambia	Rural Gambia	Rural Bangladesh
Design	Community-level randomised trial. 1989-94[194]	Randomised double-blind placebo-controlled trial, ISRCTN96502494. 1995-2000 [195]	Randomised controlled trial with factorial design (6 groups), ISRCTN16581394. 2002-2004
Randomisation	Randomisation at village level, stratified for village size. 16 intervention and 12 'control' villages	Random number tables, in blocks of four to ensure seasonality equally distributed	Computer tracking system assigned women to one of six supplementation blocks. After birth one further randomisation stage allocated breast feeding counselling.
Participants	Pregnant women in participating villages, from 20wks gestation (intervention arm). 1460 participating women	Pregnant women from 26 villages in rural Gambia Enrolled less than 20wks gestation 662 participating women	Pregnant women from DSS. Randomisation to food invitation at 8-10 wks gestation, multiple micronutrient arm at 14-15wks. 4436 participating women
Supplement	2 biscuits provided daily = 4250kJ energy, 22g protein, 56g fat, 47mg calcium, 1.8mg iron Intervention arm = biscuits from 20 wks gestation until delivery Control arm = biscuits for 20wks post partum.	3 tables daily from 20wks gestation until delivery Intervention arm = 1.5g calcium carbonate (500mg per tablet) a day Control arm = cellulose-lactose placebo	3 arms of trial: 1. Food: encouraged to access government food provision either immediately (early start) or at the time of their choosing (usual start) 2. Micronutrient supplement: 30mg iron and 400µg folic acid; 60mg iron and 400µg folic acid; multiple micronutrient supplement containing 15 micronutrients 3. Breast feeding: counselling for exclusive breastfeeding or counselling for alternative health messages.
Primary outcome	Size at birth	Blood pressure at 36 weeks gestation	Size at birth, gestational age at birth and infant mortality at 1y

1.8 Candidate's involvement

I defined the topic of this thesis and main research question in close collaboration with my supervisor Dr Sophie Moore. The two large follow-up studies (one in The Gambia and one in Bangladesh) were conducted in close collaboration with the original study principal investigators who gave full permission to use data from the original trials in this thesis. For clarity, I have outlined my specific involvement in the different aspects of this thesis below.

Chapter 2: Gambian methods

Description of the original Gambian follow-up study and statistical analysis. The fieldwork component did not take place in the timeframe of the PhD and is merely described here to allow for the interpretation of results in their appropriate context.

Chapters 3 and 4: protein-energy and calcium results

Presentation of the impact of maternal protein-energy (Chapter 3) and calcium (Chapter 4) supplementation on offspring blood pressure. All aspects of the data analysis from defining the research questions to presenting the data were conducted by me with expert guidance, where appropriate, from Dr Sophie Moore and Dr Tony Fulford (statistician).

Chapter 5: Bangladesh methods

Description of fieldwork conducted in Bangladesh to follow-up a large multifaceted nutritional intervention. I was a co-PI on this large project, in collaboration with staff from Uppsala University, Sweden and the International Centre for Diarrhoeal Disease Research, Bangladesh. I was heavily involved from the beginning of the project with the design and implementation of the follow-up study as well as the staff training and monitoring of data collection.

Chapter 6: Bangladesh results

Presentation of the impact of maternal food, micronutrient supplementation and breast feeding counselling on offspring blood pressure and kidney function. Again, I performed all aspects of the analysis, receiving expert advice where appropriate.

Chapter 2 Gambian study methods

2.1 Forward

In 2005 the UK Medical Research Council International Nutrition Group (ING), in collaboration with MRC Human Nutrition Research, Cambridge, participated in a large European Union Consortium to investigate the early life nutritional determinants of disease. The project was funded by the European Union Sixth Framework Programme for Research and Technical Development of the European Union Community and was known as the 'Early Nutrition Programming Project' (FOOD-CT-2005-007036) or 'EARNEST' (Early nutrition programming – long term follow up of efficacy and safety trials and integrated epidemiological, genetic, animal, consumer and economic research). The role of the ING was to investigate the impact of two maternal supplementation trials in The Gambia on cardiovascular disease risk factors in the offspring. A description of the original supplementation trials and the follow-up study is given in this chapter, with particular emphasis on the statistical methodology that has been used in the analysis of the data. The results of the analysis of the effect of maternal protein-energy supplementation will be described and explored in Chapter 3 and the effect of maternal calcium supplementation in Chapter 4.

2.2 Field site

2.2.1 The Gambia

The Gambia is a small country in West Africa, surrounded on three sides by Senegal and on the fourth by the Atlantic Ocean (Figure 2.1). The country is 11,295km^2 and in 2006 the population was reported to be 1.6 million [196]. Islam is the main religion and English is the official language, although there are many indigenous languages spoken [197]. World Bank statistics for 2007 estimate life expectancy as 69 years and under five mortality rate as 68 per 1000 [196]. The vegetation is

tropical savannah and the climate is characterised by a hot rainy season that runs from June to November and a dry season which covers the remaining months [197].

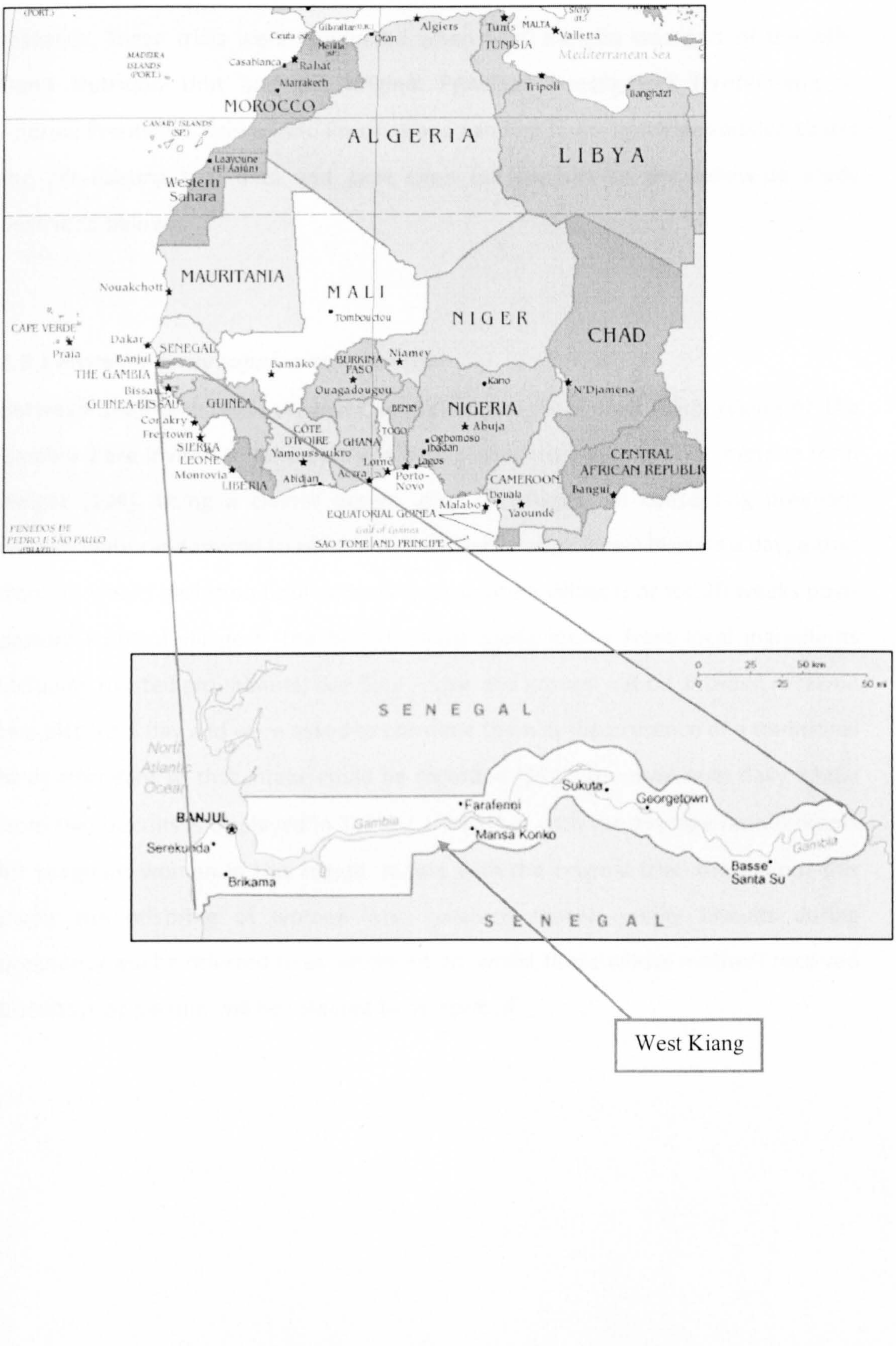
2.2.2 West Kiang

The majority of fieldwork conducted by the ING is concentrated in the rural region of West Kiang, 80km from the more urbanised coastal areas (Figure 2.1) and with a population of over 14,000 as of 2009 (Fulford, *pers. comm.*). The majority of people in the West Kiang region speak the Mandinka language and extended families live together in compounds, the average size of which is 15 people (Fulford, *pers. comm.*). The region is characterised by subsistence agriculture and the cereal crops are rice (*Oryza sativa*), millet (*Pennisetum glaucum*), and sorghum (*Sorghum bicolour*), which are predominately tended to by the women in the community. Farming activities and the availability of food show marked variation over the year in response to the seasonality of the rains. The months from June to October, when the majority of farming work is conducted and next year's harvest has yet to come in, have been termed the 'hungry season', whilst November to May is known as the 'harvest season' [194]. This seasonal pattern of energy expenditure and nutrient intake is associated with marked changes in maternal weight gain and in birth weight [198].

2.2.3 MRC Keneba

The MRC has run a research station in the village of Keneba within West Kiang since 1974, previously as part of the MRC Dunn Nutrition Unit and now as part of the ING. In parallel to their international research agenda, the MRC provides a clinical service and over the years has established strong links with the local community. More than 120 staff are employed at MRC Keneba; the fieldworkers have a wealth of experience of working in the area and are a key component of any successful project.

Figure 2.1 Map of The Gambia, located within West Africa
(reproduced from West Africa Crossroads (regional map) and University of Missouri (Gambian map) [199, 200])



2.3 Original supplementation trials

A follow-up study of two supplementation trials forms the basis of this PhD research. These trials were conducted when MRC Keneba was part of the MRC Dunn Nutrition Unit and the original Principal Investigators (Protein-energy: Andrew Prentice; Calcium: Ann Prentice and Landing Jarjou) gave permission to use the pre-existing trial data and gave their full support to the follow-up study described below.

2.3.1 Protein-energy supplementation trial

Between 1989 and 1994, pregnant women in the rural West Kiang region of The Gambia were invited to take part in a food supplementation trial to improve birth weight [194]. Using a cluster design with 28 villages, all consenting pregnant women were randomised to receive two protein-energy dense biscuits a day, either from 20 weeks gestation until delivery (intervention villages) or for 20 weeks post-partum (control villages). The biscuits were made locally from local ingredients including roasted groundnuts, rice flour, sugar and ground nut oil. Women received two biscuits a day and were asked to consume them in the presence of a traditional birth attendant so that intake could be recorded [194]. The maximum daily intake from two biscuits is displayed in Table 2.1 together with the average requirements for pregnant women in this region. In line with the original trial, throughout this thesis the offspring of women who received protein-energy biscuits during pregnancy will be referred to as 'intervention' whilst those whose mothers received biscuits post-partum will be referred to as 'control'.

Table 2.1 Nutrient intake provided by protein-energy biscuit supplement

Nutrient	Daily requirement[a]	Maximum daily intake from two biscuits
Energy (kJ)	11840[b]	4250
Protein (g)	74[c]	22
Fat (g)	62.8[d]	56
Calcium (mg)	800[e]	47
Iron (mg)	N/A[f]	1.8

[a]Average requirement for third trimester of pregnancy, based on the average age of 24y and weight of 53kg of women in the original trial.
[b]Calculated from FAO/WHO/UNU Expert Consultation [201]
[c]The safe level (meeting 95% of the population) calculated from the WHO Technical Report [202]
[d]Based on the minimal requirement of 20% of energy from fat and assuming a conversion factor of 37.7KJ=1g of fat [203]
[e]Theoretical allowance reported in FAO/WHO Expert Consultation [204]
[f]No recommend intake is given in the FAO/WHO Expert Consultation for iron [204]

Birth weight was the primary outcome of the original trial and was measured using a portable spring balance and tared sling (CMS Weighing Equipment, London) to the nearest 20g and within 48 hours of birth [194]. Birth length was measured using neonatal length mats (TALC Teaching Aids, St Albans, England) to the nearest 5mm. Maternal height was measured at baseline (20 weeks gestation) and maternal weight was measured within 48 hours of delivery to the nearest 200g [194].

The randomisation process of the trial was successful in producing two groups which did not differ in the distribution of measured characteristics known to influence birth weight (Table 2.2).

Table 2.2 Characteristics of rural Gambian women enrolled into the protein-energy supplementation trial
(adapted from Ceesay *et al* [194])

	Control group[a]	Intervention group
No of live singleton births	1037	1010
Male births (%)	50.4	49.5
Maternal age (y)	23.7 (6.4)	24.0 (6.2)
Maternal parity	4.2 (2.4)	4.3 (2.6)
Maternal height (m)	1.59 (0.06)	1.59 (0.06)
No days of prenatal supplementation	0	82 (31)

Values are means (SD), unless otherwise stated
[a]Control group received supplementation during lactation

There were 2047 live singleton births during the five years of the trial and the intervention was found to improve birth weight, by 136g overall and with a greater increase (201g) during the nutritionally poor hungry season (June – October) (Table 2.3) [194]. The odds of neonatal mortality (up to day 28, including stillbirths) was also improved by the administration of the supplement during pregnancy with an odds ratio of 0.57 (95% CI: 0.38, 0.88) [194].

Table 2.3 Effect of protein-energy supplementation to pregnant women on offspring birth anthropometry in The Gambia
(reproduced from Ceesay *et al* [194])

		Regression coefficient (95% CI)	P-value
Birth weight (g) (n=1751)[a]	All year	136 (79, 193)	<0.001
	Harvest season[b]	94 (31, 157)	<0.001
	Hungry season	201 (132, 270)	<0.001
Birth length (cm) (n=1746)[a]	All year	0.16 (-0.24, 0.56)	>0.2
	Harvest season[b]	-0.01 (-0.41, 0.39)	>0.2
	Hungry season	0.41 (-0.09, 0.91)	>0.05

Results are the mean difference in birth anthropometry between individuals born to intervention compared to control women, derived from three stage random effects modelling, adjusted for sex, primiparity and gestational age as Parkin score
[a]Missing Parkin scores for gestational age reduced sample size of analysis from 2047
[b]Harvest season defined as November – May, hungry season as June – October

2.3.2 Calcium supplementation trial

Between 1995 and 2000, staff from MRC Human Nutrition Research in Cambridge conducted a prenatal calcium supplementation trial in The Gambia to reduce pre-eclampsia. All pregnant women from 16 villages in the rural West Kiang region were invited to participate in a double-blind, randomised, placebo-controlled trial of calcium supplementation [195]. Women were randomised using published tables to receive the intervention (1500mg Ca/d as calcium carbonate) or placebo (cellulose-lactose), from 20 weeks gestation until delivery. This is well above the level of 200mg Ca/d recommended as a requirement during pregnancy [205]. Both the calcium supplement and placebo were provided daily in three identical chewable tablets. Study participants were randomised in blocks of four to ensure that women were enrolled from different seasons. Maternal antenatal care was stratified by three clinic blocks, which corresponded to different areas under the responsibility of the three midwives involved in the trial. Fieldworkers delivered the tablets to participating women on a daily basis between 5-7pm and recorded their consumption as a measure of compliance.

The primary outcome of the study was maternal blood pressure at 36 weeks gestation, measured in triplicate using the DINAMAP PRO 400 automated monitor (Critikon Ltd, UK) under standard techniques. Maternal weight was measured to the nearest 0.1kg whilst wearing light clothing but no shoes (Wylux scales: CMS Weighing Equipment Ltd, London, United Kingdom) and height was measured using a stadiometer, also whist wearing no shoes, to the nearest 0.1cm (Magnimetre stadiometer: CMS Weighing Equipment Ltd). Birth weight and length were measured by traditional birth attendants but there were a large number of missing data (34% of live singleton births), therefore for the analysis presented in this thesis, infant anthropometry at two weeks of age has been used as an estimate of birth anthropometry. At this later time point trained fieldworkers took the measurements; naked weight was measured to the nearest 0.01kg (Seca baby-weighing scale: CMS Weighing Equipment Ltd) and supine crown-heel length was measured using a length-board to the nearest 1cm (Kiddimate: Raven Equipment Ltd, Dunmow, United Kingdom).

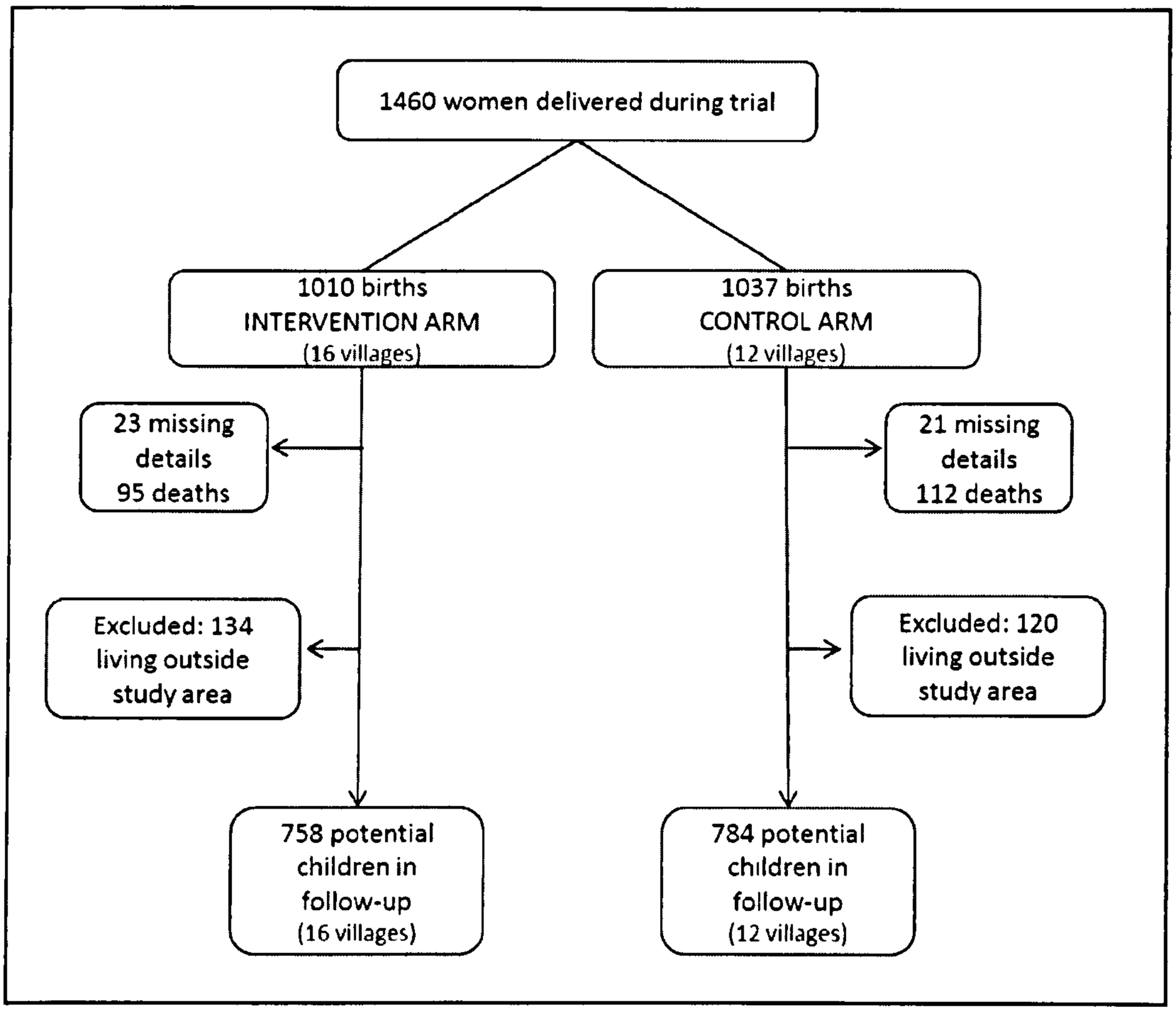
Blood pressure at 36 weeks gestation was measured in 536 women, and 546 live singleton infants were deemed to have been delivered at term and retained in the cohort. Maternal calcium supplementation was shown to have no effect on breast milk calcium concentrations, birth weight or infant bone mineral status in a sub-set of the participants who were enrolled in a more detailed sub-study [195]. Analysis of the main outcome, maternal blood pressure at 36 weeks gestation, is ongoing.

2.4 Follow-up study, methodology

2.4.1 Subjects

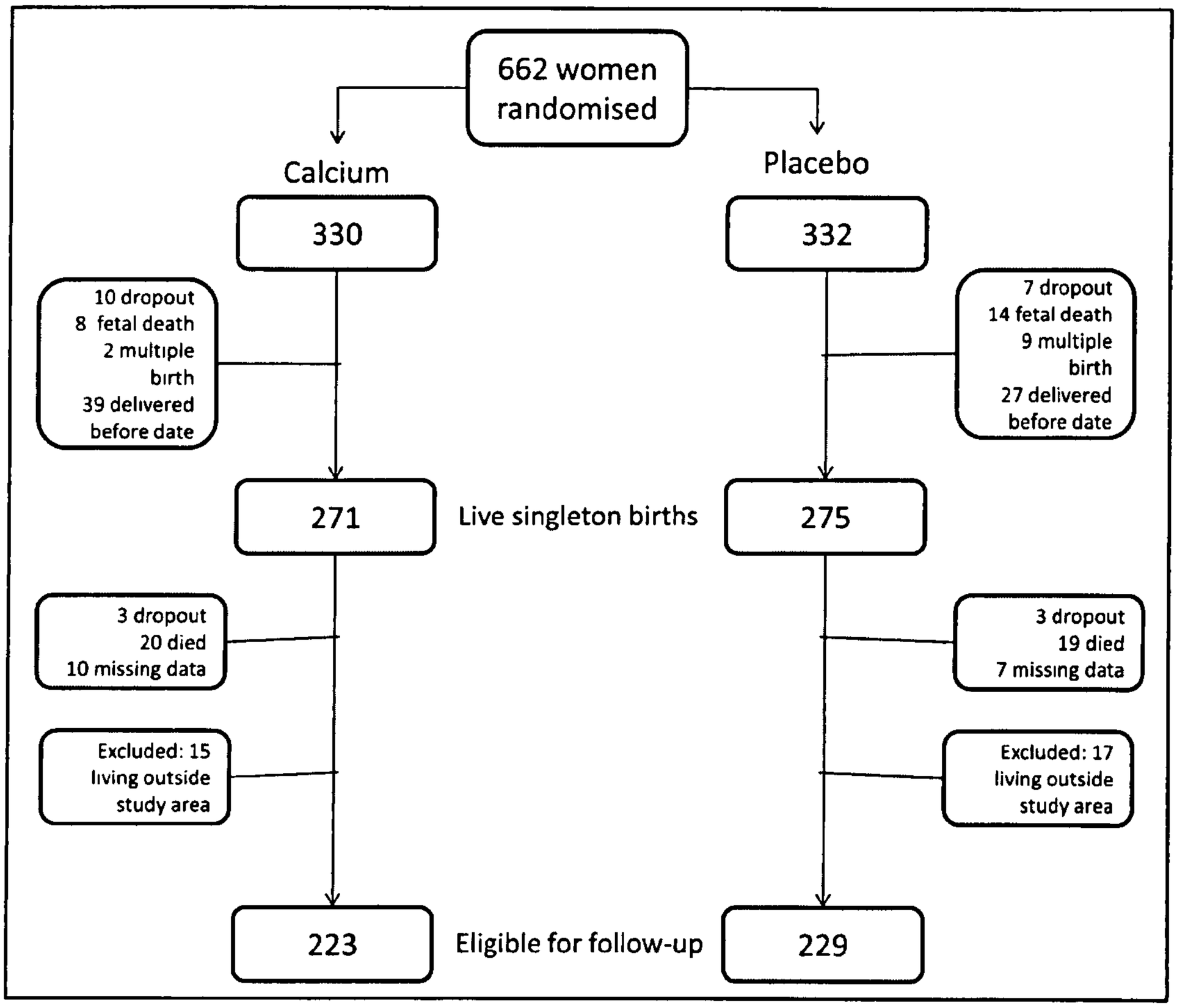
The EU-funded follow-up study was conducted between November 2005 and August 2006, and the same protocol was used for the offspring of both trials. Children born to women who had participated in the protein-energy trial were 11-17 years old at follow-up, whilst those born to women who had participated in the calcium trial were 5-10 years old. For logistical reasons, recruitment was restricted to individuals who were still resident in West Kiang and to those who had moved to urban/peri-urban areas at the coast, provided they were within one hour drive from the MRC Laboratories. From the protein-energy trial, data were missing for 44 individuals, 207 had died in the intervening years and 254 had moved outside of the study area, thus reducing the potential sample size for follow-up to 1542 (Figure 2.2). For the calcium trial, 452 children were known to be still alive and to be living within the study area and thus represented the number of children that were eligible for the follow-up (Figure 2.3).

Figure 2.2 Flow diagram of progression of offspring from protein-energy trial until time of current follow-up



Follow-up study was restricted to individuals still living in West Kiang or within an hour drive from MRC laboratories in the coastal area

Figure 2.3 Flow diagram of progression of offspring from calcium trial until time of current follow-up



Follow-up study was restricted to individuals still living in West Kiang or within an hour drive from MRC laboratories in the coastal area

Scientific approval for the study was granted by MRC The Gambia Scientific Coordinating Committee (SCC), and ethical permission was granted by the joint Gambian Government and MRC Ethical Committee as well as the London School of Hygiene and Tropical Medicine Ethics Committee. Full informed consent was obtained from the parents or guardians of the children and community support was sought through meetings with village elders and school head teachers.

2.4.2 Study protocol

In West Kiang, measurements were conducted in the subject's village of residence as far as this was possible, to minimise disruption to their day. At the coastal areas, local clinics were used as study sites. All fieldworkers were trained and were unaware of which treatment group the child's mother had belonged to. Subjects were asked to attend the study visit early in the morning (7am) in a fasted state and were provided with breakfast once all of the measurements had been obtained.

Data collected in the field were recorded onto paper copies of subject-specific data recording forms, which contained the subject's unique identifier details such as date of birth and maternal and paternal names. These details were used to check that the correct subject was recruited into the study and to ensure that the measurements were recorded on the correct form. Data was double-entered by data entry clerks at MRC Keneba within a few days of collection and any inconsistencies were checked in the field.

2.4.3 Measurements

2.4.3.1 Blood pressure

Blood pressure was measured in triplicate using the automated Omron 705IT oscillometric device (Morton Medical Ltd, London, United Kingdom) and following the manufacturer's instructions. Children were seated at rest for five minutes before the initial measurement was taken and for two minutes between each subsequent measurement. The appropriate cuff size was chosen from

measurement of the mid-arm circumference and then was placed on the upper right arm with the arm resting on a flat table at chest height. In an effort to improve the accuracy of the readings, fieldworkers were asked to continue taking measurements until three were within 5mmHg of each other, a protocol amended from recommendations of the American Heart Association [206]. Children with unusually high blood pressure readings, as defined by the United States National Heart, Lung and Blood Institute guidelines [12], were seen again on a different day and referred to a physician if readings remained high. If the blood pressure readings on the second visit were within the normal range, these new values were entered into the database and used in the analysis.

2.4.3.2 Anthropometry and body composition

Height, weight, mid-upper arm circumference (MUAC) and triceps skinfold thickness were measured by a single fieldworker in the majority (99%) of cases to minimise observer bias. Standard techniques were used for each measurement. Weight was measured to the nearest 0.1kg using daily calibrated, digital scales (Tanita digital scales: Chasmors Ltd, London, United Kingdom) whilst height was measured using a daily calibrated stadiometer (Seca Leicester stadiometer: Chasmors Ltd) to the nearest 0.1cm. Triceps skinfold thickness was measured in triplicate on the left arm using Holtain callipers (Chasmors Ltd) and the mean reading to the nearest 0.2mm was recorded. MUAC was measured on the left arm to the nearest 0.1cm using a waxed measuring tape (Teaching Aids at Low Cost (TALC): Hertfordshire, UK) whilst the subject's arm was hanging by their side.

Body composition was assessed by bioelectrical impedance analysis (BIA) using the Tanita BC-418MA (Chasmors Ltd) segmental analyser; measurements were conducted after an overnight fast and at a similar time point each day (between 8 and 10am). This analyser uses eight electrodes to measure the impedance (z) to an alternating electric current as it passes through the body and inbuilt prediction equations convert this into an estimate of body fat. These equations are based on Caucasian populations, and in a validation study they were found to not be accurate

for this population [207]. Percentage fat-free mass (%FFM) was therefore predicted from a population-specific equation that had been derived previously in this population using deuterium oxide dilution as a reference method [207].

Prediction equation:

%FFM = exp(7.659 + 0.709*ln(ht) – 0.311*ln(z) – 0.402*ln(wt) – 0.044*ln(triceps) + 0.024*sex + 0.007*age) where z is impedance (Ω), ht is height (m), wt is weight (kg) and triceps is triceps skinfold thickness (mm).

2.5 Statistical analysis

All statistical analysis was conducted using Stata 10 (Stata Corporation, College Station, Texas, USA). The analysis initially focused on the effect of the intervention (intention-to-treat followed by an as-treated analysis) before going on to explore some of the relevant observational data relating early exposures to later blood pressure. Due to the different nature of the two original trials the statistical analysis will be described separately for each, in the sections below.

2.5.1 Data manipulation, both trials

Outcome variables and continuous covariates were assessed for the normality of their distribution using histograms and quantile-quantile plots. For the primary outcome measure the mean of three measurements was taken as the estimate of systolic and diastolic blood pressure. Pulse pressure was defined as systolic – diastolic pressure, whilst mean arterial pressure (MAP) was defined as diastolic + (1/3 * pulse pressure).

Body mass index (BMI) was calculated as weight(kg)/height(m)^2. Percentage fat-free mass (%FFM) was derived from bioelectrical impedance analysis, using the population specific equation quoted previously (Section 2.4.3.2), and was used to calculate percentage fat mass (%FM=100-%FFM). Impedance data were missing for nine subjects from the protein-energy trial and six subjects from the calcium trial due to a short circuit in the analyser, which was fixed at a later date. Log-log regression analysis, described by Wells *et al* [208], was used to assess the relationship of FM and FFM with height (Ht). FM/Ht and FFM/Ht indices that were uncorrelated with height were generated by using the value of the slope parameter from the regression equation (Table 2.4). The relationship between fat and lean tissues and height was different for individuals whose mothers had participated in the two trials, reflecting the different age of the two groups of offspring. It was not possible to create prediction equations for the segmental impedance readings and therefore the Tanita system's inbuilt prediction equations were used for percentage trunk fat, which is reported as an indication of central fat distribution.

Table 2.4 Body composition indices created for analysis of offspring of the maternal protein-energy supplementation and calcium supplementation trials

Trial	Body composition estimate	Calculation[a]
Protein-energy	Fat-mass index (FMI)[b]	$FM/Ht^{4.2}$
	Lean mass index (LMI)[c]	$FFM/Ht^{2.8}$
Calcium	FMI	$FM/Ht^{1.6}$
	LMI	$FFM/Ht^{2.3}$

[a]Denotes the equation used to generate each index
[b]Index of fat mass independent of height
[c]Index of lean mass independent of height

The association between body composition and blood pressure was assessed by linear regression analysis with models adjusted for age and sex. The relative contribution of fat and lean mass was also assessed by fitting a simultaneous model containing both FMI and LMI.

2.5.2 Protein-energy trial analysis

The analysis of the protein-energy trial was restricted to individuals born at term by excluding those that were assessed as less than 37 weeks gestation by their Parkin score [209]. Independent t-tests were used to assess any differences in characteristics from the original trial between individuals who were recruited and those lost to follow-up.

2.5.2.1 Intention-to-treat analysis

An intention-to-treat analysis was used to assess the effect of maternal protein-energy supplementation (during pregnancy *vs* during lactation) on offspring blood pressure. Generalised estimating equations [210] were used to take the cluster design of the original trial into account in the analysis. Variables that were potential correlates of blood pressure were assessed by simple linear regression and those statistically associated with the outcome were included in the adjusted regression models. There were three different stages of regression models used to investigate

the impact of the maternal intervention: unadjusted (model 1), adjusted for covariates unrelated to the maternal intervention but related to blood pressure (model 2), as model 2 but additionally adjusted for height, FMI and LMI (as continuous variables) (model 3).

Model 2 was first conducted as a simple main effects model, and then any interaction of the intervention effect with age, sex, location, season of birth (defined as Fourier terms [211], see Section 2.5.2.4) and current body composition was assessed by fitting interaction terms in a sequential manner.

Children were defined as having high blood pressure if their systolic blood pressure was above the 95th centile for their age and height defined by NHBPEP reference values [12]. The impact of the maternal intervention on the odds of having high blood pressure was assessed using generalised estimating equations fitted with a binomial family.

2.5.2.2 As-treated analysis

Data on the number of biscuits consumed was available for women in the intervention arm of the protein-energy trial but was not available for women in the control arm. Women in the intervention arm were divided into those who consumed more than the median number of biscuits and those who consumed less. An as-treated analysis was then conducted comparing blood pressure in these two groups of offspring with the offspring of control women; generalised-estimated equations were used, adjusted for the same covariates as before.

2.5.2.3 Size at birth and infant growth analysis

The association between birth weight and later blood pressure was assessed using linear regression. A number of different models were fitted: firstly the unadjusted association, then adjusting for confounders (sex, age and season of birth), the final model was also adjusted for current weight. A similar analysis was conducted using

birth length as the explanatory variable and fitting height instead of weight in the final model.

For a proportion of the subjects, weight data were available for 3, 6, 9, 12 and 36 months of age. It had not been possible to recruit the entire cohort during these studies and some individuals were present at one time point but not others. As a result, records were available for 80% of the current follow-up subjects at 3 months of age, falling to 71-73% between 6 and 12 months and to 43% at 36 months. Small proportions of length data were also available from the 12 (45%) and 36 (18%) month time points. Within-population standard deviation scores (SD score: value – mean / standard deviation) were calculated for these age points as well as for the birth anthropometric variables. Magnitude of growth was defined by the change in weight SDS from birth to 3 months, 3 months to 6 months and so on. The impact of early growth on later blood pressure was assessed using linear regression analysis adjusted for confounders (age, sex and season of birth). In addition, the growth data were visualised by using life-course plots suggested by Cole [121]. For these plots the regression coefficient for the association between weight SD score at different time points and final blood pressure are plotted against age to give an indication of the shape of the relationship [121].

2.5.2.4 Season of birth analysis

A further analysis was conducted to investigate the effect of season of birth on blood pressure in adolescence. Season of birth was defined by fitting Fourier terms (sin, cos, $\sin^2$, $\cos^2$) which are smooth linear functions whose terms are approximately orthogonal to one another and inherently cyclic, making them ideal models for seasonality [211]. The impact of season of birth on blood pressure was investigated using likelihood ratio tests to assess whether a linear regression model containing season of birth terms was a better fit than a simple model containing only factors relating to current blood pressure (age, sex, height, FMI and LMI).

2.5.3 Calcium trial analysis

The analysis of the calcium trial was also restricted to infants judged to be born at term, this time by excluding those who had been assessed as under 37 weeks gestation by their Duboviz score [212] at delivery. Independent t-tests were used to assess any differences in characteristics from the original trial between those recruited and those lost to follow-up. Again for the primary outcome measure, the mean of three measurements was taken as the estimate of systolic and diastolic blood pressure, pulse pressure was defined as systolic – diastolic pressure, and mean arterial pressure (MAP) was defined as diastolic + (1/3 * pulse pressure).

2.5.3.1 Intention-to-treat analysis

An intention-to-treat analysis using linear regression was used to assess the effect of supplementation during pregnancy (calcium *vs* placebo) on blood pressure in the offspring. Variables that were potential correlates of blood pressure were assessed by simple linear regression and those that were associated were included in the adjusted regression models. There were four different stages of regression models used to investigate the impact of the maternal intervention: unadjusted (model 1), adjusted for covariates unrelated to the maternal intervention but related to blood pressure (model 2), as model 2 but additionally adjusted for height, FMI and LMI (model 3), as model 3 but additionally adjusted for maternal compliance (defined as number of observed tablets consumed/number of expected tablets) and length of time spent in the supplementation trial (model 4).

Model 2 was first conducted as a simple main effects model, then any interaction of the intervention effect with age, sex, maternal baseline blood pressure, maternal baseline BMI and child body composition were assessed by fitting interaction terms in a sequential manner.

Children were defined as having high blood pressure if their systolic blood pressure was above the 95th centile for their age and height [12]. The impact of the intervention on the odds of having high blood pressure was assessed using logistic regression adjusted for the same covariates as above.

2.5.3.2 As-treated analysis

An as-treated analysis was conducted relating the number of tablets a woman consumed during pregnancy to her offspring's blood pressure in childhood. The total number of tablets consumed was affected by the amount of time a woman spent in the study and her compliance with treatment. Compliance was defined as the number of observed tablets consumed/number expected over the study period. The as-treated analysis was controlled for the effects of compliance and of length of time in the study (their interaction was also fitted but the term was dropped as it was not significant). The calcium dose consumed by women in the original trial was regarded as the interaction between compliance, time in study and treatment group (calcium *vs* placebo). The association between calcium dose and offspring blood pressure was assessed using linear regression analysis. Different models were fitted: unadjusted (model 1), adjusted for covariates unrelated to the maternal intervention but related to blood pressure (model 2), as model 2 but additionally adjusted for height, FMI and LMI (model 3).

2.5.3.3 Size at birth and infant growth analysis

Weight (and length) at two weeks of age (adjusted for the actual age at measurement) was used as a proxy measure of birth weight, because measurements at this age were more complete and were deemed to be of greater accuracy. The association between weight at two weeks and later blood pressure was assessed using linear regression analysis. A number of different models were fitted: firstly a simple unadjusted analysis, then adjusted for confounders (age, sex, maternal weight and maternal blood pressure), the final model was also adjusted for current weight. A similar analysis was conducted using length at two weeks as the explanatory variable and fitting height rather than weight in the final model.

Anthropometric data were also available from 52 weeks of age, these data were adjusted for the actual date of measurement and then within-population standard deviation scores (SD score: value – mean / standard deviation) were calculated for the 2 week and 52 week timepoints. Growth was defined by the change in weight SDS from 2 to 52 weeks and the impact of this early growth on later blood pressure

was assessed using linear regression analysis adjusted for confounders (age, sex, maternal weight and maternal blood pressure). In addition, the growth data were visualised by using life course plots [121].

2.5.3.4 Season of birth analysis

A further analysis was conducted to investigate the effect of season of birth on blood pressure in childhood. Season of birth was defined by fitting Fourier terms as before [211]. Linear regression models were fitted with factors relating to current blood pressure (age, sex, height, FMI and LMI) and this model was then compared, using a likelihood ratio test, to one also containing season of birth terms.

Chapter 3 Follow-up of protein-energy supplementation trial

3.1 Forward

This chapter will describe in detail the results of the statistical analysis investigating the impact of maternal protein-energy supplementation on offspring blood pressure at 11-17 years of age in The Gambia. The main findings have already been published [213], although this chapter will display and explore the data in greater detail. A review of the statistical methods has been covered in Chapter 2 (Section 2.5) and should be referred to when interpreting the analysis. Regression coefficients appear as β in the text, standard deviations are denoted by SD, the chi-squared test statistic by χ^2 and confidence intervals by CI.

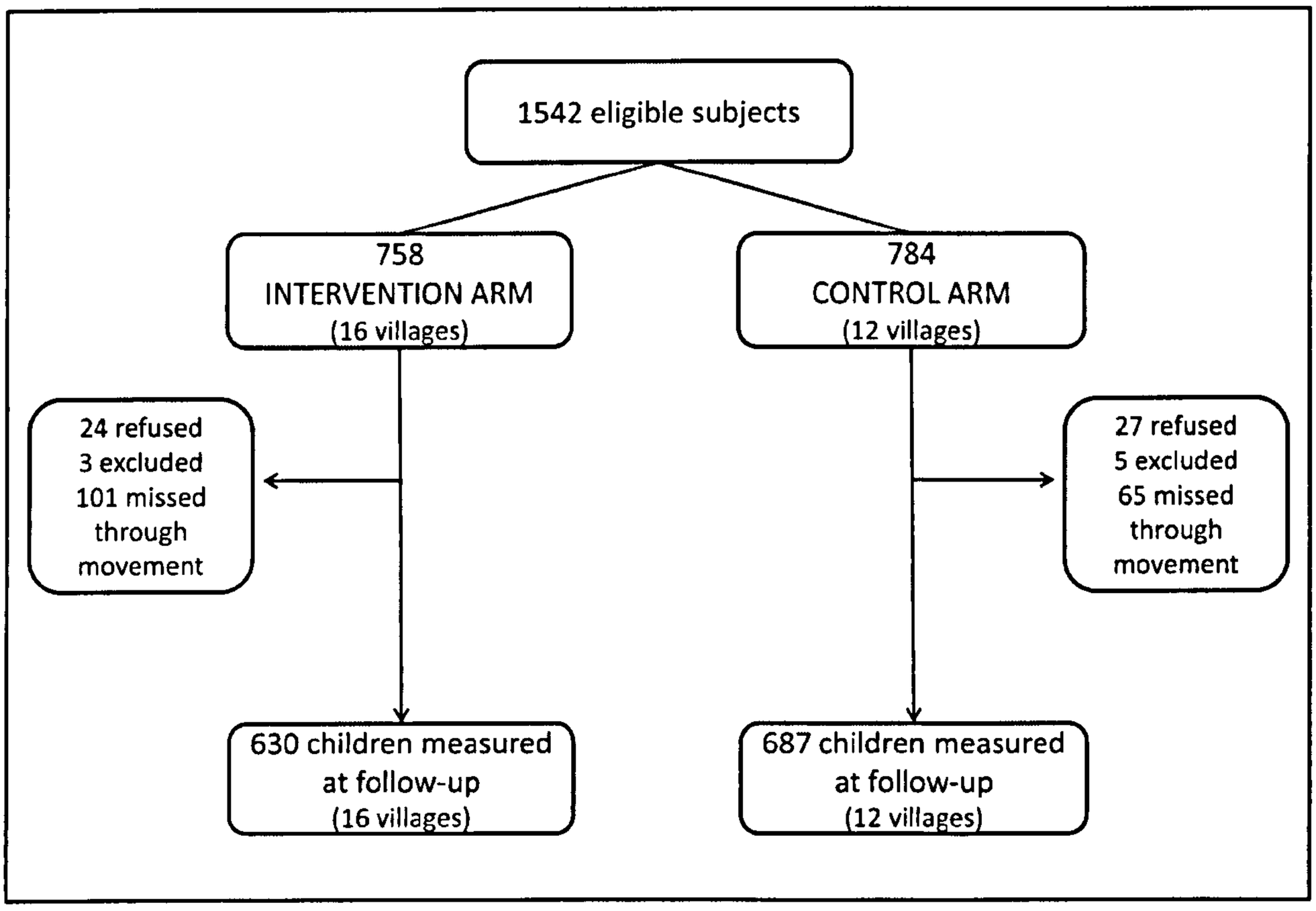
3.2 Study participants

3.2.1 General characteristics

The follow-up study recruited 1317 children, representing 64% of individuals born during the original trial and 85% of those who were eligible to participate in the follow-up (Figure 3.1). Eligible subjects were offspring still living in West Kiang or those who had moved to coastal areas that were within an hour drive from MRC facilities (see Section 2.4.1). Recruitment rates were similar between the two arms of the trial: 630 children from the intervention arm (62% of original births) and 687 children from the control arm (66%) were recruited. The main reason for non-recruitment (13% of intervention subjects and 8% of control subjects) was due to family movement within The Gambia, resulting in eligible subjects being unavailable during the study period. Eight subjects were excluded from the study: six due to a reported mental illness, one who was handicapped from polio and one who had suffered a trauma to the leg. Three percent of parents refused to give consent for

their children to take part, primarily because they were not happy for their children to give blood.

Figure 3.1 Flow diagram of offspring eligible for protein-energy trial follow-up and those recruited



There were no differences in the available characteristics between those recruited and those lost to follow-up, with the exception that recruited children were on average almost three months younger (Table 3.1).

Table 3.1 Differences between individuals recruited into protein-energy trial follow-up and those lost to follow-up[a]

	Lost to follow-up		Recruited		P-value[c]
	N[b]	Mean (SD) or percentage	N	Mean (SD) or percentage	
Age at start of follow-up (y)	686	14.0 (1.5)	1317	13.8 (1.5)	0.001
% male	686	49.3	1317	52.1	0.23
Birth weight (g)	686	2921 (415)	1317	2896 (436)	0.21
Birth length (cm)	575	49.4 (2.1)	1179	49.4 (2.3)	0.72
Maternal weight (kg)	685	53.2 (7.1)	1315	53.0 (7.0)	0.52
Maternal height (m)	524	1.6 (0.1)	1060	1.6 (0.1)	0.21
% term infants	568	97.4	1168	96.1	0.17
% primiparous mothers	637	11.8	1257	12.3	0.76

[a]Lost to follow-up defined as all live, singleton children born into the original trial who were not recruited into follow-up (ie: includes those ineligible for current follow-up)
[b]data were missing on 44 children from the original study
[c]P-value refers to t-tests for continuous data or chi-squared tests for categorical data

Forty-six individuals (19 intervention, 27 control) with a gestational age less than 37 weeks (preterm) were excluded from the analysis. Three further individuals had implausible blood pressure readings, which were removed from the analysis: two of the blood pressure readings were more than four standard deviations from the mean and one individual had identical systolic and diastolic readings, which were interpreted as a recording error. One further individual was excluded because they had ambiguous treatment allocation, resulting in a sample size of 1267 that was used as the basis for all of the following analyses.

Of this sample, 48% were girls and 22% were living in urban/semi-urban coastal areas at the time of the study. Urban subjects were on average older than their rural counterparts (mean difference: 0.7y; 95%CI: 0.5, 0.9; p<0.001), which is to be expected as children move to the urban areas to attend senior school. A greater proportion of the urban subjects were girls, 57% compared to 45% in the rural area (χ^2 test: 12.05; P: 0.001), which may reflect that they were also moving to the urban

areas for marriage. A greater proportion of urban subjects belonged to the intervention (55%) rather than control (46%) arm of the original trial (χ^2 test: 7.07; P: 0.01).

3.2.2 Anthropometry, body composition and age of menarche

Table 3.2 displays the anthropometric and body composition data for the analysed sample. Mean height for girls was 152.3cm (SD: 9.3) and the mean height-for-age Z-score (HAZ), compared to UK reference data [214], was -0.92 (SD: 1.03). Mean height for boys was 150.4cm (SD: 10.8) and the mean HAZ was -1.47 (SD: 1.10). A relatively high proportion (24%) of the children was classified as stunted, defined as a HAZ ≤-2 Z-scores of the reference population. At the other end of the spectrum, mean BMI for girls was 17.5kg/m^2 (SD: 2.8) and for boys was 16.2kg/m^2 (SD: 1.8). Using International Obesity Taskforce cut-offs for childhood BMI [215], 2.6% of girls and 0.3% of boys would be classified as overweight. All anthropometric and body composition variables increased with age, with the exception of percent trunk fat which was unrelated to the age of the individual (data not shown).

Table 3.2 Anthropometry and body composition of offspring born during Gambian protein-energy supplementation trial

	Mean (SD)	
	Girls	Boys
N	608	659
Height (cm)	152 (9)	150 (11)
Weight (kg)	41.2 (10.1)	37.2 (8.5)
Height-for-age Z-score[a]	-0.9 (1.0)	-1.5 (1.1)
BMI (kg/m^2)	17.5 (2.8)	16.2 (1.8)
BMI Z-score[a]	-1.0 (1.2)	-1.5 (1.0)
Body fat (%)	19.4 (4.5)[#n-2]	12.6 (2.9)[#n-7]
Trunk fat (%)	15.0 (4.8)[#n-4]	12.0 (2.9)[#n-7]
Fat mass index (FMI) (kg/m$^{4.2}$)	1.4 (0.4)[#n-2]	0.8 (0.3)[#n-7]
Lean mass index (LMI) (kg/m$^{2.8}$)	10.0 (1.0)[#n-2]	10.2 (0.8)[#n-7]

[a]Compared to UK reference data[214]

[#n]Denotes sample size which differs from the column header due to missing data

3.2.3 Blood pressure

Mean systolic blood pressure is shown in Table 3.3. The variance in blood pressure was similar for boys and for girls and the analysis was therefore conducted for the sexes combined, although adjusted for sex where appropriate. Blood pressure was normally distributed as assessed by quantile-quantile plots and the Shapiro-Wilk test for normal data (systolic P: 0.002; diastolic P: <0.001)

Table 3.3 Blood pressure of subjects recruited into protein-energy trial follow-up

	Mean (SD)	
	Girls	Boys
N	608	659
Systolic (mmHg)	111.1 (8.8)	109.9 (9.2)
Diastolic (mmHg)	65.5 (7.2)	63.9 (8.0)
Pulse pressure (mmHg)[a]	45.5 (6.5)	46.0 (7.2)
Mean arterial pressure (mmHg)[b]	80.7 (7.1)	79.2 (7.7)
Hypertension (%)[c]	6.4	4.6

[a]Calculated as systolic – diastolic blood pressure

[b]Mean arterial pressure calculated as diastolic + (1/3*pulse pressure)

[c]Hypertension defined as systolic blood pressure above the NHBPEP 95% centile for height and age [12]

In simple linear regression analysis, offspring systolic and diastolic blood pressures were unrelated to maternal height or parity (data not shown). Systolic blood pressure increased with child age (β: 0.9mmHg; 95% CI: 0.5, 1.2; P: <0.001) and was higher for girls compared to boys (mean difference: 1.1mmHg; 95% CI: 0.1, 2.1; P: 0.03). Diastolic blood pressure was also higher in girls (mean difference: 1.58mmHg; 95% CI: -2.42, -0.74; P: <0.001) but there was no association with age (β: 0.18mmHg; 95% CI: -0.10, 0.46; P: 0.21). The location of the individual at follow-up (rural *vs* urban) was not associated with systolic or diastolic blood pressure (data not shown). Variables included in the adjusted models were therefore restricted to age, sex and season of birth. In addition, the location of the individual (rural *vs* urban) was included as a covariate because this variable was associated with the intervention (see Section 3.2.1).

Both systolic and diastolic blood pressures were positively associated with height, weight, and BMI (Table 3.4). Systolic blood pressure was positively associated with LMI, but not with FMI or percent trunk fat. In simultaneous models including both LMI and FMI this association remained; the association with LMI was 0.95mmHg (95% CI: 0.24, 1.66; P: 0.01) and with FMI was -0.74mmHg (95% CI: -2.41, 0.93; P: 0.38). In contrast, diastolic blood pressure was positively associated with FMI, percent body and trunk fat but not associated with LMI. Again this association remained in simultaneous analysis (data not shown). BMI and height in this group of children are highly correlated (Pearson correlation coefficient: 0.5; P: <0.001) and BMI is therefore a poor index of relative weight (independent of height) in these individuals.

Table 3.4 Association between blood pressure and anthropometry/body composition for individuals in protein-energy trial follow-up

	Systolic blood pressure (mmHg) Regression coefficient (95% CI)	P-value	Diastolic blood pressure (mmHg) Regression coefficient (95% CI)	P-value
Height (cm)	0.29 (0.22, 0.35)	<0.001	0.14 (0.09, 0.20)	<0.001
Weight (kg)	0.30 (0.23, 0.37)	<0.001	0.19 (0.14, 0.25)	<0.001
BMI (kg/m^2)	0.68 (0.44, 0.92)	<0.001	0.57 (0.36, 0.77)	<0.001
Body fat (%)	0.13 (-0.01, 0.26)	0.06	0.36 (0.25, 0.47)	<0.001
FMI ($kg/m^{4.2}$)	0.67 (-0.57, 1.90)	0.29	2.83 (1.69, 3.97)	<0.001
LMI ($kg/m^{2.8}$)	0.76 (0.19, 1.34)	0.01	0.35 (-0.14, 0.84)	0.16
Trunk fat (%)	0.07 (-0.05, 0.20)	0.25	0.27 (0.16, 0.38)	<0.001

Results are the effect on blood pressure of a one unit increase in explanatory variable, derived from linear regression analysis, adjusted for age and sex

3.3 Maternal protein-energy supplementation and offspring blood pressure

3.3.1 Results of intention-to-treat analysis

There was no effect of prenatal compared to postnatal maternal protein-energy supplementation on offspring systolic, diastolic or mean arterial blood pressure in adolescence (Table 3.5). There was a suggestion that pulse pressure was related to maternal supplementation, with higher pulse pressure for individuals whose mothers received the intervention during pregnancy compared to individuals born to control mothers, although the association was borderline in all but the final model (Table 3.5).

Table 3.5 Effect of maternal protein-energy supplementation during pregnancy on offspring blood pressure at 11-17 years in The Gambia: intention-to-treat analysis

	Model 1[a] (95% CI)	P-value	Model 2[b] (95% CI)	P-value	Model 3[c] (95% CI)	P-value
Systolic pressure (mmHg)	0.46 (-1.12, 2.04)	0.57	0.47 (-1.14, 2.08)	0.57	0.59 (-0.94, 2.12)	0.45
Diastolic pressure (mmHg)	0.09 (-1.31, 1.13)	0.89	-0.01 (-1.24, 1.23)	0.99	-0.02 (-1.19, 1.15)	0.97
Pulse pressure (mmHg)	0.69 (-0.03, 1.41)	0.06	0.64 (-0.06, 1.33)	0.07	0.71 (0.10, 1.33)	0.02
Mean arterial pressure (mmHg)	0.10 (-1.20, 1.40)	0.88	0.16 (-1.16, 1.48)	0.81	0.19 (-1.07, 1.46)	0.77

Results are the difference in mean blood pressure (95% CI) for individuals born to women receiving protein-energy supplements during pregnancy (coded 1) compared to during lactation (coded 0), derived from generalised estimating equations (gee) with original village of residence (cluster) modelled as a random effect

[a]Model 1 = unadjusted

[b]Model 2 = adjusted for age, sex, rural or urban location and season of birth

[c]Model 3 = as model 2 but additionally adjusted for height, fat mass index (FMI) and lean mass index (LMI)

Only 5% of individuals had a systolic blood pressure above the NHBPEP 95% centile for their height and age [12]. There was no difference in the proportion of individuals with high systolic blood pressure in the intervention or control groups (adjusted odds ratio: 1.19; 95% CI: 0.73, 1.95; P: 0.48).

A potential source of bias in the results is the method chosen to measure blood pressure: using three measurements of blood pressure that were within 5mmHg of each other rather than taking the first three measurements. From the study records it was possible to compare the chosen method with the results that would have been obtained if only the first three blood pressure measurements had been taken. Using the first three measurements only produced a higher estimate of mean systolic (mean difference between the two methods: 0.72mmHg; 95% CI: 0.58, 0.86; P: <0.001) and diastolic (mean difference: 0.35mmHg; 95% CI: 0.24, 0.46; P: <0.001) blood pressure than the method chosen in this study. However, there was no difference in the conclusions drawn from the analysis of the effect of the maternal intervention on offspring blood pressure (Table 3.6). Therefore in all subsequent analysis the original protocol has been used to generate an estimate of blood pressure (ie: the mean of three blood pressure measurements which were within 5mmHg). It was deemed unnecessary to repeat this extra analysis for the calcium intervention reported in Chapter 4.

Table 3.6 Effect of maternal protein-energy supplementation on offspring blood pressure when using the first three measurements as an estimate of blood pressure

	Unadjusted regression coefficient (95% CI)	P-value
Systolic BP (mmHg)	0.42 (-1.27, 2.11)	0.63
Diastolic BP (mmHg)	0.09 (-1.18, 1.37)	0.89
Pulse pressure (mmHg)	0.63 (0.11, 1.15)	0.02
Mean arterial pressure (mmHg)	-0.03 (-1.40, 1.34)	0.97

Results are the difference in mean blood pressure (95% CI) for individuals born to women receiving protein-energy supplements during pregnancy (coded 1) compared to during lactation (coded 0), derived from unadjusted generalised estimating equations (gee) with original village of residence (cluster) modelled as a random effect

Another potential source of error is the incorrect inclusion of preterm individuals due to the fact that Parkin scores were missing for 148 individuals (70 intervention, 78 control), making it impossible to classify their gestational age. However, conducting the analysis of the effect of maternal supplementation on offspring blood pressure on only those individuals for whom Parkin scores were available does not alter the conclusions of the analysis, with the exception that any effect on pulse pressure is no longer apparent (Table 3.7).

Table 3.7 Effect of maternal protein-energy supplementation on offspring blood pressure for individuals with known gestational age (n=1119)

	Unadjusted regression coefficient (95% CI)	P-value
Systolic BP (mmHg)	0.44 (-1.20, 2.08)	0.60
Diastolic BP (mmHg)	-0.10 (-1.31, 1.10)	0.87
Pulse pressure (mmHg)	0.70 (-0.17, 1.58)	0.12
Mean arterial pressure (mmHg)	0.09 (-1.20, 1.39)	0.89

Results are the difference in mean blood pressure (95% CI) for individuals born to women receiving protein-energy supplements during pregnancy (coded 1) compared to during lactation (coded 0), derived from unadjusted generalised estimating equations (gee) with original village of residence (cluster) modelled as a random effect

The possibility of the intervention effect being modified by covariates was assessed by fitting interaction terms into the generalised estimating equations. There were no interactions between treatment group and age, sex, location (rural or urban), season of birth or any of the body composition variables (height, FMI, LMI or % trunk fat) (data not shown).

3.3.2 Results of as-treated analysis

For most of the blood pressure outcomes, there remained no association with the intervention when investigated using an as-treated analysis model (Table 3.8). There was a suggestion however, that children whose mothers had consumed less than the median number of biscuits during the trial had a higher pulse pressure compared to control children. The difference in pulse pressure between the low biscuit category and control group was 0.99 mmHg (95% CI: 0.31, 1.67; P: 0.004). This association was not reflected in the intervention children whose mothers had consumed more than the median number of biscuits. For these individuals there was no difference in blood pressure compared to control individuals (β: 0.16; 95% CI: -0.78, 1.10; P: 0.74).

Table 3.8 Effect of maternal protein-energy supplementation during pregnancy on offspring blood pressure at 11-17 years in The Gambia: as-treated analysis

	Biscuit categories[a]	Model 1 (95% CI)	P-value	Model 2 (95% CI)	P-value	Model 3 (95% CI)	P-value
Systolic BP (mmHg)	<average[b]	0.05 (-1.69, 1.80)	0.95	0.18 (-1.61, 1.96)	0.85	0.58 (-1.08, 2.25)	0.49
	>average[c]	0.31 (-1.33, 1.95)	0.71	0.37 (-1.26, 2.01)	0.66	0.31 (-1.25, 1.86)	0.70
Diastolic BP (mmHg)	<average	-0.76 (-2.18, 0.65)	0.29	-0.63 (-2.07, 0.80)	0.39	-0.43 (-1.85, 0.99)	0.55
	>average	0.32 (-0.96, 1.59)	0.63	0.38 (-0.92, 1.69	0.56	0.20 (-1.04, 1.45)	0.75
Pulse pressure (mmHg)	<average	1.00 (0.26, 1.74)	0.01	0.99 (0.31, 1.67)	0.004	1.12 (0.54, 1.70)	<0.001
	>average	0.15 (0.82, 1.13)	0.76	0.16 (-0.78, 1.10)	0.74	0.25 (-0.57, 1.07)	0.55
MAP (mmHg)	<average	-0.47 (-1.94, 1.01)	0.53	-0.34 (-1.84, 1.16)	0.66	-0.07 (-1.53, 1.40)	0.93
	>average	0.31 (-1.02, 1.65)	0.65	0.38 (-0.97, 1.72)	0.58	0.24 (-1.07, 1.55)	0.72

Results are the difference (95% CI) in mean blood pressure between individuals born to women consuming less than or more than the average number of protein-energy dense biscuits during pregnancy compared to women provided with biscuits during lactation. Regression coefficient is derived from generalised estimating equations (gee) with original village of residence (cluster) modelled as a random effect. Model 1 = unadjusted; model 2 = adjusted for age, sex, rural or urban location and season of birth; model 3 additionally adjusted for height, FMI and LMI

[a] baseline = control group

[b] Below average biscuit intervention group = 4 – 78 biscuits

[c] Above average biscuit intervention group = 79 – 194 biscuits

3.4 Early life exposures: observational analysis

3.4.1 Birth weight and later blood pressure

There was no overall association between birth weight and systolic blood pressure (adjusted for age, sex and season of birth) in this cohort (β: -0.52mmHg; 95%CI: -1.72, 0.68; P: 0.39), even after adjustment for current body weight (β: -1.13mmHg; 95%CI: -2.31, 0.04; P: 0.06). There was also no association between birth weight and diastolic blood pressure, both without (β: -0.004mmHg; 95%CI: -1.03, 1.02; P: 0.99) and with adjustment for current body weight (β: -0.40mmHg; 95%CI: -1.41, 0.62; P: 0.44). Adjusting birth weight for gestational age did not alter the lack of association between birth weight and systolic or diastolic blood pressure (data not shown).

Birth length was also unrelated to systolic (β: 0.19mmHg; 95%CI: -0.06, 0.43; P: 0.13) and diastolic (β: 0.11mmHg; 95%CI: -0.10, 0.32; P: 0.31) blood pressure. After adjustment for current height there remained no association between birth length and systolic (β: 0.06mmHg; 95%CI: -0.18, 0.30; P: 0.63) or diastolic (β: 0.05mmHg; 95%CI: -0.16, 0.26; P: 0.64) blood pressure. There was no association between birth weight or length and either pulse pressure or mean arterial pressure (data not shown).

The data presented in this analysis are derived from the follow-up of an intervention trial and are therefore not strictly a cohort study. Although maternal supplementation was unrelated to blood pressure it was associated with birth weight and the analysis was therefore also conducted for the different supplementation groups separately (Table 3.9). For individuals in the intervention arm of the trial only, birth weight was negatively associated with systolic blood pressure and pulse pressure. The association became more negative once the equations were adjusted for current weight (Table 3.9).

Table 3.9 Association between birth weight and blood pressure at 11-17 years for individuals in the different treatment arms of the original protein-energy supplementation trial

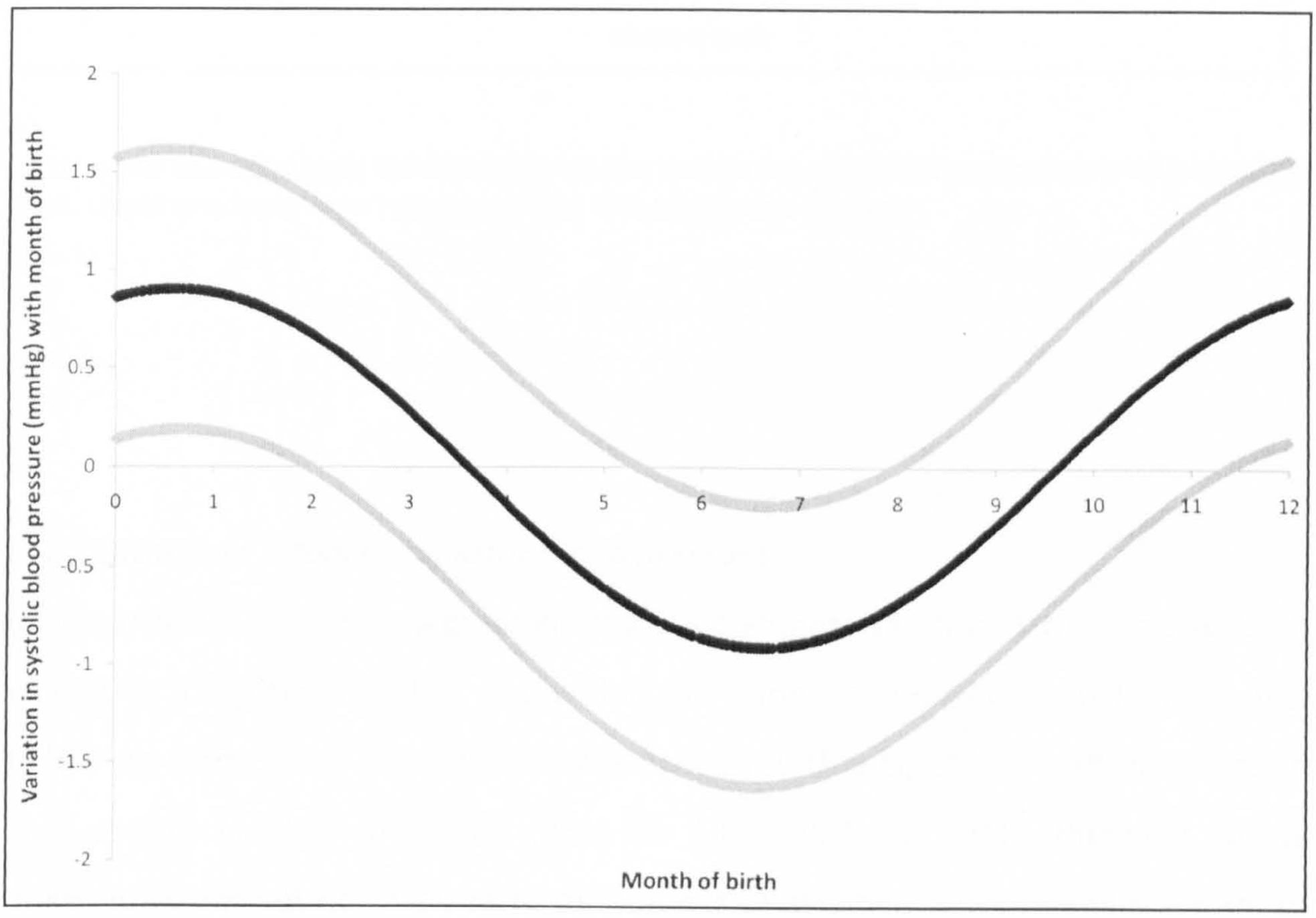
	Intervention group				Control group			
	Model 1[a]	P-value	Model 2[b]	P-value	Model 1[a]	P-value	Model 2[b]	P-value
Systolic BP (mmHg)	-2.17 (-3.91, -0.44)	0.01	-2.78 (-4.47, -1.09)	0.001	0.79 (-0.90, 2.48)	0.36	0.17 (-1.48, 1.82)	0.84
Diastolic BP (mmHg)	-0.14 (-1.66, 1.38)	0.86	-0.54 (-2.04, 0.96)	0.48	0.25 (-1.16, 1.66)	0.73	-0.14 (-1.53, 1.26)	0.85
Pulse pressure (mmHg)	-2.04 (-3.36, -0.71)	0.003	-2.24 (-3.57, -0.91)	0.001	0.54 (-0.75, 1.82)	0.41	0.31 (-0.98, 1.59)	0.64
MAP (mmHg)	-0.82 (-2.28, 0.65)	0.28	-1.29 (-2.72, 0.15)	0.08	0.43 (-0.95, 1.81)	0.54	-0.03 (-1.39, 1.32)	0.96

Results are the effect on blood pressure of a 1kg increase in birth weight, derived from linear regression analysis
[a]Model 1 = adjusted for age, sex and season of birth
[b]Model 2 = additionally adjusted for current weight

3.4.2 Season of birth and later blood pressure

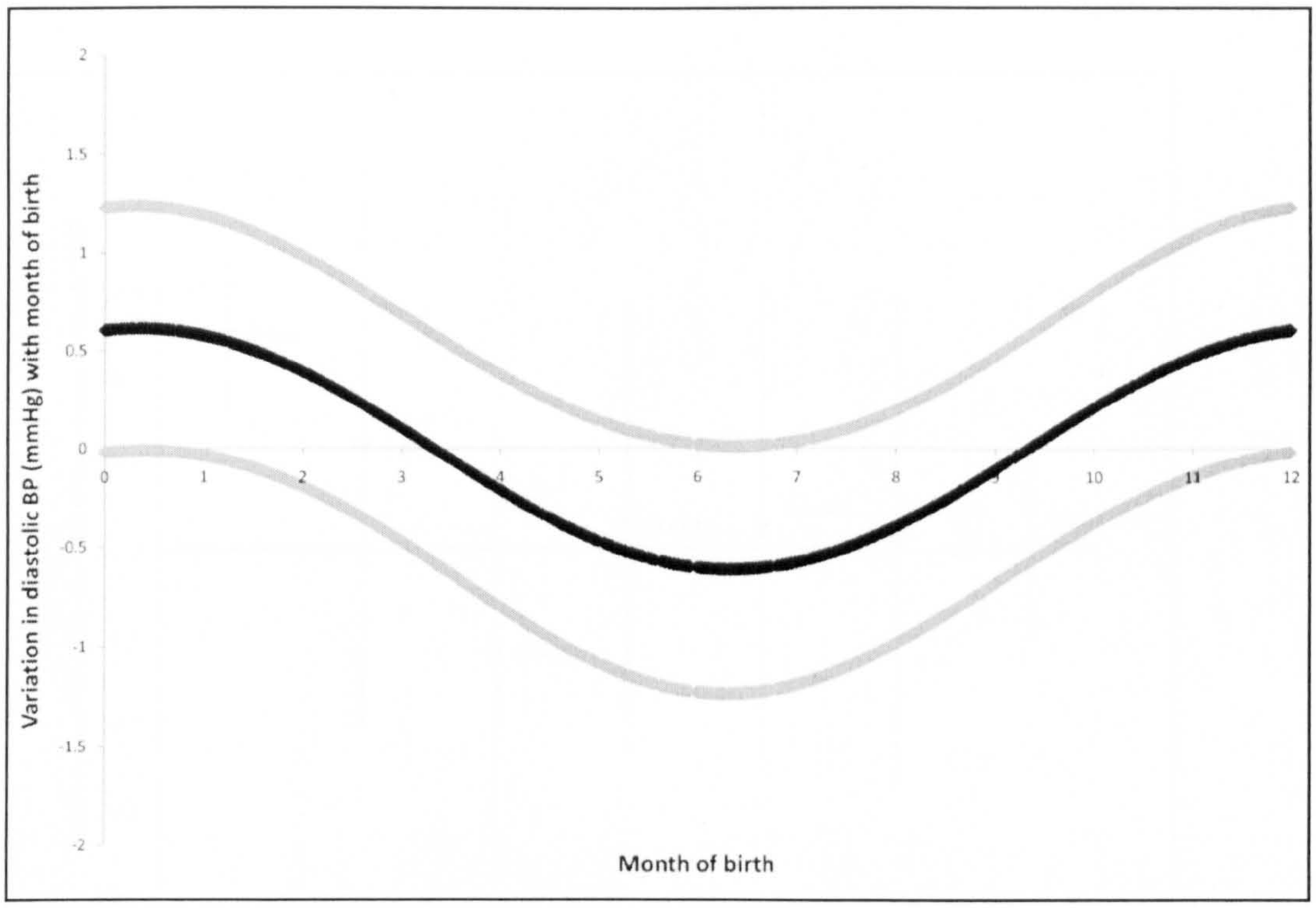
Season of birth was associated with systolic blood pressure in adolescence (Likelihood Ratio (LR) test χ^2: 6.69, P: 0.04). Individuals who had been born in June and July had lower systolic blood pressure than individuals born during the remainder of the year (Figure 3.2) after adjustment for age, sex, current height, current FMI and current LMI. There was no association between season of birth and diastolic blood pressure (LR test χ^2: 4.51; P: 0.10), although the pattern was similar (Figure 3.3). Season of birth was also unrelated to pulse pressure and mean arterial pressure (data not shown).

Figure 3.2 Variation of systolic blood pressure in Gambian adolescents aged 11-17 in relation to their month of birth



Bold middle line represents the fitted line for the variation in systolic blood pressure with month of birth. Upper and lower lines represent fitted 95% confidence interval.

Figure 3.3 Variation of diastolic blood pressure in Gambian adolescents aged 11-17 in relation to their month of birth

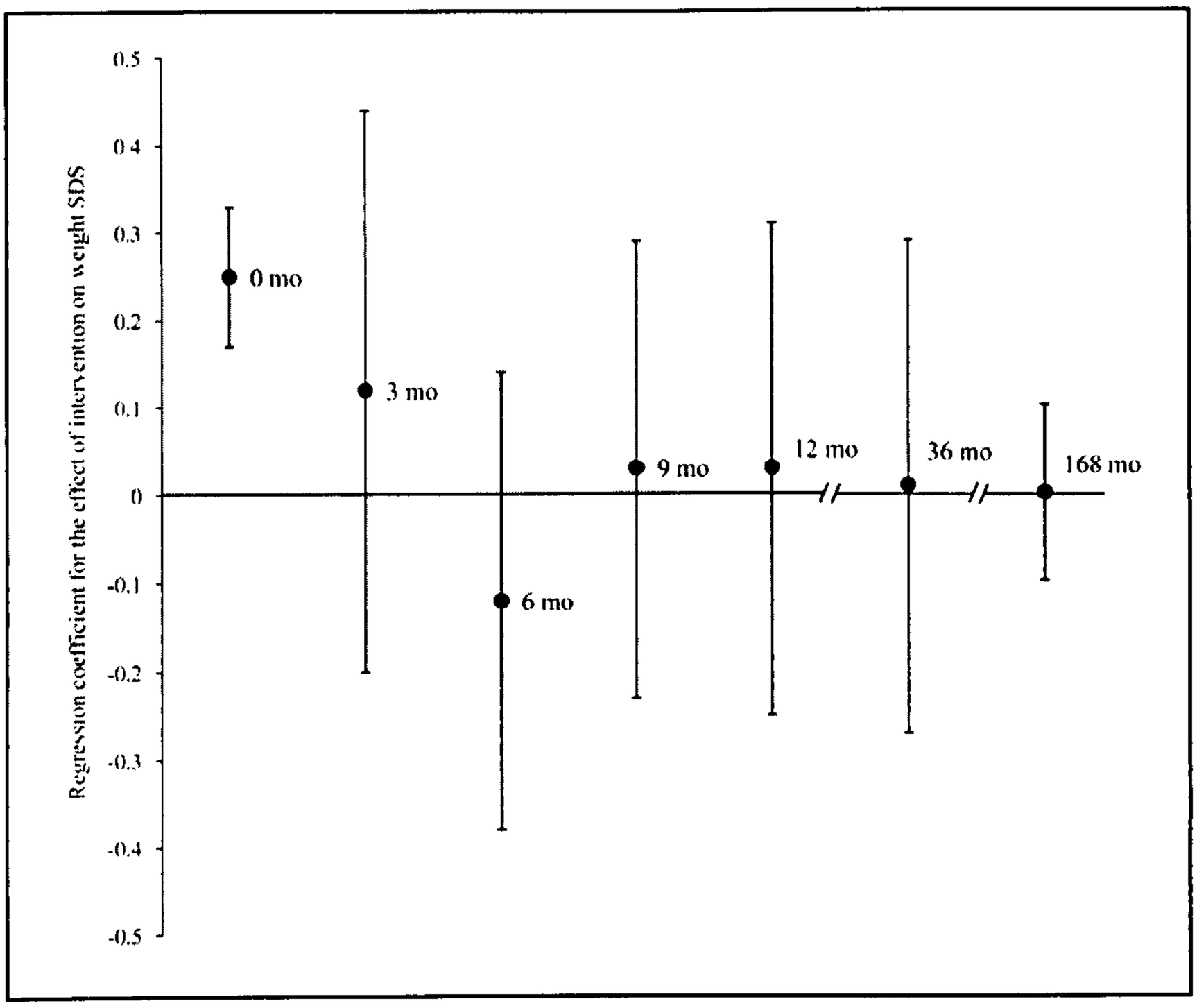


Bold middle line represents the fitted line for the variation in diastolic blood pressure with month of birth. Upper and lower lines represent fitted 95% confidence interval.

3.4.3 Growth in infancy and later blood pressure

In the original trial, the protein-energy supplement was found to increase birth weight by 136g overall [194], and within the sample of individuals included in this follow-up study there remained a difference in birth weight between intervention and control children of 97.6g (95% CI: 6.8, 188.4; P: 0.04). However, these differences did not correspond to differences in attained size in infancy for those individuals for whom there were data available (Figure 3.4) [216]

Figure 3.4 Effect of protein-energy supplementation to pregnant women on their offspring's attained size in infancy, represented by internal standard-deviation-score.



Regression coefficient and error bars (±2 x SE) are displayed for the effect of the intervention on offspring weight SDS (calculated internally) at different age points. The sample size at different age points differs (n = 2003 (birth), 840 (3mo), 870 (6mo), 848 (9mo), 874 (12mo), 527 (36mo) and 1029 (168mo)).

Growth in infancy, defined as the change in weight SD score between two time points, was unrelated to blood pressure in adolescence (Table 3.10).

Table 3.10 Relationship between growth in infancy and blood pressure at 11-17 years in Gambian adolescents

	Systolic blood pressure (mmHg)		Diastolic blood pressure (mmHg)	
Growth time period[a]	Regression coefficient (95% CI)	P-value	Regression coefficient (95% CI)	P-value
Birth – 3 months (n=1017)	0.30 (-0.17, 0.77)	0.21	0.07 (-0.33, 0.47)	0.73
3 – 6 months (n=897)	-0.07 (-0.61, 0.48)	0.82	0.06 (-0.41, 0.52)	0.82
6 – 9 months (n=911)	0.55 (-0.03, 1.12)	0.07	0.42 (-0.08, 0.93)	0.10
9 – 12 months (n=926)	0.56 (0.00, 1.12)	0.05	0.17 (-0.31, 0.65)	0.49
12 – 36 months (n=534)	-0.59 (-1.31, 0.12)	0.10	-0.27 (-0.89, 0.34)	0.38

Results are the effect on blood pressure of a one unit increase in weight standard deviation score between two time points, derived from linear regression analysis adjusted for sex, age and season of birth

[a]Exposure is change in within-sample weight standard deviation score between specified time points

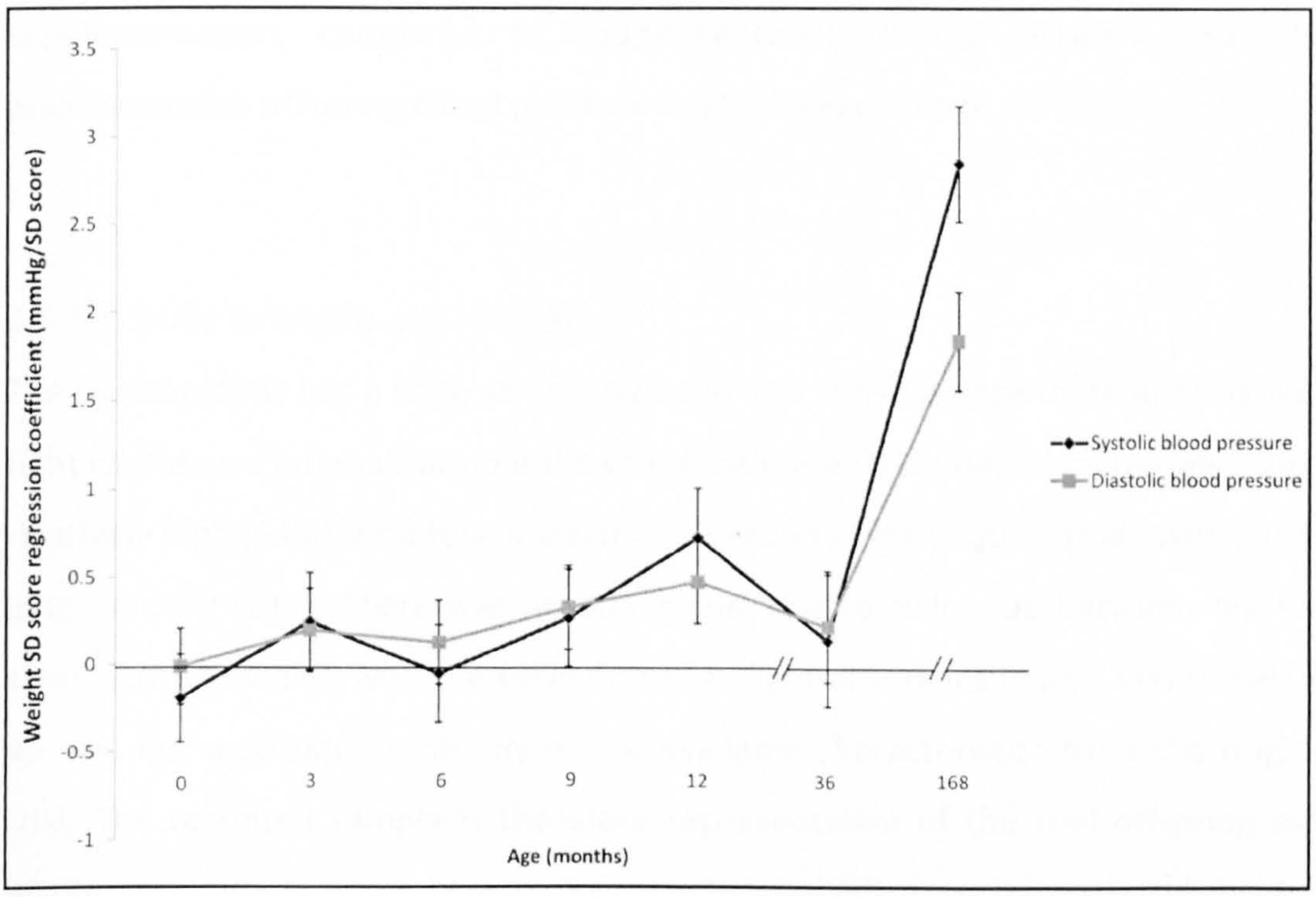
Using change in weight SD score from birth to one year as a single measure of growth over the first year, an association with blood pressure emerged; increased growth was associated with higher systolic blood pressure in adolescence after adjusting for age, sex and season of birth (β: 0.73mmHg; 95% CI: 0.27, 1.19; P: 0.002). There was a similar association for pulse pressure (β: 0.42mmHg; 95% CI: 0.06, 0.77; P: 0.02) and mean arterial pressure (β: 0.46mmHg; 95% CI: 0.07, 0.84; P: 0.02). Diastolic blood pressure however, was unrelated to change in weight SD score from birth to one year (β: 0.32mmHg; 95% CI: -0.08, 0.72; P: 0.12). Change in length SD score between birth and one year was not related to systolic (β:

0.27mmHg; 95% CI: -0.39, 0.92; P: 0.43) or diastolic (β: 0.36mmHg; 95% CI: -0.22, 0.93; P: 0.22) blood pressure in adolescence. Pulse pressure and mean arterial pressure were also not associated with change in length SD score (data not shown).

A potential source of bias in the growth data is that a number of records were missing. However, there was no difference in mean systolic blood pressure for individuals who had a weight measurement at 12 months of age and those who did not (mean difference: -1.1mmHg; 95% CI: -2.4, 0.3; P: 0.12).

Cole recommends a method for visualising the impact of growth during childhood on blood pressure in later life, termed life course plots [121]. The regression coefficient for the association between weight SD score at different time points and final blood pressure are plotted against age to give an indication of the shape of the relationship. The individual data points represent the strength of the association at a given age and the change from one age to the next represents the effect of growth [121]. For the current study, the life course plot (Figure 3.5) shows the strong association between current weight and blood pressure for both systolic and diastolic blood pressure. In infancy the association between weight and later blood pressure is less clear although there is a generally linear trend for systolic blood pressure from birth to one year, indicating that weight centile crossing at this time point has a positive effect on blood pressure in adolescence.

Figure 3.5 Life course plot of the association between growth at different ages and blood pressure in adolescence in rural Gambia



Points are regression coefficient (±2 x SE plotted as error bars) for association between weight internal SD score at different ages and blood pressure at 168 months, for systolic (black line, circle) and diastolic (grey line, square) blood pressure

3.5 Discussion

3.5.1 Maternal protein-energy supplementation

3.5.1.1 Summary of findings

These results show that in a rural area of The Gambia maternal protein-energy supplementation, compared to supplementation during lactation, was not associated with offspring blood pressure at 11-17 years of age.

3.5.1.2 Study strengths and limitations

The current study has a large sample size and as a consequence there are relatively tight confidence intervals around the effect estimate. The rates of recruitment were relatively high (64%) for a follow-up of a community intervention study with such a long time-lag [217]. There was no differential loss to follow-up between the two arms of the trial and, with the exception of a slight difference in age, loss to follow-up was not associated with any of the available characteristics from the original trial. The recruited sample is therefore representative of the trial offspring as a whole.

The main weakness of the current study is that the 'control' group received the protein-energy supplement during lactation. The current analysis could therefore be regarded as comparing the effects of improved nutrition during pregnancy with improved nutrition during lactation (Table 3.11).

Table 3.11 Study design of original protein-energy supplementation trial

	Time point at which supplement provided	
Study arm	Pregnancy	Lactation
Prenatal supplement ('intervention')	X	---
Postnatal supplement ('control')	---	X

For this analysis it has been assumed that the improved nutrition of the 'control' group would not have affected the infants, who may therefore be regarded as a true control group. To support this view there is little evidence that providing supplements to lactating women improves their breast milk quality and thus the nutrition of the growing infant. Data from a previous trial in The Gambia found no evidence that providing protein-energy and multivitamin supplements to lactating women affected their breast milk quantity or quality [218, 219]. Furthermore, the lack of an association between supplementation group and size in infancy [216] argues against an impact of supplementation during lactation; at least one that affects growth.

The age of the individuals in the current study is a limitation of the follow-up study design. Subjects were undergoing puberty at the time of recruitment, which could potentially obscure a relationship between early-life exposures and blood pressure. It is noticeable for example that the inverse association with birth weight commonly apparent in the literature is often much weaker at this age [220].

A further limitation is the setting of the study in a rural area of The Gambia where nutritional intakes tend to be marginal. Gluckman and Hanson have proposed a new paradigm to explain the DOHaD phenomenon, which has been termed the 'predictive adaptive response' [193, 221]. They suggest that the developing organism utilises cues from their current environment to predict the environment that will be faced in later life, and in response, takes the appropriate developmental pathway. If conditions in later life are mismatched to those predicted in early-life there will be a greater risk of disease [221]. Subjects in the current study were relatively lean (mean BMI: 16.2 kg/m^2 (boys) and 17.5 kg/m^2 (girls)), and it may be that their prevailing nutritional exposure remains within the confines of that programmed by their early-life experiences. It is possible that any adverse outcomes will only become apparent if individuals are exposed to a more obesogenic environment in later life.

A final limitation is the lack of data on maternal nutritional status at baseline making it impossible to investigate any interaction of the intervention with pre-pregnancy BMI. However, season of birth could be viewed as a proxy for maternal nutritional status in this study because in this population seasonality has a marked impact on maternal weight and on birth weight [198]. Furthermore, the supplement had a greater impact on birth weight when it was consumed during the hungry season [194]. Season of birth did not modify the effect of the maternal intervention on offspring blood pressure, which suggests that maternal nutritional status may have been similarly unimportant for this outcome.

3.5.1.3 Comparison with literature

The overall lack of association between pregnancy supplementation and offspring blood pressure reflects the findings of a Guatemalan community-based intervention where a balanced protein-energy supplement, provided during pregnancy and in early-life, was not associated with blood pressure at 20-29 years of age [97]. Similarly, follow-up studies of the Dutch hunger winter and the Lenningrad siege, which have been considered 'natural experiments', have shown no association between exposure to famine during *in utero* development and blood pressure in adulthood [58, 62]. Although both of these examples are of reduced, rather than supplemented, nutrition during pregnancy.

A recently published follow-up from India investigated the impact of a community-based intervention, which provided a cereal meal to pregnant and lactating women and children up to age seven years, on cardiovascular disease risk factors in the offspring [99]. The intervention was rolled-out in a stepwise manner as part of a national public health programme and the authors were able to compare individuals born into areas covered by the programme with 'control' areas which did not yet have that provision. Although there was no effect of the early-life intervention on blood pressure in adolescence, a measure of global arterial stiffness (the augmentation index) was found to be lower in individuals born into intervention compared to control areas [99]. It is important to acknowledge that

both the Guatemalan and Indian studies involved community-level interventions that provided supplements to pregnant women and to their children up to seven and six years of age, respectively. Thus, it is not possible to distinguish between the prenatal and postnatal interventions, making them not directly comparable to the study being presented here. In addition, the roll-out of the Indian intervention had not been conducted in a random manner, which should also add caution to the interpretation of its conclusions.

The effect of the Indian intervention on arterial stiffness is a finding which warrants further discussion as increased arterial stiffness, as a consequence of fetal growth restriction, has been proposed as one of the mechanisms linking early-life exposures to blood pressure in adulthood [222]. From the data presented in this thesis there was a suggestion that the maternal intervention was associated with lower pulse pressure in adolescence. Pulse pressure is determined in part by the compliance of the arteries and could therefore be viewed as a surrogate marker of arterial stiffness [223]. There should be caution on overemphasising this finding however, as there was no effect of the intervention on systolic blood pressure, which should also be affected by arterial compliance [223]. The observed association may reflect that the non-significant effects on systolic and diastolic blood pressure were operating in opposite directions; positive for systolic and negative for diastolic. As pulse-pressure is calculated by subtracting diastolic from systolic blood pressure the pulse pressure effect may be an artificial consequence of this. In addition, the effect was not observed when the analysis was restricted to those individuals for whom known gestational age was available, despite the fact that the sample size remained large. The clinical importance of pulse pressure in this age group can also be questioned, especially as the subjects are undergoing puberty and therefore experiencing different growth rates. In the Framingham cohort, pulse pressure has been shown to be an important predictor of congestive heart failure in older people [224], but its importance as a predictor of risk in younger adults is less clear [225].

The as-treated analysis also suggested a putative association between consumption of biscuits during pregnancy and pulse pressure in the offspring, although interestingly the association was only apparent in offspring whose mothers had consumed less than the median number of biscuits, compared to control children, and not in those who had consumed more. It is therefore likely that this represents a chance finding or one which reflects an inherent issue with conducting as-treated analysis: loss of randomisation. It could be that there is an unmeasured characteristic which makes these women less likely to adhere to the study protocol and which is also associated with higher blood pressure in their children.

Returning to the concept that the experimental design compared improved pregnancy nutrition with improved lactation nutrition, there are comparable experiments in mice conducted by Ozanne and Hales [226]. Pups born to dams provided with normal nutrition (20% protein) during pregnancy and suckled by dams fed a protein-restricted diet (8% protein) during lactation experienced greater longevity than pups born to protein-restricted dams but suckled by dams fed a normal diet during lactation [226]. Unfortunately there is no blood pressure data on these pups but using the same experimental design, Tarry-Adkins *et al* observed nephroprotective effects in rats suckled by dams fed a protein-restricted diet compared to controls [227].

3.5.1.4 Implications of findings

One interpretation of the null result of this analysis is that the maternal diet during pregnancy does not directly affect the development of the fetus in a way that impacts on body systems. Although birth weight was directly influenced by the maternal diet in the original trial [194], the supply of nutrients to the fetus is also influenced by placental sufficiency and maternal nutrient stores [45]. The protein-energy supplement was provided after 20 weeks gestation [194], which may be too late to have an impact on organ development, and therefore blood pressure, but at a time where it may still influence fetal size. As stated previously, an alternative

explanation is that in an environment where the prevailing exposures remain relatively frugal any effect of the intervention may not become apparent.

3.5.2 Observational analysis

3.5.2.1 Summary of findings

Birth weight was unrelated to blood pressure in adolescence overall. However, amongst individuals in the intervention arm of the trial, birth weight was negatively associated with systolic blood pressure, pulse pressure and mean arterial pressure. For every 1kg increase in birth weight, systolic blood pressure decreased by 2.17mmHg for intervention children only. Season of birth also influenced systolic blood pressure, which was lowest for individuals who were born in June and July. Increased growth during the first year of life was positively associated with systolic blood pressure, pulse pressure and mean arterial pressure, but not with diastolic blood pressure. For each unit increase in weight SD score over the first year of life, systolic blood pressure increased by 0.73mmHg at follow-up, although current weight was a much more important predictor of current blood pressure.

3.5.2.2 Study strengths and limitations

Although the study being presented in this thesis was not designed as a cohort study it still provides a useful resource with which to investigate observational associations, particularly as the intervention did not have an effect on the outcome of interest (blood pressure). The dataset can therefore be regarded as a prospective cohort who were enrolled at birth and characterised by their birth anthropometry. The impact of the intervention on birth weight makes the interpretation of birth weight effects on blood pressure challenging; hence the analysis was explored for the intervention groups separately, as well as combined. The loss to follow-up rate is fairly low for a cohort study and loss to follow-up is not associated with the exposures of interest (birth weight, season of birth) and it is unlikely to be associated with the outcome under study (blood pressure).

Issues of confounding are of much greater importance in the analysis of cohort data than they are for trial data where, providing randomisation was successful, known and unknown confounders should be equally distributed between the trial groups [95]. The current dataset suffers from a lack of detailed data on many possible confounders. For example, no data on social economic status or on the health of women during pregnancy are available, particularly their smoking status although a recent study in the region reported no smoking amongst women aged 18-30 [228].

Data on growth in infancy are relatively incomplete in this study, which could potentially introduce bias into the results under analysis. However, there was no difference in systolic blood pressure between individuals who had a weight measurement at 12 months of age and those that did not, which suggests that this bias may be minimal. Another error inherent in the growth data is that the exact age at measurement is not recorded in the database (data are recorded under the heading of 3 months, 6 months and so on without an indication of the exact date of measurement) and it is therefore not possible to know the margin of error around the quoted measurement age in infancy.

3.5.2.3 Comparison with the literature

The association between birth weight and blood pressure has been under debate for many years [50]; a summary statistic often quoted is that for an increase of 1kg in birth weight, systolic blood pressure decreases by 2mmHg [51]. In adolescents, the association is much less clear [51], and the lack of overall association between birth weight and blood pressure in this study may therefore reflect the timing of follow-up. An alternative explanation is that the association between birth weight and blood pressure in rural African populations is different from the Western data most commonly published. Inverse associations between birth weight and blood pressure have been reported for some African populations such as five year old children in Soweto, South Africa [229] and school children (6.5 years) in Harare, Zimbabwe [230], but the findings have not been universal [56] and there are no data from rural populations. Amongst individuals from the intervention arm of the

study, birth weight was inversely associated with blood pressure in a similar magnitude as is often reported; for every 1kg increase in birth weight, systolic blood pressure decreased by 2.17mmHg.

In the literature, the impact of growth in infancy on later blood pressure has been inferred from the stronger association between birth weight and blood pressure once the data are adjusted for current size. In the data presented in this thesis, individuals who experienced less growth during the first year of life also had lower blood pressure in adolescence, although the exposure window did not appear to be limited to early infancy as has been previously suggested [136].

A life course plot from Brazil depicting the association between growth in early-life and blood pressure at age 15 shows a similar pattern to the life course plot presented here [121]. In both studies, current weight was the strongest predictor of blood pressure but crossing of weight centiles from 42 months to 15 years in the Brazil cohort or 36 months to 14.5 years in the current study, was associated with raised systolic and diastolic blood pressure in adolescence. It should be acknowledged that the timing of measurements in these plots will affect the pattern of the observed association. A recent pooled analysis by Adair *et al* of data from five different cohorts in lower income countries reported no association between weight gain in infancy and blood pressure in adulthood [123]. However the oldest measurement of relative size in this combined analysis was at 48 months of age, the pattern of the association between growth after this age and later blood pressure may be similar to that reported here and from Brazil.

3.5.2.4 Implications of findings

Systolic blood pressure was lowest for individuals who were born in June and July, and highest for individuals born in December and January. In rural areas of The Gambia such as this one there is a seasonality of farming activity that leads to changes in energy balance [198]. The prevalence of small for gestational age babies experiences a nadir in June and is thought to reflect more favourable conditions

(less farming work) during gestation [198]. The seasonality of birth in relation to blood pressure may also reflect this more favourable gestation. It may be that individuals born at the middle of the year, after a gestation throughout the harvest season, exhibit body systems that have developed to their full potential and thus have lower blood pressure. It is important not to over-interpret the graphs of seasonality, which utilise smoothed data rather than the raw measurements and may therefore appear to be stronger associations than exist in reality. Furthermore, a previous observational study in this region found no association between being born in the hungry season and blood pressure in young adulthood [148]. In addition, in the current study the association was only present for systolic rather than diastolic blood pressure.

The finding that growth in infancy may be an important determinant of later blood pressure in the current population is interesting particularly as the importance of growth trajectories for cardiovascular disease risk are receiving greater attention [231]. Again the findings should not be over-interpreted, especially as the growth data are incomplete and there is no information on the exact date of measurement. Standardised methods of assessing growth are required to build up a clearer picture of the trajectories which confer greatest risk. If faster growth is a determinant of increased blood pressure as some of the data suggest, this will have important policy implications for developing countries, particularly ones experiencing the nutrition transition where the immediate benefits of increased growth may need to be balanced against potential long-term consequences [231].

Chapter 4 Follow-up of calcium supplementation trial

4.1 Forward

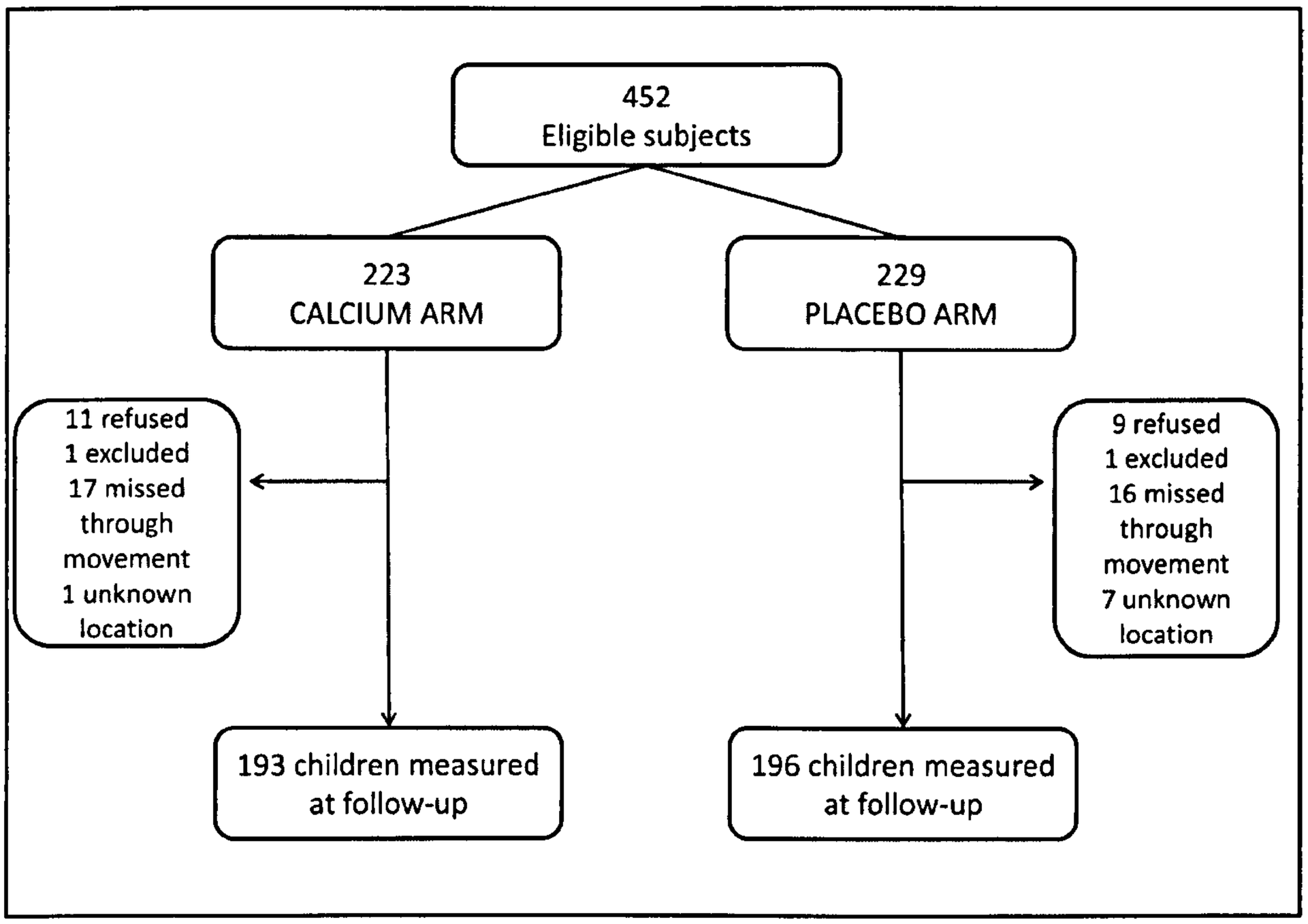
This chapter will describe in detail the results of the statistical analysis investigating the impact of maternal calcium supplementation on offspring blood pressure at 5-10 years of age in The Gambia. A review of the statistical methods employed has been covered in Section 2.5 and should be referred to when interpreting the analysis. Regression coefficients appear as β in the text, standard deviations are donated by SD, the chi-squared test statistic as χ^2 and confidence intervals by CI.

4.2 Study participants

4.2.1 General characteristics

A total of 389 children (193 born to calcium supplemented mothers and 196 born to mothers who received a placebo) were enrolled into the follow-up study, representing 86% of those eligible and 71% of the original trial births (Figure 4.1). Eligible subjects were offspring still living in West Kiang or those who had moved to coastal areas that were within an hour drive from MRC facilities (see Section 2.4.1). Recruitment rates were similar between the two arms of the trial: 193 children from the calcium supplement arm (71.2% of original trial births) and 196 from the placebo supplement arm (71.3%). The main reason for non-recruitment (7.6% of calcium supplement children and 7.0% of placebo children) was due to family movement within The Gambia, resulting in eligible subjects being unavailable during the study period. Two subjects were excluded from the study due to a reported mental illness and four percent of parents refused to give consent for their child to participate in the follow-up study.

Figure 4.1 Flow diagram of offspring eligible for calcium trial follow-up and those recruited



There were some differences in available characteristics between those recruited and those lost to follow-up (Table 4.1). The mothers of recruited individuals were 1.8 years older and had slightly lower baseline (20 weeks gestation) systolic blood pressure (-1.7mmHg) than the mothers of individuals enrolled into the follow-up study. Children enrolled into the follow-up study had been slightly shorter (-0.1cm) in length at two weeks of age compared to those lost to follow-up.

Table 4.1 Differences between recruited individuals into calcium supplementation trial follow-up and those lost to follow-up[a]

	Recruited		Lost to follow-up[a]		P-value[b]
	N	Mean (SD)	N	Mean (SD)	
Maternal age (y)	249	27.9 (7.0)	93	26.1 (6.7)	0.03
Maternal baseline[c] weight (kg)	387	55.0 (6.8)	156	55.3 (8.1)	0.4
Maternal height (cm)	389	160.1 (5.3)	157	159.7 (5.6)	0.6
Maternal baseline[c] systolic BP (mmHg)	389	101.5 (8.7)	157	103.2 (10.0)	0.04
Maternal baseline[c] diastolic BP (mmHg)	389	55.3 (7.6)	157	56.5 (6.9)	0.09
Child weight at 2wk[d] (kg)	385	3.34 (0.50)	136	3.35 (0.56)	0.1
Child length at 2wk[d] (cm)	385	50.7 (2.0)	136	50.8 (2.4)	0.08

[a]Lost to follow-up defined as all live, singleton children born during original trial who were not recruited into follow-up study (ie: includes those ineligible for current follow-up)
[b]P-value refers to a difference in mean values between those recruited and those lost to follow-up
[c]Baseline for original supplementation trial was 20 weeks gestation, prior to commencement of supplementation
[d]Anthropometry at two weeks of age used as a proxy measure for birth weight due to a large number of missing data at the earlier age point

Thirty eight children enrolled (13 calcium, 25 placebo) were classified as having a gestational age under 37 weeks (preterm), as assessed by the Dubowitz score [212] at delivery and were excluded from the analysis. One blood pressure measurement was missing as the child refused to cooperate, leaving a final sample of 350 (171 born to calcium supplemented mothers and 179 to placebo mothers) for analysis.

The analysed sample was split equally between girls and boys. Only 13% were recruited from the urban/semi-urban areas and there were no differences in characteristics (age, sex, intervention arm) between individuals recruited from West Kiang and those from urban areas (data not presented).

4.2.2 Anthropometry and body composition

Table 4.2 displays the anthropometric and body composition data for individuals included in the analysis. Mean height for girls was 118.0cm (SD: 8.3) and mean height for age Z-score was -1.0 (SD: 0.9). Mean height for boys was also 118.3cm (SD: 7.6) and their mean height for age Z-score was -1.2 (SD: 0.8). Thirteen percent of the sample fell below two Z-scores of the reference population and can therefore be regarded as stunted. Mean BMI for girls was 13.9 kg/m^2 (SD: 1.2) and for boys was 14.1 kg/m^2 (SD: 1.0). Height and weight increased with age whereas BMI, fat (FMI) and lean mass index (LMI) were unrelated to age (data not shown).

Table 4.2 Anthropometry and body composition of offspring born during Gambian calcium supplementation trial

	Mean (SD)	
	Girls	Boys
N	175	175
Height (cm)	118 (9)	118 (8)
Weight (kg)	19.6 (3.6)	19.9 (3.1)
Height-for-age Z-score[a]	-1.0 (0.9)	-1.2 (0.8)
BMI (kg/m^2)	13.9 (1.2)	14.1 (1.0)
BMI Z-score[a]	-1.4 (0.9)	-1.4 (1.0)
Body fat (%)	16.3 (2.9)[#n-3]	12.8 (2.5)[#n-4]
Fat mass index (FMI) (kg/m$^{1.6}$)	2.5 (0.6)[#n-2]	1.9 (0.4)[#n-4]
Lean mass index (LMI) (kg/m$^{2.3}$)	11.1 (0.8)[#n-1]	11.7 (0.8)[#n-4]
Trunk fat (%)	14.2 (2.8)[#n-2]	13.6 (2.7)[#n-4]

[a]Compared to UK reference data[214]
[#n]Denotes sample size which differs from the column header

4.2.3 Blood pressure

Mean systolic blood pressure for girls was 98.6mmHg (SD: 8.8) and for boys was 97.6mmHg (SD: 8.1) (Table 4.3). The variance in blood pressure was similar for boys and girls and the analysis was therefore conducted for the sexes combined, although adjusted for sex where appropriate. Blood pressure was normally distributed when assessed using quantile-quantile plots and the Shaprio-Wilk test for normality (systolic P: 0.01; diastolic P: 0.001).

Table 4.3 Blood pressure of subjects recruited into calcium trial follow-up

	Mean (SD)	
	Girls	Boys
N	175	175
Systolic (mmHg)	98.6 (8.8)	97.6 (8.1)
Diastolic (mmHg)	58.7 (7.5)	57.3 (7.3)
Pulse pressure (mmHg)[a]	39.9 (5.9)	40.4 (6.6)
Mean arterial pressure (mmHg)[b]	72.0 (7.4)	70.7 (6.9)
Hypertension (%)[c]	5.1	3.4

[a]Calculated as systolic – diastolic blood pressure
[b]Mean arterial pressure calculated as diastolic + (1/3*pulse pressure)
[c]Hypertension defined as systolic blood pressure above the NHBPEP 95% centile for height and age [12]

Using simple linear regression analysis maternal baseline (20 weeks gestation) weight was marginally but positively associated with child systolic blood pressure; for each kilogram increase in weight, offspring systolic blood pressure increased by 0.2mmHg (95% CI: 0.03, 0.29; P: 0.02). There was a very similar association between maternal weight and child diastolic blood pressure (β: 0.18mmHg; 95% CI: 0.08, 0.30; P: 0.001). Maternal baseline systolic blood pressure was also marginally associated with offspring systolic blood pressure; for each mmHg increase in maternal blood pressure, child blood pressure increased by 0.1mmHg (95% CI: 0.003, 0.208; P: 0.04). Maternal baseline diastolic blood pressure was also positively associated with offspring diastolic blood pressure; for each mmHg increase in maternal diastolic blood pressure, child blood pressure increased by 0.2mmHg (95% CI: 0.04, 0.25; P: 0.01). Maternal parity was not associated with offspring blood pressure (data not shown). The antenatal clinic attended during pregnancy (one of three) was also not significantly associated with offspring blood pressure but was retained in the model for completeness.

Both systolic (β: 1.6mmHg; 95% CI: 0.8, 2.3; P: <0.001) and diastolic (β: 0.9mmHg; 95% CI: 0.4, 1.6; P: 0.003) blood pressure increased with child age but were unrelated to season of birth, or to the location of the individual (rural or urban) at follow-up (data not shown). Variables included in the adjusted models were

therefore restricted to age, sex, antenatal clinic, maternal baseline weight and maternal baseline blood pressure.

Systolic and diastolic blood pressure were found to be positively associated with most measures of body composition, with the exception of percentage body fat for systolic blood pressure and percentage trunk fat for both systolic and diastolic blood pressure (Table 4.4). Investigating the relative contribution of fat mass index (FMI) and lean mass index (LMI) in simultaneous models including both terms different patterns were observed for systolic and diastolic blood pressure. In the simultaneous model, FMI was no longer associated with systolic blood pressure (β: 1.37mmHg; 95% CI: -0.44, 3.18; P: 0.14) but LMI remained associated (β: 1.58mmHg; 95% CI: 0.38, 2.78; P: 0.01). For diastolic blood pressure, FMI remained associated (β: 2.70mmHg; 95% CI: 1.10, 4.30; P: 0.001) whereas LMI was no longer associated (β: 0.83mmHg; 95% CI: -0.23, 1.89; P: 0.12).

Table 4.4 Association between blood pressure and anthropometry/body composition for individuals in calcium trial follow-up

	Systolic BP (mmHg)		Diastolic BP (mmHg)	
	Regression coefficient (95% CI)	P-value	Regression coefficient (95% CI)	P-value
Height (cm)	0.50 (0.31, 0.68)	<0.001	0.35 (0.18, 0.51)	<0.001
Weight (kg)	1.10 (0.76, 1.44)	<0.001	0.90 (0.59, 1.20)	<0.001
BMI (kg/m^2)	1.72 (0.96, 2.47)	<0.001	1.49 (0.82, 2.15)	<0.001
Body fat (%)	0.22 (-0.11, 0.55)	0.20	0.45 (0.15, 0.74)	0.003
FMI ($kg/m^{1.6}$)	2.30 (0.62, 3.98)	0.01	3.19 (1.71, 4.67)	<0.001
LMI ($kg/m^{2.3}$)	1.93 (0.83, 3.04)	0.001	1.53 (0.54, 2.52)	0.003
Trunk fat (%)	0.02 (-0.32, 0.36)	0.99	0.25 (-0.05, 0.56)	0.10

Results are the effect on blood pressure of a one unit increase in explanatory variable, derived from linear regression analysis adjusted for age and sex

4.3 Maternal calcium supplementation and offspring blood pressure

4.3.1 Results of intention-to-treat analysis

Maternal calcium, compared to placebo, supplementation during pregnancy did not affect offspring blood pressure at 5-10 years of age (Table 4.5). The maternal intervention was unrelated to offspring blood pressure even after adjusting for compliance to the intervention and for covariates related to blood pressure (model 4, Table 4.5).

Only four percent of children had a systolic blood pressure that was above the 95th percentile for their height and age, and the odds of having high blood pressure was not related to the maternal calcium intervention (unadjusted OR: 1.1, 95% CI: 0.4, 3.1, P: 0.86).

Table 4.5 Effect of maternal calcium supplementation during pregnancy on offspring blood pressure at 5-10 years in The Gambia: intention-to-treat analysis

	Model 1[a] (95% CI)	P-value	Model 2[b] (95% CI)	P-value	Model 3[c] (95% CI)	P-value	Model 4[d] (95% CI)	P-value
Systolic pressure (mmHg)	-0.10 (-1.89, 1.68)	0.91	-0.001 (-1.74, 1.74)	0.99	0.36 (-1.32, 2.05)	0.67	0.28 (-1.41, 1.97)	0.74
Diastolic pressure (mmHg)	0.10 (-1.46, 1.67)	0.90	0.31 (-1.21, 1.83)	0.69	0.50 (-1.02, 2.01)	0.52	0.40 (-1.12, 1.91)	0.61
Pulse pressure (mmHg)	-0.20 (-1.53, 1.12)	0.76	-0.31 (-1.62, 1.00)	0.64	-0.14 (-1.44, 1.17)	0.84	-0.13 (-1.43, 1.17)	0.84
Mean arterial pressure (mmHg)	0.03 (-1.49, 1.55)	0.97	0.20 (-1.27, 1.67)	0.79	0.45 (-1.00, 1.90)	0.54	0.35 (-1.10, 1.81)	0.63

Results are the difference in mean blood pressure (95% CI) for individuals born to women receiving calcium supplements (coded 1) compared to placebo (coded 0) during pregnancy, derived from linear regression analysis.

[a]Model 1: unadjusted

[b]Model 2: adjusted age, sex, antenatal clinic, maternal baseline weight and maternal baseline blood pressure

[c]Model 3: as model 2 but additionally adjusted height, lean mass index (LMI) and fat mass index (FMI)

[d]Model 4: as model 3 but additionally adjusted for maternal compliance and length of supplementation.

A potential source of error is the incorrect inclusion of individuals born preterm as there were a large number of missing data regarding gestational age (37%). However, restricting the analysis only to offspring with a known gestational age did not alter the conclusions (Table 4.6).

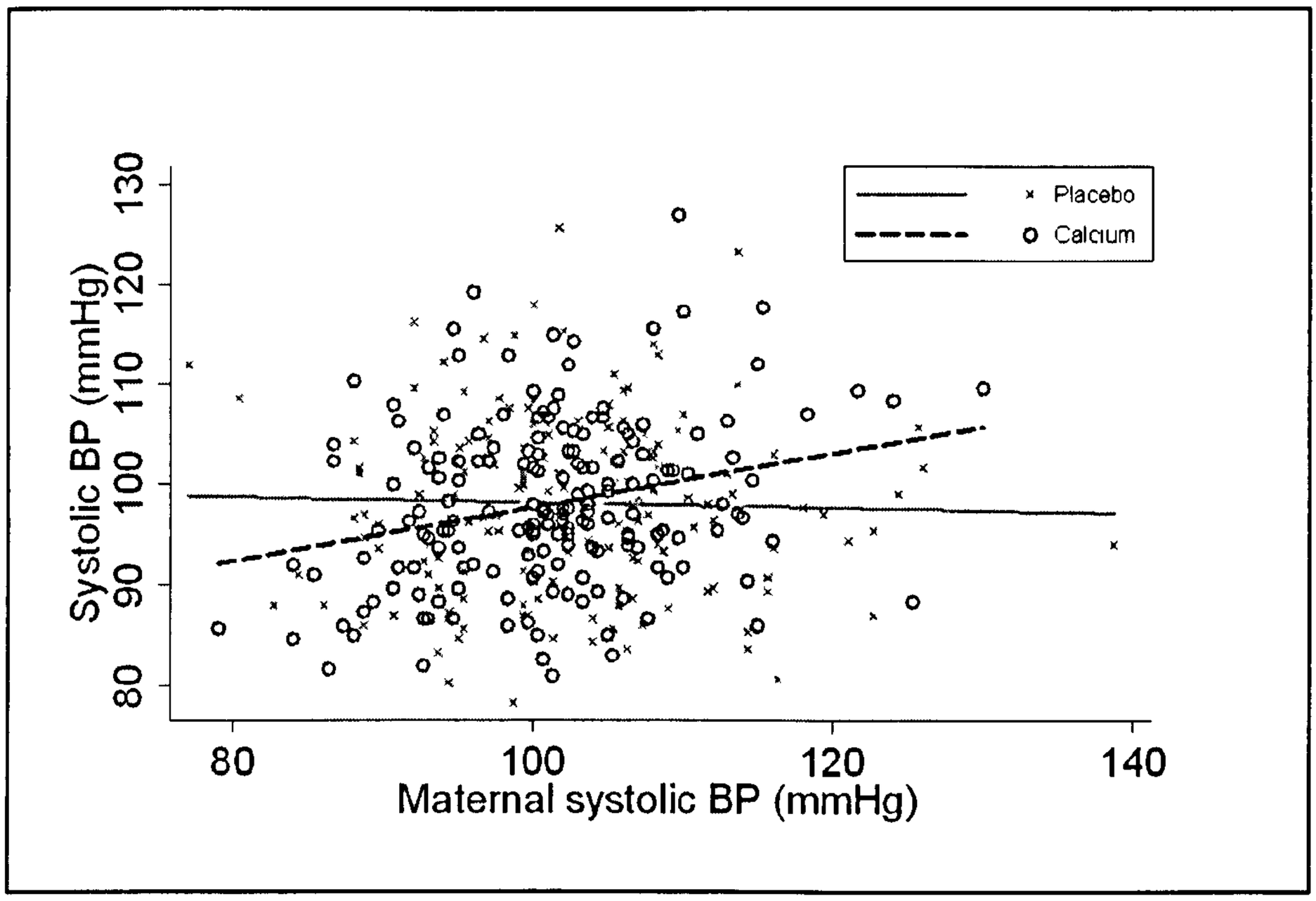
Table 4.6 Effect of maternal calcium supplementation on offspring blood pressure for individuals with known gestational age (n=221)

	Unadjusted regression coefficient (95% CI)	P-value
Systolic (mmHg)	-0.03 (-2.26, 2.19)	0.98
Diastolic (mmHg)	-0.35 (-2.34, 1.64)	0.73
Pulse pressure (mmHg)	0.32 (-1.32, 2.00)	0.71
Mean arterial pressure (mmHg)	-0.25 (-2.17, 1.68)	0.80

Results are the difference in mean blood pressure (95% CI) for individuals born to women receiving calcium supplements (coded 1) compared to placebo (coded 0) during pregnancy, derived from linear regression analysis.

The possibility of the intervention effect being modified by covariates was assessed by fitting interaction terms into the linear regression models. There was a significant interaction between maternal baseline blood pressure and the effect of the intervention on childhood systolic blood pressure (Interaction coefficient, β: 0.26mmHg; 95% CI: 0.06, 0.46; P: 0.01). Figure 4.2 graphically displays this interaction; for women with low baseline systolic blood pressure, the calcium intervention was associated with lower child systolic blood pressure compared to placebo, whereas for women with high blood pressure this association was reversed.

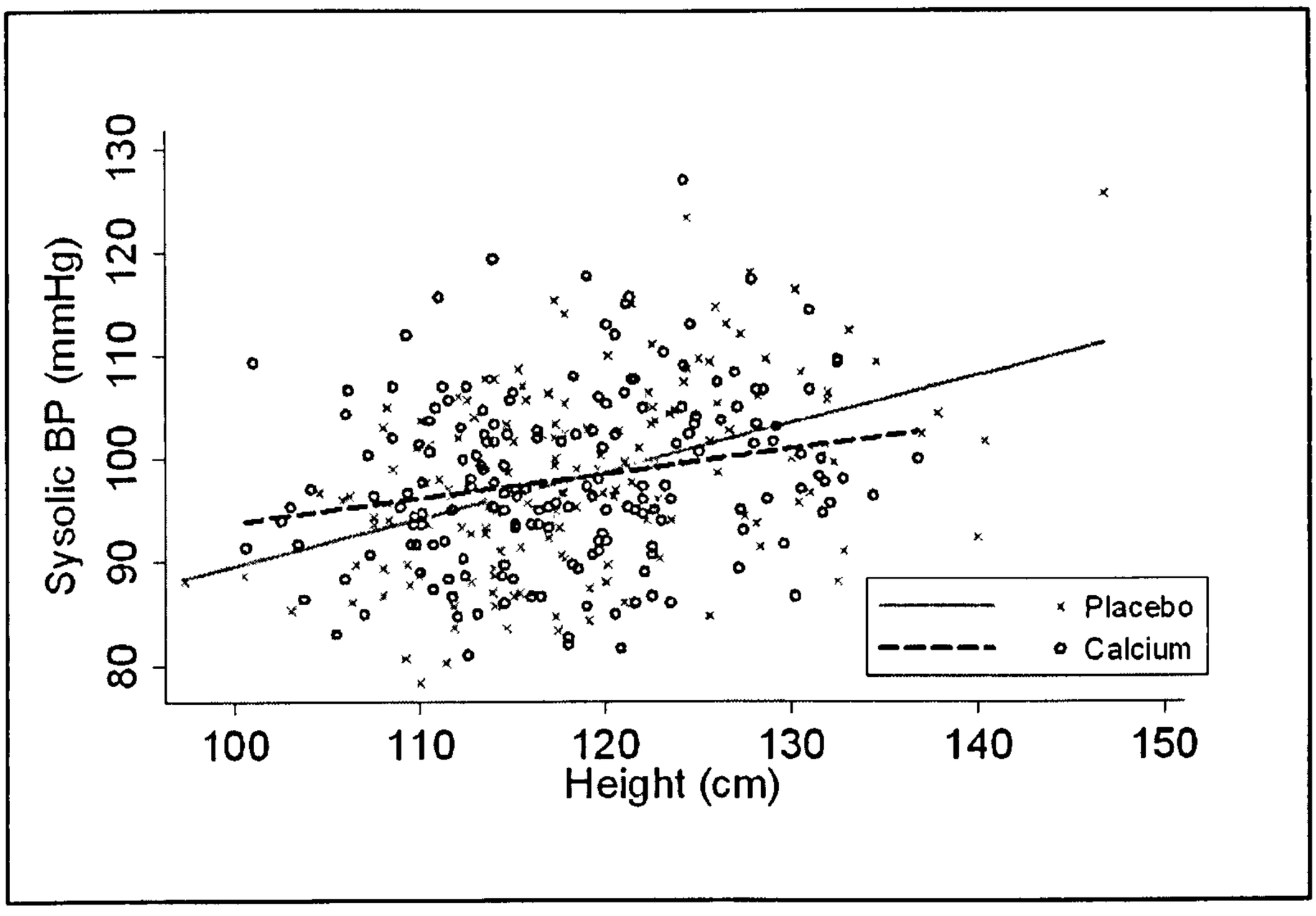
Figure 4.2 Interaction between calcium intervention and maternal blood pressure; effect on child blood pressure



Lines are lines of best fit from linear regression analysis plotted for placebo and control groups separately

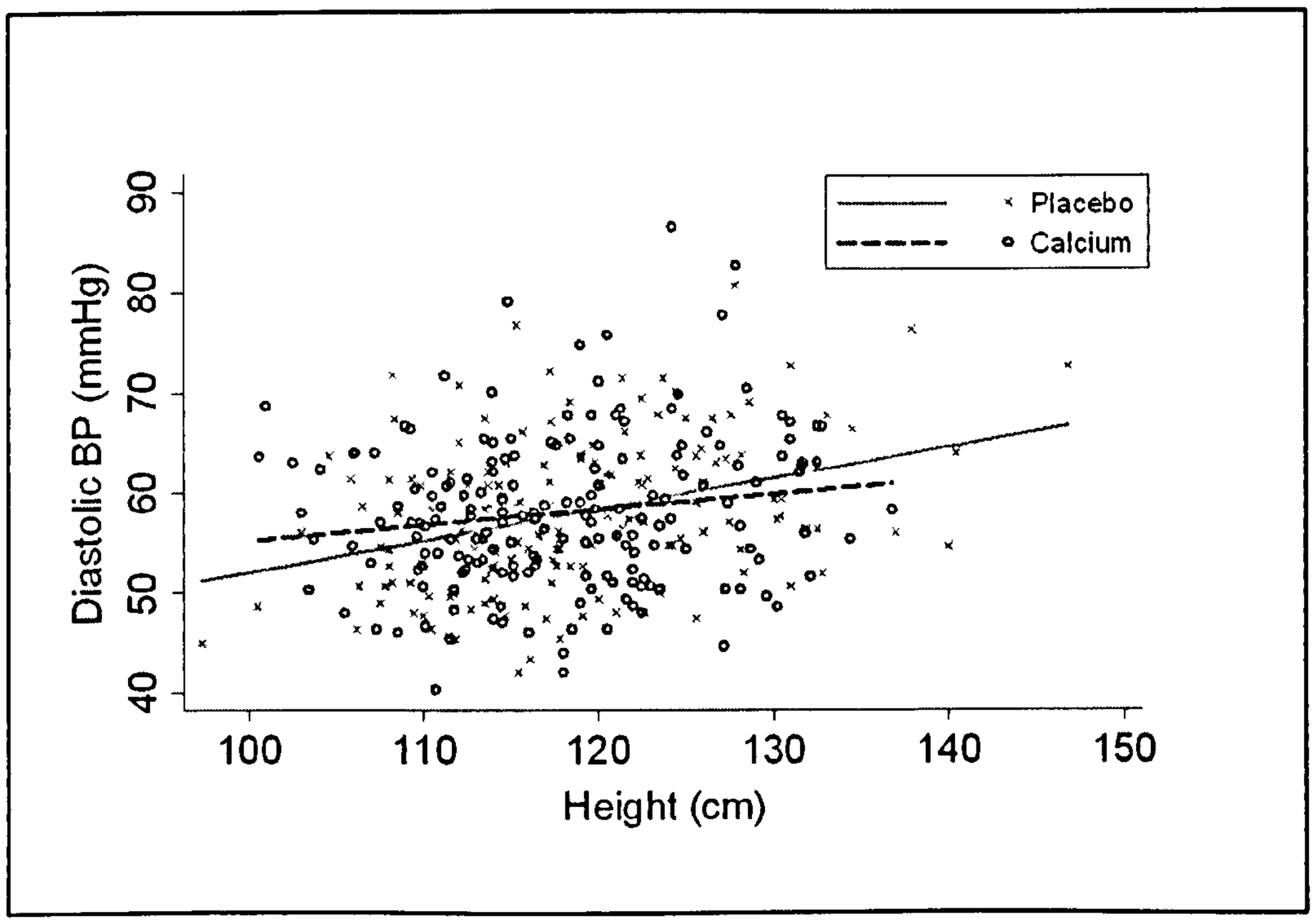
The association between maternal calcium supplementation and offspring blood pressure was not modified by an interaction with attended antenatal clinic or with maternal BMI (data not shown). There was also no interaction with age or sex of the offspring (data not shown) but there was a significant interaction between the maternal intervention and child height at follow up on offspring systolic blood pressure (Interaction coefficient, β: -0.23mmHg; 95% CI: -0.44, -0.02; P: 0.03). For shorter children, the maternal calcium intervention was associated with higher blood pressure compared to placebo, whereas for taller children it was associated with lower blood pressure (Figure 4.3). There was also the suggestion of a similar relationship for diastolic blood pressure (Interaction coefficient, β: -0.18mmH; 95% CI: -0.37, 0.01; P: 0.06) (Figure 4.4).

Figure 4.3 Interaction between maternal calcium intervention and child height; effect on child systolic blood pressure



Lines are lines of best fit from linear regression analysis plotted for placebo and control groups separately

Figure 4.4 Interaction between maternal calcium intervention and child height; effect on child diastolic blood pressure



Lines are lines of best fit from linear regression analysis plotted for placebo and control groups separately

4.2.3 Results of as-treated analysis

Mean compliance (comprising both intervention and control women) as assessed by tablet count and consumption was 98% (range: 75-100%) and there was no difference in mean compliance between the calcium and placebo arms of the trial (calcium: 98%, placebo: 98%; t-test P-value: 0.43). The mean total number of calcium tablets consumed by women in the intervention arm was 422 (range: 267-561) and the mean number of placebo tablets consumed was 415 (range: 285-588). In the as-treated analysis, there was a suggestion that women who demonstrated greater compliance had offspring with higher systolic blood pressure (β: 29.92mmHg, 95% CI: 1.10, 58.74, P: 0.04) but this was not related to the amount of the calcium supplement consumed (reflected by the 'dose consumed' term) (Table 4.7). Offspring diastolic blood pressure was also unrelated to the maternal calcium dose.

Table 4.7 Effect of maternal calcium supplementation during pregnancy on offspring blood pressure at 5-10 years in The Gambia: as-treated analysis

		Model 1[a] (95% CI)	P-value	Model 2[b] (95% CI)	P-value	Model 3[c] (95% CI)	P-value
Systolic BP (mmHg)	Compliance[d]	29.92 (1.10, 58.74)	0.04	23.33 (-6.86, 53.52)	0.13	22.69 (-6.50, 51.87)	0.13
	Duration[e]	0.52 (-1.00, 2.05)	0.50	0.46 (-1.04, 1.97)	0.55	-0.12 (-1.61, 1.37)	0.88
	Dose[f]	-0.05 (-0.47, 0.38)	0.83	-0.02 (-0.43, 0.39)	0.91	0.06 (-0.34, 0.46)	0.76
Diastolic BP (mmHg)	Compliance	18.87 (-6.36, 44.10)	0.14	11.34 (-14.96, 37.64)	0.40	11.53 (-14.56, 37.62)	0.39
	Duration	1.07 (-0.26, 2.41)	0.12	1.19 (-0.12, 2.50)	0.07	0.82 (-0.51, 2.15)	0.23
	Dose	0.01 (-0.36, 0.38)	0.94	0.06 (-0.30, 0.42)	0.75	0.10 (-0.26, 0.45)	0.60
Pulse pressure (mmHg)	Compliance	11.04 (-10.34, 32.44)	0.31	12.38 (-10.40, 35.17)	0.29	11.30 (-11.22, 33.82)	0.32
	Duration	-0.55 (-1.68, 0.58)	0.34	-0.77 (-1.90, 0.37)	0.19	-0.95 (-2.09, 0.20)	0.11
	Dose	-0.06 (-0.37, 0.25)	0.71	-0.08 (-0.39, 0.23)	0.60	-0.04 (-0.35, 0.27)	0.80
Mean arterial pressure (mmHg)	Compliance	22.55 (-1.93, 47.04)	0.07	15.28 (-10.20, 40.76)	0.24	15.20 (-9.80, 40.19)	0.23
	Duration	0.89 (-0.41, 2.18)	0.18	0.96 (-0.31, 2.23)	0.14	0.51 (-0.76, 1.79)	0.43
	Dose	-0.01 (-0.36, 0.35)	0.97	0.03 (-0.32, 0.38)	0.87	0.08 (-0.26, 0.43)	0.63

Results are the effect on child blood pressure of a one unit increase in exposure variables (compliance, duration or dose), derived from linear regression analysis

[a]Model 1: unadjusted

[b]Model 2: adjusted age, sex, antenatal clinic, maternal baseline weight and maternal baseline blood pressure

[c]Model 3: as model 2 but additionally adjusted height, LMI and FMI

[d]Compliance calculated as a proportion: observed tablet consumption/expected tablet consumption

[e]Duration refers to the amount of time a women was enrolled into the supplementation trial

[f]Calcium dose calculated as the interaction term between compliance, duration of supplementation and treatment allocation (calcium/placebo).

4.4 Early life exposures: observational analysis

4.4.1 Birth weight and later blood pressure

Weight at two weeks of age was used as a proxy for birth weight. The regression coefficient for the association between weight at two weeks and systolic blood pressure (adjusted for age, sex and maternal weight and blood pressure) was 1.31mmHg (95% CI: -0.64, 3.25, P: 0.19) and after adjustment for current weight it was -0.89mmHg (95% CI: -2.89, 1.10, P: 0.38). For the association with diastolic blood pressure the regression coefficient was 0.11mmHg (95% CI: -1.59, 1.81, P: 0.90), and adjusted for current weight was -1.64mmHg (95% CI: -3.41, 0.12, P: 0.07). There was no association between weight at two weeks and pulse pressure or mean arterial pressure (data not shown).

Length at two weeks was not related to either systolic (β: 0.04mmHg; 95% CI: -0.1, 0.09; P: 0.08) or diastolic (β: 0.03mmHg; 95% CI: -0.01, 0.07; P: 0.14) blood pressure at follow-up. After additional adjustment for current height there remained no association with either systolic (β: 0.003mmHg; 95% CI: -0.05, 0.05; P: 0.90) or diastolic (β: 0.01mmHg; 95% CI: -0.04, 0.05; P: 0.73) blood pressure. There was also no association between length at two weeks and pulse pressure or mean arterial pressure (data not shown). Unlike the analysis in Chapter 3, there was no interaction between maternal supplementation group and the birth weight – blood pressure association for the offspring (data not shown).

4.4.2 Season of birth and later blood pressure

Season of birth, adjusted for age, sex, maternal blood pressure, maternal weight and child height and body composition, was unrelated to systolic (LR text χ^2: 0.71; P: 0.69) or diastolic (LR test χ^2: 7.93; P: 0.09) blood pressure at 5-10 years of age.

4.4.3 Growth in infancy and later blood pressure

Growth in the first year of life, defined as the change in weight SD score between two and fifty-two weeks of age, was unrelated to blood pressure at 5-10 years in analysis adjusted for age, sex, maternal weight and maternal blood pressure (Table 4.8). In contrast, change in weight SD score from one year to the current study was positively associated with systolic, diastolic and mean arterial pressure.

Table 4.8 Relationship between weight change in early-life and blood pressure at 5-10 years of age in Gambian children

	Change between 2–52 weeks[a]		Change between 1–7 years[b]	
	Regression coefficient (95% CI)	P-value	Regression coefficient (95% CI)	P-value
Systolic BP (mmHg)	0.32 (-0.49, 1.13)	0.44	1.12 (0.09, 2.15)	0.03
Diastolic BP (mmHg)	0.47 (-0.23, 1.18)	0.19	0.92 (0.01, 1.82)	0.05
Pulse pressure (mmHg)	-0.19 (-0.81, 0.43)	0.55	0.21 (-0.58, 1.00)	0.60
Mean arterial pressure (mmHg)	0.43 (-0.26, 1.11)	0.22	0.98 (0.11, 1.86)	0.03

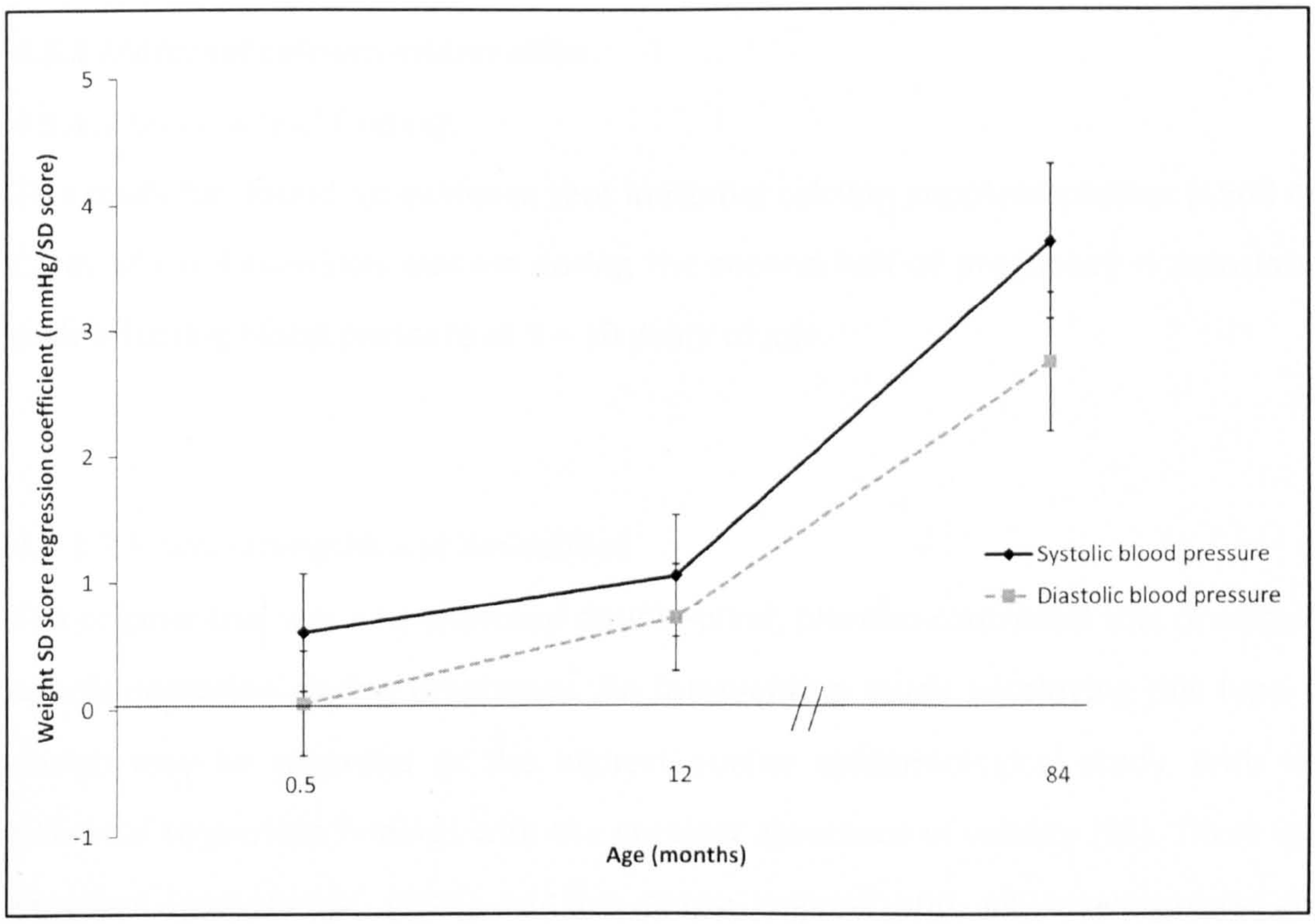
Results are the effect on blood pressure at 5-10y of a one unit increase in weight standard deviation score between the specified time points, derived from linear regression analysis adjusted for age, sex, maternal weight and maternal blood pressure

[a]Change in weight SD score between the two and 52 weeks

[b]Change in weight SD score between 1 year of age and current follow-up (average 7 years old)

The pattern of the association between growth and blood pressure at 5-10 years was further explored by creating a life course plot [121]. This reveals the importance of current weight for current blood pressure but also the positive association between growth after one year and blood pressure at follow-up (Figure 4.5).

Figure 4.5 Life course plot of the association between growth at different ages and blood pressure at 5-10 years in rural Gambia



Points represent regression coefficient (±2 x SE plotted as error bars) for the association between internal weight SD score at different ages and blood pressure at 84 months, for systolic (black line, diamond) and diastolic (grey line, square) blood pressure

Change in length SD score between two and 52 weeks was unrelated to systolic (β: 0.05mmHg; 95% CI: -0.80, 0.90; P: 0.91) and diastolic (β: 0.24mmHg; 95% CI: -0.50, 0.99; P: 0.52) blood pressure at 5-10 years. There was also no association with pulse pressure and mean arterial pressure and length change (data not shown). Change in length SD score between one and seven years was similarly unrelated to blood pressure at follow-up (data not shown).

4.5 Discussion

4.5.1 Maternal calcium intervention

4.5.1.1 Summary of findings

This study has found no evidence that maternal calcium supplementation (1500 mg Ca/d) of rural Gambian women during the second half of pregnancy is associated with offspring blood pressure at 5 – 10 years of age.

4.5.1.2 Study strengths and limitations

The original trial was a randomised double-blind, placebo controlled trial of calcium supplementation during pregnancy. An intervention study employing this type of design may be regarded as the highest quality epidemiological study, with the potential to provide findings with the greatest assurance of validity [95]. There was excellent compliance (98%) to the study regime and all investigators and participants remained blinded to the treatment allocation throughout the original study and the follow-up. The recruitment rate into the follow-up study was high (86% of all eligible individuals) and there was no differential follow-up between the two arms of the trial. In contrast, the only trial to report an overall association between maternal calcium supplementation and offspring blood pressure suffered losses to follow-up of over 90% [107]. Loss to follow-up was associated with only minor differences in characteristics from the original trial for which data were available, such as maternal baseline blood pressure and maternal age. The recruited sample is therefore representative of the trial offspring as a whole.

One potential limitation of this study is the age of the children at follow-up. Studies that have reported lower offspring blood pressure in relation to maternal calcium intake during pregnancy (either trial or observational data) have mainly included children aged two years and under [69, 71, 107], whereas two studies reporting no association were in children aged 4-7 [108] and 7-10 years respectively [70]. A study in the US reported an inverse association between maternal calcium intake and offspring systolic blood pressure at one month of age, which was no longer

apparent by twelve months, although recruitment was greatly reduced at the second follow-up [69]. It may be that any influence of maternal calcium intake is short lived and/or does not track into later childhood.

Another limitation is the difficulty of measuring blood pressure in children, even when an automated device is used. Given the design of the current study this issue should be equally distributed between the two random arms of the study and would therefore act to obscure any effect of maternal supplementation on offspring blood pressure through the additional 'noise' around the measurement of blood pressure. Given the small effect size and the tight confidence intervals presented in the analysis, it is unlikely that a greater precision of the effect estimate would have changed the conclusions of the study.

4.5.1.4 Comparison with literature

A number of studies have investigated the effect of maternal calcium intake, during pregnancy, on blood pressure in the offspring. These were recently summarised by Bergel and Barros [232], in a review that included both observational and randomised controlled trial studies. Three observational studies were included in the review [69-71], two of which were from the USA and included infants under one year of age [69, 71]. As outlined above, McGarvey *et al* observed a negative association between maternal calcium intake (from food and personal supplements) and offspring systolic blood pressure at one month, a borderline association at six months but no association at twelve months of age [69]. Gilman *et al* observed a negative association between maternal calcium intake from supplements and offspring systolic blood pressure at six months of age but no association with calcium intake from foods [71]. The third observational study was from Australia and included children of nine years of age and observed no association between maternal calcium intake from supplements and childhood blood pressure [70]. There were a number of methodological issues with these studies; they suffered from loss to follow-up (of up to 40% for the one year follow-up of McGarvey *et al* [69]) and small sample sizes. In addition, the study by

McGarvey *et al* [69] and the Australian study [70] both used retrospective dietary assessment after birth.

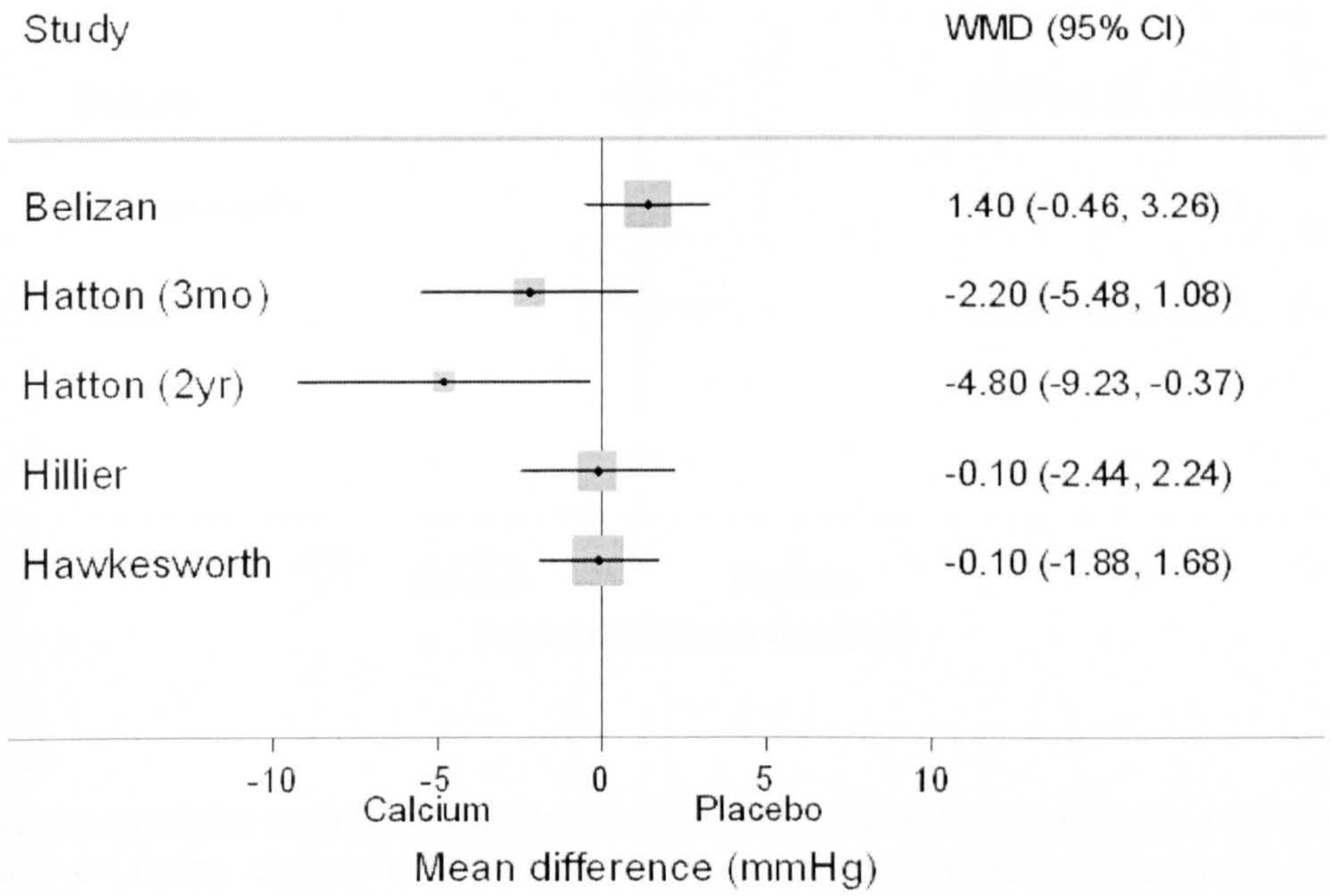
To date, three intervention studies of maternal calcium supplements have published follow-up data on offspring blood pressure. These trials are: one conducted in the US providing 2g calcium per day [109]; one from Argentina also providing 2g/d [110]; and one from Australia providing 1.8g of calcium per day [111]. The trial from the US was the only one to find an overall effect of maternal calcium supplementation on offspring blood pressure, albeit at only one of the two age points measured [107]. At three months of age 47% of targeted offspring were recruited and no association between maternal calcium supplementation and offspring systolic blood pressure was apparent. At the second follow-up the offspring were now two years old, and less than 10% of the original trial offspring were recruited but slightly lower systolic blood pressure was observed for children born to women receiving the supplement compared to placebo [107]. The follow-up of the Australian calcium supplementation trial recruited offspring who were 4-7 years old and observed no association between the maternal intervention and offspring blood pressure [108].

Despite no overall effect of maternal calcium supplementation on offspring blood pressure at 5-9 years in Argentina, there was a lower risk of having high systolic blood pressure (>90th centile for US reference data [12]) for the offspring of calcium supplemented women compared to those provided with a placebo [106]. Additionally, the Argentinean trial observed an interaction with childhood BMI; for individuals with greater BMI at follow-up (above the mean) the intervention was associated with lower blood pressure [106]. In the current study from The Gambia there was no interaction with BMI and one explanation for this could be that the Gambian children were leaner (mean BMI: 14.0kg/m^2 ±1.1) than those enrolled into the Argentinean follow-up (mean BMI: 16.2kg/m^2 ±2.4), which was restricted to offspring in the wealthier subgroup. Only seven percent of subjects in the current study would be classified as having a BMI in the top two quartiles of the Argentinean study [106].

Discrepancies in the results of calcium trials of supplementation with respect to hypertensive disorders in pregnancy have been attributed to different baseline intakes with a lower risk for women from areas of low habitual intake [233]. It is important to acknowledge that different baseline intakes may also explain the differences in the findings presented here, with average baseline intakes in the US trial of over 1000mg/d [107], compared to 300-400mg/d in The Gambia [195].

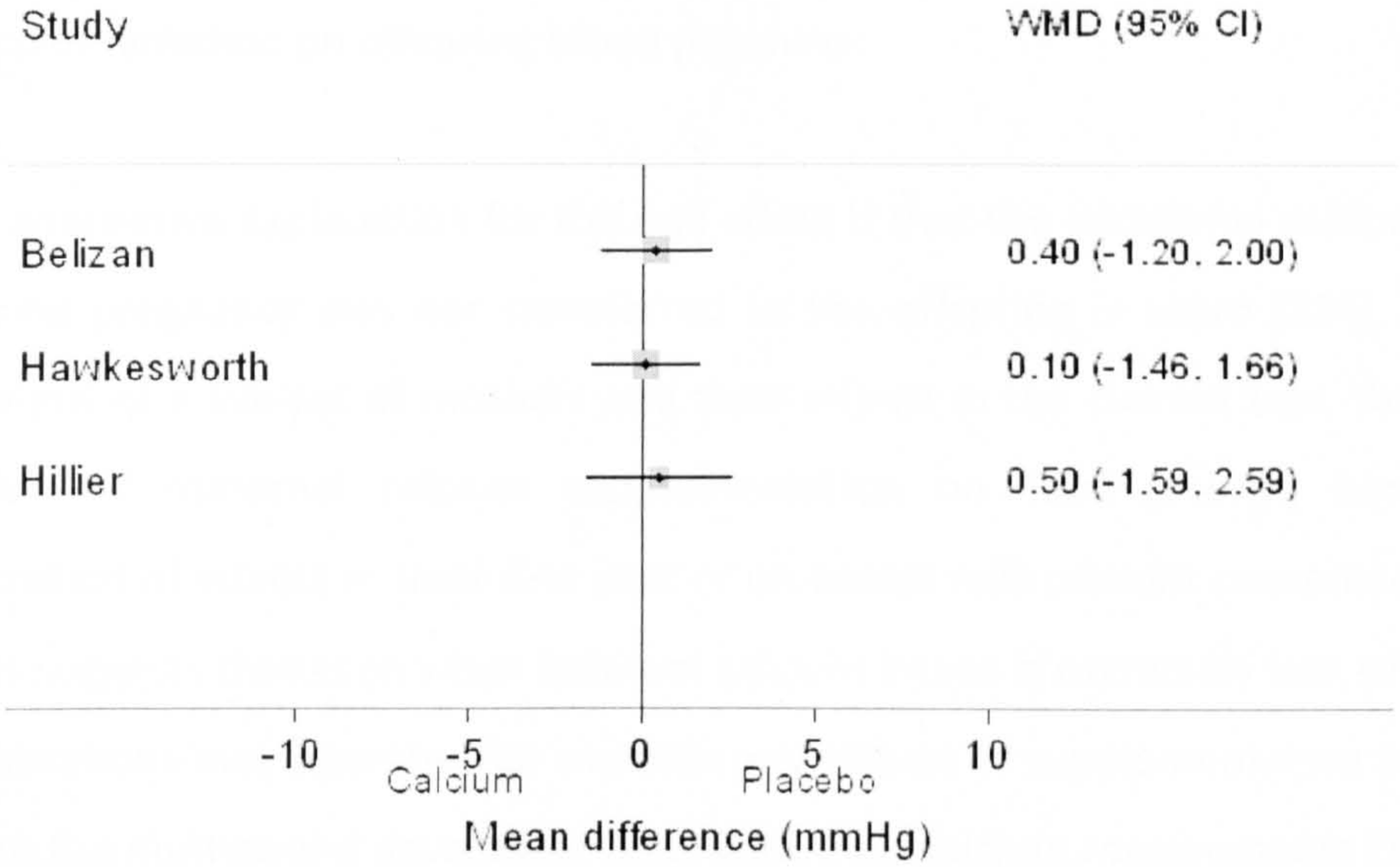
The four trials of calcium supplementation during pregnancy that have published findings on offspring blood pressure can be usefully combined into a forest plot to give a clearer indication of the effect of this exposure. A meta-analysis of the combined effect may not be appropriate however, due to the different ages of the trial offspring and the different concentrations of calcium supplement provided by the trials. Taken together, the current evidence suggests that maternal calcium supplementation during pregnancy does not affect offspring systolic (Figure 4.6) or diastolic (Figure 4.7) blood pressure.

Figure 4.6 Forest plot for the effect of maternal calcium supplementation during pregnancy on offspring systolic blood pressure



Points represent weighted mean difference (WMD) in systolic blood pressure for children born to women taking calcium compared to placebo supplements during pregnancy. Data are from unadjusted intention-to-treat analysis reported in the publications. No overall estimate of effect is included due to the different study designs of the trials and follow-up studies included.
Studies are Belizan *et al* [106] from Argentina, Hatton *et al* [107] from the US and Hillier *et al* [108] from Australia together with the current analysis (Hawkesworth).

Figure 4.7 Forest plot for the effect of maternal calcium supplementation during pregnancy on offspring diastolic blood pressure



Points represent weighted mean difference (WMD) in diastolic blood pressure for children born to women taking calcium compared to placebo supplements during pregnancy. Data are from unadjusted intention-to-treat analysis reported in the publications. No overall estimate of effect is included due to the different study designs of the trials and follow-up studies included.
Studies are Belizan *et al* [106] from Argentina, Hatton *et al* [107] from the US and Hillier *et al* [108] from Australia together with the current analysis (Hawkesworth).

4.5.1.5 Implications of findings

This data adds important findings to the question of the impact of maternal calcium supplementation on offspring blood pressure, benefitting from a high rate of recruitment and being conducted in an area of low habitual intake where it might be hypothesised that a greater impact would be observed. The findings suggest that the calcium content of the maternal diet during pregnancy does not affect offspring blood pressure, at least in this setting and this age group. It could be argued that a larger sample size would be necessary to corroborate this finding, as in order to detect a 2mmHg difference in blood pressure between the trial arms (assuming means and standard deviations similar to those reported in this study) an

intervention study would require a sample of 608 individuals. A pooled analysis of the available data was not considered appropriate for the studies that have been conducted as there is great heterogeneity in study design, especially in the age of subjects. However, the forest plots suggest there is no impact of maternal calcium supplementation on offspring blood pressure.

An alternative explanation for the null effect is that the additional calcium provided during pregnancy was not transferred to the offspring *in utero* [234, 235]. In an analysis of a sub-set of mothers and their infants in the current trial, there was no effect of maternal calcium supplementation on fetal growth, bone mineral accretion of infants in their first year or on breast milk calcium concentration [195]. This suggests that even when habitual calcium intake is extremely low, physiological adaptations may operate that override any effects of supplementation and provide both the mother and developing fetus with their calcium requirements [195].

4.5.2 Observational analysis

4.5.2.1 Summary of findings

Relative weight at one year of age and the crossing of weight SD scores from one year to the current study were positively associated with systolic blood pressure at 5-10 years. Neither anthropometry at two weeks of age (used as an indicator of birth weight) nor season of birth were associated with blood pressure at seven years of age in this group of individuals.

4.5.2.2 Study strengths and limitations

The study presented here was designed as a follow-up of a randomised controlled trial but it may also represent a useful resource with which to study these observational associations. The intervention was not demonstrated to have an effect on the exposures under investigation, such as birth weight, or on the outcome blood pressure and the data may thus be thought of as a cohort enrolled

at birth. Loss to follow-up was relatively low: 71% of individuals born into the study were recruited into the follow-up and loss to follow-up was not associated with the exposures under investigation (early-life anthropometry, season of birth) and is unlikely to be associated with the outcome of interest (blood pressure). An advantage of the data is the accuracy of the anthropometric measurements in infancy, which were conducted by trained fieldworkers and were adjusted for the actual age at measurement.

Similarly to the data in Chapter 3, the major limitation of using trial follow-up data for cohort analysis is a lack of data on well-characterised confounders. Although smoking prevalence is known to be low in this region [228], there is no data on other important confounders such as social economic status.

4.5.2.3 Comparison with the literature

A number of cohort studies have published data on the impact of early postnatal growth on later blood pressure, employing many different data analysis techniques and presenting conflicting results. The Collaborative Perinatal Project in the US reported that a change in weight SD score from birth to four months, four months to one year, one to four years and four to seven years were independently associated with increased odds of having high blood pressure (>90th percentile in the cohort) at seven years [116]. In the UK, reflecting similar findings to the Gambian data, growth (defined as weight SD score change) in the first year of life was unrelated to blood pressure at age 22 but growth from one to five years of age was positively associated with systolic and diastolic blood pressure at this age [119]. In contrast, data from Hong Kong suggests the opposite effect of growth to that normally observed; a decrease in ponderal index between six and eighteen months of age was associated with increased systolic blood pressure at 30 years [120].

A recent analysis of five cohorts from developing countries reported that higher than average weight gain between birth and four years was only associated with

early adult blood pressure because it predicted later body size, once later height and BMI were included in the analysis the association was no longer apparent [123]. With such heterogeneity of study designs, particularly the time points studied and the method of characterising growth, it is difficult to draw a conclusive picture from the literature of the association between early growth and later blood pressure.

4.5.2.4 Implications of findings

Similar to Chapter 3, the findings presented in this chapter suggest that childhood growth is a factor that influences blood pressure in later childhood. Interestingly, season of birth was not associated with blood pressure in the children under study here although it had been associated with blood pressure in the adolescent individuals presented in Chapter 3. This disparity of findings may be related to the smaller sample size involved in the analysis of the calcium cohort (350 compared to 1267). Alternatively it may be that in this region seasonal effects have lessened over time so that individual born during the protein-energy trial were exposed to greater variation than the more contemporary children from the calcium trial. An alternative explanation is that the seasonality effect was not seen because the calcium trial (unlike the protein-energy trial) included the core village of Keneba, which has been the recipient of the greatest amount of health care in the region and may thus be less susceptible to seasonal effects. However, repeating the analysis only on children born outside of Keneba only did not reveal an association between season of birth and later blood pressure, indicating that this is unlikely to be the explanation of the null effect.

Chapter 5 Bangladesh study methods

5.1 Forward

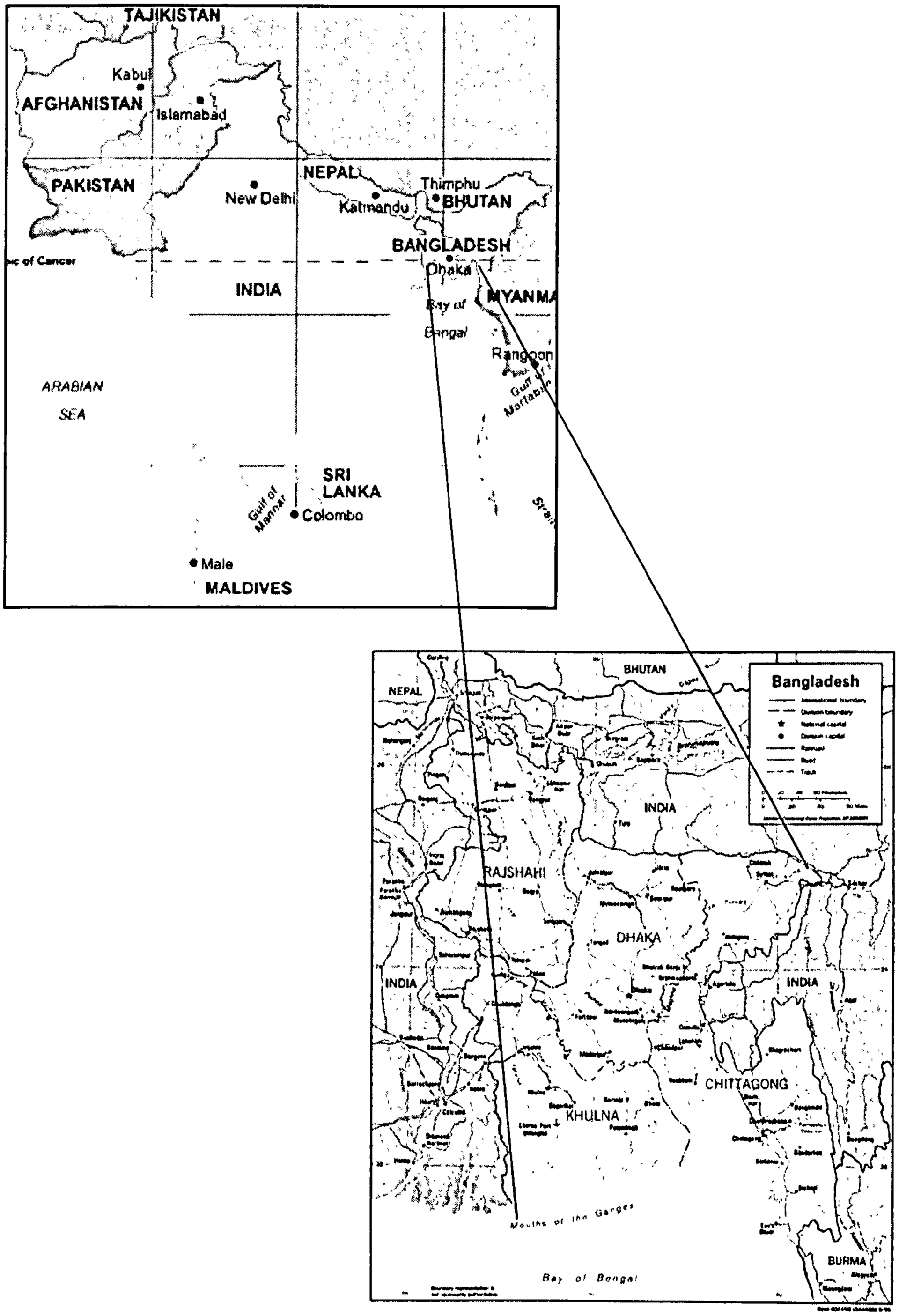
In 2006, the MRC ING was awarded an MRC Project Grant to collaborate in a follow-up study of a multiple micronutrient supplementation trial in Bangladesh. Partner institutions included the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) (PI: Dr Shams Arifeen) and Uppsala University, Sweden (PI: Professor Lars-Ake Persson). The collaborators allowed full access to data from the original trial, which is described briefly below. SH was employed full-time on this grant as a research assistant and subsequently a research fellow responsible for the implementation of the study in Bangladesh. SH was also registered as a PhD student throughout this period. The design, implementation and management of fieldwork insofar as they relate to this PhD will be outlined in this chapter and the results will be presented in Chapter 6.

5.2 Field site

5.2.1 Bangladesh

Bangladesh is situated in South Asia, bordering India and Myanmar at the head of the Bay of Bengal (Figure 5.1). It is the most densely populated major country in the world, having a population in mid 2007 of 158.6 million [236] residing in a country of 144,000 km^2 [237]. Dhaka is the capital city and is home to over 11 million people [237]. Bangladesh is located on the delta of three major rivers, the Ganges, Brahmaputra and Meghana and as a result much of the country experiences annual flooding during the seasonal monsoon rains. The official language is Bengali and the major religion is Islam (89%), with a Hindu minority of around 10% [237].

Figure 5.1 Location of Bangladesh in South Asia
(reproduced from Trinity College Dublin(regional map) [238] and Map Archive (Bangladesh map) [239])

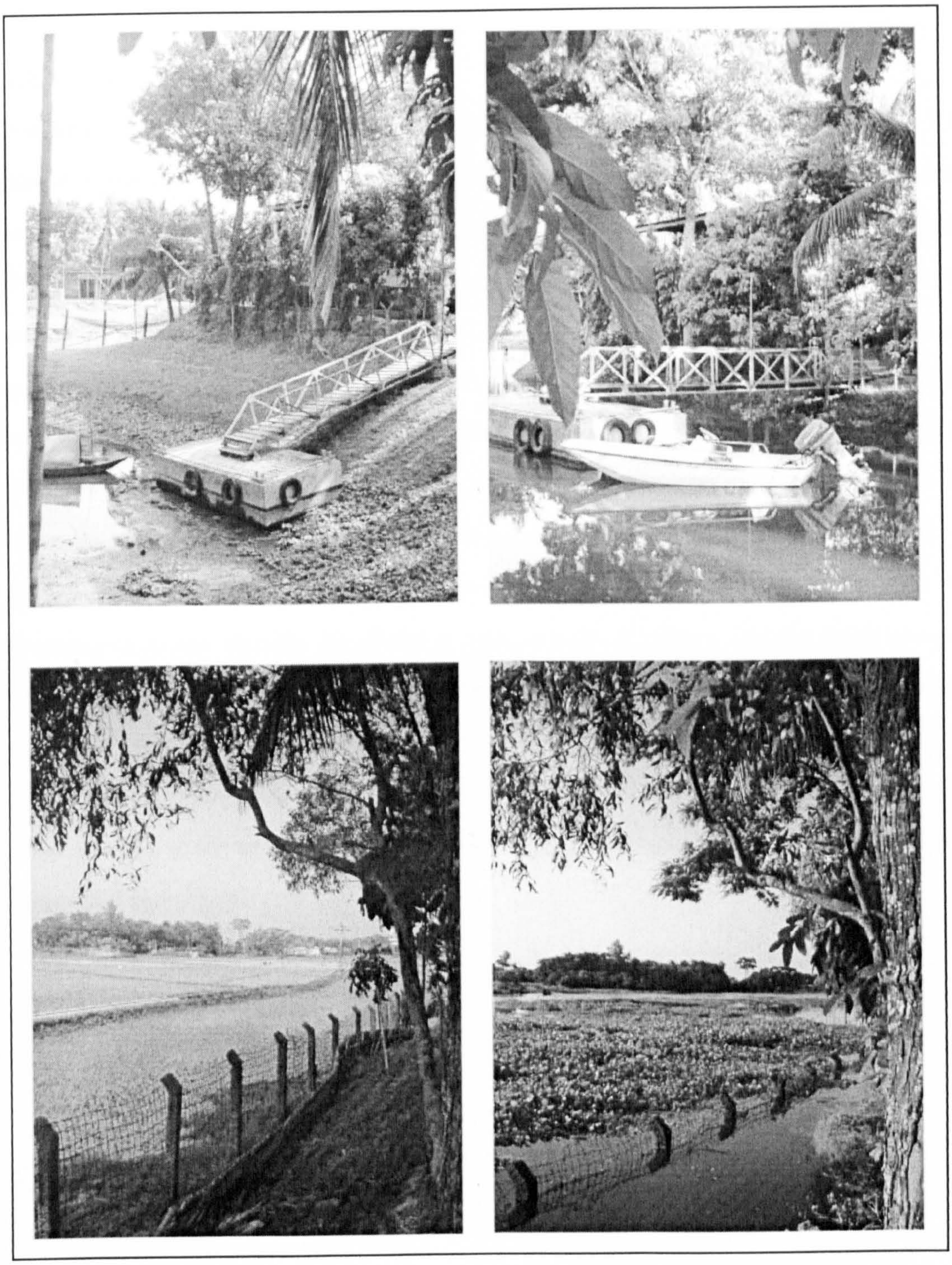


Bangladesh is lagging behind in economic development compared to its larger neighbour India, although annual economic growth and poverty indicators are slowly improving. World Bank statistics quote the Gross Domestic Product (GDP) per capita of Bangladesh in 2007 as US$42.7 compared to the Indian GDP/capita of $1042.5 [236]. Poverty and malnutrition remain widespread and there is an increasing gap between the rich and the poor. According to World Bank statistics, again for 2007, average life expectancy at birth is 64 years and infant mortality is 52/1000 live births with a much higher rate (85/1000 live births) in lower income groups [236].

5.2.2 Matlab

Matlab is a region situated around 55km south-east of Dhaka in the flood plains of Bangladesh. In common with the majority of the country, Matlab experiences a tropical climate with a hot, humid summer from March to June followed by the rains of the monsoon season which fall between July and October. The seasonal pattern of the rains is mirrored by changes in the water level and resulting changes in farming activities. During the winter season (November to March), the waters gradually retreat, revealing large tracts of cultivatable land on extremely fertile alluvial soil. The main harvest takes place during the summer months before the onset of the monsoon. Once the monsoon starts the water level rapidly rises covering the fields and preventing most forms of farming activity, although fishing thrives during this time (Figure 5.2). The local community is well adapted to the seasonal changing water levels, which rise and fall by an average of two to three metres each year. The houses and roads are all built above the level that the floods usually reach. The main crops grown in the region are rice (*Oryza sativa*), maize (*Zea mays*) and potatoes (*Solanum tuberosum*).

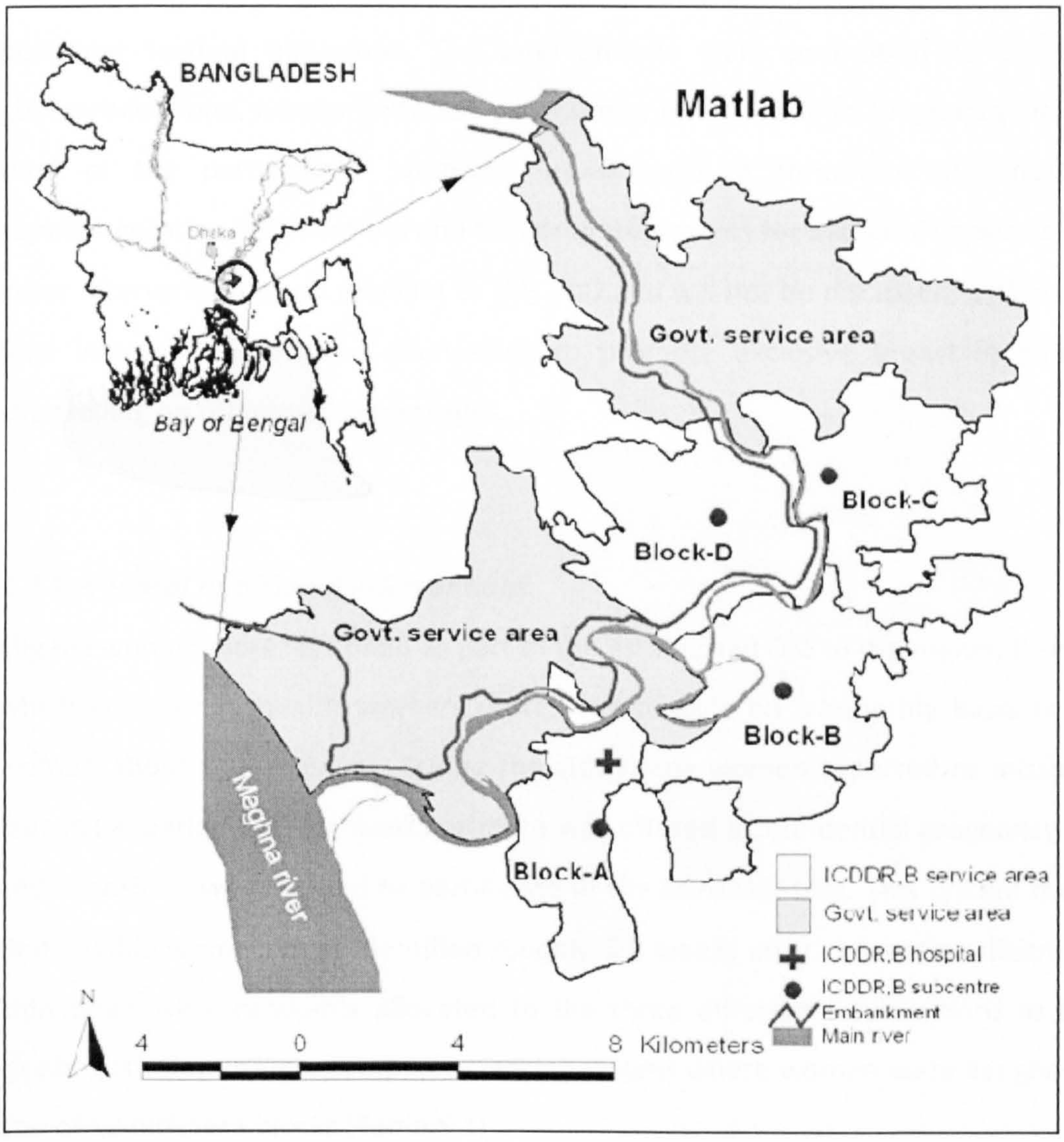
Figure 5.2 Annual change in water levels, Matlab Bangladesh



5.2.3 ICDDR,B Matlab

The International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) is a health research institution based in Dhaka. Since 1963, ICDDR,B has conducted medical research in the Matlab area basing their work around a health clinic. One of the consequences of this increased health service provision is a reduced rate of infant mortality, which is 30/1000 live births [240] compared to the national average of 52/1000 [236]. In addition to clinic activities and various field trials the centre has conducted a detailed demographic surveillance study (DSS) of the area since 1966, which is the largest longitudinal demographic data collection system in a developing country [240]. The DSS area covers sections of Matlab that receive health care provided by ICDDR,B (population of 113,128 in 2006) as well as contiguous areas outside of ICDDR,B care where residents receive government provided health care (population of 111,410 in 2006) [240]. The area of the DSS that receives medical care from ICDDR,B, and is included in any large-scale studies in the region, comprises four blocks (A,B, C and D) each of which has a small sub-centre that is used for outpatient care and as a base for some of the larger research projects (Figure 5.3).

Figure 5.3 The location of ICDDR,B demographic surveillance survey within the district of Matlab
(reproduced from ICDDR,B [240])



5.3 Original supplementation trial ("MINIMat")

The Maternal and Infant Nutrition Interventions, Matlab (MINIMat) trial was conducted by ICDDR,B between 2000 and 2004 in Matlab, Bangladesh. The study was designed to test community interventions aimed at improving birth outcomes and infant health. Three interventions targeted pregnancy and a fourth focused on postnatal feeding behaviour. The interventions were promotion to access a government food supplementation programme either early in pregnancy or at a time of the participants' choosing (usual care); a three-arm micronutrient supplementation intervention; and two drug treatments for bacterial vaginosis. The latter intervention is not relevant to this PhD and will not be discussed further. The final intervention involved counselling to promote exclusive breast feeding or counselling on other health messages.

5.3.1 Prenatal nutritional interventions

Eligible women were identified as part of the established DSS in the region, through which community health workers visited households on a monthly basis to ask women about their menses. During the study, any women reported as missing a menstrual period for two weeks or more was offered a confidential pregnancy test and if positive were invited to participate in the MINIMat trial. This system meant that eligible women were identified roughly 6-8 weeks after conception. Recruited individuals were randomly allocated to the three different interventions at 8-10 weeks gestation using a computer tracking system where women were assigned to one of 12 different blocks (Table 5.1).

Table 5.1 Randomisation to prenatal interventions in the MINIMat trial, Bangladesh

Food arm		Micronutrient arm					
		Tablet A		Tablet B		Tablet C	
	Intervention A	A1	A2	B1	B2	C1	C2
	Intervention B	D1	D2	E1	E2	F1	F2

The food supplementation arm of the MINIMat trial involved encouragement given to participants to attend government sponsored local community nutrition centres (CNC). These centres are part of the Bangladesh Integrated Nutrition Programme (BINP) and food is usually only available for pregnant and lactating women with a BMI <18.5kg/m^2 to commence at a time of their choosing; average enrolment in the program is 17 weeks gestation [241]. For the purposes of this intervention, all women were entitled to the supplement (irrespective of BMI) and were randomised to an early (8-10 weeks gestation) invitation to attend the CNC or to the usual invitation (at a time of their choosing). Women in the early arm of the intervention were given strong encouragement to enrol at the CNC as soon as possible. Once enrolled, women were eligible to receive the supplement throughout pregnancy and for two months post-partum. The food supplement was made from 80g of fried rice powder, 40g of fried pulse powder, 20g of molasses and 12ml of soybean oil and it provided 608kcal/day and 17.9g protein/day (6 days/week) [242]. On seven defined occasions during pregnancy, women were visited at home by research staff and asked about the consumption of food packets received from the CNC, this data was therefore available as an estimate of compliance.

The second nutritional intervention was micronutrient supplementation in tablet form. Women were randomised to three groups, either receiving 30mg of iron and 400µg of folic acid (Fe30F), the usual government provision of 60mg of iron and 400µg of folic acid (Fe60F), or a supplement containing the UNICEF/UNU/WHO preparation of 15 micronutrients at or above the recommended daily allowance (RDA) (Table 5.2). The tablets were identical for the three arms of the trial and were provided at 14-15 weeks gestation in containers of 200 tablets to last throughout pregnancy and for two months post-partum. The tablet containers carried equipment in the lid containing a counting device and small microprocessor (eDEM®); each time the pill-bottle was opened and closed, the time and date were recorded. At the end of the study these pill counts were aggregated and used as an indication of maternal compliance to the intervention.

Table 5.2 Nutritional content of the three micronutrient supplements provided during the MINIMat trial, Bangladesh

Supplement group		
Fe30F	Fe60F[a]	MuMs[b]
30mg Iron	60mg Iron	30mg Iron
400µg Folic acid	400µg Folic acid	400µg Folic acid
-	-	800µg RE vitamin A
-	-	200IU Vitamin D
-	-	10mg Vitamin E
-	-	70mg Vitamin C
-	-	1.4mg Vitamin B_1
-	-	1.4mg Vitamin B_2
-	-	18mg Niacin
-	-	1.9mg Vitamin B_6
-	-	2.6µg Vitamin B_{12}
-	-	15mg Zinc
-	-	2mg Copper
-	-	65µg Selenium
-	-	150µg Iodine

[a]Usual tablet composition provided to all pregnancy women in Bangladesh
[b]UNICEF/UNU/WHO recommended preparation

5.3.2 Postnatal intervention

At 30 weeks gestation, women were randomly assigned to the final intervention in the program. Participants were either allocated to receive counselling to promote exclusive breastfeeding or counselling on different health education messages in newborn care. There were seven counselling sessions, which all took place in the home: two occurred before delivery, one just after delivery and then again at 1, 2, 3 and 5 months postpartum. Breast feeding messages concentrated on emphasising the importance of exclusive breastfeeding for six months, whilst the control group

received more general health messages about newborn care. Counsellors were trained using WHO material modified from a previous intervention study in the region, and provided emotional and practical support to the participating women and family members [243].

5.3.3 Trial protocol and measurements

Women attended clinic visits for data collection at four time points during their pregnancy: baseline at 8-10 weeks gestation, then 14-15 weeks, 19-20 weeks and 30-31 weeks. After birth, both the participating women and the newborn children were seen each month until the child was six months old and every two months thereafter until they were one year old. Additional measurements were also taken during infancy, including thymic size assessment and child development indices. The main outcome measures of the original trial were fetal growth, birth weight and maternal haemoglobin and the publication of these findings is in progress. Table 5.3 summarises the measurements taken during the original trial. Only some of these measurements are of direct relevance to this PhD and are described in the sections below.

Table 5.3 Measurements conducted in the original MINIMat trial, Bangladesh

Stage	Measure	Time point
Pre-pregnancy	Maternal weight	Routine DSS surveillance
Pregnancy	Pregnancy identification: urine pregnancy test	6-8 wk gestation
	Fetal ultrasound: gestational age + growth	8-10, 14-15, 19-20 & 30-31 weeks gestation
	Maternal anthropometry (weight, height, MUAC) + blood pressure	
	Maternal urine collection	
	Maternal morbidity	
	Maternal venous blood collection	14-15 & 30-31 wk gest
	Maternal dietary intake (recall) and food security	8-10, 22-23 & 30-31 wk gestation
	Interviews on stress, violence, toxic exposures and work load	
Birth	Birth anthropometry (weight, length, head circumference)	Within 72h of birth
	Maternal and newborn health	
	Cord blood and placenta samples	
Infancy	Infant anthropometry (weight and length)	Monthly to 6 mo, then every two months until 24 mo
	Infant morbidity (diarrhoea, respiratory tract infections)	
	Infant feeding (recall)	
	Infant thymus size by ultrasound	1, 8, 24 & 52 weeks
	Infant blood sample	6 months
	Breast milk samples	8, 24, 52 weeks
	Infant motor milestone assessment	Monthly from 3-12 mo
	Infant psycho-motor assessment	7 months
	Infant language assessment	12 months
	Maternal blood sample	12 months
	Maternal anthropometry (weight)	2, 6 & 12 mo post-partum
Childhood 1-3y	Child anthropometry (weight and height)	15, 18, 21 & 24 months
	Child dietary intake (recall)	
	Child morbidity (diarrhoea and respiratory tract infections)	
	Child motor milestone assessment	15 & 18 months
	Child psycho-motor assessment	18 months
	Child language assessment	18 months
	Maternal anthropometry (weight)	24 months

5.3.3.1 Prenatal measurements

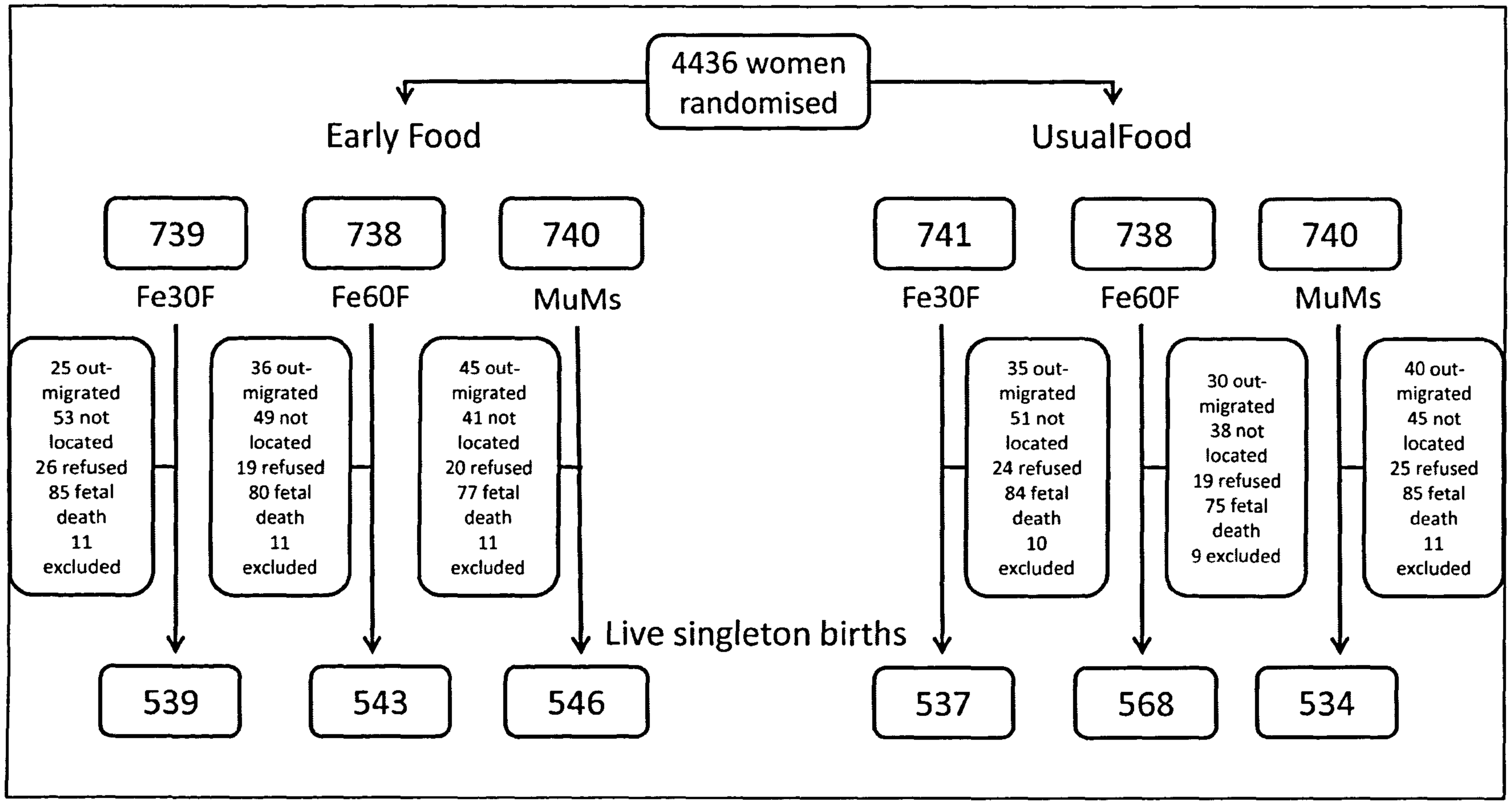
Maternal weight and height were measured at the baseline clinic visit (8-10 weeks gestation) by trained fieldworkers, and additional weight measurements were made throughout pregnancy. Weight was measured on electronic scales (UNISCALE) to the nearest 100g. Data on maternal age were available from the ongoing DSS as was information on parity. Detailed interviews were conducted at baseline to collect data on household and personal assets owned and on level of parental education.

Venus blood samples were collected at two different time points during pregnancy; 14-15 weeks (baseline for micronutrient arm) and 30-31 weeks gestation. At these time points, haemoglobin was assessed on all women in the field using a haemocue®. Maternal blood pressure was measured at four time points in pregnancy (8-10, 14-15, 19-20 and 30-31 weeks gestation); at these visits a nurse made a single assessment of systolic and diastolic blood pressure using a mercury sphygmomanometer.

5.3.3.2 Postnatal measurements

In total, 4436 women were randomised to take part in the MINIMat trial and between May 2002 and June 2004 there were 3267 live singleton births recorded (Figure 5.4). Anthropometric measurements were conducted on the infants within 72 hours of birth, either at the subject's home or the subcentre. Birth weight, length, head circumference and knee-heel length were measured at this time by fieldworkers. Infant weight and length were also measured every month after birth until the child was six months of age and thereafter every two months until they were twelve months of age. Up to six months of age, all infant weights (including birth weight) were measured by beam scales, which are accurate to 10g. All maternal weights and infant weights beyond six months of age were measured with electronic scales (UNISCALE), accurate to 100g. Locally manufactured, collapsible length boards, which are precise to 1mm, were used to measure the recumbent length of the infant.

Figure 5.4 Flow diagram of women enrolled into the original MINIMat trial and their subsequent offspring



Early and usual food represent food invitation intervention arms. Fe30F represents women randomised to receive 30mg iron and 400µg folic acid; Fe60F represents women randomised to receive 60mg iron and 400µg folic acid; MuMs represents women randomised to receive a supplement containing 15 micronutrients

In addition to measurements of infant anthropometry, the post-partum visits also involved the administration of a questionnaire to assess infant feeding practices both using a 24h recall and questions relating to feeding during the previous seven days. This self-report of breast feeding practices was found to have a good level of accuracy when compared to infant breast milk consumption measured by the dose-to-mother deuterium dilution technique in a sub-sample of the participating mother-infant pairs [244].

5.4 Follow-up study, methodology

5.4.1 Subjects

A detailed follow-up of the children born during the MINIMat trial was conducted between May 2007 and February 2009, when they were 4.5 years old. The follow-up study was coordinated by ICDDR,B with funding and expertise also provided by Uppsala University and LSHTM. All of the 3267 individuals comprising the live singleton births during the original trial were eligible for recruitment. Scientific approval was granted by the Scientific Research Committee of ICDDR,B and ethical approval was provided by the ICDDR,B Ethical Research Committee as well as the ethical committees of the participating universities. Full informed consent was obtained from the parents or guardians of the children prior to enrolment in the study.

5.4.2 Study protocol

Children were invited to participate within two weeks of reaching 4.5 years old, although if missed at this time point they were invited again at a later date. Fieldworkers collected the children and their mother, or guardian, early in the morning to bring them to the study sub-centre in a fasted state. Clinic visits were conducted in each of the four sub-centers of the Matlab study area, with additional questionnaires administered during a home visit. Two teams of fieldworkers, supervised by a medical doctor, conducted the clinic visits and alternated on a

weekly basis which clinics they were located in. Children born during the first half of the MINIMat trial (May 2002 – mid June 2003) comprised 'Group A' and participated in a reduced protocol, whilst the remaining children (Group B: June 2003 – June 2004) participated in a protocol that required two clinic visits (21 days apart) with a larger number of assessments at each (Table 5.4). It was not possible to conduct the detailed protocol on all offspring due to the costs involved; children born in the latter half of the trial were chosen for the detailed study as they had also participated in a detailed study at one year old which included assessment of thymus size amongst other outcomes.

Table 5.4 Assessments conducted during MINIMat 4.5 follow-up relating to current thesis

	Group A	Group B
Anthropometry	X	X
Blood pressure	X	X (twice[a])
Cystatin c	X	X (sub-sample only)
Kidney size	-	X
Maternal sitting ht	-	X

[a]Conducted on two separate occasions, 21 days apart

Eligible individuals were identified from their unique DSS number and location details, and because they had been extensively followed-up since birth and were therefore well known to the fieldworkers. Recorded data were checked by the Medical Officer at the end of each visit and sent to Dhaka to be entered by ICDDR,B staff at the data records office there.

5.4.3 Measurements

5.4.3.1 Blood pressure

Blood pressure was measured in triplicate using an automated oscillometric device (Omron 705IT, Morton Medical Ltd), with the cuff placed on the right arm and the arm resting on a table at chest height. The first measurement was taken after the

subject had been seated at rest for five minutes and there was one minute between each subsequent measurement. Group B children were seen on two separate occasions, 21 days apart, to gain a more accurate estimate of the habitual blood pressure of the subject with blood pressure assessed in the same manner.

5.4.3.2 Anthropometry, body composition and morbidity

Anthropometric measurements included height, weight, skinfold thickness, mid-upper arm circumference and head circumference. Measurements were taken by trained nurses and only four individuals held this post during the study period. Height was measured to the nearest 0.1cm with the subject wearing no shoes using a daily-calibrated Seca Leicester stadiometer (Chasmors Ltd). Weight was measured on a daily-calibrated digital scale (Tanita, Chasmors Ltd) to the nearest 0.1kg, again with the subject wearing no shoes. Four skinfold thickness sites were assessed using Holtain callipers (Chasmors Ltd): triceps, biceps, superilliac and sub-scapular sites were all measured in triplicate to the nearest 1mm.

Total body composition was assessed using a foot-to-foot bioelectrical impedance analyser (Tanita TBF-300MA, Chasmors Ltd). Subjects stood on the scale with bare feet after being asked to void their urine. The analyser produces fat mass and fat-free mass values that are based on inbuilt equations derived in Caucasian populations and for use in children over seven years old. A validation study was conducted using deuterium dilution as a reference method to assess the accuracy of the analyser in this population. However, the laboratory results are still being analysed and in the meantime the raw impedance data has been used (see Section 5.5.1), as this provides a proxy measure of lean mass that may be more accurate than the inbuilt equations in this setting.

For children in group B only, maternal sitting height was recorded using the same stadiometer as for child height but with the stadiometer placed on the seat of a hard bench against a wall. Mothers were asked to sit on the base of the

stadiometer with as much as possible of their thighs supported and height measured in the standard way.

When children first arrived at the subcentre their parents were asked general health questions including whether they were feeling well on the study day and if the children had experienced diarrhoeal episodes in the preceding two weeks. Both of these were thought to have potential implications for blood pressure measurement on the study day and were therefore used in the analysis.

5.4.3.3 Kidney function

Plasma Cystatin C (CysC) is a non-glycosylated protein produced in lysosomes and cleared exclusively by the kidneys [159]. It has recently been proposed as an estimate of glomerular filtration rate that may be more accurate than the more commonly used creatinine [245]. It has been suggested that it may be particularly useful in studies involving children where creatinine is thought to be less accurate due to the variations in body size and muscle mass [159]. In a recent study of Colombian children CysC was found to have greater specificity and sensitivity than serum creatinine, leading the authors to conclude that CysC was a better indicator of kidney function in children [159]. In the current follow-up study, fasted blood samples were collected from participating children using lithium heparin treated collection tubes for later analysis of a number of biomarkers. Blood samples were transported to the Matlab laboratory within two hours of collection for separation of plasma by centrifugation at 3000rpm for five minutes. Plasma aliquots were then separated and frozen in a -70°C freezer. At the end of the study, frozen plasma aliquots were transported to the hospital laboratory in Uppsala, Sweden where CysC was analysed using the immunoturbidimetric analysis described by Flodin *et al* [246].

Group B children took part in a more detailed protocol at the clinic visits, including sonographic assessment of the size of the kidney, liver and spleen (Figure 5.5 and 5.6). Ultrasonography was conducted using a convex scanner (Toshiba SSA 320A

Justavision-200, Toshiba Medical Systems, Japan) at a frequency of 3.5Mhz by two highly skilled medical doctors who had undergone extensive training and standardisation. Single measurements of kidney size (length, width and depth) and two alternative methods of assessing volume were conducted on each participant in the vertical dorsal view. Assessments were made on the right kidney first, followed by the left. Length was measured as the long axis of the kidney, taking a line that passed through the hilus (Figure 5.7). In the same view, width was measured as the shortest axis of the kidney. From the length measurement position, the probe was turned 90° anti-clockwise and the diameter of the largest round section was measured to obtain an estimate of depth. Using the measurements of length, width and depth obtained, the internal software of the ultrasound machine provided an estimate of kidney volume. As an alternative estimate of volume the ellipsoid function was also used; in the length view the sonographer placed an ellipsoid shape around the kidney and adjusted the size to best fit the kidney shape (Figure 5.7). Again the internal software used these dimensions to estimate kidney volume.

Figure 5.5 Medical officer conducting ultrasound measurements

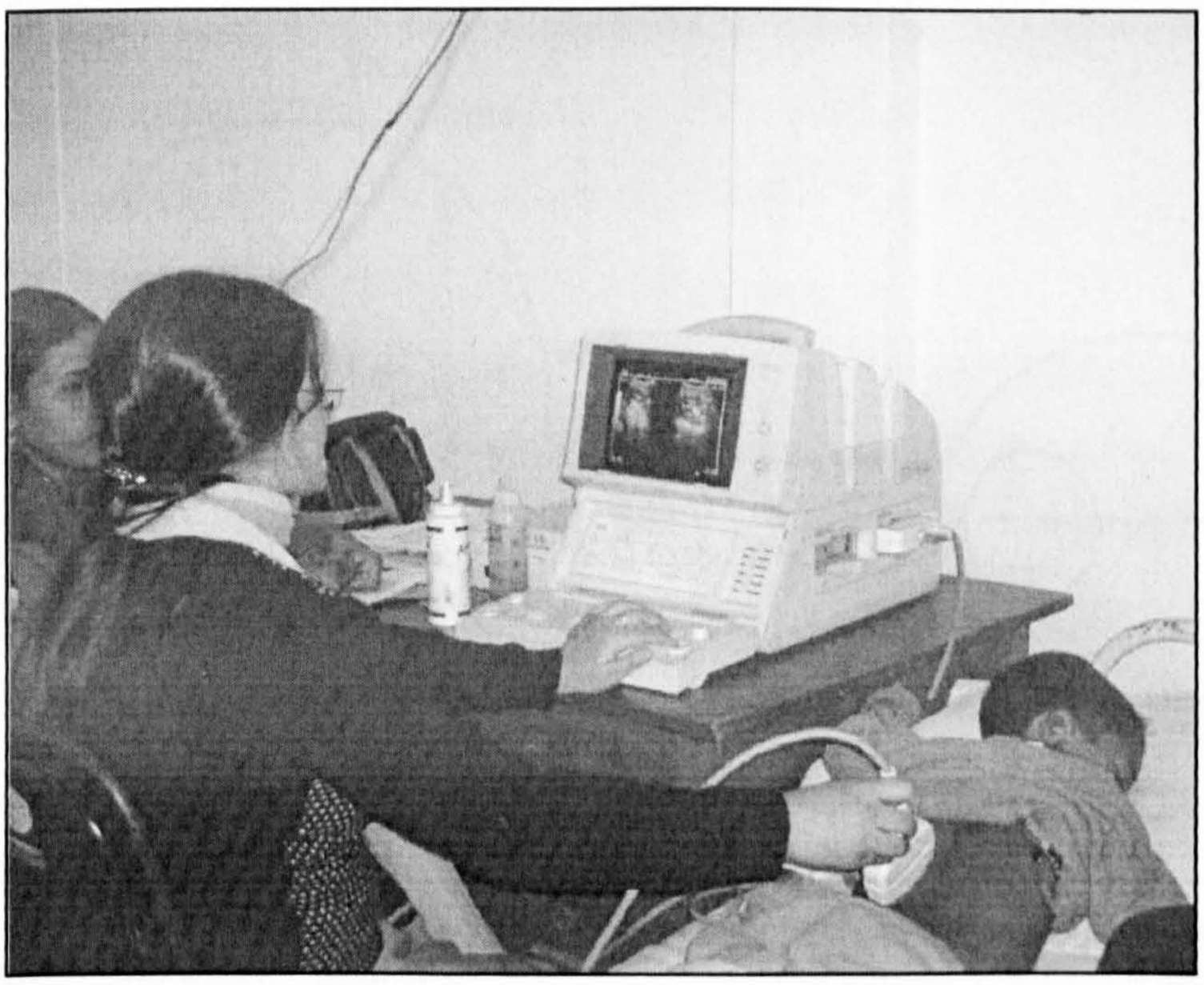


Figure 5.6 Kidney ultrasound image taken during MINIMat 4.5 follow-up

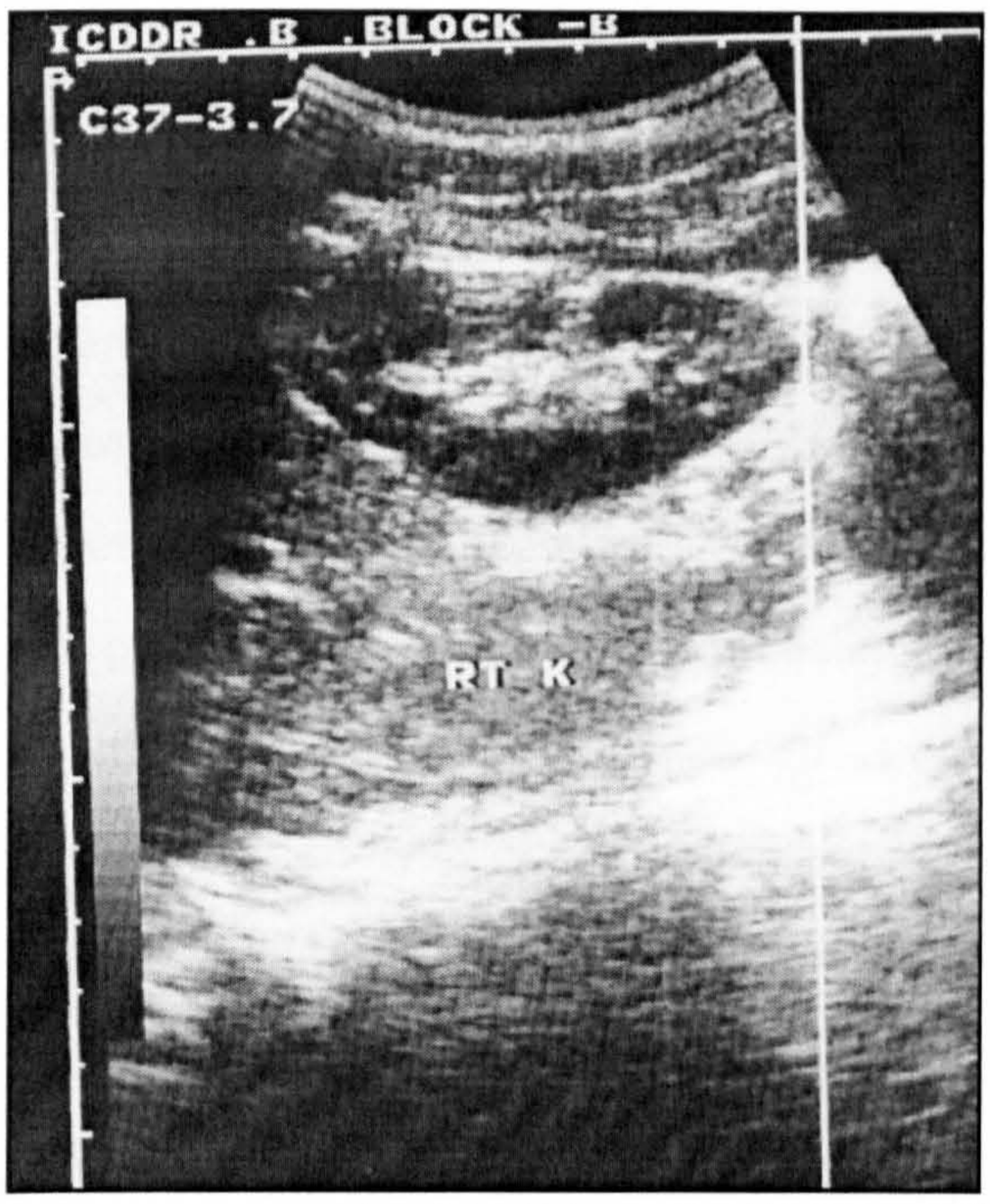
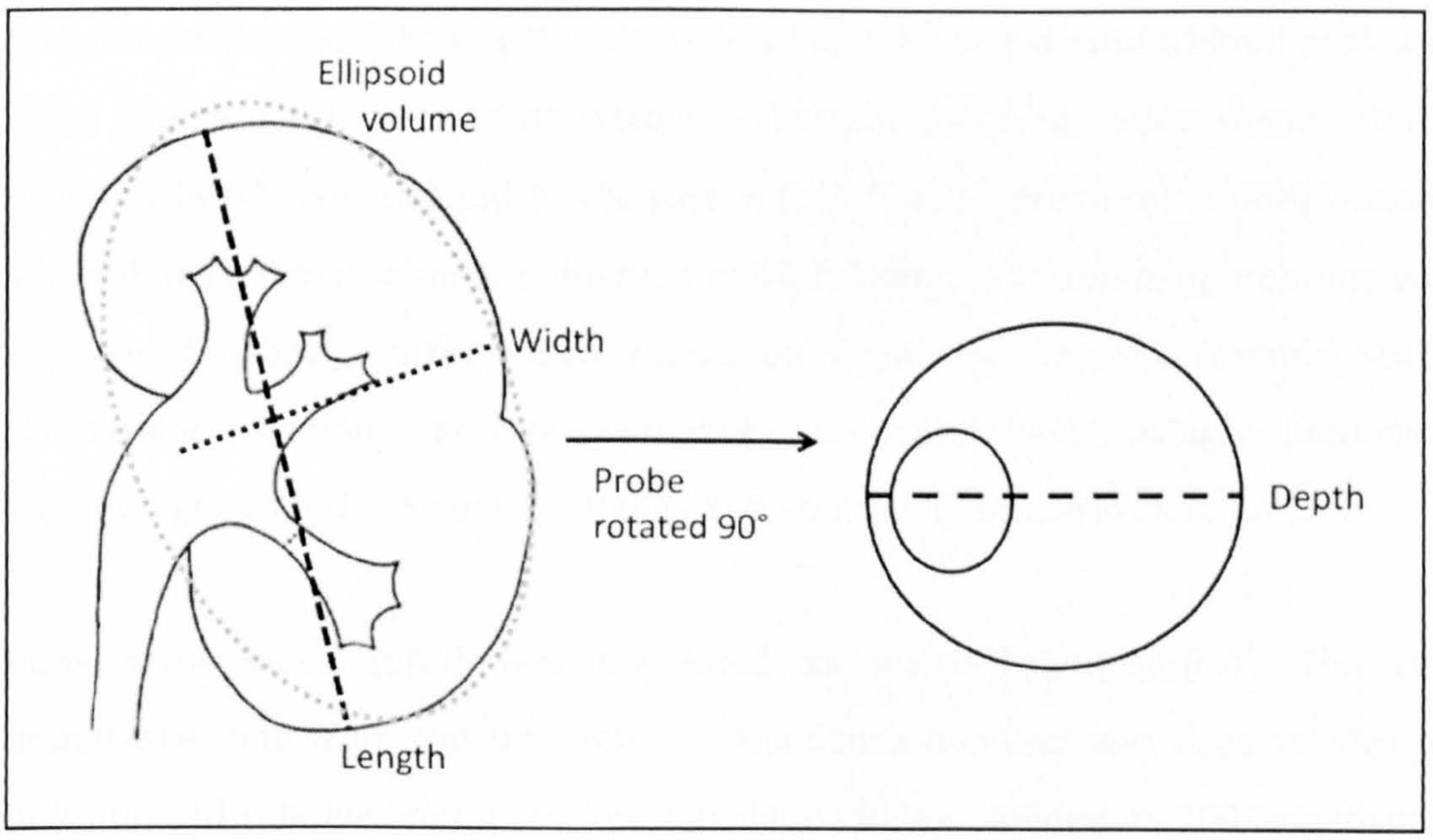


Image represents the right kidney of a MINIMat follow-up subject: length, width and ellipsoid volume were measured in this view

Figure 5.7 Diagram of kidney dimension measurement by ultrasound in MINIMat trial follow-up



Schematic representation of the different kidney size measurements conducted: length, width, depth and ellipsoid volume

5.5 Statistical analysis

All statistical analysis was conducted using Stata 10 (Stata Corporation, College Station, Texas, USA). The effects of the prenatal interventions were assessed by intention-to-treat analysis and then by an as-treated analysis. Both analyses focused on the effect of food and micronutrient supplementation separately and together, on blood pressure and then on kidney function outcomes. Analysis of the effect of randomisation to counselling for exclusive breast feeding or for other health messages was also assessed as an intention-to-treat followed by as-treated analysis. An analysis of the observational data of the association between early-life factors and later blood pressure and kidney function was also conducted. All of the analysis was restricted to infants born at term (>37 weeks gestation) as assessed by the timing of their mothers' last menstrual period (LMP method).

5.5.1 Data manipulation

Outcome variables and continuous covariates were assessed for the normality of their distribution using histograms and quantile-quantile plots. The mean of three measurements was taken as the estimate of systolic and diastolic blood pressure. Pulse pressure was defined as systolic – diastolic pressure, whilst mean arterial pressure (MAP) was defined as diastolic + (1/3 * pulse pressure). Kidney volume was defined as total volume of the right and left kidney, and where appropriate was adjusted for body surface area calculated from the Haycock formula [247]. Glomerular filtration rate was calculated from plasma CysC using a prediction equation generated in Swedish patients and applicable for use in children [248].

Body mass index (BMI) was calculated as weight(kg)/height(m)^2. The raw impedance data from the bioelectrical impedance analyser was used to give an indication of relative lean mass. An impedance index, defined as 100/impedance, was created as this has been shown by Wells *et al* to have a good correlation with lean body mass assessed by the four component model [249]. Skinfold measurements were used to give an indication of body fat, again retaining the data

in their raw form rather than using prediction equations. For each site (biceps, triceps, subscapular and suprailiac) the three measurements were combined into an average value. Body fat was then calculated as the sum of the four skinfold sites, whilst relative trunk fat was calculated as the ratio of central skinfold thickness (subscapular + suprailiac) to limb skinfold thickness (biceps + triceps).

Socio-economic status (SES) was assessed by the creation of a wealth index using principal component analysis in a method described by Filmer and Prichett [250]. Variables included in the index were land, construction materials of house walls, ownership of household assets, numbers of sarees or shalwer-kameez owned for ceremonial use and pairs of shoes or sandals owned. The explanation of these variables and a more detailed description of the creation of the wealth index in this population is given by Saha *et al* [251]. The wealth index score (ranging from -6 to +4) was kept as a continuous variable for this analysis.

5.5.2 Intention-to-treat analysis

Independent t-tests were used to assess any differences in characteristics from the original trial between those recruited and those lost to follow-up. Linear regression was used to investigate the effect of the maternal interventions on offspring blood pressure and kidney function. Variables that were potential correlates of blood pressure and kidney function were assessed by simple linear regression analysis and those that were associated were included in the adjusted regression models. There were three stages of regression models used to investigate the impact of the maternal interventions: unadjusted (model 1), adjusted for covariates unrelated to the maternal intervention but related to blood pressure/kidney function (model 2), as model 2 but additionally adjusted for height, BMI, impedance index, body fat, morbidity on the study day and the reporting of diarrhoea in past 2 weeks (model 3).

The analysis was firstly conducted to investigate the impact of the food intervention, with the variable divided into early or usual start of food

supplementation. The micronutrient intervention was assessed in two different ways; comparing multiple micronutrient supplementation (MuMs) to iron and folate supplementation and comparing high iron dose (60mg) with low iron dose (30mg) (Table 5.5). This method of analysis was chosen as the most effective method of answering two important research questions: is there an impact of multiple micronutrient supplementation and is there an impact of providing a high iron dose. The linear regression models were fitted with both terms but the coefficients are reported separately in the results.

Table 5.5 Categorising micronutrient intervention from MINIMat trial for analysis

Original rancomisation code[a]	New code for analysis[b] Multiple micronutrients (MuMs)	High iron dose (highFe)
Fe30F	0	0
Fe60F	0	1
MuMs	1	0

[a]Original micronutrient arm of the intervention. Fe30F represents women randomised to receive 30mg iron and 400µg folic acid; Fe60F represents women randomised to receive 60mg iron and 400µg folic acid; MuMs represents women randomised to receive 15 multiple micronutrients (of which iron concentration was 30mg)
[b]Variable is recoded to represent multiple micronutrients or high iron dose: individuals coded 0 ('control') or 1 (receiving intervention).

The combined effect of the food and multiple micronutrient interventions was assessed by fitting interaction terms between the food and MuMs interventions (food*MuMs) and between the food and iron interventions (food*highFe) together in a model. The final analysis investigated the impact of counselling for exclusive breast feeding, which was fitted as a binary variable again using linear regression analysis and investigating the impact on offspring blood pressure and kidney function via the same models as described above.

Children were defined as having high blood pressure if their systolic blood pressure was above the 95th centile for their age and height [12]. The impact of the three

interventions on the odds of having high blood pressure was assessed using logistic regression adjusted for the same covariates as above.

Interactions

For each intervention (food, micronutrient and breast feeding counselling) it was also necessary to model potential interactions with co-variates to assess if the effects of the interventions were modified by other factors. Interaction terms were added in model 2 in a sequential manner to investigate any modification of the intervention effect with sex, SES, maternal baseline blood pressure, maternal baseline BMI and offspring body composition (height, impedance index, body fat and BMI). If an interaction term was found to be significant the relationship was investigated graphically to assess how biologically meaningful the association was likely to be.

5.5.3 As-treated analysis

The number of food packets consumed by participating women was available from home visits conducted at weeks 14, 18, 19, 22, 26, 30 and 34 of gestation. The total number of packets consumed during pregnancy was calculated from this data based on maternal recall at each visit. A linear regression analysis was conducted to investigate the association between total food packets consumed and offspring blood pressure with a separate analysis to investigate any effect on offspring kidney function. The as-treated analysis was conducted using the same models (1-3) as the intention-to-treat analysis described above.

For the micronutrient intervention the total number of tablets consumed was available from the pill count provided by the eDEM® lid technology. Although women had been provided with micronutrient tablets that lasted for two months post partum the level of encouragement they had been given to take the tablets after birth was unclear. It therefore did not seem appropriate to define compliance as observed/expected tablet consumption. Instead, the effect of tablet consumption (irrespective of randomisation) on later blood pressure was modelled

by linear regression analysis using total pill consumption as the exposure variable. The effect of the micronutrient dose (multiple micronutrient tablets compared to iron and folate only or high compared to low iron tablets) was then included in the same model by fitting an interaction between the number of tablets consumed and the randomisation variable. Again, the analysis was conducted first as an unadjusted model and then adjusting the model for co-variates related to blood pressure and finally adjusting for body size and composition.

Data on breast feeding practices and on the provision of other foods and liquids to the child were available from monthly interviews conducted until the children were six months of age and bimonthly from six to twelve months. At each visit, researchers asked about feeding practices in the preceding month in 15 day intervals. Breast feeding was then categorised in line with WHO recommendations into exclusive breast feeding (breast milk only), predominant breast feeding (breast milk plus other liquids including water and juice) and partial breast feeding (breast milk plus foods and other milks) [252]. The as-treated analysis utilised this categorical variable on infant feeding practices as the exposure variable and different models (unadjusted and adjusted) were fitted as before. Breast feeding practices changed markedly between four and size months of age, falling from 60% to 30% of women reporting to be exclusively breast feeding. Thus the as-treated analysis of the effect of different infant feeding types was conducted for both of these time points separately.

5.5.4 Observational analysis

5.5.4.1 Maternal growth in childhood

Maternal leg length can be used as a proxy indicator of the mother's own nutritional exposures in early-life as leg growth is affected to a much greater degree than trunk length [253]. Maternal leg length was calculated as height–sitting height and the association between maternal leg length and offspring blood pressure and kidney function was assessed using linear regression analysis. Firstly an unadjusted

analysis was conducted, followed by analysis adjusting for variables associated with the outcome of interest.

5.5.4.2 Maternal micronutrient status

The only maternal micronutrient status data that was available on the majority of women was haemoglobin concentration, as this had been assessed in the field at weeks 14 and 30 gestation. The association between maternal haemoglobin concentration in pregnancy, separately for each time point, and offspring blood pressure and kidney function was assessed using linear regression analysis. Firstly an unadjusted analysis was conducted followed by analysis adjusted for factors associated with current blood pressure and kidney function. As the iron intervention may have directly impacted on maternal haemoglobin by week 30 gestation, an interaction between the intervention and maternal haemoglobin on offspring blood pressure and kidney function was assessed for this time point by fitting an interaction term. An additional analysis was conducted to investigate the association between anaemia during pregnancy (defined as haemoglobin <11g/dl) and offspring blood pressure and kidney function. Again this analysis was conducted for both time points (week 14 and week 30) separately.

5.5.4.3 Birth anthropometry

The association between birth weight and length and later blood pressure and kidney function was assessed using linear regression analysis. Two models were fitted, with the first adjusted for confounders (age, sex, SES and maternal blood pressure) and the second also adjusted for current weight or current height.

5.5.4.4 Season of birth

A further analysis was conducted to investigate the effect of season of birth on blood pressure and kidney function in childhood. Season of birth was defined by fitting Fourier terms (sin, cos, sin^2, cos^2) in the same manner as for the analysis of the Gambian data (Chapter 2). Linear regression models were fitted with factors relating to current blood pressure and this model was then compared, using a likelihood ratio test, to one also containing season of birth terms. This analysis was repeated for the association of season of birth and child kidney function.

5.5.4.5 Growth in infancy

Anthropometric data on infant weights and lengths were available monthly from birth until the child was six months old and every two months until they were two years old. Weight and length standard deviation scores (SD scores) were calculated in comparison to the WHO reference data [254]. Due to the large number of data it was felt more appropriate to restrict the analysis to two time points. Growth was therefore defined as the change in weight and length SD score from birth to one year of age and from one to two years of age. The impact of this early growth pattern on blood pressure at 4.5 years was assessed using linear regression analysis adjusted for confounders. In addition, the association between the more complete growth data and later blood pressure was visualised by using life course plots [121].

A similar analysis was conducted to investigate the impact of early growth on later kidney function. Any relationship between growth and kidney volume was explored with the outcome variable unadjusted for current body surface area as this was would be closely related to early size and to growth.

Chapter 6 Follow-up of maternal food, micronutrient and breast feeding interventions

6.1 Forward

This Chapter presents the analysis of the effect of three interventions during pregnancy and lactation on offspring blood pressure and kidney function at 4.5 years of age in rural Bangladesh. The interventions were: an invitation to enroll in a food supplementation programme early in pregnancy or at the usual time (around 17 weeks gestation); a tablet-based multiple micronutrient or iron and folate supplement; and randomisation to receive counselling for exclusive breast feeding or for other health messages. A review of the statistical methods has been covered in Section 5.5 and should be referred to when interpreting the analysis. Regression coefficients appear as β in the text, standard deviations are denoted by SD, the chi-squared test statistic by χ^2 and confidence intervals by CI.

6.2 Study participants

6.2.1 General characteristics

The follow-up study successfully recruited 2526 children, representing 77% of the live, singleton individuals born during the trial (Figure 6.1). Recruitment rates were similar across the six supplement arms of the trial, and the reasons for loss to follow-up were also similar in their distribution. The main cause of loss to follow-up was individuals who could not be located during the follow-up study, accounting for 39% of the losses. Refusal by the parents or guardians of the children, primarily due to concerns about the taking of blood, accounted for 28% of losses. Out-migration (between birth and the follow-up) accounted for 22% of the losses and the further 11% of individuals had died in the period between birth and 4.5 years of age.

Figure 6.1 Flow diagram of offspring born during the MINIMat trial and those recruited into the current follow-up study

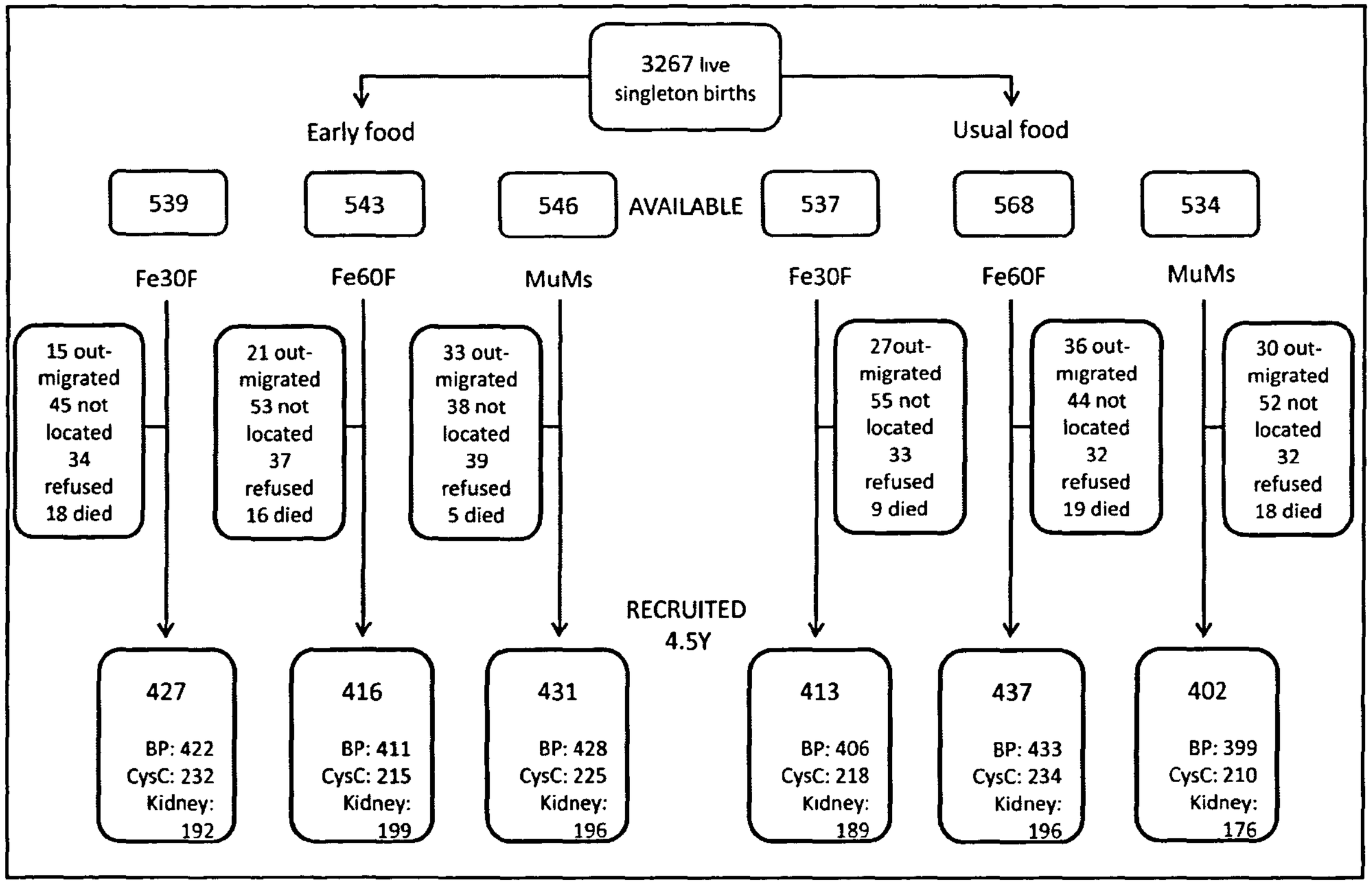


Diagram represents the flow of trial subjects from birth to the current follow-up study at 4.5 years of age.
Early food: maternal randomisation to access food early in pregnancy; Usual food: maternal randomisation to access food at the usual time in pregnancy
Fe30F: maternal randomisation to receive 30mg iron and 400µg folate; Fe60F: maternal randomisation to receive 60mg iron and 400µ folate; MuMs maternal randomisation to receive multiple micronutrient tablets. BP: number of individuals recruited for whom blood pressure was measured; CysC: individuals with Cystatin C measurements; kidney: individuals with kidney size measurements via ultrasound

Table 6.1 Difference between individuals recruited into the MINIMat trial follow-up and those lost to follow-up

	Early food			Usual food			All recruited[a]	Lost to follow-up
	Fe30F	Fe60F	MuMs	Fe30F	Fe60F	MuMs		
N	427	416	431	413	437	402	2526	1910
Maternal age	26.4	26.7	27.1	26.9	26.5	26.2	26.6	25.8[#n-14]
(years)	(5.8)	(6.0)	(6.3)	(6.0)	(6.0)	(5.7)	(6.0)	(5.9)
Maternal height	149.6[#n-1]	150.1	150.0	150.0	149.8[#n-3]	149.7	149.9[#n-4]	149.7[#n-1]
(cm)	(5.7)	(5.1)	(5.2)	(5.4)	(5.0)	(5.4)	(85.3)	(5.4)
Maternal weight	45.13[#n-3]	45.48	45.12	45.15	45.24[#n-2]	45.44[#n-3]	45.26[#n-8]	45.54[#n-1]
(kg), wk 8 gestation	(6.73)	(7.03)	(6.70)	(7.26)	(6.8)	(6.38)	(6.82)	(7.04)
Maternal BMI	20.15[#n-3]	20.18	20.04	20.01	20.15[#n-3]	20.26[#n-3]	20.13[#n-9]	20.30[#n-2]
(kg/m^2)	(2.63)	(2.80)	(2.67)	(2.72)	(2.73)	(2.44)	(2.67)	(2.71)
% women in low;	36.4;	35.8;	39.9;	40.2;	32.8;	34.3;	36.6;	37.2;
high third of SBP	21.8[#n-1]	25.2[#n-3]	24.4	25.8[#n-2]	22.6[#n-4]	26.0[#n-2]	24.3[#n-12]	24.0[#n-3]
Wealth index	-0.4[#n-18]	0.02[#n-20]	-0.24[#n-16]	0.07[#n-20]	-0.06[#n-20]	0.05[#n-19]	-0.04[#n-113]	0.05[#n-115]
	(2.27)	(2.24)	(2.47)	(2.28)	(2.30)	(2.18)	(2.29)	(2.38)
Maternal baseline	11.70[#n-32]	11.61[#n-31]	11.65[#n-32]	11.73[#n-39]	11.58[#n-31]	11.82[#n-26]	11.68[#n-191]	11.62[#n-789]
Hb (g/dl)	(1.27)	(1.31)	(1.27)	(1.19)	(1.26)	(1.31)	(1.27)	(1.31)
Maternal education	7.2[#n-133]	7.4[#n-122]	7.1[#n-152]	7.2[#n-132]	7.2[#n-145]	7.3[#n-124]	7.2[#n-808]	7.7[#n-562]
(years)	(2.6)	(2.7)	(2.7)	(2.8)	(2.7)	(2.6)	(2.7)	(2.8)
Mat primiparity (%)	31.4	31.7	28.5	29.5	28.7[#n-1]	32.1	30.3[#n-1]	38.8[#n-14]

Values are means (SDs) or percentages where indicated

[#n-x]sample size differs from column total by x amount; [a]All individuals recruited into current follow-up study

Early food: maternal randomisation to access food early in pregnancy; Usual food: maternal randomisation to access food at the usual time in pregnancy; Fe30F: maternal randomisation to receive 30mg iron and 400µg folate; Fe60F: maternal randomisation to receive 60mg iron and 400µ folate; MuMs: maternal randomisation to receive multiple micronutrient tablets

Amongst those individuals enrolled into the follow-up, maternal baseline characteristics remained equally distributed between the six nutritional supplementation arms of the trial (Table 6.1). There were small differences in characteristics between mothers whose children were recruited into the study and those lost to follow-up. Children lost to follow-up were more likely to have been the first born (χ^2: 34.6; P:<0.001). There was also a small difference in maternal age and years spent at school; women whose children were lost to follow-up were on average around nine months younger (mean difference: -0.8y; 95%CI: -1.2, -0.5; P: <0.001) and had spent six months longer in education (mean difference: 0.5y, 95%CI: 0.3, 0.7; P: <0.001), than women whose children were recruited.

Gestational age was estimated from the mother's last menstrual period (LMP method) and 191 individuals had been recruited who were deemed to have been born before 37 weeks (pre-term). These subjects were excluded from the analysis resulting in a sample size of 2335 that forms the basis of all subsequent results.

6.2.2 Anthropometry and body composition

The mean age at follow-up was 4.6 years (range: 4.5, 5.4) and 50.5% of recruited subjects were boys. The anthropometric characteristics of the recruited individuals are displayed in Table 6.2. Lean mass (or fat-free mass) was estimated by the impedance index (100/impedance) as recommended by Wells *et al* [249]. There were occasional technical faults with the Tanita technology, primarily as it was designed for use in older (7y+) well nourished children; impedance data are therefore missing for 224 children. The sum of four skinfold sites is reported as an estimate of body fatness, whist the ratio of trunk to limb skinfolds is reported as an estimate of fat distribution.

Table 6.2 Anthropometry and body composition of offspring born during the MINIMat trial, Bangladesh

	Mean (SD)	
	Girls	Boys
N	1157	1178
Age (years)	4.6 (0.1)	4.6 (0.1)
Height (cm)	99.9 (26.8)	100.5 (4.3)
Weight (kg)	13.4 (1.6)	14.1 (1.7)
BMI (kg/m^2)	13.6 (1.0)	13.9 (1.0)
Impedance index (100/R)	0.13 (0.01)$^{\#n-171}$	0.14 (0.01)$^{\#n-53}$
Height-for-age z-score (HAZ)[a]	-1.69 (0.87)$^{\#n-10}$	-1.53 (0.95)$^{\#n-11}$
Weight-for-height z-score (WHZ)[a]	-1.33 (0.80)$^{\#n-10}$	-1.29 (0.87)$^{\#n-11}$
BMI-for-age z-score (BMIZ)[a]	-1.25 (0.77)$^{\#n-10}$	-1.14 (0.85)$^{\#n-11}$
Whole body fat[b]	23.2 (4.5)$^{\#n-11}$	21.5 (4.2)$^{\#n-10}$
Trunk fat[c]	0.86 (0.13)$^{\#n-11}$	0.83 (0.13)$^{\#n-10}$
Triceps-for-age z-score[a]	-0.71 (0.81)$^{\#n-19}$	-0.47 (0.85)$^{\#n-18}$
Subscapular-for-age z-score[a]	-0.70 (0.92)$^{\#n-18}$	-0.70 (1.03)$^{\#n-18}$

$^{\#n-x}$Sample size differs from column total by x amount
[a]Compared to WHO Multicentre Growth Reference Study data [254]
[b]Calculated as the sum of four skinfold thickness measurements (biceps, triceps, subscapular and suprailiac)
[c]Calculated as the ratio of central skinfolds (subscapular and suprailiac) : limb skinfolds (biceps and triceps)

The height-for-age (HAZ) and weight-for-height (WHZ) Z-scores demonstrate that this is a marginally nourished population; 34% of recruited children were stunted (<-2 HAZ) and 19% were categorised as wasted (<-2 WHZ) compared to WHO standards [254]. The skinfold thickness Z-scores however, indicate these individuals may have relatively more body fat for their size, as the values are less reduced relative to the reference population than those for height.

6.2.3 Blood pressure

Blood pressure was normally distributed and the first, second and third readings were highly correlated (r: >0.65). The mean of the three readings was used as an estimate of average blood pressure. Twenty three subjects had missing blood pressure measurements as the child had failed to cooperate with the medical team, leaving a sample size of 2312 for all subsequent analysis. Mean systolic blood pressure was 91.1mmHg (SD: 7.5) for girls and 91.4mmHg (SD: 7.7) for boys. Mean diastolic blood pressure was 55.0mmHg (SD: 6.4) for girls and 53.8mmHg (SD: 6.5) for boys (Table 6.3). Half of the individuals (n=1058) recruited into the follow-up had triplicate blood pressure readings taken on two separate occasions, 21 days apart (explained in Section 5.4.3). The correlation coefficient between mean systolic blood pressure at the first and second visit was r: 0.59, and for diastolic blood pressure it was r: 0.52. For the remainder of this section, blood pressure will refer to that measured on the first visit only as this involves the greater sample size, returning to the issue of additional measurements in Section 6.3.

Table 6.3 Blood pressure of subjects recruited into the MINIMat trial follow-up

	Mean (SD)	
	Girls	Boys
N	1140	1172
Systolic BP (mmHg)	91.1 (7.5)	91.4 (7.7)
Diastolic BP (mmHg)	55.0 (6.4)	53.8 (6.5)
Pulse pressure (mmHg)[a]	36.1 (5.5)	37.6 (5.8)
Mean arterial pressure (mmHg)[b]	67.0 (6.3)	66.3 (6.3)
Hypertension (%)	3.4	1.7

[a]Calculated as systolic – diastolic blood pressure

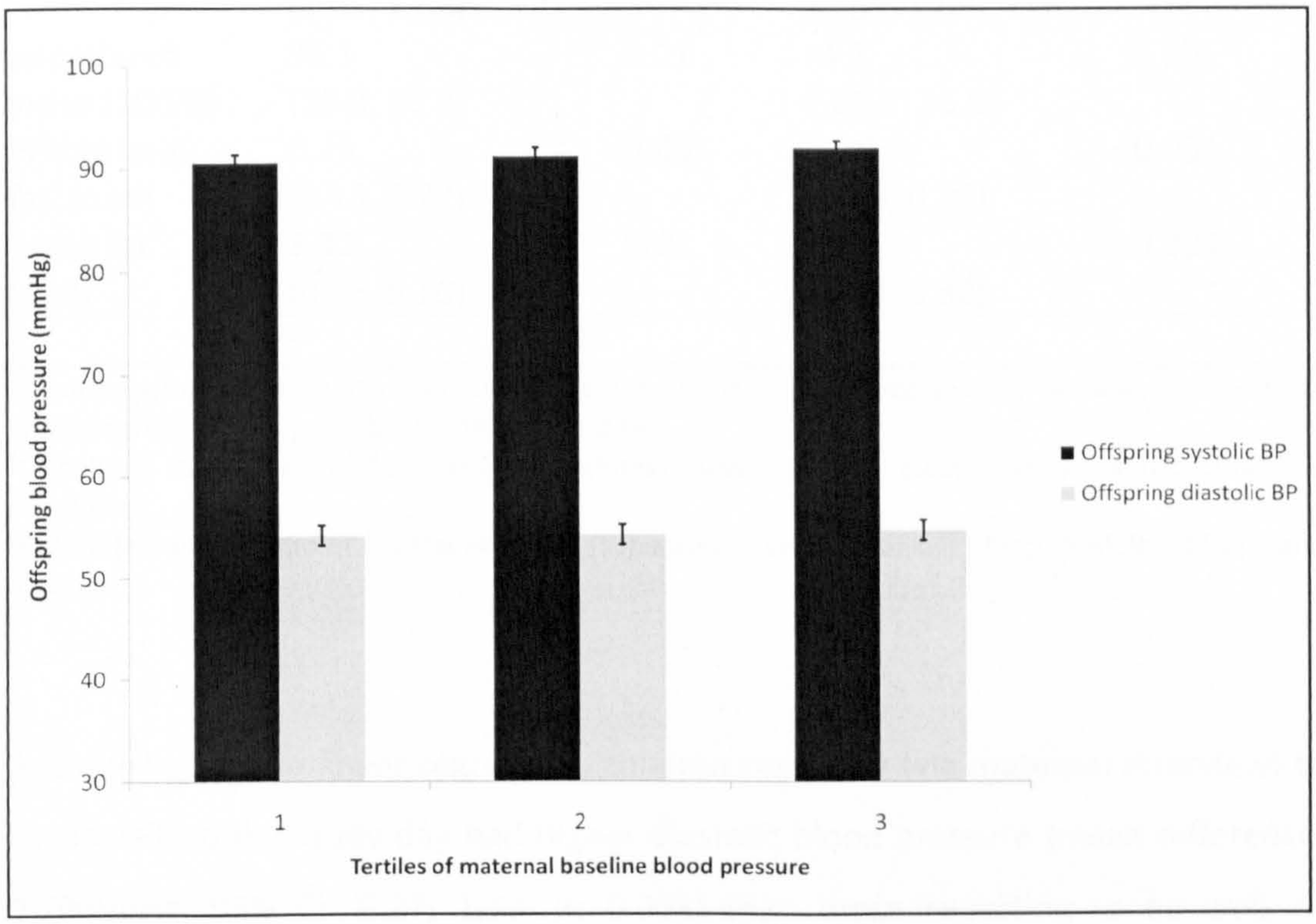
[b]Mean arterial pressure calculated as diastolic + (1/3*pulse pressure)

[c]Hypertension defined as systolic blood pressure above the NHBPEP 95% centile for height and age [12]

Blood pressure was unrelated to maternal age, maternal height or early (week 8 gestation) pregnancy BMI in simple linear regression analysis (data not shown). Systolic, but not diastolic, blood pressure was positively associated with maternal wealth index: for each point increase in wealth index, systolic blood pressure

increased by 0.18mmHg (95%CI: 0.04, 032; P: 0.01). Systolic blood pressure also increased marginally with tertiles of maternal systolic blood pressure (ANOVA: 6.55; P: 0.002), but there was no association between maternal and child diastolic blood pressure (ANOVA: 1.37; P: 0.25) (Figure 6.2).

Figure 6.2 Association between maternal and child blood pressure in MINIMat trial follow-up



Bars: mean offspring blood pressure for each tertile of maternal blood pressure
Error bars: ± 2 x S.E

Both systolic and diastolic blood pressure at 4.5 years were positively associated with current height and weight and with estimates of lean and fat mass (Table 6.4). The one exception was that no association was observed between diastolic blood pressure and impedance index.

Table 6.4 Association between blood pressure and anthropometry/body composition for individuals in the MINIMat trial follow-up

	Systolic BP (mmHg)		Diastolic BP (mmHg)	
	Regression coefficient (95% CI)	P-value	Regression coefficient (95% CI)	P-value
Height (cm)	0.27 (0.19, 0.34)	<0.001	0.16 (0.10, 0.22)	<0.001
Weight (kg)	0.96 (0.78, 1.15)	<0.001	0.58 (0.43, 0.74)	<0.001
BMI (kg/m^2)	1.36 (1.05, 1.68)	<0.001	0.81 (0.55, 1.08)	<0.001
Impedance index (100/R)	35.1 (10.8, 59.2)	0.01	-4.1 (-25.1, 16.8)	0.70
Whole body fat[a] (mm)	0.20 (0.13, 0.27)	<0.001	0.20 (0.14, 0.26)	<0.001
Trunk fat[b] (mm)	3.32 (0.95, 5.70)	0.01	4.36 (2.34, 6.38)	<0.001

Results are the effect on blood pressure of a one unit increase in explanatory variable, derived from linear regression analysis adjusted for age and sex

[a]Calculated as the sum of four skinfold thickness measurements (biceps, triceps, subscapular and suprailiac)

[b]Calculated as the ratio of central skinfolds (subscapular and suprailiac) : limb skinfolds (biceps and triceps)

Again using simple linear regression, children reporting (via maternal interview) to be unwell on the study day had higher diastolic blood pressure (mean difference: 0.79mmHg; 95% CI: 0.27, 1.32; P: 0.003) than those reporting to be well. In contrast, systolic blood pressure was unrelated to reported health on the study day (data not shown). Children reporting to have had diarrhoea in the past two weeks had lower systolic (mean difference: -1.15mmHg; 95% CI: -2.07, -0.24; P: 0.01) and diastolic (mean difference: -0.81mmHg; 95% CI: -1.59, -0.03; P: 0.04) blood pressure than children reporting no illness. This appeared to be partly explained by the lower weight exhibited by these children; once the association between diarrhoea and systolic blood pressure was adjusted for current weight it became weaker (mean difference: -0.81; 95% CI: -1.71, 0.08; P: 0.08).

6.2.4 Kidney function

There were 1077 individuals recruited into the study visits that included ultrasound measurements. Of these children, eight failed to cooperate sufficiently to have ultrasound performed and one had measurements that were greater than four SDs of the mean and were therefore regarded as a recording error. This resulted in a sample size of 1068 that forms the basis of the kidney size analysis. Kidney volume (right plus left kidney) measured by the length, width, depth method was highly correlated with kidney volume measured by the ellipsoid method (r: 0.92), suggesting that using either method in further analysis would be appropriate. The results from the ellipsoid measurement have been used in all further analysis. Total kidney volume was higher for boys (65.2cm^3; SD: 11.3) compared to girls (63.1cm^3; 10.8) (P: 0.002).

In simple linear regression analysis kidney volume was unrelated to maternal baseline BMI, maternal height or wealth index (data not shown). All estimates of anthropometry and body composition were positively associated with kidney volume, with the exception of trunk fat (Table 6.5).

Table 6.5 Association between kidney volume and anthropometry/body composition for individuals in the MINIMat trial follow-up

	Kidney volume (cm^3)	
	Regression coefficient (95% CI)	P-value
Height (cm)	1.25 (1.11, 1.39)	<0.001
Weight (kg)	3.29 (2.94, 3.65)	<0.001
BMI (kg/m^2)	2.89 (2.23, 3.56)	<0.001
Impedance index (100/R)	169.4 (115.2, 223.7)	<0.001
Whole body fat[a](mm)	0.35 (0.20, 0.50)	<0.001
Trunk fat[b] (mm)	-0.78 (-6.37, 4.80)	0.78

Results are the effect on kidney volume of a one unit increase in explanatory variable, derived from linear regression analysis adjusted for age and sex

[a]Calculated as the sum of four skinfold thickness measurements (biceps, triceps, subscapular and suprailiac)

[b]Calculated as the ratio of central skinfolds (subscapular and suprailiac) : limb skinfolds (biceps and triceps)

Kidney volume was adjusted for body surface area (kidney volume/BSA) calculated from the Haycock formula [247]; adjusted kidney volume was 103.8 (SD: 15.5) cm^3/m^2 for boys and 104.0 (SD: 15.9) cm^3/m^2 for girls. This estimate of kidney volume was used in all further analysis with the exception of certain observational associations (Section 6.7). Adjusted kidney volume was not associated with either systolic (β: 0.002mmHg; 95% CI: -0.12, 0.13; P: 0.97) or diastolic (β: -0.12mmHg; 95% CI: -0.28, 0.03; P: 0.12) blood pressure. Adjusted kidney volume was also unrelated to reporting to be unwell on the study day or to have experienced diarrhoea in the preceding two weeks (data not shown).

Glomerular filtration rate (GFR), calculated from plasma Cystatin C (CysC), was used as an alternative estimate of kidney function. CysC had been measured on individuals recruited into the first half of the study protocol, n: 1225. One child had a CysC measurement that was more than four SDs of the mean, and therefore deemed to be a recording error, which was removed from further analysis leaving 1224 individuals. Using simple linear regression, GFR was not related to maternal height or baseline BMI (data not shown). GFR was associated with wealth index; for each point increase in wealth index, GFR increased by 1.15ml/min/1.73m^2 (95% CI: 0.31, 2.00; P: 0.01). GFR was unrelated to age (data not shown) or sex in simple linear regression analysis; mean GFR for boys was 159.7 (SD: 36.0) ml/min/1.73m^2 and for girls it was 157.1 (34.1) ml/min/1.73m^2 (P: 0.19). GFR was positively associated with both height and weight and marginally negatively associated with impedance index (Table 6.6). There was no association between GFR and reporting to be unwell on the study day or to have had recent diarrhoeal episodes (data not shown). GFR was also unrelated to systolic and diastolic blood pressure (data not shown).

Table 6.6 Association between glomerular filtration rate and anthropometry/body composition for individuals in the MINIMat trial follow-up

	Glomerular filtration rate (ml/min/1.73m^2)	
	Regression coefficient (95% CI)	P-value
Height (cm)	0.54 (-0.09, 0.99)	0.02
Weight (kg)	1.21(-0.004, 2.42)	0.05
BMI (kg/m^2)	0.40 (-1.66, 2.46)	0.71
Impedance index (100/R)	-131.4 (-278.6, 15.9)	0.08
Whole body fat[a] (mm)	0.10 (-0.38, 0.58)	0.69
Trunk fat[b] (mm)	3.73 (-11.77, 19.22)	0.64

Results are the effect on GFR of a one unit increase in explanatory variable, derived from linear regression analysis adjusted for age and sex

[a]Calculated as the sum of four skinfold thickness measurements (biceps, triceps, subscapular and suprailiac)

[b]Calculated as the ratio of central skinfolds (subscapular and suprailiac): limb skinfolds (biceps and triceps)

Due to the design of the follow-up study into two separate groups, only a small number of individuals (n=143) had measurements of both kidney size and of GFR. For these individuals there was a weak correlation between the two proxy measures of kidney function, r: 0.28. For every 1cm^3/m^2 increase in kidney volume, GFR increased by 0.12ml/min/1.73m^2 (95% CI: 0.05, 0.19; P: 0.001).

6.3 Maternal nutritional interventions and offspring blood pressure

6.3.1 Invitation to food supplementation

6.3.1.1 Intention-to-treat analysis

The invitation to food supplementation intervention was successful in producing two groups of women who received different amounts of food during pregnancy. Mean food packet consumption in the early invitation group was 95 (SD: 41) compared to 64 (SD: 35) packets in usual invitation arm. Information on food packet consumption was taken at regular intervals throughout pregnancy and women in the early food invitation arm appeared to access the food service earlier than their counterparts in the usual invitation arm (Table 6.7).

Table 6.7 Percentage of women reporting to have never received food packets during the MINIMat trial, at different time points in pregnancy

	Early invitation (%) (N=1173)[a]	Usual invitation (%) (N=1162)[a,b]
Week 14 gestation	31	92
Week 18 gestation	12	57
Week 22 gestation	6	24
Week 26 gestation	5	15
Week 30 gestation	4	12
Week 34 gestation	3	10

[a]Data only refers to those individuals whose children were recruited into the follow-up study, although the data are representative of the entire cohort

The invitation to attend the government food supplementation programme early in pregnancy was associated with lower diastolic and mean arterial blood pressure in the offspring (Table 6.8). In unadjusted analysis, early invitation to food supplementation was associated with a 0.58mmHg lower diastolic blood pressure at 4.5 years of age. The timing of the food invitation was not associated with systolic or pulse pressure in the offspring. Adjusting the analysis for co-variates did not alter the results significantly.

Table 6.8 Effect of maternal food intervention on offspring blood pressure at 4.5 years in Bangladesh: intention-to-treat analysis

	Model 1[a] (95% CI)	P-value	Model 2[b] (95% CI)	P-value	Model 3[c] (95% CI)	P-value
Systolic pressure (mmHg)	0.46 (-0.16, 1.08)	0.15	0.42 (-0.21, 1.05)	0.19	0.57 (-0.08, 1.21)	0.09
Diastolic pressure (mmHg)	0.58 (0.06, 1.11)	0.03	0.59 (0.05, 1.13)	0.03	0.74 (0.18, 1.30)	0.01
Pulse pressure (mmHg)	-0.12 (-0.59, 0.34)	0.60	-0.17 (-0.64, 0.30)	0.48	-0.17 (-0.67, 0.33)	0.50
Mean arterial pressure (mmHg)	0.54 (0.03, 1.06)	0.04	0.53 (0.01, 1.06)	0.05	0.68 (0.14, 1.22)	0.01

Results are the difference in mean blood pressure (95% CI) for individuals born to women invited to receive food supplements early in pregnancy (coded 0) compared to the usual time (coded 1), derived from linear regression analysis

[a]Model 1 = unadjusted

[b]Model 2 = adjusted for age, sex, wealth index, thirds of maternal BP and season of birth fitted as Fourier terms [211]

[c]Model 3 = as model 2 but additionally adjusted for height, BMI, 100/R, body fat, diarrhoea in the past 2 weeks and feeling well on the study day

Only 2.5% of individuals had a systolic blood pressure above the 95th percentile for their height and age [12]. There was no difference in the proportion of individuals with high blood pressure in the early or usual food invitation groups (adjusted odds ratio: 1.47; 95% CI: 0.86, 2.49; P: 0.16).

Half of the recruited offspring had a more reliable estimate of their habitual blood pressure because they were measured in triplicate on two separate occasions, on average 21 days apart. Restricting the analysis to only those individuals who had these two measures of blood pressure, produced a less significant association with diastolic and mean arterial blood pressure, albeit with a reduced sample size (Table 6.9).

Table 6.9 Effect of MINIMat food intervention on child blood pressure measured on two separate occasions (n=1064)

	Unadjusted regression coefficient (95% CI)	P-value
Systolic (mmHg)	0.58 (-0.22, 1.37)	0.15
Diastolic (mmHg)	0.62 (-0.05, 1.30)	0.07
Pulse pressure (mmHg)	-0.04 (-0.60, 0.52)	0.88
Mean arterial pressure (mmHg)	0.61 (-0.06, 1.27)	0.08

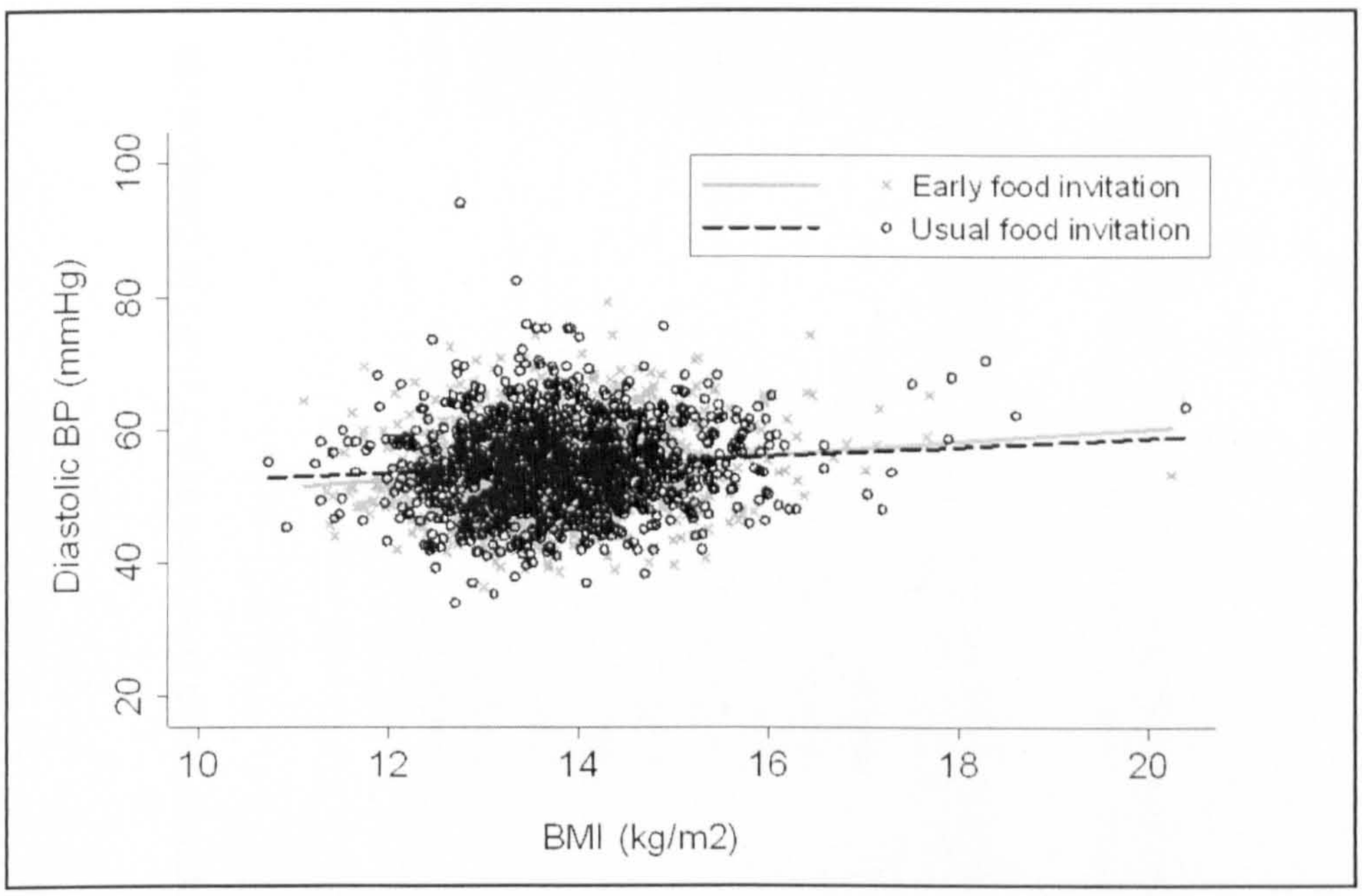
Results are the difference in mean blood pressure (95% CI) for individuals born to women invited to receive food supplements early in pregnancy (coded 0) compared to the usual time (coded 1), derived from linear regression analysis

Interactions

The potential for the effect of the food intervention to be modified by covariates was assessed on the entire dataset by fitting interaction terms between the intervention variable and relevant covariates. The effect of the food intervention was not modified by sex, wealth index, maternal baseline BMI, child impedance index, body fat or height for any of the blood pressure measures. There was a

significant interaction between the food intervention and child BMI for the effect on diastolic blood pressure (Interaction coefficient, β: -0.67mmHg; 95% CI: -1.26, -0.08; P: 0.03), although the graphical representation reveals this to be of questionable biological significance (Figure 6.3).

Figure 6.3 Interaction between food intervention and child BMI in the MINIMat trial: effect on child diastolic blood pressure



Lines are lines of best fit from linear regression analysis plotted for early and usual food invitation arms separately

6.3.1.2 As-treated analysis

The mean number of food packets consumed during pregnancy in the early intervention arm was 95 (range: 0-156) and in the usual invitation arm was 64 (range: 0-141) (mean difference: 30 packets; 95% CI: 27, 33; P: <0.001). Using the number of food packets consumed as the exposure variable, rather than randomisation to food intervention, there was no association with offspring blood pressure (Table 6.10).

Table 6.10 Effect of maternal food intervention on offspring blood pressure at 4.5 years in Bangladesh: as-treated analysis

	Model 1[a] (95% CI)	P-value	Model 2[b] (95% CI)	P-value	Model 3[c] (95% CI)	P-value
Systolic pressure (mmHg)	-0.003 (-0.011, 0.004)	0.41	-0.003 (-0.01, 0.01	0.52	-0.001 (-0.01, 0.01)	0.77
Diastolic pressure (mmHg)	-0.004 (-0.10, 0.003)	0.25	-0.004 (-0.01, 0.002)	0.19	-0.004 (-0.01, 0.003)	0.28
Pulse pressure (mmHg)	0.001 (-0.005, 0.006)	0.83	0.002 (-0.004, 0.008)	0.53	0.003 (-0.004, 0.01)	0.41
Mean arterial pressure (mmHg)	-0.004 (-0.010, 0.003)	0.26	-0.004 (-0.01, 0.003)	0.25	-0.003 (-0.01, 0.004)	0.39

Results are the effect on child blood pressure of a one packet increase in food packet consumption by their mother during pregnancy, derived from linear regression analysis

[a]Model 1 = unadjusted

[b]Model 2 = adjusted for age, sex, wealth index, thirds of maternal BP and season of birth fitted as Fourier terms [211]

[c]Model 3 = as model 2 but additionally adjusted for height, BMI, 100/R, body fat, diarrhoea in the past 2 weeks and feeling well on the study day

6.3.2 Micronutrient tablet intervention

6.3.2.1 Intention-to-treat analysis

The micronutrient intervention was judged separately as a multiple micronutrient compared to iron and folic acid intervention, and a higher (60mg) compared to lower (30mg) iron intervention (Table 6.11).

Table 6.11 Categorising the MINIMat micronutrient intervention for analysis

Original randomisation code[a]	New code for analysis[b]	
	Multiple micronutrients (MuMs)	High iron dose
Fe30F	0	0
Fe60F	0	1
MuMs	1	0

[a]Original micronutrient arm of the intervention: Fe30F represents women randomised to receive 30mg iron and 400µg folic acid; Fe60F represents women randomised to receive 60mg iron and 400µg folic acid; MuMs represents women randomised to receive 15 multiple micronutrients (of which iron concentration was 30mg)

[b]Variable is recoded to represent multiple micronutrients or high iron dose: individuals coded 0 ('control') or 1 (receiving intervention).

In unadjusted analysis there was no association between the maternal randomisation to multiple micronutrient supplementation and offspring blood pressure (Table 6.12). In an analysis adjusted for factors relating to offspring blood pressure, including current body composition, there was an effect of the multiple micronutrient intervention on diastolic blood pressure with 0.65mmHg (95%CI: 0.06, 1.24; P-value: 0.03) higher diastolic blood pressure compared to children whose mothers had received iron and folate only. There was no effect of maternal supplementation with high compared to low iron during pregnancy on offspring blood pressure in either unadjusted or adjusted analysis (Table 6.13).

The proportion of individuals with systolic blood pressure above the 95th percentile for their height and age [12] was not different between the two multiple micronutrient groups (adjusted odds ratio: 1.11; 95% CI: 0.65, 1.92; P: 0.70) or between the high or low iron groups (adjusted odds ratio: 0.88; 95% CI: 0.50, 1.54; P: 0.65).

Table 6.12 Effect of maternal multiple micronutrient supplementation on offspring blood pressure at 4.5 years in Bangladesh: intention-to-treat analysis

	Model 1[a] (95% CI)	P-value	Model 2[b] (95% CI)	P-value	Model 3[c] (95% CI)	P-value
Systolic pressure (mmHg)	0.05 (-0.71, 0.81)	0.89	0.01 (-0.67, 0.68)	0.98	0.17 (-0.51, 0.85)	0.62
Diastolic pressure (mmHg)	0.56 (-0.09, 1.21)	0.09	0.42 (-0.15, 0.99)	0.15	0.62 (0.03, 1.21)	0.04
Pulse pressure (mmHg)	-0.50 (-1.08, 0.07)	0.08	-0.41 (-0.91, 0.09)	0.11	-0.45 (-0.98, 0.07)	0.09
Mean arterial pressure (mmHg)	0.39 (-0.25, 1.02)	0.23	0.28 (-0.27, 0.84)	0.32	0.47 (-0.10, 1.04)	0.11

Results are the difference in mean blood pressure (95% CI) for individuals born to women receiving multiple micronutrient tablets during pregnancy (coded 1) compared to iron and folate only (coded 0), derived from linear regression analysis

[a]Model 1 = unadjusted but also fitted with high/low iron variable; [b]Model 2 = adjusted for age, sex, wealth index, thirds of maternal BP and season of birth; [c]Model 3 = as model 2 but additionally adjusted for height, BMI, 100/R, body fat, diarrhoea in past 2 weeks and feeling well on the study day

Table 6.13 Effect of maternal iron supplemenation on offspring blood pressure at 4.5 years in Bangladesh: intention-to-treat analysis

	Model 1[a] (95% CI)	P-value	Model 2[b] (95% CI)	P-value	Model 3[c] (95% CI)	P-value
Systolic pressure (mmHg)	-0.03 (-0.71, 0.81)	0.93	0.05 (-0.62, 0.72)	0.89	-0.04 (-0.72, 0.64)	0.91
Diastolic pressure (mmHg)	0.27 (-0.38, 0.92)	0.41	0.04 (-0.53, 0.61)	0.89	-0.02 (-0.57, 0.61)	0.95
Pulse pressure (mmHg)	-0.30 (-0.87, 0.27)	0.30	0.01 (-0.49, 0.51)	0.98	-0.05 (-0.58, 0.47)	0.85
Mean arterial pressure (mmHg)	0.17 (-0.46, 0.80)	0.60	0.04 (-0.51, 0.60)	0.88	-0.001 (-0.57, 0.57)	0.99

Results are the difference in mean blood pressure (95% CI) for individuals born to women receiving a high iron dose during pregnancy (60mg iron, coded 1) compared to a lower iron dose (30mg Iron coded 0), derived from linear regression analysis

[a]Model 1 = unadjusted but also fitted with MuMs variable; [b]Model 2 = adjusted for age, sex, wealth index, thirds of maternal BP and season of birth; [c]Model 3 = As model 2 but additionally adjusted for height, BMI, 100/R, body fat, diarrhoea in past 2 weeks and feeling well on the study day

Looking only at individuals who had blood pressure measured on two separate occasions there remained no association between either of the micronutrient interventions and offspring blood pressure (Table 6.14).

Table 6.14 Effect of MINIMat micronutrient intervention on child blood pressure measured on two separate occasions (n=1064)

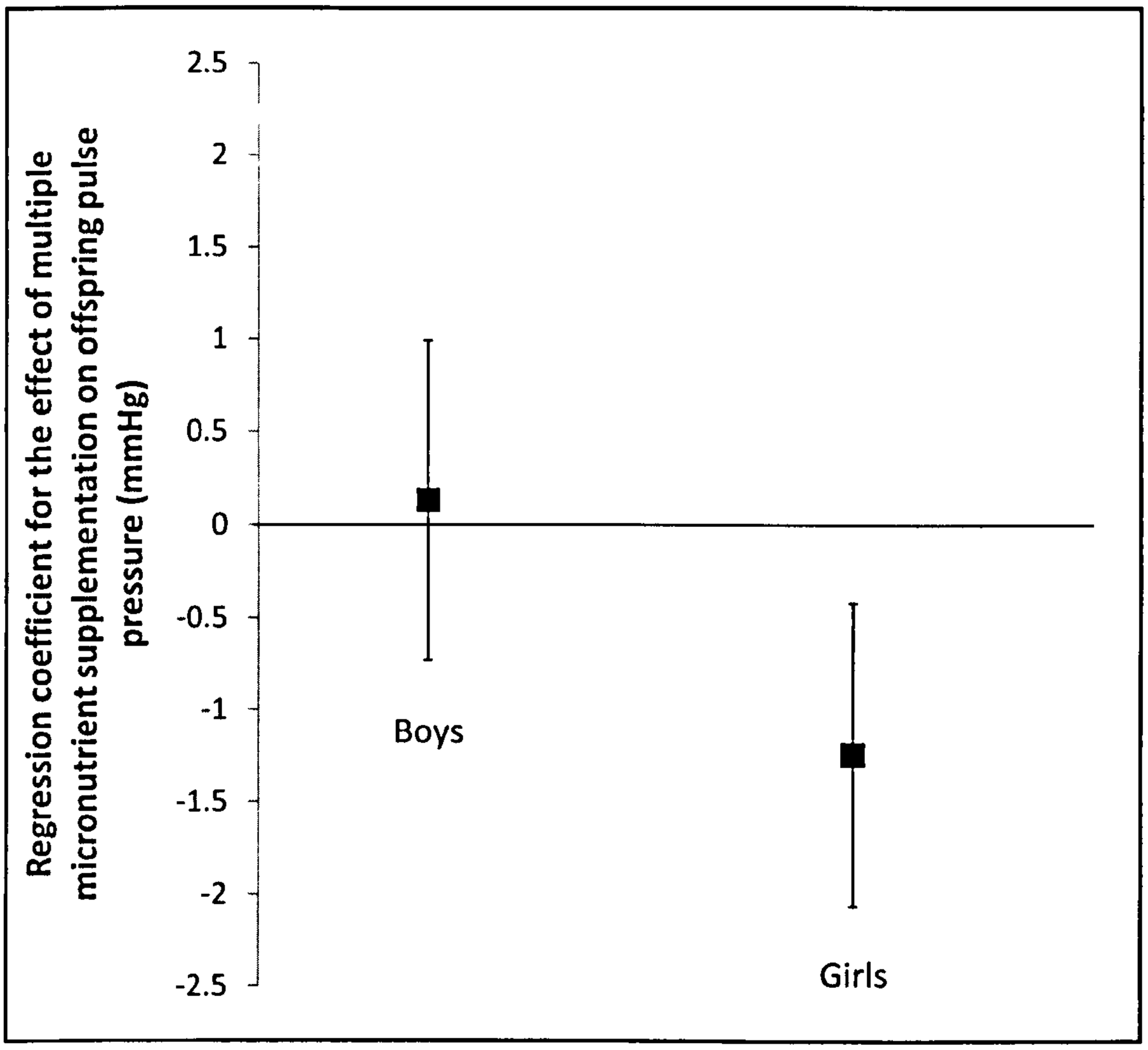
	MuMs intervention[a]		Fe intervention[b]	
	Unadjusted regression coefficient (95% CI)	P-value	Unadjusted regression coefficient (95% CI)	P-value
Systolic (mmHg)	0.02 (-0.96, 1.00)	0.98	-0.39 (-1.36, 0.58)	0.43
Diastolic (mmHg)	0.06 (-0.77, 0.89)	0.89	-0.24 (-1.06, 0.59)	0.57
Pulse pressure (mmHg)	-0.04 (-0.73, 0.64)	0.90	-0.15 (-0.83, 0.53)	0.66
MAP (mmHg)	0.04 (-0.78, 0.87)	0.92	-0.29 (-1.10, 0.53)	0.49

Results are mean difference (95% CI) in blood pressure between individuals whose mothers received the micronutrient intervention compared to iron and folate only (MuMs intervention) or the difference in blood pressure between individuals whose mothers received a high iron (60mg) compared to low iron (30mg) dose during pregnancy (Fe intervention)
[a]MuMs: multiple micronutrient supplement compared to iron and folate only
[b]Fe: high (60mg) iron supplement compared to low (30mg) iron supplement

Interactions

The effects of the multiple micronutrient and iron interventions on offspring blood pressure were not modified by any of wealth index, maternal baseline BMI, child height, impedance index, body fat or BMI (data not shown). No interaction of the multiple micronutrient intervention was observed for sex and systolic (Interaction coefficient, β: -0.36mmHg; 95% CI: -1.92, 1.20; P: 0.65) or diastolic (Interaction coefficient, β: 1.00mmHg; 95% CI: -0.33, 2.33; P: 0.14) blood pressure. However, the effect of the multiple micronutrient intervention on offspring pulse pressure was different for boys and girls (Interaction coefficient, β: -1.36mmHg; 95% CI: -2.52, -0.19; P: 0.02). For girls the intervention was associated with a decrease in pulse pressure whilst for boys there was no effect (Figure 6.4).

Figure 6.4 Effect of maternal multiple micronutrient intervention during pregnancy on offspring pulse pressure at 4.5 years differs by sex



Points are the difference in mean pulse pressure for individuals whose mothers received a multiple micronutrient supplement during pregnancy compared to those whose mothers received iron and folate only, derived from unadjusted linear regression and plotted for the sexes separately.
Error bars: ± 2 x S.E.

Combined interventions

The effect of the food and micronutrient interventions combined was assessed by adding two interaction terms, one between the food and MuMs intervention and one between the food and Fe intervention. There appeared to be no interaction between the interventions in relation to offspring blood pressure (Table 6.15).

Table 6.15 Interaction between maternal food and micronutrient interventions on child blood pressure in MINIMat follow-up

	MuMs*food intervention		Fe*food intervention	
	Interaction term regression coefficient (95% CI)[a]	P-value	Interaction term regression coefficient (95% CI)[a]	P-value
Systolic (mmHg)	-0.18 (-1.50, 1.13)	0.78	-0.01 (-1.32, 1.30)	0.98
Diastolic (mmHg)	-0.89 (-2.01, 0.23)	0.12	0.71 (-0.40, 1.83)	0.21
Pulse pressure (mmHg)	0.71 (-0.28, 1.70)	0.16	-0.73 (-1.71, 0.26)	0.15
Mean arterial pressure (mmHg)	-0.66 (-1.75, 0.44)	0.24	0.47 (-0.62, 1.56)	0.40

Results are the interaction term coefficient (MuMs*food or Fe*food), derived from linear regression analysis

6.3.2.2 As-treated analysis

Obtained pill count, from the electronic pill bottle lid technology known as eDEM®, was used for the as-treated analysis of the micronutrient intervention. The mean number of tablets consumed for women in the Fe30F group was 81 (range: 5-163), for women in the Fe60F group it was 80 (range: 0-176) and for women in the MuMs arm it was slightly lower at 76 (range: 0-143) (ANOVA: 4.12; P: 0.02). Again there was no association with offspring blood pressure in relation to the number of multiple micronutrient or iron-folate tablets consumed during pregnancy (Table 6.16).

Table 6.16 Effect of maternal micronutrient supplementation on offspring blood pressure at 4.5 years in Bangladesh: as-treated analysis

	Variable[a]	Model 1[b]	P-value	Model 2[c]	P-value	Model 3[d]	P-value
Systolic BP (mmHg)	Tablets	0.002 (-0.02, 0.02)	0.78	-0.004 (-0.02, 0.02)	0.68	-0.01 (-0.03, 0.01)	0.46
	MNS dose	0.01 (-0.01, 0.04)	0.36	0.01 (-0.01, 0.04)	0.31	0.02 (-0.004, 0.047)	0.12
	Fe dose	-0.001 (-0.03, 0.02)	0.96	0.01 (-0.02, 0.03)	0.63	0.01 (-0.01, 0.04)	0.33
Diastolic BP (mmHg)	Tablets	-0.0001 (-0.02, 0.02)	0.99	-0.0003 (-0.02, 0.02)	0.97	-0.004 (-0.02, 0.01)	0.59
	MNS dose	0.01 (-0.01, 0.03)	0.49	0.01 (-0.02, 0.03)	0.58	0.02 (-0.01, 0.04)	0.16
	Fe dose	-0.004 (-0.02, 0.02)	0.72	-0.001 (-0.02, 0.02)	0.93	0.01 (-0.01, 0.03)	0.50
Pulse pressure (mmHg)	Tablets	0.003 (-0.01, 0.02)	0.70	-0.003 (-0.02, 0.01)	0.62	-0.003 (-0.02, 0.01)	0.73
	MNS dose	0.004 (-0.01, 0.02)	0.67	0.01 (-0.01, 0.03)	0.47	0.01 (-0.01, 0.02)	0.59
	Fe dose	0.003 (-0.02, 0.02)	0.74	0.01 (-0.01, 0.03)	0.45	0.01 (-0.01, 0.02)	0.60
Mean arterial pressure (mmHg)	Tablets	0.001 (-0.01, 0.01)	0.92	-0.001 (-0.02, 0.01)	0.85	-0.01 (-0.02, 0.01)	0.51
	MNS dose	0.01 (-0.01, 0.03)	0.40	0.01 (-0.01, 0.03)	0.68	0.02 (-0.004, 0.039)	0.11
	Fe dose	-0.003 (-0.02, 0.02)	0.79	0.001 (-0.02, 0.02)	0.89	0.01 (-0.01, 0.03)	0.39

Results are the effect on child blood pressure of a one tablet increase in maternal consumption of supplementation tablets during pregnancy, derived from linear regression analysis

[a]Tablets: coefficient for the association between total number of tablets consumed and offspring blood pressure (irrespective of intervention arm); MNS dose: coefficient for the association between multiple micronutrient dose (fitted as interaction between tablets consumed and MuMs intervention variable) and offspring BP; Fe dose: coefficient for the association between high iron dose (fitted as interaction between tablets consumed and Fe intervention variable) and offspring BP

[b]Model 1 = unadjusted;

[c]Model 2 = adjusted for age, sex, wealth index, thirds of maternal BP and season of birth;

[d]Model 3 = as model 2 but additionally adjusted for height, BMI, 100/R, body fat, diarrhoea in past 2 weeks and feeling well on the study day

6.3.3 Breast feeding counselling intervention

6.3.3.1 Intention-to-treat analysis

Of the 2526 individuals recruited into the follow-up study, 43% had been born to mothers receiving counselling for exclusive breast feeding (Figure 6.5). Loss to follow-up was equally distributed between the two arms of the trial and there were no differences in baseline characteristics between individuals who were given counselling on general health messages and those who were given counselling promoting exclusive breast feeding (Table 6.17).

Figure 6.5 Flow of participants into the MINIMat 4.5 follow-up by maternal counselling intervention arm

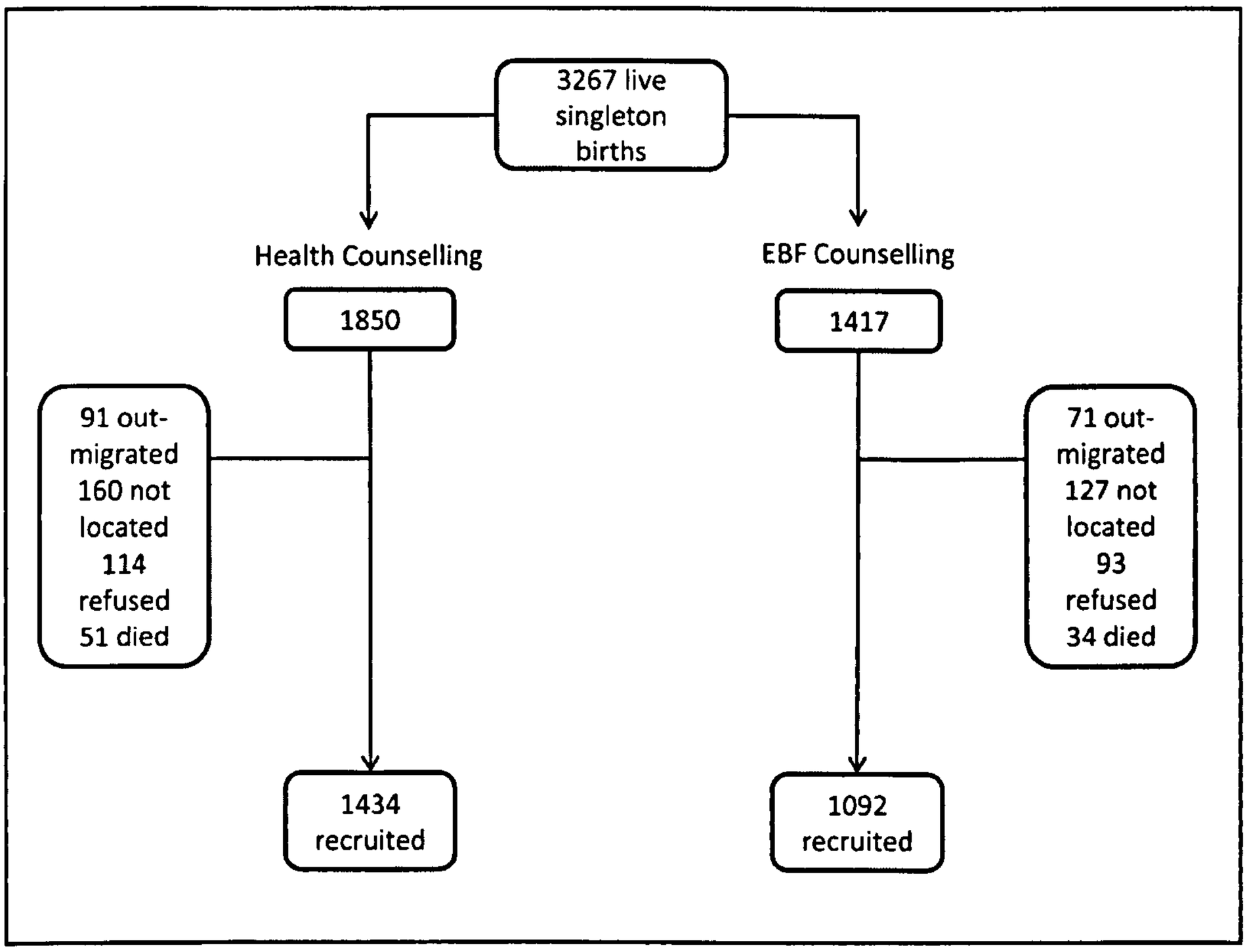


Table 6.17 Baseline characteristics for MINIMat trial follow-up individuals by maternal breast feeding counselling intervention arm

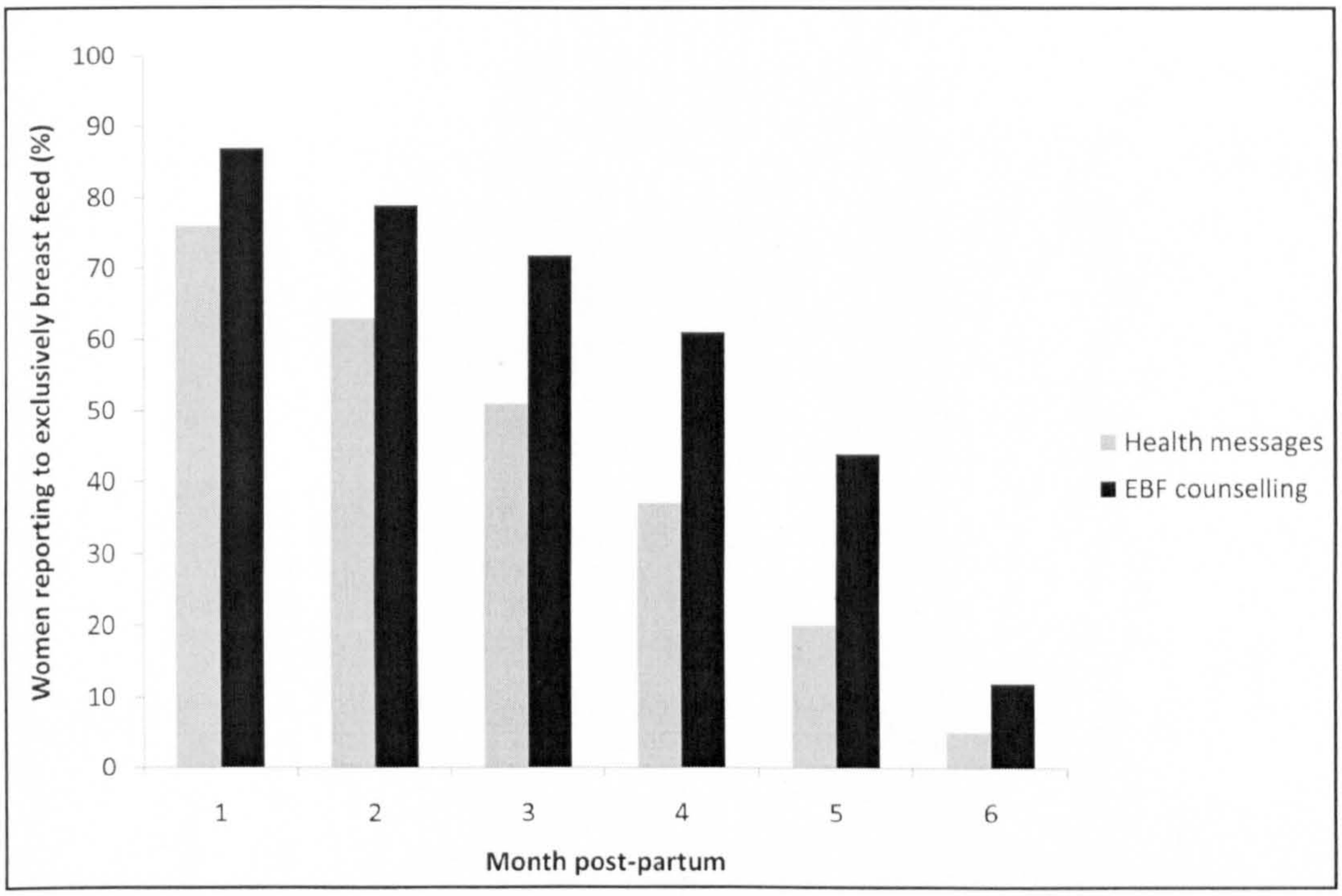
	Health message counselling	EBF counselling
N	1434	1092
Maternal age (y)	26.6 (5.9)	26.7 (6.1)
Maternal height (cm)	150.0 (5.3)[#n-2]	149.8 (5.3)[#n-2]
Maternal weight, 8 wk gestation (kg)	45.4 (6.9)[#n-5]	45.0 (6.7)[#n-3]
Maternal BMI (kg/m^2)	20.1 (2.6)[#n-5]	20.2 (2.7)[#n-4]
Maternal education (years)	7.3 (2.7)[#n-462]	7.2 (2.6)[#n-346]
Wealth index	0.01 (2.33)[#n-59]	-0.11 (2.25)[#n-54]
Primiparous mothers (%)	30.9	29.5[#n-1]

Values are means (SD) or percentages

[#n-x]sample size differs from column header by x amount

The breast feeding counselling intervention was successful in promoting exclusive breast feeding amongst women in the intervention arm. Although the percentage of women reporting to exclusively breast fed declined with child age, the proportion was consistently higher in the intervention arm (Figure 6.6).

Figure 6.6 Effect of breast feeding counselling on the adoption of this practice post-partum



Bars: percentage of women reporting to provide only breast milk at each time point
Data derived from questionnaires asking about breast feeding and the provision of other foods and liquids in the preceding month.
Data presented only for individuals recruited into the current follow-up

There was no impact of counselling for exclusive breast feeding compared to counselling for other health messages on child blood pressure at 4.5 years of age using intention-to-treat analysis (Table 6.18). There was also no difference between the two counselling intervention groups in the proportion of individuals with systolic blood pressure above the 95th percentile for their height and age [12] (adjusted odds ratio: 1.08; 95% CI: 0.64, 1.85; P: 0.77).

Table 6.18 Effect of maternal breast feeding counselling intervention on offspring blood pressure at 4.5 years in Bangladesh: intention-to-treat analysis

	Model 1[a] (95% CI)	P-value	Model 2[b] (95% CI)	P-value	Model 3[c] (95% CI)	P-value
Systolic pressure (mmHg)	-0.03 (-0.65, 0.60)	0.93	0.08 (-0.57, 0.73)	0.80	0.02 (-0.65, 0.68)	0.96
Diastolic pressure (mmHg)	0.34 (-0.83, 0.10)	0.21	0.43 (-0.13, 0.98)	0.13	0.35 (-0.22, 0.92)	0.23
Pulse pressure (mmHg)	-0.67 (-0.84, 0.10)	0.13	-0.34 (-0.83, 0.14)	0.16	-0.33 (-0.84, 0.18)	0.20
Mean arterial pressure (mmHg)	0.22 (-0.30, 0.74)	0.41	0.31 (-0.23, 0.85)	0.26	0.24 (-0.31, 0.79)	0.40

Results are the difference in mean blood pressure (95% CI) for individuals born to women receiving counselling for exclusive breast feeding (coded 1) compared to women receiving counselling for general health messages (coded 0), derived from linear regression analysis

[a]Model 1 = unadjusted

[b]Model 2 = adjusted for age, sex, wealth index, thirds of maternal BP and season of birth

[c]Model 3 = as model 2 but additionally adjusted for height, BMI, 100/R, body fat, diarrhoea in past 2 weeks and feeling well on the study day

Investigating the effect of the breast feeding intervention only on those individuals for whom blood pressure was measured on two separate occasions there remained no association with blood pressure (Table 6.19)

Table 6.19 Effect of breast feeding counselling intervention on child blood pressure measured on two separate occasions (n=1064)

	Unadjusted regression coefficient (95% CI)	P-value
Systolic (mmHg)	-0.04 (-0.83, 0.76)	0.95
Diastolic (mmHg)	-0.002 (-0.68, 0.67)	0.99
Pulse pressure (mmHg)	-0.03 (-0.59, 0.52)	0.90
MAP (mmHg)	-0.01 (-0.68, 0.65)	0.97

Results are the difference in mean blood pressure (95% CI) for individuals born to women receiving counselling for exclusive breast feeding (coded 1) compared to women receiving counselling for general health messages (coded 0), derived from linear regression analysis

Interactions

There were no interactions between the breast feeding counselling intervention and sex, maternal baseline BMI, child height, impedance index, BMI or body fat on the effect on child blood pressure (data not shown). There was a suggestion that the effect of the intervention on offspring systolic blood pressure was modified by maternal wealth index (Interaction coefficient, β: -0.28mmHg; 95% CI: -0.56, 0.003; P: 0.05). However, graphical representation of this interaction revealed it to be of limited biological significance (data not shown). There was also a suggestion that child impedance index modified the effect of the breast feeding counselling intervention on offspring systolic blood pressure (Interaction coefficient, β: -55.7mmHg; 95% CI: -105.0, -6.4; P: 0.03), although again the graphical representation failed to reveal a biologically meaningful association (data not shown).

6.3.3.2 As-treated analysis

Data on infant feeding practices were collected in 15 day intervals from birth to 12 months of age. At each time point, women were asked if they had breast fed and if they had provided other liquids and foods to their infants in the preceding 15 days. Feeding practice was categorised according to WHO guidelines as exclusive (breast milk only), predominant (breast milk plus other liquids such as water or juice) or partial (breast milk plus food or other milk) [252]. At four months of age 60% of individuals were still being exclusively breast fed whereas this proportion fell to 30% at six months of age. The analysis was therefore conducted for both time points as practices diverged so much between these ages, despite recommendations to continue to exclusively breast feed until six months of age.

There was no difference in blood pressure between those who were exclusively or predominantly breast fed at four months of age (Table 6.20). There was a suggestion that individuals who were only partially breast fed at four months of age had lower systolic and mean arterial blood pressure than those who were exclusively breast fed. However, once the analysis was adjusted for co-variates relating to blood pressure this association was no longer apparent.

There was no association between infant feeding at six months of age and blood pressure at 4.5 years in either unadjusted or adjusted analysis (Table 6.21).

Table 6.20 Effect of maternal breast feeding counselling intervention on offspring blood pressure at 4.5 years in Bangladesh: as-treated analysis for feeding practices at four months old

	Feeding type	Model 1[a] (95% CI)	P-value	Model 2[b] (95% CI)	P-value	Model 3[c] (95% CI)	P-value
Systolic pressure	Predominant	0.30 (-0.41, 1.01)	0.40	0.37 (-0.36, 1.11)	0.32	0.35 (-0.40, 7.09)	0.37
(mmHg)	Partial	-1.01 (-2.00, -0.02)	0.05	-0.83 (-1.84, 0.19)	0.11	-0.35 (-1.41, 0.71)	0.52
Diastolic pressure	Predominant	0.24 (-0.36, 0.85)	0.43	0.22 (-0.40, 0.85)	0.49	0.22 (-0.43, 0.87)	0.50
(mmHg)	Partial	-0.78 (-1.63, 0.06)	0.07	-0.73 (-1.59, 0.14)	0.10	-0.58 (-1.49, 0.34)	0.22
Pulse pressure	Predominant	0.06 (-0.48, 0.59)	0.83	0.15 (-0.40, 0.70)	0.60	0.12 (-0.45, 0.70)	0.67
(mmHg)	Partial	-0.23 (-0.97, 0.52)	0.55	-0.10 (-0.86, 0.66)	0.80	0.23 (-0.59, 1.05)	0.67
Mean arterial	Predominant	0.26 (-0.33, 0.86)	0.38	0.27 (-0.34, 0.88)	0.38	0.26 (-0.36, 0.89)	0.41
pressure (mmHg)	Partial	-0.86 (-1.68, -0.04)	0.04	-0.76 (-1.61, 0.08)	0.08	-0.50 (-1.39, 0.39)	0.27

Results are the difference in mean blood pressure (95% CI) for children at 4.5 years whose mothers had reported to be predominantly breast feeding (breast milk plus other liquids) or partially breast feeding (breast milk plus food and other milk) compared to exclusively breast feeding (nothing in addition to breast milk) when the child was four months old, derived from linear regression analysis

[a]Model 1 = unadjusted

[b]Model 2 = adjusted for age, sex, wealth index, thirds of maternal BP and season of birth

[c]Model 3 = as model 2 but additionally adjusted for height, BMI, 100/R, body fat, diarrhoea in past 2 weeks and feeling well on the study day

Table 6.21 Effect of maternal breast feeding counselling intervention on offspring blood pressure at 4.5 years in Bangladesh: as-treated analysis for feeding practices at six months old

	Feeding type	Model 1[a] (95% CI)	P-value	Model 2[b] (95% CI)	P-value	Model 3[c] (95% CI)	P-value
Systolic pressure	Predominant	-0.07 (-0.85, 0.72)	0.87	-0.09 (-0.89, 0.72)	0.83	-0.18 (-1.0, 0.64)	0.67
(mmHg)	Partial	-0.19 (-0.95, 0.57)	0.62	-0.22 (-1.01, 0.56)	0.58	0.02 (-0.78, 0.82)	0.96
Diastolic pressure	Predominant	-0.09 (-0.76, 0.58)	0.80	-0.09 (-0.78, 0.60)	0.79	-0.07 (-0.78, 0.64)	0.85
(mmHg)	Partial	-0.13 (-0.79, 0.52)	0.69	-0.18 (-0.85, 0.48)	0.59	0.01 (-0.69, 0.70)	0.99
Pulse pressure	Predominant	0.02 (-0.57, 0.61)	0.95	0.01 (-0.60, 0.61)	0.99	-0.11 (-0.74, 0.52)	0.74
(mmHg)	Partial	-0.06 (-0.63, 0.52)	0.85	-0.04 (-0.63, 0.55)	0.89	0.02 (-0.62, 0.63)	0.96
Mean arterial	Predominant	-0.08 (-0.73, 0.57)	0.81	-0.09 (-0.76, 0.58)	0.79	-0.10 (-0.79, 0.58)	0.77
pressure (mmHg)	Partial	-0.15 (-0.79, 0.48)	0.64	-0.20 (-0.85, 0.46)	0.55	0.01 (-0.66, 0.68)	0.98

Results are the difference in mean blood pressure (95% CI) for children at 4.5 years whose mothers had reported to be predominantly breast feeding (breast milk plus other liquids) or partially breast feeding (breast milk plus food and other milk) compared to exclusively breast feeding (nothing in addition to breast milk) when the child was six months old, derived from linear regression analysis

[a]Model 1 = unadjusted

[b]Model 2 = adjusted for age, sex, wealth index, thirds of maternal BP and season of birth

[c]Model 3 = as model 2 but additionally adjusted for height, BMI, 100/R, body fat, diarrhoea in past 2 weeks and feeling well on the study day

6.4 Maternal food and micronutrient supplementation and offspring kidney function

6.4.1 Invitation to food supplementation

6.4.1.1 Intention-to-treat analysis

The maternal food intervention (early pregnancy *vs* usual invitation to join the food supplementation programme) was not associated with kidney volume or glomerular filtration rate (GFR) in the offspring at 4.5 years of age in unadjusted or adjusted analysis (Table 6.22).

Interactions

The effect of the food intervention on kidney function was not modified by any of the variables tested: sex, maternal baseline BMI, wealth index, season of birth, height, impedance index, body fat or BMI (data not shown).

6.4.1.2 As-treated analysis

The number of food packets consumed during pregnancy were also unrelated to offspring kidney function at 4.5 years of age, in both unadjusted and adjusted analysis (Table 6.23).

Table 6.22 Effect of maternal food intervention on offspring kidney function at 4.5 years in Bangladesh: intention-to-treat analysis

	Model 1[a] (95% CI)	P-value	Model 2[b] (95% CI)	P-value	Model 3[c] (95% CI)	P-value
Kidney volume (cm^3/m^2)	-0.08 (-1.97, 1.80)	0.93	0.02 (-1.88, 1.93)	0.98	0.47 (-1.52, 2.47)	0.64
GFR[d] (ml/min/1.73m^2)	-1.76 (-5.70, 2.17)	0.38	-1.80 (-5.66, 2.05)	0.36	-1.08 (-5.12, 2.96)	0.60

Results are the difference in mean kidney function parameter (95% CI) for individuals born to women invited to receive food supplements early in pregnancy (coded 0) compared to at the usual time (coded 1), derived from linear regression analysis

[a]Model 1 = unadjusted; [b]Model 2 = adjusted for age, sex, wealth index and season of birth; [c]Model 3 = as model 2 but additionally adjusted for height, BMI, 100/R, and body fat

[d]GFR: glomerular filtration rate

Table 6.23 Effect of maternal food intervention on offspring kidney function at 4.5 years in Bangladesh: as-treated analysis

	Model 1[a] (95% CI)	P-value	Model 2[b] (95% CI)	P-value	Model 3[c] (95% CI)	P-value
Kidney volume (cm^3/m^2)	-0.003 (-0.03, 0.02)	0.76	-0.01 (-0.03, 0.01)	0.29	-0.01 (-0.04, 0.01)	0.26
GFR[d] (ml/min/1.73m^2)	-0.03 (-0.08, 0.02)	0.21	-0.02 (-0.07, 0.03)	0.36	-0.03 (-0.08, 0.03)	0.32

Results are the effect on child kidney function of a one packet increase in food packet consumption by their mother during pregnancy, derived from linear regression analysis

[a]Model 1 = unadjusted; [b]Model 2 = adjusted for age, sex, wealth index and season of birth; [c]Model 3 = as model 2 but additionally adjusted for height, BMI, 100/R, and body fat

[d]GFR: glomerular filtration rate

6.4.2 Micronutrient tablet intervention

6.4.2.1 Intention-to-treat analysis

Maternal multiple micronutrient supplementation was also not associated with offspring kidney function (Table 6.24). Maternal high iron compared to low iron dose was not associated with kidney volume but there was a suggestion of an association with glomerular filtration rate, albeit only in the adjusted analysis (Table 6.25). Individuals whose mothers had received 60mg of iron during pregnancy had, on average, a 4.98ml/min/1.73m^2 (95% CI: 0.30, 9.67; P: 0.04) higher GFR at 4.5 years of age than those whose mothers received 30mg of iron in analysis adjusted for co-variates related to GFR but unrelated to the maternal intervention.

Interactions

The effect of the maternal micronutrient interventions on offspring kidney function were not shown to be modified by any of the variables tested: sex, wealth index, maternal baseline BMI, season of birth, height, body fat, impedance index or BMI (data not shown).

Table 6.24 Effect of maternal multiple micronutrient supplementation on offspring kidney function at 4.5 years in Bangladesh: intention-to-treat analysis

	Model 1[a] (95% CI)	P-value	Model 2[b] (95% CI)	P-value	Model 3[c] (95% CI)	P-value
Kidney volume (cm^3/m^2)	-0.98 (-3.29, 1.34)	0.41	-1.75 (-4.09, 0.60)	0.14	-1.49 (-3.93, 0.94)	0.23
GFR[d] ($ml/min/1.73m^2$)	1.28 (-3.57, 6.13)	0.60	2.07 (-2.68, 6.81)	0.39	3.78 (-1.21, 8.76)	0.14

Results are the difference in kidney function parameter (95% CI) for individuals born to women receiving multiple micronutrient tablets during pregnancy (coded 1) compared to iron and folate only (coded 0), derived from linear regression analysis

[a]Model 1 = unadjusted; [b]Model 2 = adjusted for age, sex, wealth index + season of birth; [c]Model 3 = as model 2, additionally adjusted for height, BMI, 100/R, and body fat

[d]GFR: glomerular filtration rate

Table 6.25 Effect of maternal iron intervention supplementation on offspring kidney function at 4.5 years in Bangladesh: intention-to-treat analysis

	Model 1[a] (95% CI)	P-value	Model 2[b] (95% CI)	P-value	Model 3[c] (95% CI)	P-value
Kidney volume (cm^3/m^2)	-0.10 (-2.39, 2.20)	0.93	-0.54 (-2.88, 1.80)	0.65	-0.84 (-3.30, 1.63)	0.51
GFR ($ml/min/1.73m^2$)	4.42 (-0.37, 9.20)	0.07	4.98 (0.30, 9.67)	0.04	4.82 (-0.08, 9.71)	0.05

Results are the difference in mean blood pressure (95% CI) for individuals born to women receiving a high iron dose during pregnancy (60mg iron, coded 1) compared to a lower iron dose (30mg Iron coded 0), derived from linear regression analysis

[a]Model 1 = unadjusted; [b]Model 2 = adjusted for age, sex, wealth index + season of birth; [c]Model 3 = as model 2, additionally adjusted for height, BMI, 100/R, and body fat

[d]GFR: glomerular filtration rate

Combined intervention

There was no interaction between the maternal food and micronutrient interventions in relation to offspring kidney function (Table 6.26).

Table 6.26 Interaction between food and micronutrient interventions on child kidney function in MINIMat follow-up

	MuMs*food intervention		Fe*food intervention	
	Interaction regression coefficient (95% CI)[a]	P-value	Interaction regression coefficient (95% CI)[a]	P-value
Kidney volume (cm^3/m^2)	-0.89 (-4.90, 3.11)	0.66	-2.80 (-6.76, 1.17)	0.17
GFR[a] ($ml/min/1.73m^2$)	-1.88 (-10.28, 6.52)	0.66	1.02 (-7.28, 9.32)	0.81

Results are the interaction term coefficient (MuMs*food or Fe*food), derived from linear regression analysis

[a]GFR: glomerular filtration rate

6.4.2.2 As-treated analysis

There was a small negative effect of maternal consumption of multiple micronutrient tablets on offspring kidney volume (Table 6.27). Displayed graphically however, it is apparent that there is very little association, either for the iron and folate only or multiple micronutrient groups (Figure 6.7).

Table 6.27 Effect of maternal micronutrient supplementation on offspring kidney function at 4.5 years in Bangladesh: as-treated analysis

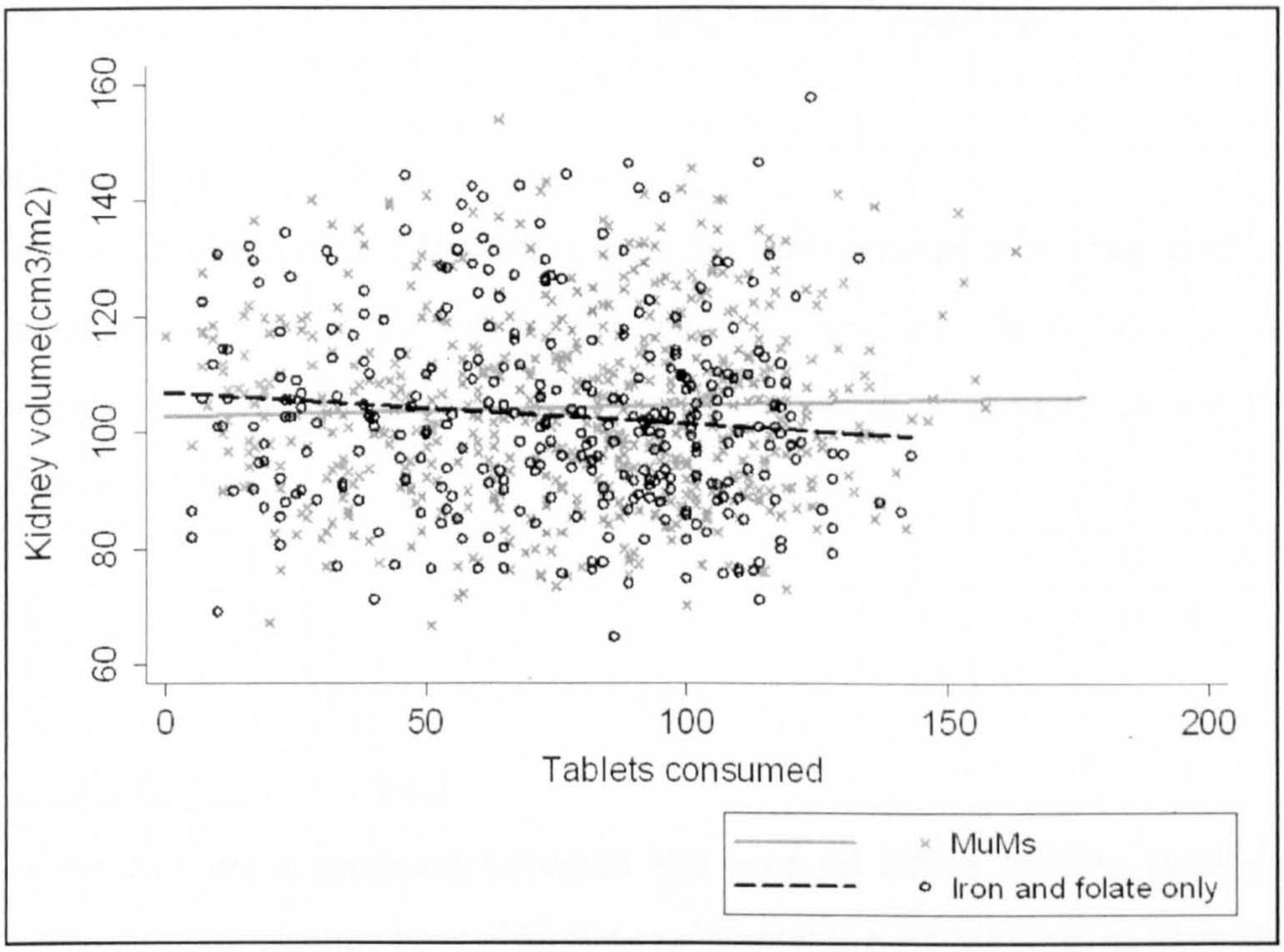
	Variable	Model 1[d]	P-value	Model 2[e]	P-value	Model 3[f]	P-value
Kidney vol (cm^3/m^2)	Tablets[a]	0.05 (0, 0.10)	0.05	0.03 (-0.02, 0.09)	0.21	0.03 (-0.03, 0.08)	0.30
	MNS dose[b]	-0.11 (-0.78, -0.03)	0.01	-0.07 (-0.15, 0)	0.05	-0.08 (-0.16, -0.01)	0.04
	Fe dose[c]	-0.07 (-0.14, 0.01)	0.07	-0.06 (-0.12, 0.02)	0.17	-0.03 (-0.11, 0.04)	0.40
Glomerular filtration rate ($ml/min/1.73m^2$)	Tablets	0.001 (-0.11, 0.11)	0.98	-0.03 (-0.13, 0.08)	0.64	-0.03 (-0.14, 0.08)	0.65
	MNS dose	0.10 (-0.06, 0.25)	0.22	0.12 (-0.03, 0.27)	0.13	0.11 (-0.04, 0.27)	0.15
	Fe dose	-0.02 (-0.17, 0.13)	0.83	-0.03 (-0.13, 0.08)	0.64	0.004 (-0.15, 0.16)	0.96

Results are the effect on child kidney function parameter of a one tablet increase in maternal consumption of supplementation tablets during pregnancy, derived from linear regression analysis

[a]Coefficient for the association between total number of tablets consumed and offspring kidney function; [b]Coefficient for the association between multiple micronutrient dose (fitted as interaction between tablets consumed and intervention variable) and offspring kidney function; [c]Coefficient for the association between high iron dose (fitted as interaction between tablets consumed and intervention variable) and offspring kidney function

[d]Model 1 = unadjusted; [e]Model 2 = adjusted for age, sex, wealth index and season of birth; [f]Model 3 = as model 2 but additionally adjusted for height, BMI, 100/R and body fat

Figure 6.7 Graphical representation of the effect of maternal micronutrient intervention on offspring kidney volume: as treated analysis



Lines are lines of best fit from linear regression analysis, plotted for multiple micronutrient and iron and folate arms of the trial separately

6.4.3 Breast feeding counselling intervention

6.4.3.1 Intention-to-treat analysis

Counselling for exclusive breast feeding was not associated with offspring kidney function (Table 6.28). There was a slight suggestion of an effect on glomerular filtration rate, which was lost after adjustment for co-variates.

Interactions

The effect of the breast feeding counselling intervention on kidney function was not modified by any of the following covariates: sex, wealth index, season of birth, maternal baseline BMI, child BMI, height, body fat or impedance index (data not shown).

6.4.3.2 As-treated analysis

There was no association between the type of infant feeding (exclusive breast feeding, predominant breast feeding or partial breast feeding) at four (Table 6.29) or six (Table 6.30) months of age and kidney function at 4.5 years.

Table 6.28 Effect of maternal breast feeding counselling intervention on offspring kidney function at 4.5 years in Bangladesh: intention-to-treat analysis

	Model 1[a] (95% CI)	P-value	Model 2[b] (95% CI)	P-value	Model 3[c] (95% CI)	P-value
Kidney volume (cm^3/m^2)	-0.80 (-2.68, 1.07)	0.40	-0.54 (-2.44, 1.36)	0.58	-0.46 (-2.46, 1.53)	0.65
GFR[d] ($ml/min/1.73m^2$)	3.71 (-0.30, 7.73)	0.07	1.31 (-2.77, 5.39)	0.53	1.29 (-2.99, 5.58)	0.55

Results are the difference in mean kidney function parameter (95% CI) for individuals born to women receiving counselling for exclusive breast feeding (coded 1) compared to women receiving counselling for general health messages (coded 0), derived from linear regression analysis

[a]Model 1 = unadjusted

[b]Model 2 = adjusted for age, sex, wealth index and season of birth

[c]Model 3 = as model 2 but additionally adjusted for height, BMI, 100/R, and body fat

[d]GFR: glomerular filtration rate

Table 6.29 Effect of maternal breast feeding counselling intervention on offspring kidney function at 4.5 years in Bangladesh: as-treated analysis for infant feeding practices at four months old

	Feeding type	Model 1[a] (95% CI)	P-value	Model 2[b] (95% CI)	P-value	Model 3[c] (95% CI)	P-value
Kidney volume (cm^3/m^2)	Predominant	0.46 (-1.67, 2.59)	0.67	-0.23 (-2.41, 1.95)	0.84	-0.60 (-2.88, 1.67)	0.60
	Partial	0.69 (-2.38, 3.75)	0.66	0.37 (-2.75, 3.48)	0.82	-0.18 (-3.55, 3.19)	0.92
GFR[d] ($ml/min/1.73m^2$)	Predominant	-0.32 (-4.84, 4.20)	0.89	-1.17 (-5.63, 3.28)	0.61	-2.22 (-6.88, 2.44)	0.35
	Partial	-1.56 (-7.89, 4.77)	0.63	-1.53 (-7.73, 4.66)	0.63	-2.54 (-9.17, 4.09)	0.45

Results are the difference in mean kidney function parameter (95% CI) for children at 4.5 years whose mothers had reported to be predominantly breast feeding (breast milk plus other liquids) or partially breast feeding (breast milk plus food and other milk) compared to exclusively breast feeding (nothing in addition to breast milk) when the child was four months old, derived from linear regression analysis

[a]Model 1 = unadjusted

[b]Model 2 = adjusted for age, sex, wealth index and season of birth

[c]Model 3 = as model 2 but additionally adjusted for height, BMI, 100/R and body fat

[d]GFR: glomerular filtration rate

Table 6.30 Effect of maternal breast feeding counselling intervention on offspring kidney function at 4.5 years in Bangladesh: as-treated analysis for infant feeding practices at six months old

	Feeding type	Model 1[a] (95% CI)	P-value	Model 2[b] (95% CI)	P-value	Model 3[c] (95% CI)	P-value
Kidney volume (cm^3/m^2)	Predominant	1.05 (-1.36, 3.46)	0.39	0.97 (-1.49, 3.42)	0.44	0.27 (-2.28, 2.83)	0.84
	Partial	-0.52 (-2.82, 1.78)	0.67	-0.49 (-2.83, 1.84)	0.68	-0.80 (-3.26, 1.65)	0.52
GFR[d] ($ml/min/1.73m^2$)	Predominant	-3.25 (-8.19, 1.69)	0.20	-2.05 (-6.93, 2.83)	0.41	-3.42 (-8.52, 1.67)	0.19
	Partial	-1.54 (-6.39, 3.32)	0.54	-1.32 (-6.10, 3.45)	0.59	-1.75 (-6.76, 3.26)	0.49

Results are the difference in mean kidney function parameter (95% CI) for children at 4.5 years whose mothers had reported to be predominantly breast feeding (breast milk plus other liquids) or partially breast feeding (breast milk plus food and other milk) compared to exclusively breast feeding (nothing in addition to breast milk) when the child was six months old, derived from linear regression analysis

[a]Model 1 = unadjusted

[b]Model 2 = adjusted for age, sex, wealth index and season of birth

[c]Model 3 = as model 2 but additionally adjusted for height, BMI, 100/R and body fat

[d]GFR: glomerular filtration rate

6.6 Early life exposures and later blood pressure: observational analysis

The impact of early-life factors on later blood pressure and kidney function for children in the MINIMat trial follow-up will be assessed in the following sections. Maternal factors (leg length and haemoglobin during pregnancy) will be assessed first, followed by birth anthropometry and season of birth, finally focusing on the impact of infant growth.

6.6.1 Maternal sitting height

There was no association between maternal leg length, used as a proxy indicator of maternal early-life nutritional exposures [255], and child blood pressure at 4.5 years of age (Table 6.31).

Table 6.31 Association between maternal leg length and child blood pressure at 4.5 years in Bangladesh

	Unadjusted regression coefficient (95% CI)	P-value	Adjusted regression coefficient (95% CI)[a]	P-value
Systolic pressure (mmHg)	0.09 (-0.06, 0.23)	0.24	0.03 (-0.18, 0.23)	0.79
Diastolic pressure (mmHg)	0.04 (-0.09, 0.17)	0.52	0.01 (-0.17, 0.19)	0.91
Pulse pressure (mmHg)	0.05 (-0.07, 0.16)	0.42	0.02 (-0.15, 0.18)	0.85
Mean arterial pressure (mmHg)	0.06 (-0.07, 0.18)	0.37	0.02 (-0.16, 0.19)	0.85

Results are the effect on child blood pressure of a one centimeter increase in maternal leg length, derived from linear regression analysis

[a]Model adjusted for age, sex, wealth index, season of birth, thirds of maternal blood pressure, maternal schooling, feeling well on study day, diarrhoea in past 2 weeks, height, BMI, body fat and 100/R

6.6.2 Maternal haemoglobin

Maternal haemoglobin levels at baseline (week 14 gestation) were positively associated with childhood blood pressure, both before and after adjustment for appropriate confounders (Table 6.32). In adjusted analysis, for each 1g/dl increase in maternal haemoglobin, childhood systolic blood pressure increased by 0.42mmHg (95%CI: -0.09, 0.75; P: 0.01) and a similar association was apparent for diastolic blood pressure.

Table 6.32 Association between maternal haemoglobin during pregnancy (week 14 gestation) and offspring blood pressure at 4.5 years in Bangladesh

	Unadjusted regression coefficient (95% CI)	P-value	Adjusted regression coefficient (95% CI)[a]	P-value
Systolic pressure (mmHg)	0.24 (-0.01, 0.50)	0.06	0.42 (0.09, 0.75)	0.01
Diastolic pressure (mmHg)	0.31 (0.09, 0.53)	0.01	0.45 (0.16, 0.73)	0.002
Pulse pressure (mmHg)	-0.07 (-0.26, 0.13)	0.50	-0.02 (-0.28, 0.23)	0.86
Mean arterial pressure (mmHg)	0.29 (0.07, 0.50)	0.01	0.44 (0.16, 0.71)	0.002

Results are the effect on child blood pressure of a 1g/dl increase in maternal haemoglobin, derived from linear regression analysis

[a]Model adjusted for age, sex, wealth index, season of birth, thirds of maternal blood pressure, maternal schooling, feeling well on the study day, diarrhoea in past 2 weeks, height, BMI, body fat and 100/R

In later pregnancy (week 30 gestation) there was still the suggestion of a positive association between maternal haemoglobin and offspring systolic blood pressure, but this was lost after adjustment for confounding variables (Table 6.33). There was no interaction between maternal high or low iron supplementation and haemoglobin at 30 weeks gestation on offspring blood pressure (data not shown).

Table 6.33 Association between maternal haemoglobin (week 30 gestation) during pregnancy and offspring blood pressure in Bangladesh

	Unadjusted regression coefficient (95% CI)	P-value	Adjusted regression coefficient (95% CI)[a]	P-value
Systolic pressure (mmHg)	0.28 (0.01, 0.54)	0.04	0.26 (-0.09, 0.61)	0.15
Diastolic pressure (mmHg)	0.17 (-0.06, 0.40)	0.14	0.13 (-0.17, 0.43)	0.39
Pulse pressure (mmHg)	0.11 (-0.10, 0.31)	0.31	0.13 (-0.15, 0.40)	0.36
Mean arterial pressure (mmHg)	0.21 (-0.02, 0.43)	0.07	0.17 (-0.12, 0.46)	0.24

Results are the effect on child blood pressure of a 1g/dl increase in maternal haemoglobin, derived from linear regression analysis

[a]Model adjusted for age, sex, wealth index, season of birth, thirds of maternal blood pressure, maternal schooling, feeling well on the study day, diarrhoea in past 2 weeks, height, BMI, body fat and 100/R

Twenty-nine percent of individuals had a haemoglobin concentration below 11g/dl at week 14 gestation, suggesting anaemia according to WHO guidelines [256]. Children born to mothers that were anaemic during pregnancy had lower systolic (mean difference β: -1.23mmHg; 95% CI: -2.17, -0.29; P: 0.01) and diastolic (β: -1.10mmHg; 95% CI: -1.91, -0.29; P: 0.01) blood pressure than those born to non-anaemic women, in analyses adjusted for appropriate confounders. Anaemia at 30 weeks gestation was not associated with offspring blood pressure (data not shown).

6.6.3 Birth anthropometry

Neither birth weight nor birth length were associated with blood pressure at 4.5 years of age until adjustment for current weight (Table 6.34). Once this variable was added to the model there became a strong inverse association between birth weight and both systolic and diastolic blood pressure and between birth length and systolic blood pressure. There was no interaction between the food or micronutrient interventions and the impact of birth weight or length on later blood

pressure (data not shown). Adjusting birth weight and length for gestational age produced very similar results (data not shown).

Table 6.34 Association between birth anthropometry and childhood blood pressure at 4.5years for individuals born during the MINIMat trial

	Systolic BP (mmHg)		Diastolic BP (mmHg)	
	Regression coefficient (95% CI)	P-value	Regression coefficient (95% CI)	P-value
Birth weight (kg)	-0.63 (-1.51, 0.26)	0.17	0.23 (-0.52, 0.98)	0.55
Adjusted birth weight (kg)[a]	-2.53 (-3.46, -1.60)	<0.001	-1.02 (-1.82, -0.22)	0.01
Birth length (cm)	-0.07 (-0.21, 0.07)	0.34	0.03 (-0.10, 0.15)	0.67
Adjusted birth length (cm)[b]	-0.23 (-0.38, -0.08)	0.002	-0.09 (-0.22, 0.04)	0.18

Results are the effect on child blood pressure of a one unit increase in birth weight or length, derived from linear regression analysis adjusted for age, sex wealth index and maternal blood pressure

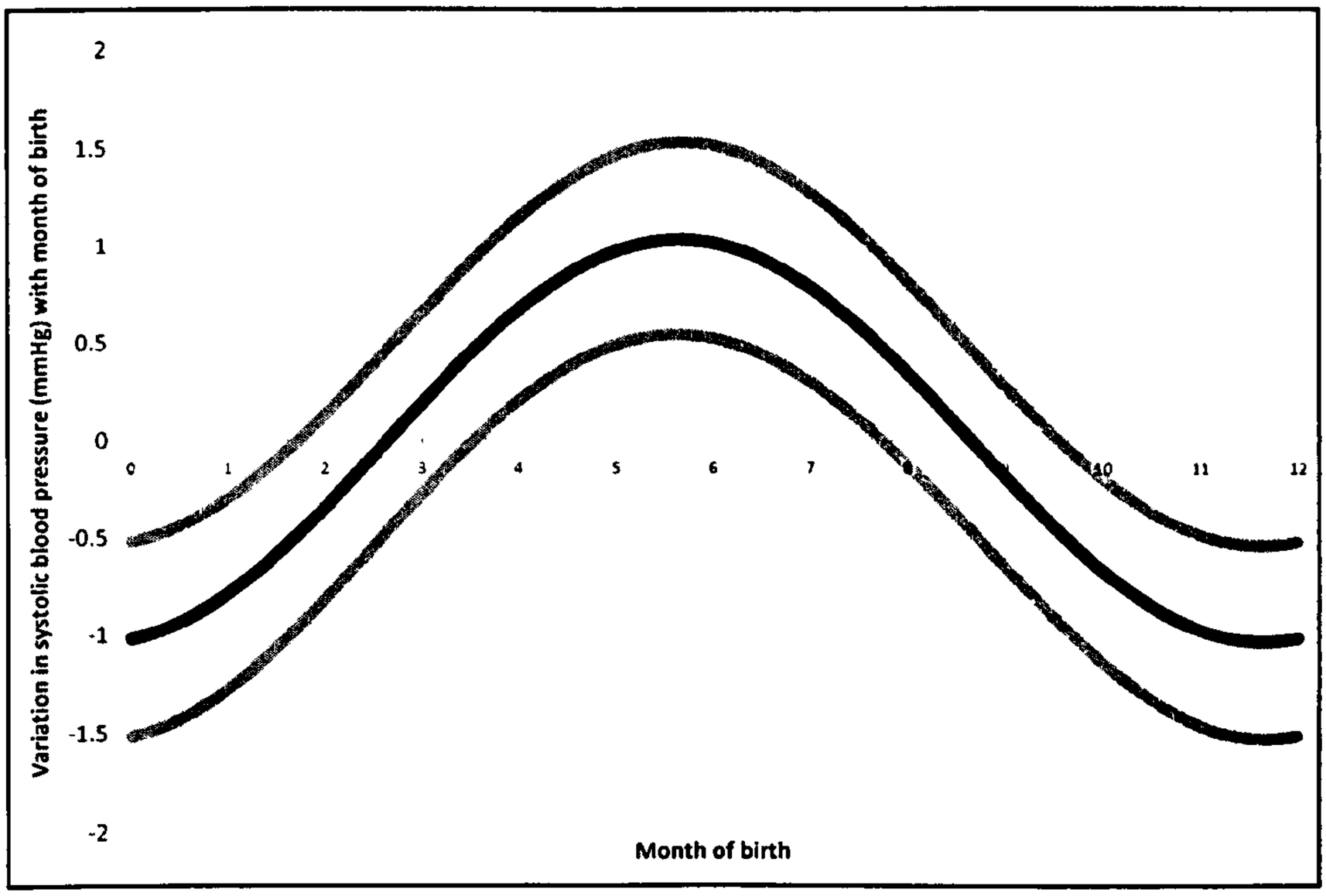
[a]Additionally adjusted for weight at follow-up

[b]Additionally adjusted for height at follow-up

6.6.4 Season of birth

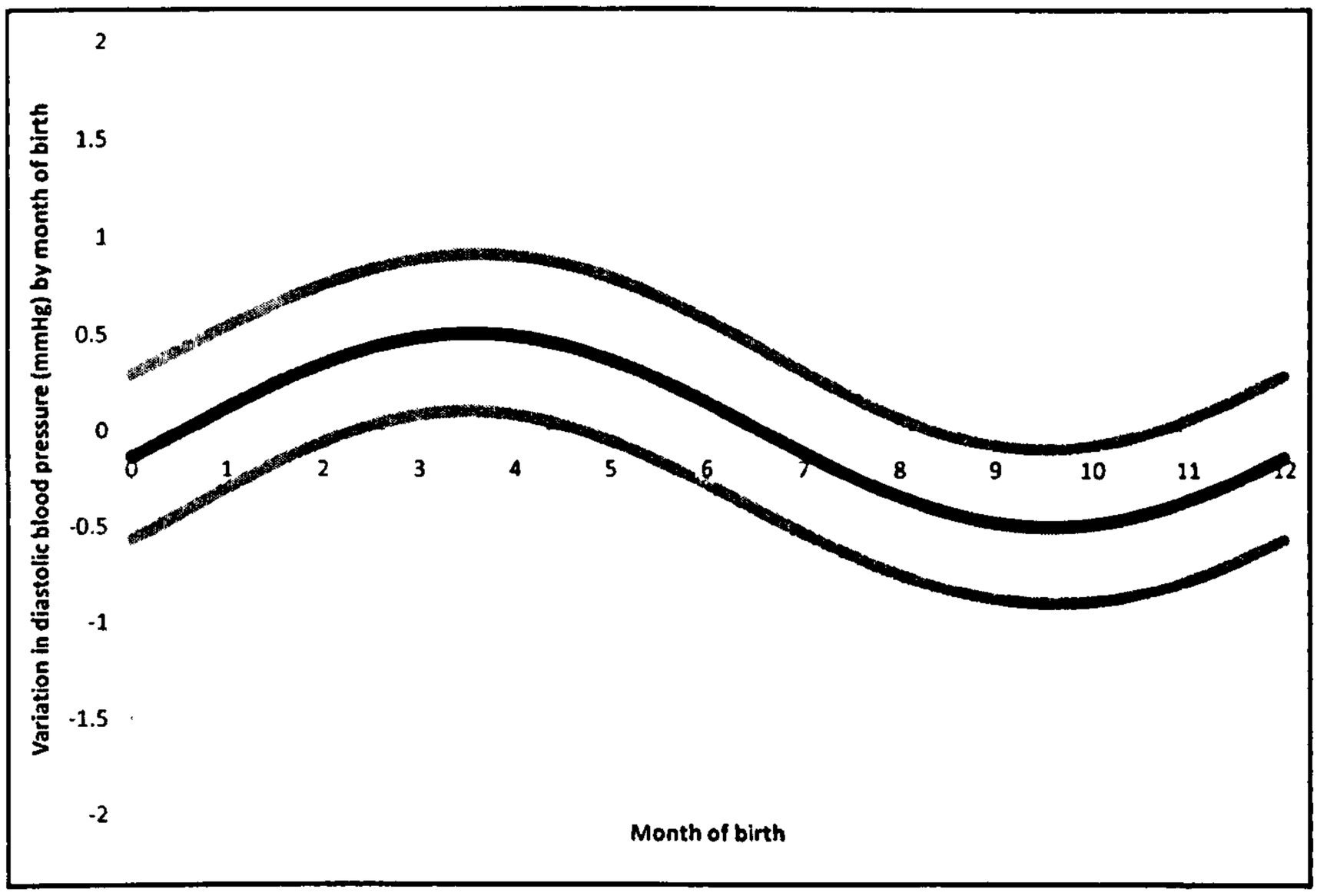
Season of birth was associated with offspring systolic blood pressure (LR test χ^2: 14.94; P: 0.001), adjusted for age, sex, wealth index, maternal blood pressure, BMI, height and impedance index. Systolic blood pressure was highest for those born during May and June and lowest for those born in November and December (Figure 6.8). There was only a very minimal and borderline association between month of birth and diastolic blood pressure (LR test χ^2: 6.77; P: 0.03) (Figure 6.9).

Figure 6.8 Variation of systolic blood pressure in Bangladesh children aged 4.5 years in relation to their month of birth



Bold middle line represents the fitted line for the variation in systolic blood pressure with month of birth. Upper and lower lines represent fitted 95% confidence interval.

Figure 6.9 Variation of diastolic blood pressure in Bangladesh children aged 4.5 years in relation to their month of birth



Bold middle line represents the fitted line for the variation in diastolic blood pressure with month of birth. Upper and lower lines represent fitted 95% confidence interval.

6.6.5 Infant growth

Infant weight and length had been measured at monthly visits from birth to one year, and bimonthly to two years of age, providing substantial data with which to investigate the association between early infant growth and systolic blood pressure in childhood. Change in weight SD score between birth and one year of age was positively associated with systolic and diastolic blood pressure at 4.5 years of age, even after adjustment for current BMI (Table 6.35). In contrast, change in weight SD score between one and two years was not associated with either systolic or diastolic blood pressure.

Table 6.35 Association between growth in infancy and blood pressure at 4.5 years in Bangladesh

Growth time period	Systolic BP (mmHg) Regression coefficient (95% CI)	P-value	Diastolic BP (mmHg) Regression coefficient (95% CI)	P-value
0-12 months	0.88 (0.58, 1.17)	<0.001	0.60 (0.35, 0.85)	<0.001
0-12 months plus current size[a]	0.40 (0.09, 0.72)	0.01	0.27 (0.01, 0.54)	0.05
12-24 months	0.35 (-0.26, 0.97)	0.26	-0.20 (-0.72, 0.33)	0.46
12-24 months plus current size[a]	0.19 (-0.42, 0.79)	0.54	-0.32 (-0.84, 0.20)	0.23

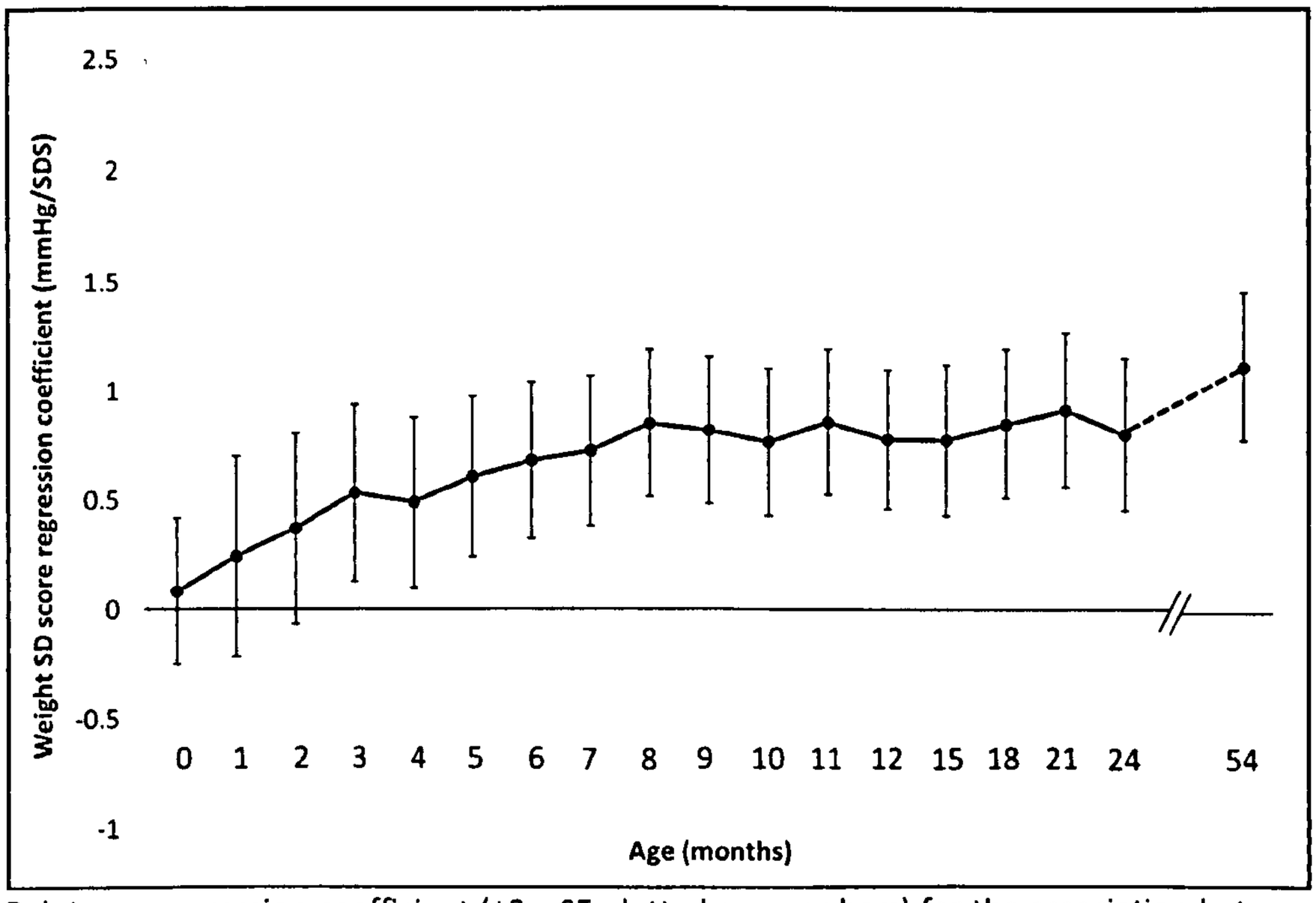
Results are the effect on blood pressure of a one unit increase in weight standard deviation score (calculated relative to WHO standards [254]) between the specified time points, derived from linear regression analysis adjusted for age, sex, wealth index and tertiles of maternal blood pressure
[a]Analysis additionally adjusted for BMI and height at 4.5 years

The association between growth in infancy and blood pressure at 4.5 years of age can be visualised by plotting the regression coefficients for the association between weight SD score and blood pressure against age in a life course plot [121]. These plots (Figure 6.10 and 6.11) indicate that early increase in weight SD score (before six months of age) is positively associated with blood pressure in later childhood. They also indicate that there is a relatively flat relationship between growth in the second year of life and blood pressure in later childhood.

Figure 6.10 Life course plot for the association between growth in infancy and systolic blood pressure at 4.5 years of age in Bangladesh

Points are regression coefficient (±2 x SE plotted as error bars) for the association between systolic blood pressure at 54 months and weight SD score (compared to WHO reference data [254]) at different ages

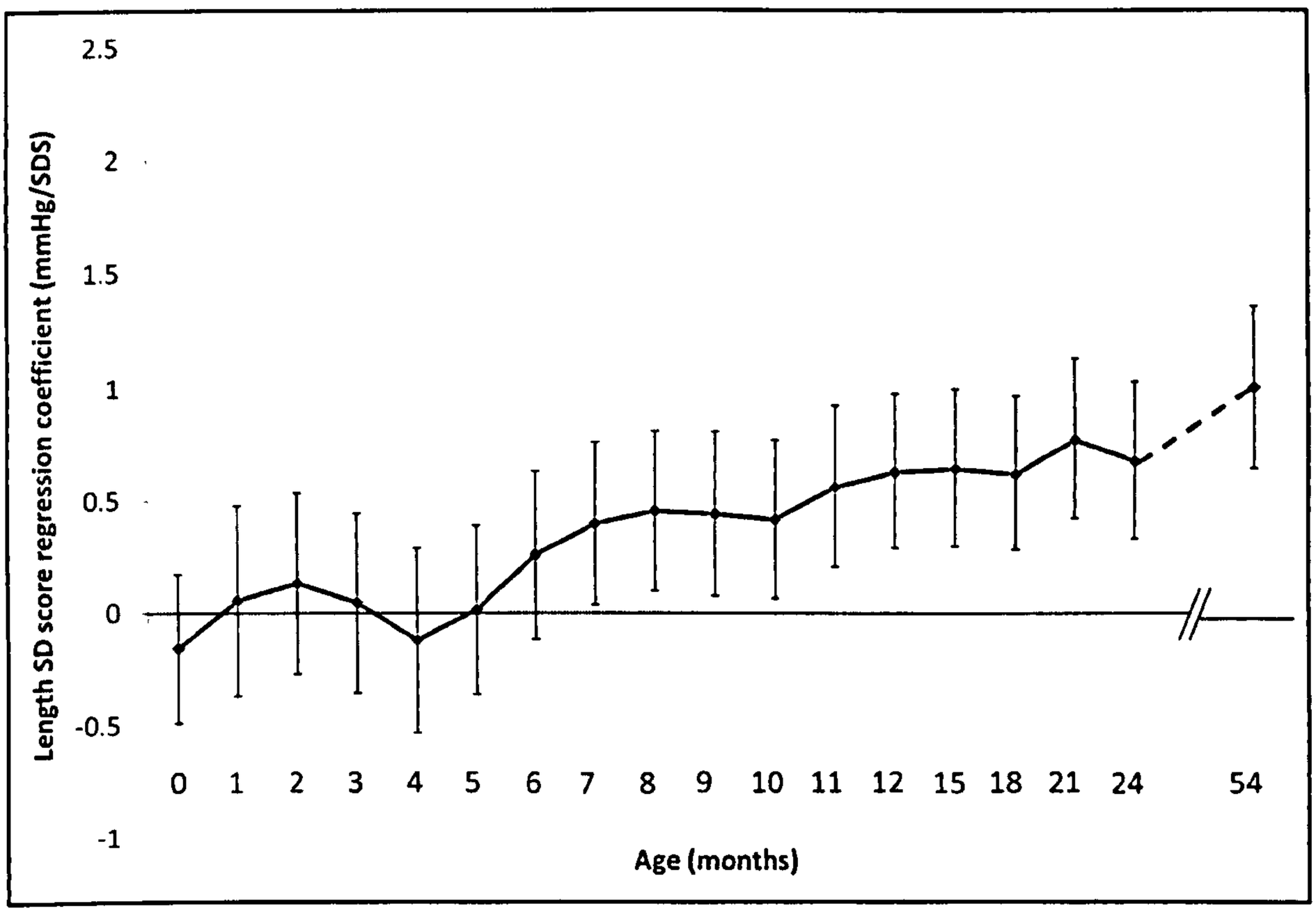
Figure 6.11 Life course plot for the association between growth in infancy and diastolic blood pressure at 4.5 years of age in Bangladesh



Points are regression coefficient (±2 x SE plotted as error bars) for the association between diastolic blood pressure at 54 months and weight SD score (compared to WHO reference data [254] at different ages

Change in length SD score between birth and one year of age was also positively associated with systolic (β: 0.71mmHg; 95% CI: 0.39, 1.02; P: <0.001) blood pressure even after adjustment for current height (β: 0.43mmHg; 95% CI: 0.09, 0.77; P: 0.01). Diastolic blood pressure was similarly associated with change in length SD score between birth and one year (β: 0.47mmHg; 95% CI: 0.20, 0.74; P: 0.001), but the association became borderline once it was adjusted for current height (β: 0.25mmHg; 95% CI: -0.04, 0.54; P: 0.09). In common to the weight change pattern there was no association between change in length SD score from one to two years for both systolic (β: 0.01mmHg; 95% CI: -0.60, 0.62; P: 0.98) and diastolic (β: -0.25mmHg; 95% CI: -0.77, 0.27; P: 0.34) blood pressure. This was again due to the flatter relationship between length gain and later blood pressure in the second year of life (Figure 6.12).

Figure 6.12 Life course plot for the association between linear growth in infancy and systolic blood pressure at 4.5 years of age in Bangladesh



Points are regression coefficient (±2 x SE plotted as error bars) for the association between systolic blood pressure at 54 months and length SD score (compared to WHO reference data [254] at different ages

6.7 Early life exposures and later kidney function: observational analysis

6.7.1 Maternal leg length

There was no association between maternal leg length and child kidney volume at 4.5 years of age in either unadjusted analysis (β: 0.16cm^3/m^2; 95% CI: -0.15, 0.48; P: 0.31) or in models adjusted for age, sex and wealth index (β: 0.14cm^3/m^2; 95% CI: -0.20, 0.48; P: 0.43). It was not possible to test the association between maternal leg length and glomerular filtration rate (GFR) due to missing data on maternal sitting height from the first half of the study period, which corresponds to the same individuals for whom GFR was measured.

6.7.2 Maternal haemoglobin

Maternal haemoglobin during pregnancy was unrelated to measures of kidney function in their offspring at 4.5 years of age in either unadjusted or adjusted analysis (Table 6.36 and 6.37). In addition, maternal anaemia (Hb: <11g/dl) at 14 or 30 weeks gestation was not associated with kidney volume or GFR (data not shown).

Table 6.36 Association between maternal haemoglobin (week 14 gestation) during pregnancy and offspring kidney function at 4.5 years in Bangladesh

	Unadjusted regression coefficient (95% CI)	P-value	Adjusted regression coefficient (95% CI)[a]	P-value
Kidney volume (cm^3/m^2)	-0.48 (-1.27, 0.31)	0.23	-0.23 (-1.20, 0.74)	0.64
GFR[b] ($ml/min/1.73m^2$)	-0.89 (-2.47, 0.69)	0.27	-0.35 (-2.55, 1.86)	0.76

Results are the effect on child kidney function of a 1g/dl increase in maternal haemoglobin, derived from linear regression analysis

[a]models adjusted for age, sex, wealth index, season of birth, height, BMI 100/R and body fat

[b]GFR: glomerular filtration rate

Table 6.37 Association between maternal haemoglobin during pregnancy (week 30 gestation) and offspring kidney function at 4.5 years in Bangladesh

	Unadjusted regression coefficient (95% CI)	P-value	Adjusted regression coefficient (95% CI)[a]	P-value
Kidney volume (cm^3/m^2)	0.19 (-0.63, 1.02)	0.65	-0.44 (-1.44, 0.57)	0.40
GFR[b] ($ml/min/1.73m^2$)	1.57 (-0.14, 3.28)	0.07	0.02 (-2.38, 2.42)	0.99

Results are the effect on child kidney function of a 1g/dl increase in maternal haemoglobin, derived from linear regression analysis
[a]models adjusted for age, sex, wealth index, season of birth, height, BMI, 100/R and body fat
[b]GFR: glomerular filtration rate

6.7.3 Birth anthropometry

Kidney volume, unadjusted for body surface area, was positively associated with birth weight and length both before and after adjustment for current size (Table 6.38). In contrast, glomerular filtration rate was not associated with either birth weight or length, either before or after adjustment.

Table 6.38 Association between birth anthropometry and kidney function at 4.5 years for individuals born during the MINIMat trial, Bangladesh

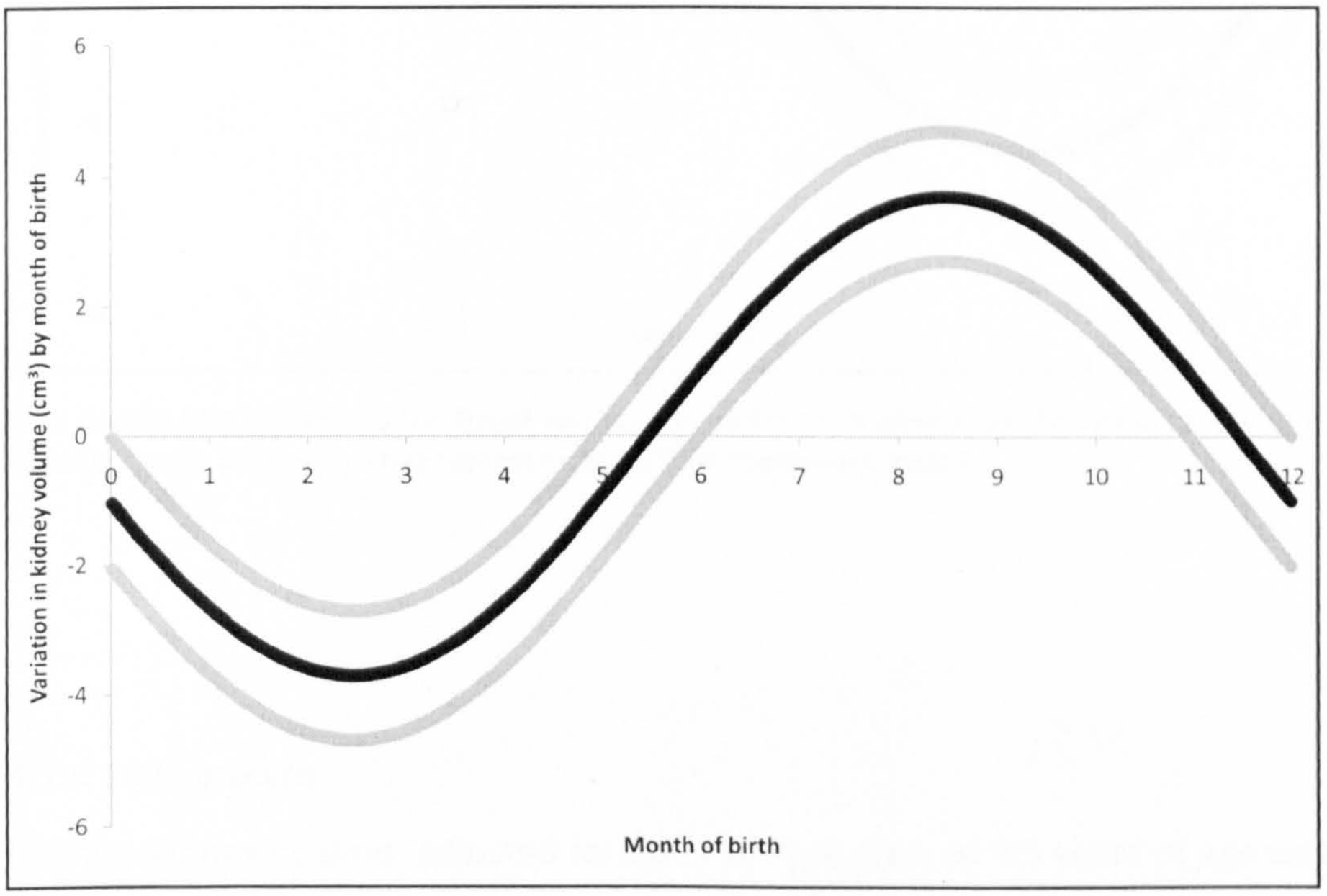
	Kidney volume (cm^3) Regression coefficient (95% CI)	P-value	Glomerular filtration rate ($ml/min/1.73m^2$) Regression coefficient (95% CI)	P-value
Birth weight (kg)	7.37 (5.50, 9.25)	<0.001	4.23 (-1.21, 9.68)	0.13
Adjusted birth weight (kg)[a]	2.59 (0.77, 4.41)	0.01	3.48 (-2.46, 9.42)	0.25
Birth length (cm)	1.41 (1.07, 1.75)	<0.001	0.52 (-0.31, 1.35)	0.22
Adjusted birth length (cm)[b]	0.52 (0.18, 0.86)	0.002	0.30 (-0.59, 1.18)	0.51

Results are the effect on child kidney function of a one unit increase in birth weight or length, derived from linear regression analysis adjusted for age, sex and wealth index
[a]Additionally adjusted for weight at follow-up
[b]Additionally adjusted for height at follow-up

6.7.4 Season of birth

Season of birth, adjusted for age, sex, height, BMI, body fat and impedance index, was associated with kidney volume at 4.5 years of age (LR test χ^2: 58.3; P: <0.001). Kidney volume was highest for individuals born in August and lowest for those born in February (Figure 6.13).

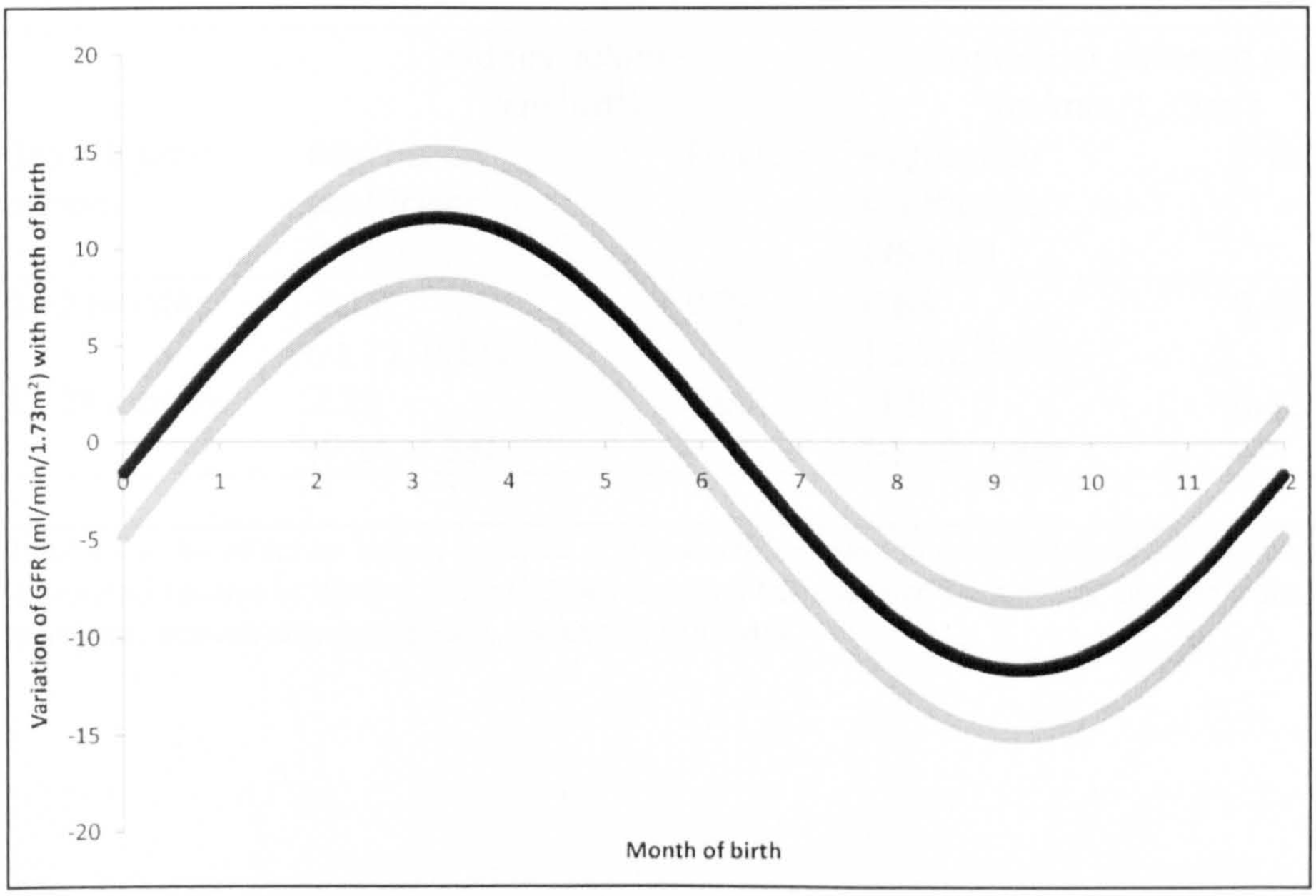
Figure 6.13 Variation of kidney volume in Bangladesh children aged 4.5 years in relation to their month of birth



Bold middle line represents the fitted line for the variation in kidney volume with month of birth. Upper and lower lines represent fitted 95% confidence interval.

A mirror image pattern was seen for the association between glomerular filtration rate and month of birth (LR test X^2: 48.7; P: <0.001). Individuals born in February and March had the highest GFR, whilst those born in August and September had the lowest (Figure 6.14).

Figure 6.14 Variation of glomerular filtration rate in Bangladesh children at 4.5 years of age in relation to their month of birth



Bold middle line represents the fitted line for the variation in glomerular filtration rate with month of birth. Upper and lower lines represent fitted 95% confidence interval.

6.7.5 Child growth

Relative kidney volume, adjusted for body surface area, at 4.5 years of age was not associated with change in weight SD score between birth and one year of age (Table 6.39). Weight change between one and two years of age was positively associated with later kidney volume however, even after adjustment for current BMI (β: 2.27cm^3/m^2; 95% CI: 0.21, 4.30; P: 0.03). Change in length SD score between birth and one year of age (β: -0.57cm^3/m^2; 95% CI: -1.17, 0.56; P: 0.32) or between one and two years of age (β: 1.18cm^3/m^2; 95% CI: -0.86, 3.22; P: 0.26) was not associated with kidney volume at 4.5 years. GFR was also not associated with change in length SD score in early-life (data not shown).

Table 6.39 Association between growth in infancy and kidney function at 4.5 years in Bangladesh

	Kidney volume (cm^3/m^2)		Glomerular filtration rate ($ml/min/1.73m^2$)	
Growth time period	Regression coefficient (95% CI)	P-value	Regression coefficient (95% CI)	P-value
0-12 months	-0.80 (-1.71, 0.11)	0.09	0.68 (-1.23, 2.60)	0.48
12-24 months	2.29 (0.24, 4.33)	0.03	-1.92 (-5.69, 1.86)	0.32

Results are the effect on kidney function of a one unit increase in weight standard deviation score (calculated relative to WHO standards [254]) between the specified time points, derived from linear regression analysis adjusted for age, sex and wealth index

6.8 Discussion

This Chapter reported the impact of three separate maternal nutritional interventions provided perinatally in a rural area of Bangladesh on offspring blood pressure and kidney function. In the following sections, the results of the three interventions will be discussed separately, followed by a discussion of the observational analyses.

6.8.1 Maternal food intervention

6.8.1.1 Summary of findings

An invitation to receive food supplements earlier in pregnancy than is the usual practice was associated with lower diastolic and mean arterial blood pressure in the offspring at 4.5 years of age. The same intervention was not associated with offspring systolic blood pressure or with kidney volume or glomerular filtration rate (GFR).

6.8.1.2 Study strengths and limitations

This was a large follow-up study where 77% of individuals born during the maternal trial were successfully recruited. Loss to follow-up was evenly distributed between the six prenatal nutritional intervention arms (two food intervention arms and three micronutrient tablet arms). There were only very marginal differences in characteristics measured during the original trial between individuals who were recruited and those who were lost to follow-up. As a result, the recruited sample should be representative of the trial offspring as a whole. The sample size of the follow-up study (n=2312 for blood pressure analysis; n=1068 for kidney volume; n=1224 for GFR) was relatively large and the resulting effect estimates exhibited tight confidence intervals, promoting confidence in the results reported. Researchers in the original trial and the follow-up study were blind to the treatment

allocation of participating women, although it was not possible to keep allocation from the participants themselves due to the nature of this intervention.

During the follow-up, the measurement of outcome variables was conducted by fully-trained staff using standard operating protocols that were created to maximise accuracy in the setting. For the measurement of blood pressure an automated oscillometric device was used, and measurements were made in triplicate following the manufacturer's guidelines. Just over half of the individuals recruited had an additional blood pressure estimate, again in triplicate, on average 21 days after the first measurement. This provided an estimate of habitual blood pressure that should be more accurate than that obtained on a single occasion. Estimates of kidney size and volume were conducted by trained sonographers who had extensive practice and standardisation prior to the commencement of the fieldwork.

A particular strength of this study was the measurement of kidney function in addition to blood pressure assessment. The blood pressure system is tightly regulated by the renal system and alterations in kidney size and function have been suggested as one of the mechanisms linking suboptimal conditions during development to raised blood pressure in later life [160, 257, 258]. It is difficult to compare the kidney volumes for children in this study with the wider literature as there are no published reference data for kidney volumes in this age group. In agreement with a Danish study of ten year old children, gender differences in kidney volume were explained by differences in body size and there was a strong association between lean body mass and kidney volume [259]. Kidney volumes of children in this study were similar to those of 18 month old infants in Copenhagen, Denmark (n=631) that were on average 63.7cm^3 [260]. Kidney volume/body surface area ratio was similar to healthy 28 month old children in Turkey, which were reported to be on average 109cm^3/m^2 [261].

The main limitation with the original study design is that women were only randomised to encouragement to access food provision, rather than provided with

the food supplement itself. Some women would therefore be unlikely to access the supplement at all, although only 54 individuals reported not to have had a single food packet during their pregnancy and the majority (76%) of these were in the usual food invitation arm. The intervention design had been adopted as a method of evaluating the existing government nutritional provision during pregnancy, but as a result lacked a true 'control' arm. However, the data on food packet consumption during pregnancy did demonstrate that the intervention successfully created two distinct groups; women in the early invitation arm accessed the service much earlier in pregnancy and consumed on average 30 more packets during pregnancy. One packet provided 608 kcal and 17.9g of protein that, provided the food was consumed by the participating women, would have led to a substantial difference in nutritional intake per day between the two groups. This leads us to another limitation of the design, although women were asked whether they had consumed food packets at regular time points during their pregnancy the food was not consumed in the presence of field staff and could therefore have been given to family members or added to family meals.

In addition to the limitations of the original trial, the follow-up study also had some inherent limitations that affect the ability to correctly interpret the findings presented here. As with the other studies presented in this thesis, the age of follow-up represents a limitation of the design. The children may be too young to observe any differential effects on blood pressure and/or kidney function. In common with the Gambian studies reported in Chapters 4 and 5 it could also be argued that in a rural area of Bangladesh, where the children experience a high degree of wasting and stunting, it would be unlikely that the interventions would result in demonstrable effects on cardiovascular risk factors such as blood pressure. Any effect may thus only become apparent if these individuals are exposed to more 'Western' lifestyles as they develop.

A limitation of the design of the follow-up study in relation to kidney function is that only a small number of individuals (n=143) had estimates of both kidney volume and GFR. Initially the analysis of Cystatin C (CysC) was planned for the entire

sample but due to cost constraints a smaller number of samples were analysed, and the choice of these individuals was the responsibility of the original trial PIs, who conducted the CysC analysis in combination with other markers. The original PIs required data on certain individuals who were born between 2001 and 2002, which resulted in only a small overlap in these markers of kidney function as the ultrasound estimates were mainly conducted on individuals born between 2002 and 2003. The main consequence of this decision is the difficulty of interpreting effects observed on one aspect of kidney function that are not seen on the other, which may merely reflect the different individuals involved. However, the randomised design of the intervention should minimise any potential differences in children born earlier rather than later in the trial, suggesting that this concern may have only minimal impact on the results. For the individuals for whom both measurements were available there was only a marginal correlation between these markers, suggesting that they are measuring different aspects of kidney function, as would be expected.

Another major challenge of the follow-up design is the correct interpretation of differences in GFR between individuals as a result of the intervention. According to the hyperfiltration theory proposed by Brenner *et al*, fetal undernutrition resulting in a reduced number of nephrons will initially cause the remaining nephrons to have an increased load in compensation [155]. It is therefore debatable what the overall effect on total GFR would be in this model, particularly in childhood. Indeed Bagby has suggested that single nephron hyperfiltration will initially lead to *increased* total GFR [160], although no human data exists with which to test this hypothesis. Thus although GFR was chosen as a useful marker of kidney function and one that is used commonly to detect early renal disease [262], its applicability as a marker of kidney function response to altered prenatal nutrition may be questioned in this age group. Kidney volume relative to body size may thus be a more robust marker of any functional alterations experienced in the kidney in response to early-life conditions. It should be noted that whilst this limitation is of academic interest it does not affect the interpretation of the results presented here

as there was no association (either reduced or enhanced) between invitation to food supplementation and offspring GFR.

6.8.1.3 Comparison with the literature

It is challenging to compare the finding that a maternal invitation to enter a food supplementation program early in pregnancy was associated with lower diastolic blood pressure with the wider literature due to the specific design of this particular trial, including the lack of a control group. The impact of protein-energy supplementation during pregnancy on offspring blood pressure has been discussed elsewhere in this thesis (Section 3.5.1.3) and has in general been shown to have no effect [97, 99, 213]. However, these designs are not directly comparable with the study presented here.

One of the factors that suggest caution in the over-interpretation of this finding is that the number of food packets consumed during pregnancy was not associated with offspring diastolic or mean arterial pressure. Whilst there are well-established limitations of as-treated analyses, primarily the loss of randomisation, it would be assumed that the intervention effect would operate through a greater consumption of food packets. Furthermore, the effect seen in the intention-to-treat analysis was only of weak significance which, given the large sample size, suggests that any real effects are very subtle.

The lack of an association between the invitation to access the food supplementation programme and offspring kidney function is also difficult to compare with the wider literature as no published studies have investigated the impact of the maternal diet on these parameters in humans. The majority of animal data focus on the effects of nutrition restriction during fetal development on subsequent renal function, rather than enhanced nutrition, and may not be directly relevant to the human situation. In addition, most of the animal data use models of protein restriction during pregnancy followed by a normal postnatal diet [79, 263]. Hoppe *et al* investigated the impact of a low protein diet in rats that was provided

throughout gestation and for 135 days postnatally, postulating that this may be more reflective of the human diet in marginally nourished populations [132]. The resulting offspring had a reduced number of nephrons but their mean arterial pressure was lower, rather than higher, than rat pups exposed to a normal protein diet [132]. In contrast, the data presented in this chapter has revealed lower mean arterial pressure for individuals exposed to higher protein intake, but no effects on the markers of kidney function studied. Again in contrast to the null effect on kidney volume reported here, animal studies have demonstrated changes in kidney weight relative to body weight in response to protein-calorie restriction [257]. Cross-species comparisons are challenging due to the inherent biological differences and the specific techniques that can be used, which could explain the conflicting findings. For example, estimation of kidney volume via ultrasound in humans has a greater error associated with it than the direct measurement of organ weight possible in animal experiments.

6.8.1.4 Implications of findings

The potential effect of the intervention on offspring blood pressure was not reflected in altered kidney function, suggesting that changes were operating outside of the renal system or that the estimates of renal function used in this study were not able to pick up subtle differences between intervention groups.

Early invitation to access the food supplementation programme was associated with only a 0.58mmHg decrease in diastolic blood pressure. Thus, even if this result is robust, the effect size is too small to have large public health implications. Although it could be argued that this small difference will become larger in magnitude as the participants age [48]. If additional health benefits were found for this intervention then the potential advantageous effect on blood pressure would represent an added benefit. However, a recent cost-effectiveness analysis of the Bangladesh Integrated Nutrition Programme revealed the food provision to be of debatable value for money [264]. The authors demonstrated that the cost of the food supplement alone was around US$0.20 per participant per day, arguing that

from a purely cost-per-calorie standpoint it would be possible to purchase over three times as many calories from the local market for the same price [264]. Their analysis thus questions the sustainability of this government nutrition programme, and the lack of any large benefit in terms of offspring blood pressure would also discourage the roll-out of the intervention to target this aspect of cardiovascular health.

6.8.2 Maternal micronutrient intervention

6.8.2.1 Summary of findings

Maternal supplementation with multiple micronutrients during pregnancy, compared to supplements containing iron and folate only, was associated with raised offspring diastolic blood pressure at 4.5 years of age in the analysis presented here. However, the association was only strongly apparent in the adjusted analysis and was not observed in the analysis involving two repeat measurements of blood pressure, even though the sample size remained greater than 1000, suggesting the finding may be a chance occurrence. Maternal multiple micronutrient supplementation was not related to systolic blood pressure but there was a suggestion that for girls only, the supplement was associated with lower pulse pressure. Supplementation with 60mg of iron compared to 30mg of iron during pregnancy was not associated with offspring blood pressure

The maternal multiple micronutrient supplement was not associated with offspring kidney volume or GFR. There was a suggestion that providing a high (60mg) versus lower (30mg) iron dose during pregnancy resulted in raised offspring GFR, although the finding was of very borderline significance.

6.8.2.2 Study strengths and limitations

In addition to the general study strengths outlined in Section 6.8.1.2, the micronutrient arm of the trial benefitted from being a true, double blind

intervention. The original trial and follow-up researchers, field staff and participants remained blind as to the treatment allocation of participating children.

A limitation specific to the micronutrient intervention is the absence of a true control group provided with a placebo. This design was used because it is Bangladesh Government policy to provide 60mg of iron and 400µg of folic acid to pregnant women and it would therefore be unethical to provide a true placebo arm. Similar designs have been adopted in other trials [103], which allows the results presented here to be directly comparable with the literature. The design of the intervention is further compromised by its unbalanced design; there is no multiple micronutrient arm that also contains 60mg of iron (Table 6.40). As a consequence, any effect of the MuMs arm could be confounded by the iron treatment arm and it is not possible to look at the interaction between these different treatments.

Table 6.40 Unbalanced design of the MINIMat intervention

	400µg folate	MuMs
30mg iron	Yes	Yes
60mg iron	Yes	No

An additional limitation from the original trial is the pill-count data that was derived from the bottles themselves, which recorded each time the lid was opened. It has been assumed in the analysis that this pill count accurately reflects the intake of participating women, whereas the tablets could have been shared with other members of the family or the lid merely opened and closed to generate data.

6.8.2.3 Comparison with the literature

Two studies have to date published directly comparable follow-up data on the blood pressure of offspring born during a maternal multiple micronutrient

supplementation trial. Both of these trials were set in Nepal: one conducted by Osrin *et al* compared the UNICEF/UNU/WHO preparation of 15 micronutrients (of the same concentrations as in the current Bangladesh study) with 60mg of iron and 400µg of folate [101], whilst the second was a follow-up of a five-arm supplementation trial conducted by Christian *et al* [102]. This second study provided different combinations of micronutrients to the participating women: the control group received vitamin A (1000µg retinol retinol equivalents) only, in addition to vitamin A the intervention groups received either folic acid (400µg); folic acid and iron (60mg); folic acid, iron and zinc (30mg); or these four micronutrients with an additional ten (10µg vitamin D, 10mg vitamin E, 1.6mg thiamine, 1.8mg riboflavin, 2.2mg vitamin B6, 2.6µg vitamin B12, 100mg vitamin C, 64µg vitamin K, 20mg niacin, 2mg copper and 100mg of magnesium) [102]. Both supplementation trials demonstrated an increase in birth weight and a reduction in low birth weight (proportion of infants <2500g) for women in the multiple micronutrient arms of the trial [101, 102]. However, the trial by Christian *et al* also found a beneficial effect on birth weight of folic acid and iron in comparison to vitamin A and no demonstrable additional benefit of the multiple micronutrient supplement [102].

Both trials were recently followed-up and the cardiovascular health of the offspring was assessed [103, 104]. For the trial conducted by Osrin *et al* the offspring were 2.5 years of age at follow-up and 83% (n=917) of the original live singleton born individuals were recruited [103]. At follow-up there remained a significant difference in weight between the two groups and systolic blood pressure was found to be on average 2.5mmHg lower in children born to women in the multiple micronutrient arm compared to the iron and folate arm [103].

The follow-up of the trial conducted by Christian *et al* enrolled individuals who were now 6-8 years old and recruited 85% (n=3524) of live born individuals from the original trial [104]. There was no difference in systolic or diastolic blood pressure between the five groups of the trial [104]; findings that are in line with the analysis presented in this chapter.

All three micronutrient supplementation trials (two from Nepal and the data from Bangladesh presented here) are large, well conducted intervention studies with high rates of follow-up and it is therefore important to consider explanations for the different findings observed. Firstly the current study participants and the participants in the follow-up of the Christian *et al* trial were older than those in the first Nepalese trial and it is possible that any effects of maternal nutritional supplementation on offspring blood pressure are only short-lived. Another explanation could be a difference in the populations as the Osrin *et al* trial recruited women from a more urban environment than the rural Bangladesh setting and the more rural setting of the second Nepalese study. An explanation for the null effects of the Bangladesh trial could be that the supplement was less effective in this setting; there was no effect of the multiple micronutrient supplement on birth weight for example (Persson, unpublished). However, both Nepalese trials were shown to impact on birth weight [101, 102]. Finally it could be that the reduction in infant blood pressure observed in the first Nepalese study was due to chance.

The authors of the first Nepalese trial follow-up suggest that the beneficial effect of multiple micronutrient supplementation on blood pressure could be due to the difference in iron composition of the two tablets, 60mg compared to 30mg in the MuMs tablet [103]. They suggest that the high iron dose may be detrimental, leading to raised blood pressure in this group. The design of the MINIMat trial allows this hypothesis to be investigated and the analysis has shown no effect of high compared to low iron dose on offspring blood pressure or kidney function in this population. Two large cohort studies have investigated the impact of the general diet during pregnancy, including iron intake, on offspring blood pressure at three years [72] and 7.5 years respectively [73]. In the US, maternal iron intake from personal supplements, but not from food, was positively associated with offspring systolic blood pressure at three years whereas in the UK there was no association between iron intake (from foods or iron-only supplements) in pregnancy and offspring blood pressure at 7.5 years [73]. It should be noted that these studies are not directly comparable with each other; the UK researchers

restricted their analysis to iron-supplements whereas the US study included iron from multiple micronutrient supplements.

As stated previously, there is very little human data on the association between maternal nutritional interventions or maternal diet during pregnancy and offspring kidney function. However, the follow-up of the second Nepalese multiple micronutrient trial, originally conducted by Christian *et al*, contained urinary measurements of the microalbumin: creatinine ratio in the offspring [104]. Microalbuminuria (microalbumin: creatinine ratio ≥3.4mg/mol) is an established marker of kidney dysfunction [265], but in Bangladesh the analysis of both microalbumin and creatinine in urine have been hampered by the highly dilute nature of the urines collected in this region (Vahter, *pers. comm.*). In the Nepalese study, there was no difference in the mean microalbumin: creatinine ratio for individuals born to women in any of the intervention arms but there was a lower risk of microalbuminuria (microalbumin: creatinine ratio ≥3.4mg/mol) for individuals whose mothers had received folic acid in addition to vitamin A or folic acid, iron and zinc in addition to vitamin A compared to controls (vitamin A alone) [104]. Interestingly there was no difference in the odds of microalbuminuria in the other two intervention arms which both contained folic acid, iron and vitamin A.

A few animal studies have focused on the impact of vitamin A as this is known to be essential for organogenesis [266]. Studies have demonstrated that retinoic acid, the metabolically active form of vitamin A, is fundamental to mammalian nephrogenesis [267]. Even a relatively mild (50%) vitamin A deficiency in pregnant rats leads to a 20% reduction in nephron number in their offspring [268]. In protein-restricted rats (restricted throughout pregnancy and lactation), the administration of retinoic acid during gestation prevented the loss of nephrons associated with the low protein diet [269], suggesting that it might have been possible to witness an interaction between the food and micronutrient interventions here, although none were observed. However, the direct relevance of the animal data to human studies is questionable as many additional factors must be considered when investigating the impact of a community-based supplementation trial. In particular, the

prevalence of vitamin A deficiency (VAD) in this population is not known; a recent survey from a northern rural district in Bangladesh reported that 18.5% of pregnant women had VAD (serum retinol <0.70µmol/l) [270]. There are no studies of vitamin A status and nephron number in humans but a recent pilot study from Bangalore, India failed to find a correlation between maternal retinol levels during pregnancy and offspring kidney volume ascertained within two days of birth [271], which is more in line with the findings presented in this thesis.

6.8.2.4 Implications of findings

This study corroborates the findings of a multiple micronutrient supplementation trial in Nepal, suggesting that there is no effect on offspring blood pressure [104]. A separate Nepalese trial did observe an impact of multiple micronutrient supplementation on offspring blood pressure, albeit in a younger age group [103]. There do not appear to be any effects of high iron compared to a low iron dose in this setting in terms of offspring blood pressure and kidney function. The impact of micronutrient supplementation on offspring kidney function requires further research; data presented in this thesis suggests no effect whilst one of the Nepalese trials reported a reduction in microalbuminuria [104].

6.8.3 Breast feeding intervention

6.8.3.1 Summary of findings

Maternal randomisation to receive counselling for exclusive breast feeding, compared to counselling for general health messages, was not associated with offspring blood pressure or kidney function. The as-treated analysis suggested that individuals who were partially breast fed (foods and other milks in combination to breast milk) at four months of age had lower systolic blood pressure at 4.5 years, although the association was lost after adjustment for confounders.

6.8.3.2 Study strengths and limitations

In addition to the general MINIMat trial design strengths and limitations outlined in Section 6.8.1.2, the main strength of the breast feeding counselling intervention is the randomised nature of the trial; the majority of the literature relies on observational data. Again, the follow-up sample size was large, recruitment rates were similar between both arms of the intervention and loss to follow-up was not associated with any baseline characteristics. The intervention was successful in promoting breast feeding practices in the targeted women with 61% still exclusively breast feeding four months after birth compared to 37% in the control group.

A limitation of the intervention is that it would be unethical to randomly assign women not to breast feed and therefore the trial focussed on counselling that promoted exclusive breast feeding, which is one step removed from the hypothesis being tested.

6.8.3.3 Comparison with the literature

The lack of an effect of breast feeding counselling on offspring blood pressure is in accordance with the follow-up of the Promotion of Breastfeeding Intervention Trial (PROBIT) in Belarus [138]. This was a large multicentre cluster-randomised controlled trial using the steps of the WHO and UNICEF-developed Baby-Friendly Hospital Initiative and targeting more than 17,000 women [137]. The intervention was successful at increasing the rate of breast feeding, with infants in intervention areas more likely to be breastfed exclusively at three and six months compared to infants in control areas [137], but there was no difference in blood pressure between these two groups of children at 6.5 years of age [138]. A small trial of preterm infants in the UK randomly assigned to receive banked breast milk or formula milk also revealed no difference in blood pressure at 7.5-8 years of age [135]. However, when the same subjects were followed-up at 13-16 years of age, those in the breast milk group had on average 3.2mmHg lower diastolic blood pressure and 4.1mmHg lower mean arterial pressure than those in the preterm formula group, although it should be noted that only 25% (n: 130) of the original

trial participants were recruited into this second follow-up [134]. The disparity in the results between these trials could suggest that an association between breast feeding and lower blood pressure will become apparent in the PROBIT and MINIMat trial offspring once they reach adolescence. Alternatively the difference between the trials could reflect the target individuals, with the Belarus and Bangladesh trials being more representative of their wider populations than a small trial recruiting pre-term infants.

There has been an ongoing debate in the literature on the effect of breast feeding on long-term blood pressure, with the majority of data derived from cohort studies. A systematic review and meta-analysis published in 2003 by Owen *et al* identified 25 studies for inclusion that compared exclusively breast fed with exclusively bottle fed individuals, and reported a small protective effect of breast feeding on systolic blood pressure from their meta-analysis (-1.10mmHg; 95% CI: -1.79, -0.42) [124]. However, the authors also found some evidence of publication bias with smaller studies (<300 individuals) showing a stronger association and studies with over 1000 subjects showing a non-significant association [124]. In 2005, Martin *et al* published a review that included only studies reporting blood pressure after 12 months of age and identified 15 studies to include [125]. Again there was a small protective effect of breast feeding in infancy on later systolic blood pressure (-1.4mmHg; 95% CI: -2.2, -0.6). Only four studies included in the review had more than 1000 participants and again the effect was much smaller for these studies and only of borderline significance (-0.4mmHg; 95% CI: -0.9, 1.0) [125].

The most recent review and meta-analysis of this topic was conducted by Horta *et al* for the WHO and published in 2007 [126]. The review included 30 studies and the analysis suggested that there was a small protective effect of breast feeding on both systolic (-1.21 mmHg; 95% CI: -1.72, -0.70) and diastolic (-0.49 mmHg; 95% CI: -0.87 to -0.11) blood pressure [126]. In addition to issues of publication bias related to study size, the authors raised concerns that residual confounding may explain the results, as the effect estimates were often attenuated after adjustment for measured confounders [126]. Randomised controlled trial data, such as that

presented here, may provide more valid results as the data should be independent of confounding both by known and unknown factors [95].

The reviews of the predominantly observational literature include very few studies from developing countries, which could explain the differences between these findings and the data from Bangladesh. One large cohort from a semi-urban area in Brazil recently reported that breast feeding was not associated with systolic or diastolic blood pressure at 15 years [272]. The comparison between exclusively breast fed and exclusively bottle fed infants, which is most common in the literature, is also not directly comparable with the situation in rural Bangladesh where very few infants do not receive any breast milk at all. From the Bangladesh data it was possible to investigate the separate effects of different categories of breast feeding, from exclusive to predominant to partial feeding. Interestingly it appeared that the individuals with lower systolic blood pressure at follow-up were those who were already receiving foods and other milks in combination with breast milk by four months of age. Singhal *et al* have proposed that formula fed infants will grow faster than their breast fed counterparts and that this difference explains the higher blood pressure often reported for these individuals [136]. In rural Bangladesh it may be that exclusive and predominantly breast fed individuals exhibit increased growth compared to partially breast fed infants due to the quality of complementary foods that are introduced. Therefore this difference in growth profiles (which is the opposite to individuals in developed country settings) may explain the differences in blood pressure revealed by this study.

Very few studies have investigated the impact of infant feeding on kidney function. A small Danish study observed a non-significant trend towards increased kidney volume (adjusted for current body surface area) from fully breast fed to partially breast fed and then formula fed individuals at three months of age [260]. However, a second follow-up in the same cohort revealed there was no longer an association between feeding type at three months and kidney volume at 18 months of age [260].

6.8.3.4 Implications of findings

These data add to the debate on the long-term health benefits of breast feeding. Data from the two community-based randomised trials in this field suggest that duration of exclusive breast feeding may not be related to blood pressure in later childhood. Concerns regarding residual confounding have been an issue for observational studies in this area and the trial data are therefore particularly valuable to investigate the impact of breast feeding on long-term cardiovascular health.

6.8.4 Observational analysis

6.8.4.1 Summary of findings

A number of early-life factors were found to be associated with blood pressure and kidney function at 4.5 years of age. Maternal baseline haemoglobin (week 14 gestation) was positively associated with offspring systolic and diastolic blood pressure. Birth weight and length were inversely associated with kidney volume but not with GFR. Systolic and diastolic blood pressure were inversely associated with birth weight only after adjustment for current size. Season of birth was also associated with systolic blood pressure, kidney volume and GFR. Increased growth (weight and length) in the first year of life was positively associated with blood pressure and kidney volume but not with GFR.

6.8.4.2 Study strength and limitations

In common with The Gambian studies it should be recognised that this observational data comes from the follow-up of a randomised trial, which was not established as a cohort study. However, with this caveat in mind, the data can usefully be viewed as a cohort enrolled at birth and followed-up 4.5 years later. The maternal interventions were not associated with the early-life exposures that have been studied, such as maternal baseline haemoglobin or season of birth. To date, the preliminary trial analysis has also revealed no association between the maternal

interventions and birth anthropometry (Persson, *unpublished*). As has been demonstrated in this chapter, there was also little effect of the maternal interventions on offspring blood pressure and kidney function, suggesting that it is appropriate to conduct these observational analyses without taking the treatment allocation into account.

One of the strengths of this data set from the standpoint of cohort analysis is that there is available information on potential confounding factors such as wealth index that was missing from The Gambian studies presented earlier in this thesis.

6.8.4.3 Comparison with the literature

Maternal leg length has been suggested as a useful proxy indicator of a mother's own nutritional exposures as a child, and thus a way of investigating intergenerational effects of sub-optimal nutrition [253]. Maternal leg length has been shown to be positively associated with the birth weight of her children in two retrospective cohort studies based in the UK [255, 273]. In the analysis presented in this thesis, the association between maternal leg length and offspring blood pressure was used to investigate the impact of maternal nutritional experiences in early-life on the development of offspring body systems, but no association was observed.

There was a strong positive association between maternal haemoglobin in early pregnancy and offspring systolic and diastolic blood pressure. In analysis adjusted for confounders, for every 1g/dl increase in maternal haemoglobin at week 14 gestation, offspring systolic blood pressure increased by 0.42mmHg (95% CI: 0.09, 0.75). In addition, anaemia at 14 weeks gestation (but not at 30 weeks) was associated with lower blood pressure in the offspring. In line with this finding, data from the ALSPAC cohort in the UK suggested that anaemia in pregnancy may be associated with lower offspring systolic blood pressure at seven years of age, although the association was lost after adjustment for confounders [73]. In Project Viva in the US, there was no association between haemoglobin concentration in

pregnancy and offspring blood pressure, despite a positive association between iron intake from supplements and offspring systolic blood pressure [72]. In contrast, experiments in rats have demonstrated that iron deficiency in pregnancy is associated with raised offspring blood pressure [89, 274, 275]. Interestingly, two of these rat experiments initially observed lower blood pressure in the offspring of anaemic dams at three [274] and six [89] weeks postpartum, but that in the longer-term persistently higher blood pressure was observed. It could therefore be that a later follow-up of this cohort would reveal a different relationship to the one reported here. Potential mechanisms to explain the link between iron deficiency in pregnancy and offspring blood pressure remain unclear. Anaemia induced at the final stages of pregnancy in sheep has been shown to have dramatic effects on the fetal coronary tree, increasing the coronary blood flow and maintaining this adaptation into adulthood [276]. It is possible that permanent changes in cardiac output as a result of anaemia during fetal development could have long-term implications for blood pressure.

The observed inverse association between birth weight and later blood pressure is in close agreement with the literature. The effect size for the association was a 2.5mmHg decrease in systolic blood pressure for every 1kg increase in birth weight, which reflects the often-quoted average association of -2mmHg for a 1kg increase [51]. The inverse relationship was only apparent after adjustment for current size, which has been interpreted as suggesting that the change in size between birth and the current time point is the more important exposure, rather than size at birth [53]. Indeed this is revealed in the analysis of the effect of changing weight standard deviation scores in the first year of life and in the life course plots, which emphasised the positive association between crossing weight centiles in early infancy and blood pressure at 4.5 years. As discussed in previous chapters, there is currently little consensus in the literature on how best to characterise growth. A recent pooled analysis from five cohorts based in developing countries investigated the effect of conditional weight (an indication of an individual's deviation from their expected size at a given age) in childhood on later blood pressure [123]. The analysis initially revealed a positive association with conditional weight at different

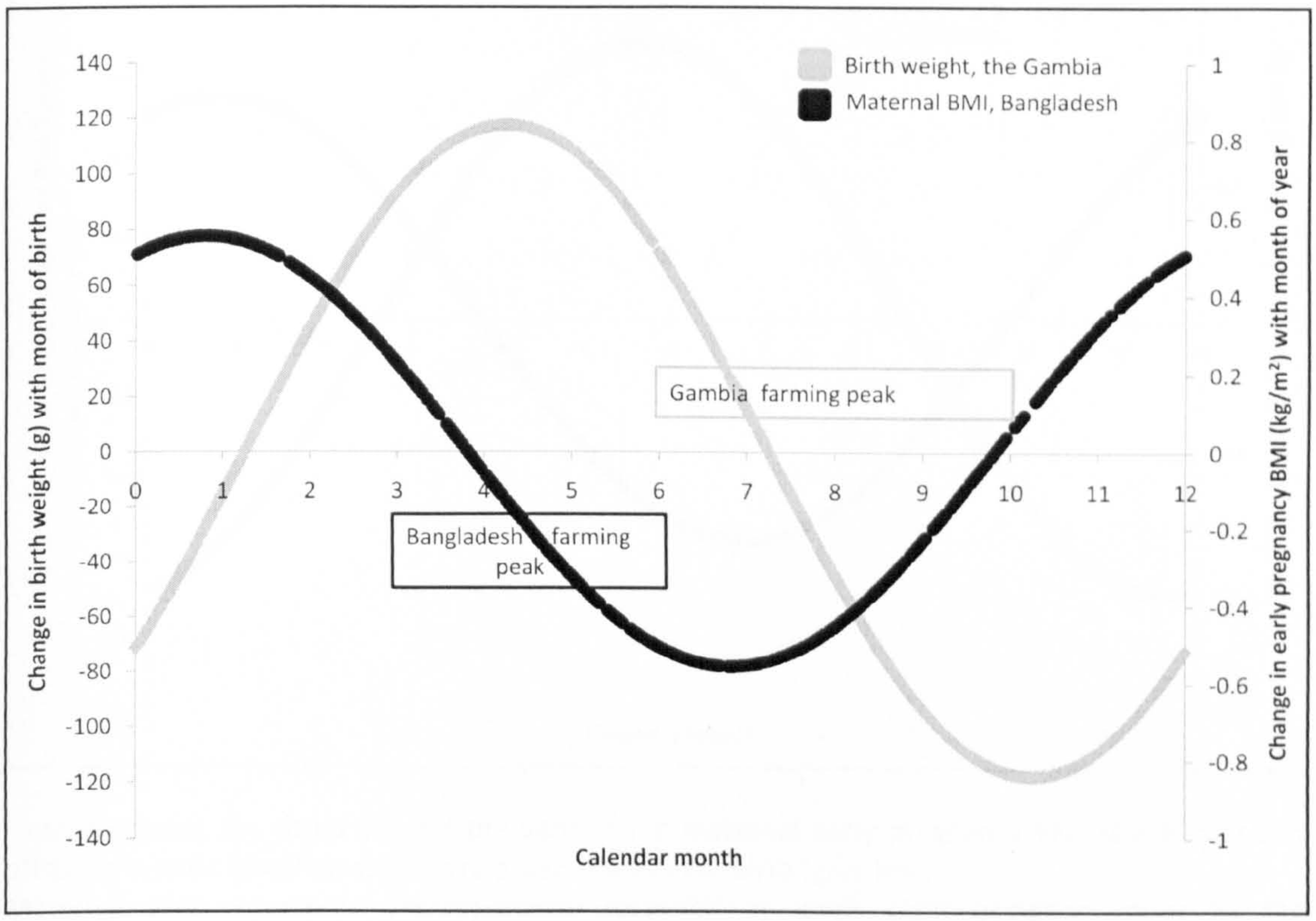
time points and early adult blood pressure although this was lost after adjustment for current BMI, which indicated that early growth only predicted adult blood pressure through its association with adult size [123]. In contrast, the association between growth in infancy and blood pressure in this Bangladesh study remained even after adjustment for current size.

The association between birth weight and kidney function has been reported for a number of studies, with conflicting results. Low birth weight Aboriginal children in Australia had lower kidney volume than normal birth weight children [172], a finding in agreement with the analysis presented here of the positive association between birth weight and kidney volume. In contrast, birth weight was not associated with kidney volume or glomerular filtration rate in Swedish children [277]. A recent systematic review by White *et al* investigated the association between birth weight and chronic kidney disease (CKD) [174]. From the 31 identified eligible studies, half reported an association between low birth weight and risk of CKD whilst half reported no association [174]. It is therefore currently difficult to draw firm conclusions from the existing literature.

Season of birth can be used as a proxy indicator of suboptimal conditions during development in areas where there are marked changes in food availability and farming activities. A previous analysis of the effect of season of birth on survival in this region of Bangladesh was conducted by Moore *et al* and revealed no association with adult mortality, in contrast to similar data from The Gambia [278]. The analysis presented in this thesis however, suggests that subtle health differences may occur between individuals in relation to their month of birth. Interestingly, the observed pattern between the month of birth and systolic blood pressure was almost the direct opposite of that reported in Section 3.4.3 for the Gambian data. This could be explained by the different pattern of food and activity in these two countries. In Bangladesh, the greatest farming activity occurs in the middle of the year before the monsoon starts in June and July, whilst in The Gambia the farming peak is from June to October. Variations in maternal BMI and in birth weight in these areas reflect the different patterns of activity; women joining the

MINIMat trial exhibited the lowest BMI when they were enrolled just after the peak farming activities (Figure 6.15). In The Gambia the lowest birth weights (no data on maternal early pregnancy BMI is available) occur just after the peak in farming activities.

Figure 6.15 Seasonal pattern of farming activities, maternal BMI and birth weight in rural Gambia and Bangladesh

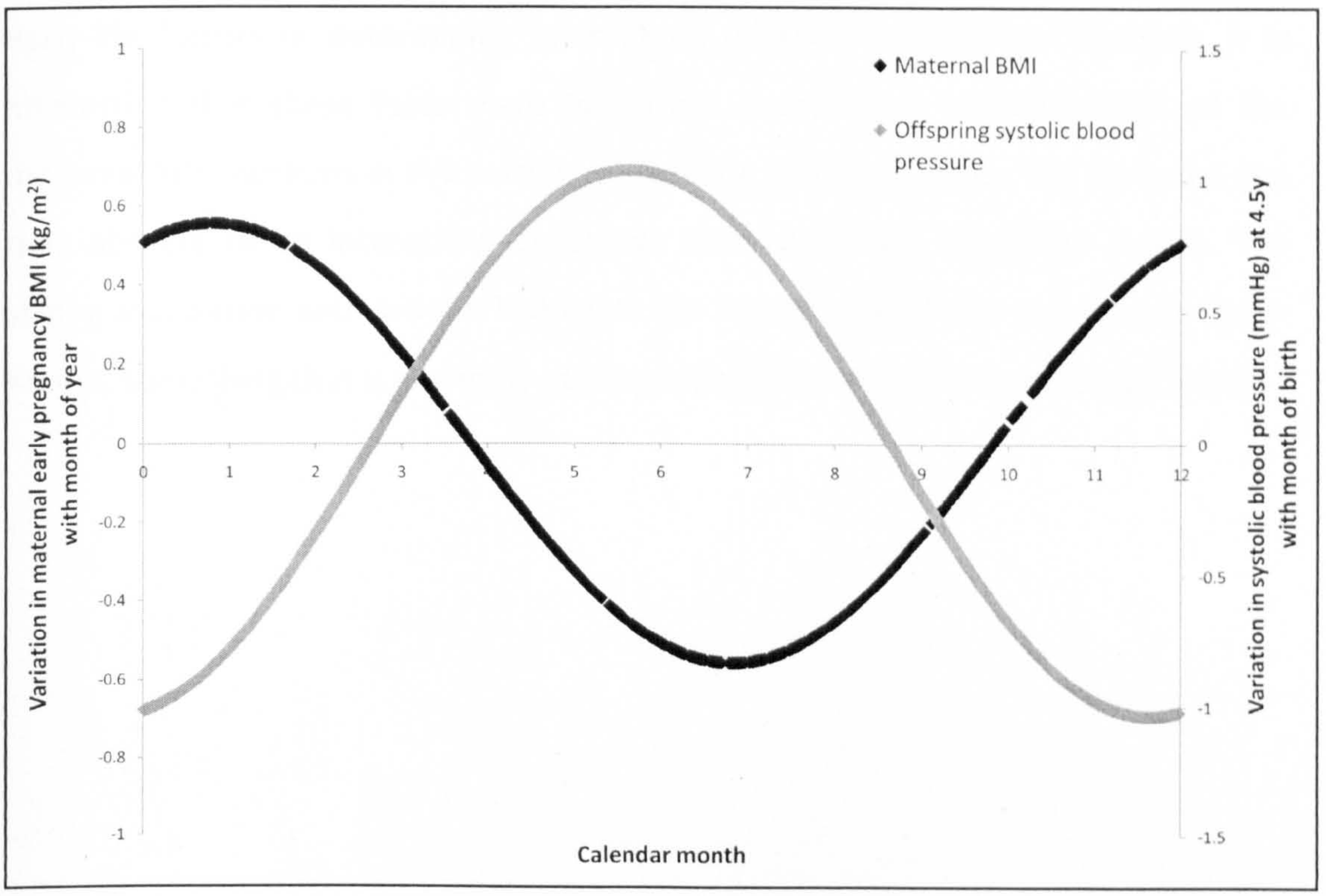


Lines represent the fitted line for the variation in birth weight in The Gambia (grey line) and early pregnancy BMI in Bangladesh (black line) with month of the year
The Gambia: birth weight restricted to control group from protein-energy supplementation trial only [194], pre-intervention maternal BMI was not available from this dataset
Bangladesh: maternal BMI represents anthropometry recorded at week eight gestation, prior to the implementation of the intervention. Birth weight was considered inappropriate for this trial as it may have been influenced by the maternal food intervention inasmuch as the effect of seasonality could have been reduced by the intervention

Interestingly, the seasonal pattern of maternal BMI variation in Bangladesh reflects the association between season of birth and blood pressure in the offspring of these women (Figure 6.16). Individuals born in the middle of the year may exhibit

the highest systolic blood pressure at follow-up as they have been born in the 'hardest' part of the year as evidenced by the variation in maternal BMI.

Figure 6.16 Seasonal variation in maternal BMI and in blood pressure in relation to month of birth



Lines represent the fitted line for the variation in maternal early pregnancy BMI (black line) and offspring systolic blood pressure in relation to season of birth (grey line)
Maternal BMI represents anthropometry recorded at week eight gestation, prior to the implementation of the intervention
Offspring systolic blood pressure was measured at 4.5 years

It should be acknowledged that the use of Fourier terms to model the effect of seasonality as an exposure by necessity smoothes the raw data so that the pattern may appear more robust than the reality. In addition, the actual size of the association between month of birth and blood pressure reported here is very small with the 95% confidence interval only spanning 1mmHg, which again cautions against the over-interpretation of these intriguing patterns.

6.8.4.4 Implications of findings

The observational analyses presented in this Chapter emphasise the importance of early-life factors in determining later blood pressure and kidney function. It is interesting that these factors are important despite the limited impact of the maternal interventions in this setting, suggesting that focusing on the maternal diet may obscure other interesting exposures that can affect long-term health. The strong association with growth highlights the importance of this exposure for later health, something that is currently gaining recognition in the literature [123, 231].

Chapter 7 General discussion, implications and future research

7.1 Forward

This Chapter will summarise the main findings from this PhD thesis, focusing on results that span all three follow-up studies. The data will be interpreted and future research suggested before a final discussion of the importance of this data in the wider context of the Developmental Origins of Health and Disease (DOHaD) research field.

7.2 Summary of results

7.2.1 Intervention analysis

The purpose of this PhD was to investigate the impact of maternal nutritional interventions during pregnancy on offspring blood pressure. The research question falls under the general umbrella of DOHaD, specifically on the importance of nutrition in early-life for later cardiovascular health. Historically, this research field has relied heavily on observational data reporting the association between birth weight and later health [45, 49] and on animal experiments that may not be directly relevant to the human situation [77, 79]. In contrast, the research presented in this thesis utilised trials that were originally conducted to improve maternal and infant health, in order to investigate the impact of the maternal diet on offspring blood pressure from the standpoint of a randomised trial. Intervention data such as these provide two main advantages over the conventional literature, producing effects that should be independent of confounding, including by possible genetic effects, and removing the necessity to assess maternal nutritional intake using dietary assessment techniques that are often prone to error.

The data presented were from follow-up studies of protein-energy supplementation in The Gambia, calcium supplementation also in The Gambia and a large multifaceted intervention of food, micronutrient supplementation and breast feeding counselling in Bangladesh (the MINIMat study). Each trial provided a different study design with which to investigate the impact of the maternal diet. The Gambian protein-energy trial follow-up involved older individuals who could be hypothesised to be more likely to demonstrate any long-term effects, but there were interpretation issues with the original trial design as the 'control' participants received supplements during lactation. The calcium trial provided the most robust data; recruiting offspring from a randomised double-blind placebo-controlled trial and with a number of directly comparable studies already in the literature. The follow-up of the MINIMat trial in Bangladesh enabled the hypothesis to be tested in a different setting and allowed for the exploration of kidney function (volume and glomerular filtration rate (GFR)) effects that were postulated to exist even in the absence of any effect on blood pressure. In addition, the Bangladesh study provided data on a breast feeding intervention that added another interesting angle to the PhD research.

Overall, the results revealed little evidence that any of these interventions influenced offspring blood pressure or kidney function, with the exception of a slight decrease in diastolic blood pressure in response to an invitation to access food supplementation early in pregnancy in rural Bangladesh (Table 7.1).

Table 7.1 Impact of maternal nutritional interventions provided during pregnancy on offspring blood pressure and kidney function

	Intervention[a]	N	Age (y)	Mean difference (95% CI)[b]	P-value
Systolic blood pressure (mmHg)	Protein-energy	1267	11-16	0.46 (-1.12, 2.04)	0.57
	Calcium	350	5-10	-0.10 (-1.89, 1.68)	0.91
	Food invitation	2312	4.5	0.46 (-0.16, 1.08)	0.15
	MuMs	2312	4.5	0.05 (-0.71, 0.81)	0.89
	60Fe	2312	4.5	-0.03 (-0.71, 0.81)	0.93
	Breast feeding counselling	2312	4.5	-0.03 (-0.65, 0.60)	0.93
Diastolic blood pressure (mmHg)	Protein-energy	1267	11-16	0.09 (-1.31, 1.13)	0.89
	Calcium	350	5-10	0.10 (-1.46, 1.67)	0.90
	Food invitation	2312	4.5	0.58 (0.06, 1.11)	0.03
	MuMs	2312	4.5	0.56 (-0.09, 1.21)	0.09
	60Fe	2312	4.5	0.27 (-0.38, 0.92)	0.41
	Breast feeding counselling	2312	4.5	0.34 (-0.83, 0.10)	0.21
Kidney volume (cm^3/m^2)	Food invitation	1068	4.5	-0.08 (-1.97, 1.80)	0.93
	MuMs	1068	4.5	-0.98 (-3.29, 1.34)	0.41
	60Fe	1068	4.5	-0.10 (-2.39, 2.20)	0.93
	Breast feeding counselling	1068	4.5	-0.80 (-2.68, 1.07)	0.40
Glomerular filtration rate ($ml/min/1.73m^2$)	Food invitation	1224	4.5	-1.76 (-5.70, 2.17)	0.38
	MuMs	1224	4.5	1.28 (-3.57, 6.13)	0.60
	60Fe	1224	4.5	4.42 (-0.37, 9.20)	0.07
	Breast feeding counselling	1224	4.5	3.71 (-0.30, 7.73)	0.07

[a]Interventions are, in order: protein-energy biscuits provided during pregnancy compared to during lactation; 1500mg of calcium carbonate or matched placebo during pregnancy; invitation to access a food supplementation program early in pregnancy (coded 0) or at the usual time (coded 1); supplement containing 15 multiple micronutrients compared to iron and folate only during pregnancy and for 2 months post-partum; supplement containing 60mg of iron compared to 30mg of iron during pregnancy and for 2 months post-partum; counselling for exclusive breast feeding compared to counselling for general health messages provided during six sessions perinatally.

[b]Difference in mean blood pressure (95% CI) for individuals born to mothers receiving the intervention compared to controls, derived from unadjusted analysis

7.2.2 Observational analysis

The observational analyses were conducted as a secondary aim to utilise the data to their full potential, allowing comparisons of these populations with the wider literature and exploring exposures other than prenatal nutrition. This was felt to be particularly valid as the interventions did not affect the exposures under investigation, with the exception of an increase in birth weight associated with protein-energy supplementation in The Gambia that was accounted for in the analysis.

There was some heterogeneity in the observational findings from these studies (Tables 7.2 and 7.3). Season of birth was associated with systolic blood pressure for adolescents in The Gambia and for the Bangladesh cohort, although there were distinct patterns of association in each setting. Birth weight and length unadjusted for current size were not related to later blood pressure in any of the three groups. However, once birth weight was adjusted for current weight there was a strong inverse association for individuals in Bangladesh and the suggestion of an association for adolescents in The Gambia. This implicates postnatal growth as an important exposure and indeed the change in weight standard deviation score from birth to one year was found to be positively associated with later systolic blood pressure in both Bangladesh and the older Gambian cohort. Change in length SD score was also positively associated with systolic blood pressure in Bangladesh. None of the exposures relating to birth weight or the first year of life were associated with systolic or diastolic blood pressure for the younger Gambian children but this may reflect the smaller sample size (n=350) of this group.

Table 7.2 Summary of observational analyses relating early-life exposures to later systolic blood pressure (mmHg)

		Regression coefficient (95% CI)	P-value
Maternal			
Maternal leg length (cm)	Bangladesh 4.5y	0.03 (-0.18, 0.23)	0.79
Maternal hb, wk14 (g/dl)	Bangladesh 4.5y	0.42 (0.09, 0.75)	0.01
Birth			
Birth weight (kg)	The Gambia, 11-16y	-0.52 (-1.72, 0.68)	0.39
	The Gambia, 5-10y	1.31 (-0.64, 3.25)	0.19
	Bangladesh, 4.5y	-0.63 (-1.51, 0.26)	0.17
Birth weight (kg) adjusted for current weight	The Gambia, 11-16y	-1.13 (-2.31, 0.04)	0.06
	The Gambia, 5-10y	-0.89 (-2.89, 1.10)	0.38
	Bangladesh, 4.5y	-2.53 (-3.46, -1.60)	<0.001
Birth length (cm)	The Gambia, 11-16y	0.19 (-0.06, 0.43)	0.13
	The Gambia, 5-10y	0.04 (-.01, 0.09)	0.08
	Bangladesh, 4.5y	-0.07 (-0.21, 0.07)	0.34
Birth length (cm) adjusted for current height	The Gambia, 11-16y	0.06 (-0.18, 0.30)	0.63
	The Gambia, 5-10y	0.00 (-0.05, 0.05)	0.90
	Bangladesh, 4.5y	-0.23 (-0.38, -0.08)	0.002
Season of birth	The Gambia, 11-16y	LR test χ^2: 6.69[a]	0.04
	The Gambia, 5-10y	LR text χ^2: 0.71[a]	0.69
	Bangladesh, 4.5y	LR test χ^2: 14.94[a]	0.001
Infant			
Change in weight SDS from 0-12m	The Gambia, 11-16y	0.73 (0.27, 1.19)	0.002
	The Gambia, 5-10y	0.32 (-0.49, 1.13)	0.44
	Bangladesh, 4.5y	0.88 (0.58, 1.17)	<0.001
Change in length SDS from 0-12m	The Gambia, 11-16y	0.27 (-0.39, 0.92)	0.43
	The Gambia, 5-10y	0.05 (-0.80, 0.90)	0.91
	Bangladesh, 4.5y	0.71 (0.39, 1.02)	<0.001

Results are the effect on systolic blood pressure of a one unit increase in the explanatory variable, derived from linear regression analysis adjusted for appropriate confounders

[a]Results of likelihood ratio tests comparing models with or without season of birth as an exposure

Table 7.3 Summary of observational analyses relating early-life exposures to later diastolic blood pressure (mmHg)

		Regression coefficient (95% CI)[a]	P-value
Maternal			
Maternal leg length (cm)	Bangladesh, 4.5y	0.01 (-0.17, 0.19)	0 .91
Maternal Hb, wk14 (g/dl)	Bangladesh 4.5y	0.45 (0.16, 0.73)	0.002
Birth			
Birth weight (kg)	The Gambia, 11-16y	-0.004 (-1.03, 1.02)	0.99
	The Gambia, 5-10y	0.11 (-1.59, 1.81)	0.90
	Bangladesh, 4.5y	0.23 (-0.52, 0.98)	0.55
Birth weight (kg)	The Gambia, 11-16y	-0.40 (-1.14, 0.62)	0.44
adjusted for current	The Gambia, 5-10y	-1.64 (-3.41, 0.12)	0.07
weight	Bangladesh, 4.5y	-1.02 (-1.82, -0.22)	0.01
Birth length (cm)	The Gambia, 11-16y	0.11 (-0.10, 0.32)	0.31
	The Gambia, 5-10y	0.03 (-0.01, 0.07)	0.41
	Bangladesh, 4.5y	0.03 (-0.10, 0.15)	0.67
Birth length (cm)	The Gambia, 11-16y	0.05 (-0.16, 0.26)	0.64
adjusted for current	The Gambia, 5-10y	0.01 (-0.04, 0.05)	0.73
height	Bangladesh, 4.5y	-0.09 (-0.22, 0.04)	0.18
Season of birth	The Gambia, 11-16y	LR test χ^2: 4.51[a]	0.10
	The Gambia, 5-10y	LR test χ^2: 7.93[a]	0.09
	Bangladesh, 4.5y	LR test χ^2: 6.77[a]	0.03
Infant			
Change in weight	The Gambia, 11-16y	0.32 (-0.08, 0.72)	0.12
SDS	The Gambia, 5-10y	0.47 (-0.23, 1.18)	0.19
from 0-12m	Bangladesh, 4.5y	0.60 (0.35, 0.85)	<0.001
Change in length	The Gambia, 11-16y	0.36 (-0.22, 0.93)	0.22
SDS	The Gambia, 5-10y	0.24 (-0.50, 0.99)	0.52
from 0-12m	Bangladesh, 4.5y	0.47 (0.20, 0.74)	0.001

Results are the effect on diastolic blood pressure of a one unit increase in the explanatory variable, derived from linear regression analysis adjusted for appropriate confounders

[a]Results of likelihood ratio tests comparing models with or without season of birth as an exposure

For the Bangladesh cohort only it was possible to investigate the impact of early-life factors on later kidney function (Table 7.4). Kidney volume was positively associated with birth weight, length and the crossing of weight or length centiles in the first year of life. Glomerular filtration rate was unrelated to any of the factors studied with the exception of season of birth.

Table 7.4 Summary of observational analyses relating early-life exposures to kidney function at 4.5 years
(data from Bangladesh only)

		Regression coefficient (95% CI)	P-value
Kidney volume (cm^3/m^2)	Maternal leg length (cm)	0.14 (-0.20, 0.48)	0.43
	Maternal Hb, wk14 (g/dl)	-0.23 (-1.20, 0.74)	0.64
	Birth weight (kg)	7.37 (5.50, 9.25)	<0.001
	Birth weight (kg) adjusted for current weight	2.59 (0.77, 4.41)	0.01
	Birth length (cm)	1.41 (1.07, 1.75)	<0.001
	Birth length (cm) adjusted for current height	0.52 (0.18, 0.86)	0.002
	Season of birth	LR test χ^2: 58.3[a]	<0.001
	Change in weight SDS from 0-12m	1.05 (0.41, 1.69)	0.001
	Change in length SDS from 0-12m	1.64 (0.85, 2.43)	<0.001
Glomerular filtration rate ($ml/min/1,73m^2$)	Maternal Hb (g/dl)	-0.35 (-2.55, 1.86)	0.76
	Birth weight (kg)	4.23 (-1.21, 9.68)	0.13
	Birth weight (kg) adjusted for current weight	3.48 (-2.46, 9.42)	0.25
	Birth length (cm)	0.52 (-0.31, 1.35)	0.22
	Birth length (cm) adjusted for current height	0.30 (-0.59, 1.18)	0.51
	Season of birth	LR test X^2: 48.7[a]	<0.001
	Change in weight SDS from 0-12m	0.68 (-1.23, 2.60)	0.48
	Change in length SDS from 0-12m	1.24 (-0.63, 3.11)	0.20

Results are the effect on kidney function of a one unit increase in the explanatory variable, derived from linear regression analysis adjusted for appropriate confounders
[a]Results of likelihood ratio tests comparing models with or without season of birth as an exposure

7.3 Interpretation and future research

7.3.1 Prenatal nutrition

7.3.1.1 Interpretation

In the main, the findings suggest that prenatal nutrition, at least those aspects studied here, does not affect offspring blood pressure. The three follow-up studies had large sample sizes, good rates of follow-up and experienced no differential loss to follow-up. Therefore we can be fairly sure that the results reported are not due to chance or bias and it is important to consider alternative explanations for these null results, some of which are diagrammatically summarised in Figure 7.1.

Firstly, there may be general limitations of the design of this PhD research that could explain the null results of the interventions. The lack of a true control group is an issue for all of the studies with the exception of the calcium supplementation trial, which thus represents the data with greatest validity. It could also be that the supplements were provided too late in pregnancy to affect the development of body systems. Both the Gambian interventions provided supplements after 20 weeks gestation, whilst the Bangladesh food intervention could be accessed from 8-10 weeks gestation. In humans, nephrogenesis occurs throughout gestation [257] however, suggesting that nutritional insults operating up until the process is complete at 34 weeks could be expected to have an effect.

The timing of the interventions in the longer perspective of the maternal life course may also not be the most appropriate for long-term health implications in the offspring. Fetal nutrition is affected by maternal nutrient stores and placental function, which are in turn influenced by the mother's own lifetime nutritional exposures. This concept has been illustrated by studies investigating intergenerational effects of diet restriction during pregnancy. A number of experiments in rats have shown that the effects of a low-protein diet during pregnancy lead to raised blood pressure [279, 280] and reduced nephron number [280] in the second, as well as the first, generation. In this thesis the impact of

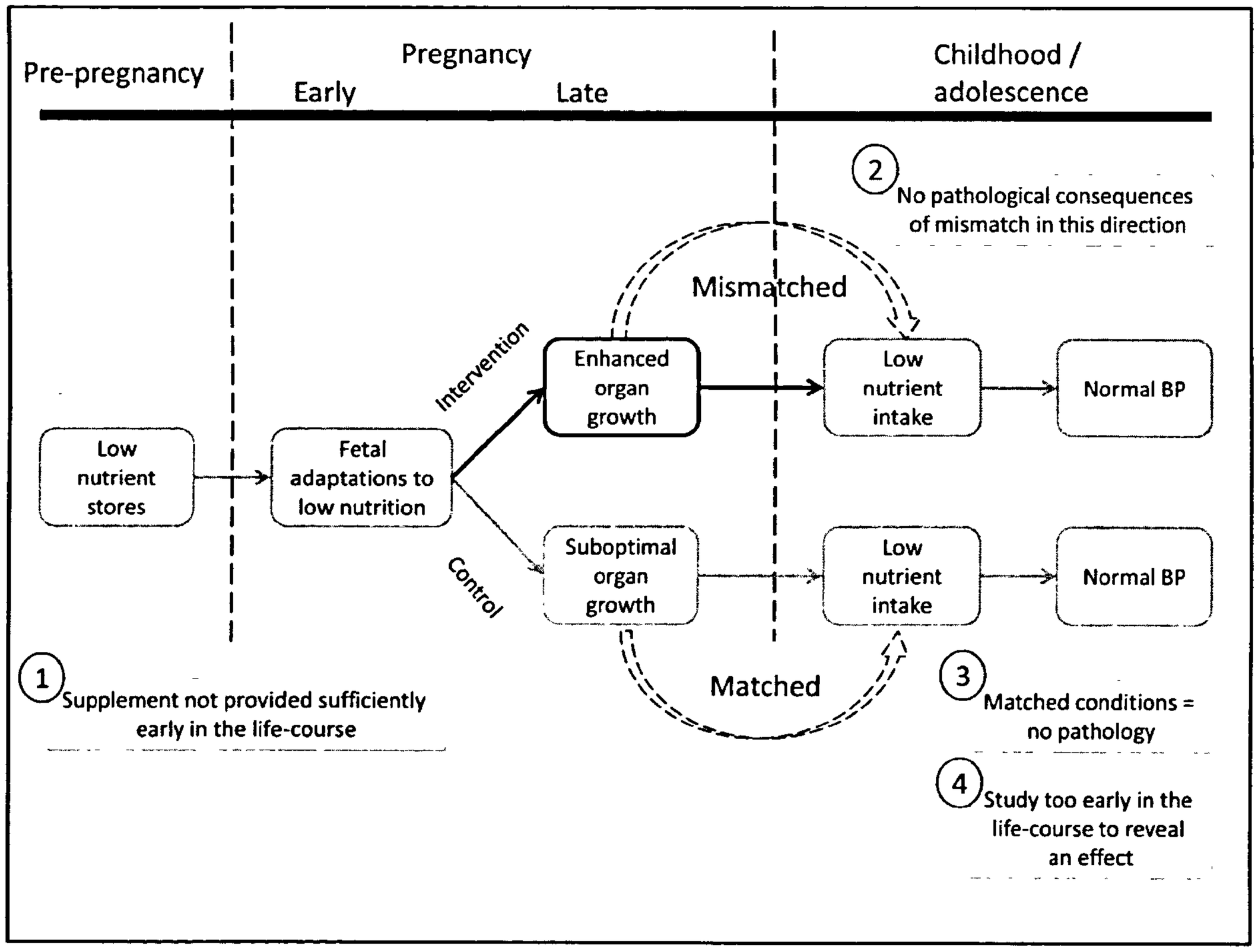
maternal leg length was used as a proxy estimate of maternal nutritional exposures in early life, but was not found to be associated with offspring blood pressure or kidney function.

There are two other key limitations of the follow-up studies presented in this thesis that have been discussed in previous chapters. The first is the age of the subjects at follow-up, who may be too young to reveal differences in blood pressure between the trial arms. The second is the setting of the studies in rural areas of developing countries where the subjects are, in the main, not exposed to the constituents of a 'Western' lifestyle that may be important for any disease susceptibility to appear. Authors in the animal field have suggested that the 'thrifty phenotype' programmed by undernutrition *in utero* is associated with an increased appetite as well as structural changes in organ systems [160]. The effects on appetite control in humans are not fully understood, but the relatively poor diet quality in these rural settings may nullify any inherent differences in appetite and hence partly explain the similarity of risk factor profiles between the trial arms.

Gluckman and Hanson have proposed the "Predictive Adaptive Response" theory to bring together the importance of exposures operating both pre- and post-natally [193]. According to this theory, the developing fetus will utilise cues from the uterine environment to predict the environment that will be experienced after birth and will use this information to take the appropriate developmental pathway [193]. Only if there is a mismatch between the uterine information and conditions experienced postnatally, does an increased susceptibility to disease occur [193]. In the three intervention studies presented here it could be argued that maternal supplementation during pregnancy provides conditions that are better than those experienced postnatally in these rural farming communities. Applying the predictive adaptive response theory, children in the intervention arm of the trial could therefore be at risk of raised blood pressure as there will be a mismatch between the expected conditions and those experienced. However, no difference in blood pressure was demonstrated and it may be that a mismatch in this direction will not incur disease susceptibility as organ systems have merely developed to a fuller

potential than is required. Again this lack of effect may point to the timing of the supplement; as highlighted by Kuzawa it is unlikely that the maternal diet during pregnancy is an important cue to the developing fetus as this exposure is variable and influenced by temporary changes in the environment that will not provide information on habitual conditions to be experienced after birth [281].

Figure 7.1 Conceptual diagram to explain the null effect of maternal nutritional supplementation on offspring blood pressure (proposed explanations represented numerically)



Explanations: 1: timing of supplementation; 2 & 3: mismatch theory not applicable in these settings; 4: timing of follow-up studies

Another possible explanation for the null findings is that the interventions themselves may not have significantly altered the maternal diet. Although the Gambian protein-energy supplement did improve birth weight [194], no effect of the calcium intervention was observed on breast milk calcium concentrations, birth weight or infant bone mineral status in the sub-set of participants studied [195], and neither the Bangladesh food or micronutrient interventions impacted on maternal weight gain in pregnancy or birth weight (Persson, unpublished). Whilst randomised controlled trials represent the 'gold-standard' study design in epidemiological research [95], there are many logistical issues that can impact on their effectiveness in a community setting. For example, individuals provided with food supplements may simply add these to their family's meal rather than consume them themselves (although this possibility was absent in the Gambian trials). Alternatively, they may consume less of their normal diet in response to being given a supplement. If a nutritional intervention is ineffective, for whatever cause, its usefulness as a design with which to investigate the impact of the maternal diet on offspring blood pressure can be questioned.

In general, trials of nutritional supplementation during pregnancy have demonstrated disappointingly modest effects on birth weight, which has often been the primary outcome. A recent systematic review and meta-analysis of the effect of multiple micronutrient supplementation during pregnancy on birth outcomes included eight studies that compared multiple micronutrients (MuMs) with iron and folate only [282]. The analysis revealed a positive effect of MuMs compared to iron and folate on birth weight and a reduction in the proportion of individuals born low birth weight [282]. However, the effect was of fairly modest size, with a weighted mean increase in birth weight of 54.02g (95% CI: 36.22, 71.83), and there was some heterogeneity in the results with three out of the eight studies (the review did not include the MINIMat data) showing no effect [282]. One explanation for the small effect size of these large interventions could be that the micronutrients are not fully available in tablet form and that a food-based supplement may be more effective [283]. Trials of protein-energy food

supplementation have also been shown to have only a modest impact on birth weight [284], with the exception of the Gambian trial that was the basis of one of the chapters in this thesis [194]. This has led some researchers to suggest that a food-based fortified supplement also containing a number of micronutrients may be the most effective method of improving nutrition during pregnancy [283]. This hypothesis was recently tested by Huybregts *et al* in Burkina Faso where pregnant women were provided with a food-based supplement fortified with the same 15 multiple micronutrients that were provided in the MINIMat trial [283]. Disappointingly, the fortified food-based supplement was found to increase birth length, but there was no difference in birth weight between intervention arms [283].

As described in Chapter 1, the observational data on the impact of the diet during pregnancy on offspring blood pressure is limited and often reports conflicting results [63, 65-68]. In contrast, the large amount of animal data in this field has consistently shown that reduced nutrition *in utero*, including global undernutrition, a restricted protein diet and iron deficiency, leads to raised offspring blood pressure in a range of species [75-80]. It is therefore interesting to consider why the human data presented here have shown no effect of manipulating the maternal diet. There are clearly some challenges for extrapolating from one species to another and it may not be surprising that the findings do not translate. Moreover, animal experiments occur in a tightly controlled environment whereas human interventions are placed in a real world setting where other factors operate to dilute any potential effect of the intervention. Another key difference between the human and animal interventions is that the latter have focussed on *restricting* nutrients, whereas studies of human pregnancy are designed to increase intake relative to control or placebo, therefore making the results difficult to compare. Finally, the incremental nutrient requirements for human reproduction are very low compared to other species, and the developing fetus is protected by a number of evolved adaptations [94]. It may not therefore be surprising that it does not appear possible to replicate the animal findings in the human situation using the tools of epidemiology.

7.3.1.2 Future research

The conceptual diagram in Figure 7.1 helps to visualise the various proposed explanations for the null effect of the interventions. In order to explore these ideas further, additional research projects should be conducted. Firstly it would be interesting to follow these same cohorts of children into the future when they are themselves adults to see if differences in risk factors are apparent at a later age. An important aspect to study in these individuals as they develop will be the pace of rural development and/or the choice to remain in the rural area or to travel to more urban environments where they will be increasingly influenced by 'Western' diet and physical activity patterns. An increasing dichotomy of exposures may reveal any underlying differences in body systems that are not visible in the current study, where individuals in the control groups remain 'matched' to their environments. An inherent challenge would be that the more distant the subjects are from the original study site the harder they will be to trace and recruit into future research projects, creating major logistical difficulties.

To investigate the concept that the supplement was not provided at a time point that would optimally affect offspring body systems it would be interesting to study children whose mothers had received supplements at a different time. The simplest study design would be to focus on the periconceptual period as a number of trials have investigated this time point, including one in the West Kiang region of The Gambia [ISRCTN 13687662] [285]. It would also be interesting to look at individuals who were born to women who had been provided with supplements earlier in their life course, perhaps during their own childhood, in order to investigate the intergenerational cycle of improved nutrition.

This thesis has only focused on one aspect of offspring health that could potentially be affected by maternal nutrition during pregnancy. It will be important to look at other aspects of disease susceptibility such as body composition, lipid profiles, immune response, cognitive development and human capital in these children. The follow-up of the Gambian trials have to date revealed no effect of either the protein-energy or calcium interventions on body composition [216] [Goldberg,

unpublished] and the analysis of other outcomes is ongoing. Currently only two publications have reported the impact of the MINIMat interventions on offspring health; one revealing a small positive impact of the maternal nutritional interventions on offspring motor skills in infants born to women of low BMI only [286], whilst the second reported no impact on offspring thymic size as an indicator of immune development [287]. Follow-up studies of other maternal nutritional interventions have provided some evidence for long-term effects on outcomes other than blood pressure. For example, the Guatemalan protein-energy intervention (INCAP) has been associated with taller stature and enhanced cognitive performance in individuals from villages that received the 'Atole' compared to 'Fresco' supplement [98]. The Indian protein-energy intervention was also associated with taller stature at follow-up of subjects aged 13-18 years and with more favourable measures of insulin resistance [99].

7.3.2 Growth

7.3.2.1 Interpretation

The lack of an association between prenatal nutrition and later blood pressure forces us to consider alternative explanations for the apparent inverse associations between birth weight and blood pressure that are so prevalent in the literature and are generally interpreted as relating to inadequate fetal nutrition [49]. Lucas *et al* proposed that it was the change in size between birth and later life that was the explanatory factor, rather than fetal growth restriction *per se* [53]. Recently there has been increasing research focused on the impact of early postnatal growth rates on the development of cardiovascular disease in later life and on the potential for increased understanding to inform prevention strategies [231]. In this thesis, relative change in weight emerged as a predictor of later blood pressure in all three studies. This is a relatively new area of research, which currently remains hampered by methodological issues of how best to define growth and how to interpret an exposure that is fundamentally related to later size, and therefore to later blood pressure.

Despite these research issues a number of observational studies have now reported an association between growth in early life and later blood pressure [117, 119, 122, 288-292], although the different statistical methods employed and the time points studied act to obscure any coherent pattern of association. Recently, Ben-Shlomo *et al* published an analysis where the impact of early growth was modelled in detail by creating individual growth curves based on 14 separate measurements of weight and length between birth and five years [122]. The analysis revealed that weight gain between 0-5 months and between 1.9-5 years was independently and positively associated with systolic blood pressure at 25 years, independent of adult adiposity [122]. Using the same data set but conducting an analysis that utilised relative weight at three time points (3mo, 1.5y and 5y) only, no association with later blood pressure was observed [122]. This highlights a common methodological issue in the literature where studies contain only a few measurements of size in infancy at arbitrarily chosen time points, making it difficult to interpret any null results reported.

The majority of data that has investigated the impact of early growth on later blood pressure has been derived from developed countries where there may be very different implications for catch-up growth. A recent analysis of data from five developing cohorts provides a valuable addition to the field; the association between relative weight in early to mid-childhood (48 months) in these cohorts and early adult blood pressure was entirely explained by the contribution of early weight to later size [123]. This study did suggest however, that weight gain after mid-childhood may be important for later raised blood pressure [123]. This highlights an important issue within the growth data, namely that there may be different disease patterns associated with weight gain at different time points, which, if proven, would have important policy implications. Some studies have reported that weight gain in the first year of life is associated with later raised blood pressure [122, 291], whilst others suggest that the aetiologically important period may be in later childhood [123, 290].

Even if growth in early life emerges as an important risk factor for later cardiovascular disease, the implications are challenging. This is particularly true in developing countries where poor early growth, manifested by low birth weight and/or wasting in the first few years of life, has been shown to be associated with infant mortality and morbidity and with reduced human capital in later life [293-295]. The implication is that catch-up growth for individuals who are born small is therefore beneficial, although the direct evidence on the benefits of early catch-up growth is limited [296]. If catch-up growth is beneficial in the short-term but may confer later disease risk, the issue of growth promotion may become one of priority, as discussed by Victora and Barros [296]. For example, should programmes intervene to promote growth in early life for the short-term improvements in health, if there are known long-term consequences in terms of cardiovascular disease risk factors such as hypertension and obesity? A related issue is that countries, and regions within those countries, are at different stages along the nutrition transition [297], which will make a global strategy difficult to define.

7.3.2.2 Future research

The impact of early growth on later health outcomes is in need of further well-designed studies in order to understand the phenomenon further. One of the first steps should be agreed standard methods for characterising growth as an exposure, whether this be to utilise life-course plots as recommended by Cole [121] or more complex modeling of growth trajectories as suggested by Ben-Schlomo *et al* [122]. Without standardised measures it is very challenging to interpret any null results that are published and to build up a picture of the pattern of growth that may confer the greater or lesser risk.

In order to further understand the apparent association between growth and later blood pressure it will be important to develop intervention designs that can test this hypothesis from the standpoint of a randomised controlled trial. Some preliminary data has been obtained from a trial of infant feeding, which was shown to impact on growth [136]. Infants born at term but small for gestational age in five

UK hospitals were randomly assigned to receive standard formula or a nutrient-enriched formula (with 28% more protein) until they were nine months old [136]. Those receiving the enriched formula experienced a greater increase in weight Z-score, between enrolment and nine months, than those on standard formula and also had raised diastolic blood pressure at 6-8 years, although there was no difference in systolic blood pressure [136]. It would be interesting to follow-up other intervention trials that have impacted on growth, either via infant feeding or other mechanisms, in more representative populations in order to investigate the association with later blood pressure.

Most studies in the literature have focused on the effects of weight change rather than linear growth and it will be important to be able to investigate both in future research in this area. In this thesis linear growth appeared to have either no effect or a reduced effect on later blood pressure compared to change in weight. Some studies have provided suggestive evidence that the timing of weight gain may be related to different patterns of body composition with early growth promoting lean mass and later growth promoting fat gain [298-300]. A greater understanding of the implications of the type and timing of growth will be important and requires further research.

In regions that have undergone rapid nutrition transitions such as the Indian sub-continent and South America, there is emerging a pattern of adult body shape that has been termed "stunted-obese" [296]. In these regions, intergenerational and early-life nutritional deprivation have promoted a high prevalence of stunting, whereas recent nutritional improvements are manifest in the laying down of fat, particularly centrally-distributed fat [231]. Indian babies have been shown to be small but with relatively well preserved fat mass compared to UK infants, a phenotype that researchers have termed the 'thin-fat' baby [301]. If linear gain is shown to be associated less with later disease risk than early weight gain this may have important policy implications for these settings as it may represent an opportunity to break the intergenerational cycle of stunting. It will be important to develop interventions that are able to promote linear growth in the absence of

inappropriate weight gain, which will themselves require research into the appropriate diets, delivery and target age.

Currently there is very little understood about the potential mechanisms linking growth rate in infancy and later blood pressure and this area also requires further research [122]. It may be that different underlying mechanisms are operating in different settings; individuals born low birth weight who experience rapid postnatal catch-up growth may be more susceptible to disease as their organ systems are not developed to their full potential and can therefore not process the increased demand that greater size provides [302]. For individuals born appropriate for their gestational age and with well developed organ systems there may be different mechanisms operating that explain the link between growth and blood pressure. Given the association between rapid weight gain in early childhood and increased risk of being overweight [303, 304], this could also explain the link to increased blood pressure. However, the association with obesity is unlikely to entirely explain the phenomenon as some data reveal an association with infancy weight gain that is independent of later adiposity [122].

7.4 The wider context

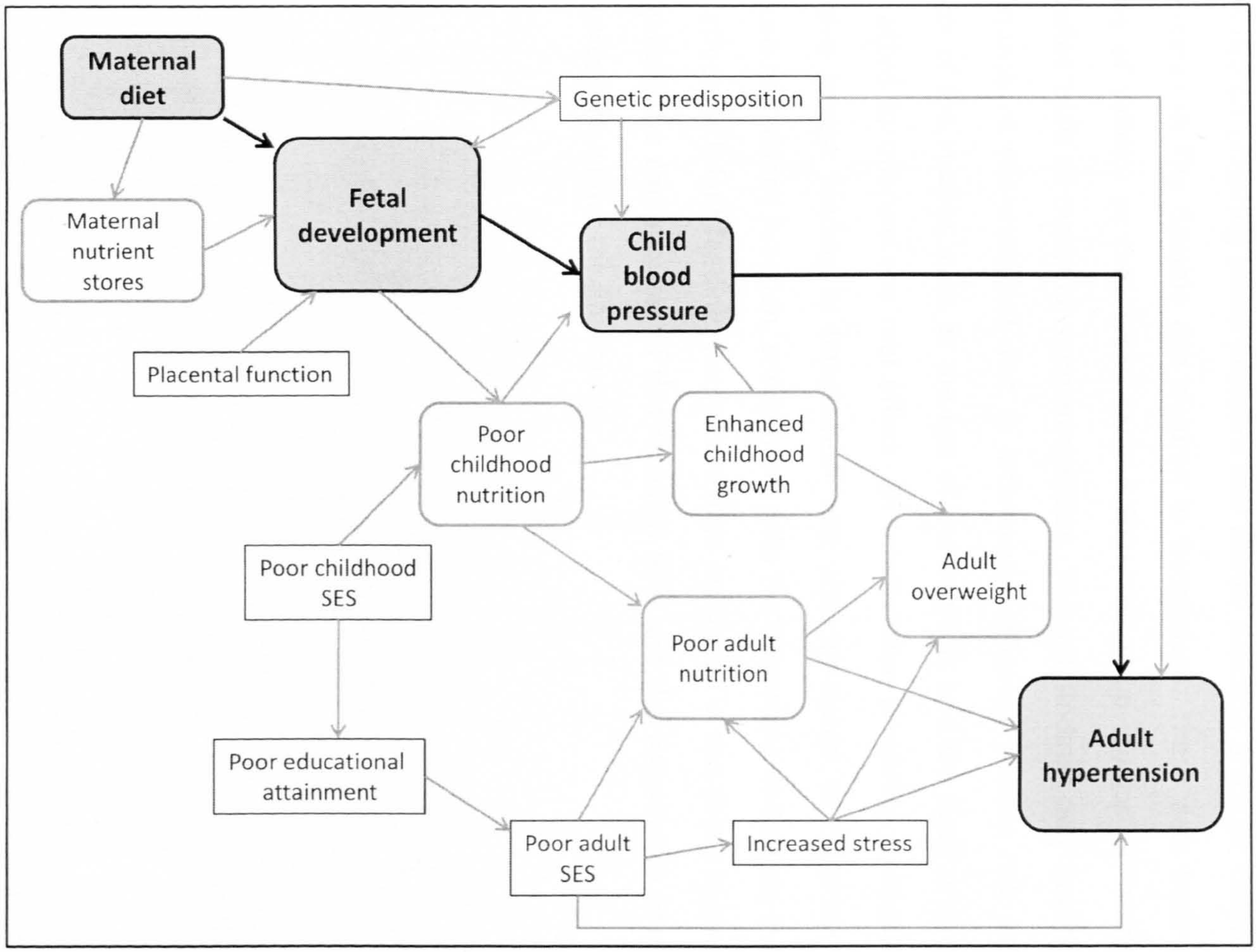
The null effect of maternal supplementation on offspring blood pressure reported in this thesis provides important information on the effect of fetal environment on long-term cardiovascular health. It suggests that either the maternal diet is not an important feature of the early-life environment that influences disease susceptibility or that the chosen study designs were too simplistic to answer what is fundamentally a more complex research question. These results should stimulate research into what other factors of the early-life environment (nutritional or other) might be operating that do have a marked effect on the disease profile of an individual.

In general, the field of DOHaD has helped to highlight the importance of the fetal and early postnatal life as a key stage in the life course that can have long-term implications for health. These concepts are important to public health and they are beginning to filter through to key stakeholders and to influence public health policy, albeit mainly in the developed countries where the majority of studies have been conducted. For example, the British Medical Association recently published a report on the importance of early-life nutrition that will help to inform medical practitioners in the UK on the most recent research in this field [305]. A recent survey of European Government stakeholders in infant nutrition found a high level of recognition and understanding of the concept of nutritional programming, although this knowledge had yet to filter through to relevant policy changes [306]. Funding bodies are also increasingly recognising the need for further research into early-life impacts, such as the European Union funded Early Nutrition Programming project and the World Cancer Research Fund call for proposals on the impact of the early-life period on cancer risk.

Whilst the DOHaD literature has helped to highlight the importance of critical windows of development in the aetiology of disease, it is important to remember that these exposures do not operate in isolation but are also affected by exposures

throughout the life course. The discipline of life course epidemiology is currently undergoing a revival in popularity and can be a useful tool for understanding the complex causes of disease that encompass both biological and social exposures in a temporal relationship [307]. If we consider the impact of the maternal diet during pregnancy on the development of adult hypertension through a life course perspective it becomes apparent that we have only focused on a very narrow pathway within a very complex web of exposures (Figure 7.2). Even this simplistic diagram does not take into account intergenerational effects or the wider social context and underlying causes. Unraveling the complex association between fetal development and the exposures that both feed into it and built on it in terms of later health outcomes will require multi-disciplinary research including trial data, such as those presented here, together with well-conducted observational studies and animal experiments in order to investigate potential mechanisms. Only with the entire complement of these, and with a life course perspective, will it be possible to build a complete disease model and to use this to accurately inform public health policy.

Figure 7.2 Simplified diagram of exposures across the life course that may influence hypertension in adulthood



Solid boxes: pathway investigated in this thesis; rounded boxes: nutritionally-related exposures and outcomes; square boxes: non-nutritional influences

7.5 Conclusion

This thesis has demonstrated that there is no robustly detectable impact of protein-energy, calcium or multiple micronutrient supplementation provided during pregnancy on offspring blood pressure and kidney function amongst children and adolescents in The Gambia and children in Bangladesh. These data have a high degree of validity as they were derived from follow-up studies of randomised controlled trials of maternal supplementation. The null findings may question the importance of the maternal diet in influencing offspring disease risk although aspects of the studies, such as the age of the subjects and the rural setting, may have acted to obscure any real effect. An intervention providing counselling for exclusive breast feeding in Bangladesh was also unrelated to offspring blood pressure and kidney function, which feeds into the important debate on the long-term effects of infant feeding. Overall the data presented in this thesis have helped to highlight the complexities of this research field and the importance of viewing exposures throughout the life course.

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Publications

The following publications have, to date, arisen from this PhD research:

Hawkesworth, S., Prentice, A.M., Fulford, A.J.C. & Moore, S.E. Maternal protein-energy supplementation does not affect adolescent blood pressure in The Gambia. *Int. J. Epidemiol.*, 2009, **38**: 119-27

Hawkesworth, S. Conference on "Multidisciplinary approaches to nutritional problems". Postgraduate Symposium. Exploiting dietary supplementation trials to assess the impact of the prenatal environment on CVD risk. *Proc. Nutr. Soc.*, 2009, **68**, 78-88

Hawkesworth, S., Sawo, Y., Fulford, A.J.C., Goldberg, G.R., Jarjou, L.M.A., Prentice, A. & Moore, S.E. Effect of maternal calcium supplementation on offspring blood pressure in 5-10 year old rural Gambian children. *Am. J. Clin. Nutr.*, **submitted**