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Paediatric Asthma and Wheeze: Early Life Origins

by

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ABSTRACT

FACULTY OF MEDICINE, HEALTH AND LIFE SCIENCES
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PAEDIATRIC ASTHMA AND WHEEZE: EARLY LIFE ORIGINS

Katharine Claire Pike

Epidemiological evidence suggests poor fetal growth is associated with poor later respiratory health, including the important childhood disorders asthma and wheeze. Factors creating a suboptimal early environment may persistently alter the structure and function of the immune and respiratory systems.

This thesis explored the early life origins of paediatric asthma and wheeze in a large, prospective mother-child cohort. In particular, the contributions of maternal nutrition, and fetal and postnatal growth were assessed.

The mothers of healthy, term infants had their body composition, lifestyle and diet characterised before and during pregnancy. Serum anti-oxidants, vitamin D and polyunsaturated fatty acids were measured in late pregnancy. Fetal growth was recorded by longitudinal ultrasound scans. 1548 children were followed to age 3 years and 469 were seen at 6 years. Wheeze was assessed by questionnaire and skin prick testing and detailed lung function measures were performed.

Late pregnancy fetal growth faltering was associated with an increased risk of atopy and atopic wheeze at age 3 years, and greater weight and adiposity gain in the first year of life were associated with increased risk of wheeze. In those children seen at 6 years, greater maternal adiposity, before and during pregnancy, was associated with increased risk of non-atopic wheeze. Lower maternal pre-pregnancy vitamin D intake was associated with increased risk of childhood asthma, recent wheeze, and non-atopic wheeze. Lower maternal serum 25(OH) vitamin D in late pregnancy was also associated with childhood asthma and wheeze. Higher late pregnancy energy-adjusted vitamin A intake was associated with higher forced expiratory volumes but also greater bronchial hyperresponsiveness. Higher arachidonic acid status during pregnancy was associated with markers of atopy and greater risk of atopic wheeze.

These results suggest early life factors contribute to paediatric asthma and wheeze. Optimising the early environment may reduce the burden of childhood wheeze.

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AUTHOR'S DECLARATION

The clinical data for this study were taken from studies conducted within the Southampton Women's Survey. I was jointly responsible for collection of respiratory data within the Developmental Influences upon Respiratory Health 6-year Follow-up component of this survey.

The data analysis and interpretation represents my own work.

The British Lung Foundation sponsored the six year respiratory follow up and paid for my salary as a research fellow.

ABBREVIATIONS

Respiratory

FENO	Fractional Exhaled Nitric Oxide
MDI	Metered Dose Inhaler

Lung Function

Crs	Respiratory System Compliance
$FEF_{50\%}$	Forced Expiratory Flow at 50% of vital capacity
$FEF_{25-75\%}$	Forced Expiratory Flow Between 25% and 75% of vital capacity
FEV_1	Forced Expiratory Volume in the first second of exhalation
$FEV_{0.4}$	Forced Expiratory Volume in the first 0.4 seconds of exhalation
FVC	Forced Vital Capacity
Raw	Airways Resistance
sGaw	Specific Airways Conductance
V'_{maxFRC}	Maximal Expiratory Flow at Functional Residual Capacity

Bronchodilator Response (BDR)

ΔFEV_1	Change in FEV_1
$\Delta FEV_1(l)$	Absolute change in FEV_1
$\Delta FEV_1(init)$	Change in FEV_1 as a percentage of the initial FEV_1
$\Delta FEV_1(pred)$	Change in FEV_1 as a percentage of the predicted FEV_1
$\Delta FEV_1(init\text{-}pred)$	Change in FEV_1 as a percentage of the baseline deficit in FEV_1

Bronchial Hyperresponsiveness (BHR)

DRS	Dose-Response Slope
PC_{20}	Concentration provoking 20% decline in lung function
PD_{20}	Dose provoking 20% decline in lung function

Immunology

CBMC	Cord Blood Mononuclear Cell
EGF	Epidermal Growth Factor
IgE	Immunoglobulin E
IGF	Insulin-like Growth Factor

IL	Interleukin
TGF	Transforming Growth Factor
Th1	T-helper cell type 1
Th2	T-helper cell type 2

Statistics

CI	Confidence Interval
CV	Coefficient of Variation
IQR	Interquartile Range
ICC	Intraclass Correlation Coefficient
ROC	Receiver Operator Chracteristic
RR	Relative Risk
SD	Standard Deviation

Organisations

ALSPAC	Avon Longitudinal Study of Parents And Children
ATS	American Thoracic Society
BTS	British Thoracic Society
EAACI	European Academy of Allergy and Clinical Immunology
ERS	European Respiratory Society
ISAAC	International Study of Asthma and Allergies in Childhood
LISA	Influences of Lifestyle-related factors on the Immune System and development of Allergies in childhood
LREC	Local Research Ethics Committee
MRC	Medical Research Council
MoBa	Norwegian Mother and Child population-based cohort study
SIDRIA	Studi Italiani sui Disturbi Respiratori e l'Ambiente (Italian Studies on Respiratory Disorders in Childhood and the Environment)
SWS	Southampton Women's Survey

Other

DHA	Docosahexaenoic Acid
DXA	Dual energy X-ray Absorptiometry

EFA	Essential Fatty Acid
EPA	Eicosapentanoic Acid
FAMEs	Fatty Acid Methyl Esters
FFQ	Food Frequency Questionnaire
HPLC	High Performance Liquid Chromatography
GP	General Practitioner
LCP	Long chain Polyene
PUFA	Polyunsaturated Fatty Acid
SPT	Skin Prick Test
SNPs	Single Nucleotide Polymorphisms
USS	Ultrasound Scan

DEDICATION

Dedications are best 'gushed' in a pink dress clutching a statuette, but until such time as my dramatic talents are recognised…and there has been some drama…I want to thank my 'early life influences', the 'old people', who were there at the beginning and have stayed to the end. Thanks particularly to mum for pointing out somewhere around page 300 that you 'don't think it's as simple as that!'. It's fair to say this thesis has not been all about fish and citrus, coffee and wine were also of extreme importance. I'm grateful to Claire, Claire and Rosie who have played a major role in maintaining my intake, although the Henley Pimms deserves special mention!

Chapter One

Introduction & Background

1.1 INTRODUCTION

1.1.1 Asthma and childhood wheeze

Asthma is a chronic disorder affecting the conducting airways. Inflammation and structural changes occur in the airway wall which cause variable airflow limitation, cough and wheeze (Tattersfield *et al.*. 2002). A dramatic increase in asthma prevalence occurred during the second half of the twentieth century and, although this increase appears to have plateaued, asthma remains the most common chronic childhood disorder. The lifetime prevalence of asthma in British children is in the region of 25% and a similar proportion of British children report wheezing within the last 12 months (Kaur *et al.*. 1998). In a proportion of cases, respiratory ill health persists into adulthood (Godden *et al.*. 1994). Asthma can cause considerable limitation of children's activities and interruption to schooling. Deaths due to asthma are rare but can occur in the paediatric age group (National Asthma Campaign 2001). Asthma and wheeze constitute a considerable financial burden due to medication, hospitalisation, sick leave, disability, and other indirect costs (Office of Health Economics 2003).

1.1.2 Childhood wheeze phenotypes

The clinical symptoms recognised as asthma probably arise from a number of related syndromes rather than a single illness (Martinez & Helms 1998; Stein *et al.*. 1997). Although there is considerable overlap between the different 'wheezing syndromes', the characteristics and early life histories of those children who suffer transient symptoms appear to differ from those in whom the problem persists. Three broad clinical wheeze

phenotypes are recognised: atopic asthma, non-atopic asthma and viral-associated wheeze.

Children with atopic asthma demonstrate cutaneous sensitisation to one or more allergens and have episodes of wheeze often in association with exercise or exposure to allergen. Early onset atopic asthma is associated with severe disease and persisting airway abnormalities; this group tends to have persistent disease (Martinez 2002). Children with non-atopic asthma experience wheeze between viral respiratory tract infections in a similar fashion to the atopic asthma phenotype but these children do not demonstrate atopic sensitisation. Many with non-atopic asthma are thought to outgrow their disease during childhood (Martinez 2002). In viral-associated wheeze, symptoms occur only with viral upper respiratory tract infections. There may be pre-existing airway abnormalities (Martinez 2002) but a family or personal history of atopy is not usual. Although this phenotype is usually outgrown by 5 years of age, there is concern that children with transient viral-associated wheeze may develop chronic obstructive pulmonary disease in adulthood (Burrows *et al.* 1977; Silverman & Kuehni 2007).

1.1.3 Early life origins of asthma and childhood wheeze

In recent years, well-designed and adequately powered longitudinal studies have been conducted to investigate the increased prevalence of atopic disorders and to characterise the origins and evolution of the childhood wheeze phenotypes (Arshad *et al.* 2005; Henderson *et al.* 2008; Martinez 2002). These epidemiological studies have provided data which suggest respiratory morbidity, in both childhood and adult life, is associated with early onset of symptoms and early impairment of lung function. This has led to the proposal that genetic and environmental factors might predispose to wheezing disorders as a consequence of altered immune or airway development (Figure 1). Genetic changes at a population level cannot explain the increase in asthma prevalence observed in the last few decades as this increase has occurred too rapidly. Environmental changes must be considered and, as most cases of asthma are diagnosed before 6 years of age (Yunginger *et al.* 1992), early environmental exposures are of likely importance in the inception of paediatric asthma and wheeze

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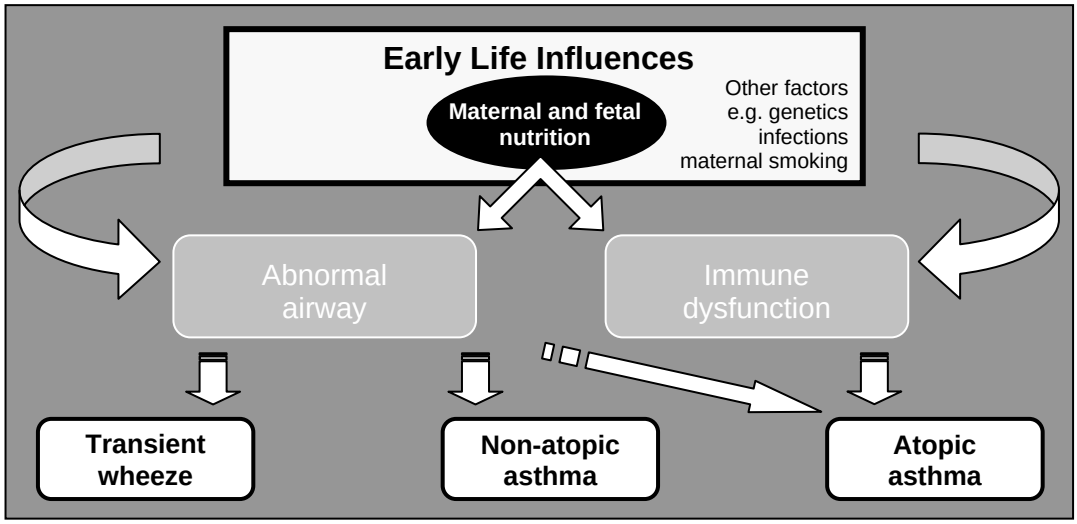
Figure 1 Development of the childhood wheeze phenotypes



1.2 EARLY LIFE ORIGINS OF HEALTH AND DISEASE

1.2.1 The developmental origins of health and disease hypothesis

The 'developmental origins of health and disease' hypothesis evolved following the observation that regions of the UK that had high infant mortality in the early twentieth century also had high death rates from coronary heart disease and respiratory disease some sixty or so years later (Barker & Osmond 1986). Follow up of individuals whose weight had been documented at birth led to the discovery of associations between lower birthweight and increased rates of cardiovascular and respiratory disease in adulthood (Barker *et al.* 1990; Barker *et al.* 1991). These observations were interpreted as evidence for the induction of metabolic or endocrine changes by early environmental influences. This process, whereby early life influences affect later health, is sometimes referred to as 'programming' or 'developmental induction'.

Initial work concentrated on fetal life, although subsequent studies have demonstrated that the sensitive periods during which the early environment can have long lasting health effects encompass the time from conception, through gestation and into postnatal life. Epidemiological and experimental evidence suggests factors capable of invoking developmental induction before birth include maternal dietary intake, maternal body composition and maternal endocrine status. There is also evidence for transgenerational effects, whereby factors such as the mother's own birthweight, for example, may influence the long-term health of her offspring (Emanuel *et al.* 1992).

1.2.2 Criticisms of the developmental origins hypothesis

Early criticisms of the developmental origins hypothesis claimed that the link between birthweight and adult disease could be explained by continuation into adulthood of the adverse events that had caused growth restriction. However, there is now strong evidence against this argument. In several studies, data on adult lifestyle factors, notably smoking, employment, diet, alcohol consumption and exercise were collected; allowing for these lifestyle factors had little effect on the association between birthweight and coronary heart disease (Rich-Edwards *et al.* 1997). It has also been argued that the associations between size at birth and later disease could primarily reflect genetic influences. However, birth size has been demonstrated to have only a modest genetic component and instead is believed to be principally determined by the quality of the intrauterine environment (Brooks *et al.* 1995). Finally, the strength of the relationship between birthweight and specific outcomes has been questioned. However, this reflects a misunderstanding of the factors responsible for developmental induction. Birthweight is a crude proxy for developmental exposures, such as maternal diet and body composition, and associations are likely to be much stronger for factors directly effecting developmental induction than they are for marker variables such as birthweight.

1.2.3 Evolutionary basis of developmental induction

The consistency of the long-term effects of developmental induction responses across species and within the normal range of fetal growth suggests the developmental origins phenomenon has a physiological rather than a pathological basis. The link between early life environment and adult disease may have an underlying evolutionary explanation. The predictive adaptive responses theory suggests that an evolutionary advantage is conferred if the developing organism can predict conditions in the postnatal environment and is then able to alter its development to optimise survival within this predicted environment (Gluckman & Hanson 2004).

The predictive adaptive responses theory suggests that the long-term consequences may be harmful if there is a 'mismatch' between the actual and predicted postnatal environment. 'Mismatch' may occur between the fetal nutrient demand, largely determined by the early fetal growth trajectory, and the materno-placental capacity to meet this demand. 'Mismatch' may also occur if maternal disease or impaired placental

function lead the fetus to adjust its development inappropriately (Edwards *et al.* 2001; Naeye 1987). Alternatively, maternal influences may act via alterations in the fetal endocrine milieu or the placental vasculature, to effect developmental plastic responses, which effectively 'mismatch' the fetus to its adult environment.

1.3 NORMAL RESPIRATORY & IMMUNE DEVELOPMENT

1.3.1 Lung development

Lung buds emerge from the ventral foregut at 4 weeks of gestation. Subsequent lung development and maturation are characterised by several distinct phases (Hislop 2001). During the embryonic phase the lung buds branch to form lobar and segmental airways. Then, in the pseudoglandular phase, between 5 and 17 weeks' gestation, the pre-acinar airways are formed by branching into the surrounding mesenchyme. Also during this phase, airway wall cells differentiate to form cartilage, glandular tissue and epithelial cell types. The lung then enters the canalicular stage, characterised by mesenchymal thinning, epithelial differentiation and surfactant protein expression. From 27 weeks' gestation the lung enters the alveolar phase. During this final stage of lung morphogenesis thin-walled alveoli and an integrated capillary network are formed. There is further multiplication of alveoli postnatally and, whilst the adult number is reached by 2-3 years of age, increases in size and surface area continue until after adolescence (Galambos & Demello 2008).

1.3.2 Immune development

The capacity to mount an immune response is present from as early as 22 weeks of gestation (Jones *et al.* 1996). Stem cells are present in the human yolk sac at 21 days and the first lymphocytes are seen the thymus by the ninth week of gestation (Migliaccio *et al.* 1986). Lymphocytes are detectable in the lungs and other organs, including the gut, by 14 weeks. Circulating B cells with surface immunoglobulin M can be detected from 12 weeks onwards (Bofill *et al.* 1985). The likely route of antenatal sensitisation is through the gut during the second trimester (Jones *et al.* 2001), there is also evidence of exposure to antigens via the fetal circulation in the third trimester. This possibly occurs as a consequence of active transport of immunoglobulin G (IgG) antibodies across the placenta complexed with antigens (Casas & Bjorksten 2001). The mechanisms controlling allergen sensitisation and tolerance during pregnancy are unclear, although a number of studies have shown high mononuclear cell proliferative responses at birth to

be associated with a higher probability of allergic disease in later childhood (Kondo *et al.* 1992). There is some evidence that higher IgG concentrations to specific antigens lessen the chance of sensitisation (Casas & Bjorksten 2001). Normal pregnancy is characterised by a suppression of maternal cell-mediated responses to fetopaternal antigens. Tissues of the fetoplacental unit secrete cytokines similar to those associated with a T-helper 2 (Th2) response. These cytokines promote ongoing pregnancy by suppressing maternal T-helper 1 (Th1) activity.

1.4 EARLY LIFE ORIGINS OF PAEDIATRIC ASTHMA & WHEEZE

Asthma may be one of a number of chronic diseases originating in the perinatal period as a consequence of 'developmental programming'. Epidemiological studies provide strong evidence that a suboptimal intrauterine environment can affect later respiratory health.

Long-term changes in lung function and health are known to follow preterm birth (Hoo *et al.* 2002) and maternal smoking (Stick *et al.* 1996). Evidence is also accruing which suggests impaired fetal nutrition and growth may have deleterious effects on respiratory health (Lucas *et al.* 2004; Maritz *et al.* 2001; Seaton *et al.* 1994). Similarly, adverse factors in the early postnatal environment, for example tobacco smoke, can also lead to persistent alteration of lung structure and function (Stocks & Dezateux 2003).

Development of the immune system is likely to be vulnerable to perinatal insults in a similar manner to the developing airway. The immune system is finely balanced, to ensure an appropriate response to an invading pathogen while minimising any risk of injury to the host (Chaplin 2003). It is thought that if this balance is disturbed, such that the childhood immune system deviates towards a Th2 response, an atopic phenotype develops (Jarvis & Burney 1998). Within the developing fetus a weak Th2 response normally develops (Wegmann *et al.* 1993). Those infants that fail to convert from a fetal Th2 to a mature Th1 immunophenotype are more likely to develop atopy (Prescott *et al.* 1999). This failure of transition probably only occurs in genetically susceptible individuals exposed to environmental risk factors (Holt *et al.* 2000).

The early environment appears to be particularly important. For example, infants born to atopic mothers are far more likely to develop early onset atopic disease than those born to an atopic father (Ruiz *et al.* 1992). This may represent a predominantly epigenetic mechanism but it is known that potentially significant differences exist between the intrauterine environments provided by atopic and non-atopic mothers. The amniotic fluid of atopic mothers has higher levels of both IgE and interleukin 10 (IL10), for example, than that of non-atopic women (Warner *et al.* 1994). It has also been proven that *in utero* insults such as maternal smoking can affect the responsiveness of cord blood mononuclear cells to allergens (Devereux *et al.* 2002).

Those babies who grow less well appear to be more likely to suffer poor respiratory health in childhood. Evidence from clinical studies shows that lower birthweights are associated with impaired infant lung function (Lucas *et al.* 2004). Moreover, associations have been demonstrated between neonatal anthropometric measurements and subsequent atopy (Gregory *et al.* 1999). Together these findings suggest that aspects of the *in utero* environmental are significant determinants of later health and that factors influencing fetal growth might also affect respiratory and immune development. Fetal growth is dependent upon the uptake of nutrients from a complex maternal supply line; nutrients supplied to the fetus reflect placental function and maternal physiological adaptations to pregnancy as well as maternal intake and absorption of nutrients and fetal demand (Bloomfield & Harding 1998). Although the link between fetal and maternal nutrition is indirect, the idea that maternal diet might influence respiratory and immune development has received some support from animal studies (Maloney *et al.* 1982; Wignarajah *et al.* 2002) and generated much interest regarding the possibility of therapeutic dietary manipulation (Prescott *et al.* 2007; Tyson *et al.* 1999).

1.4.1 Early patterns of growth and childhood respiratory health

Evidence from clinical studies shows that lower birthweights are associated with impaired infant lung function (Friedrich *et al.* 2006; Lucas *et al.* 2004) Impairment is more pronounced in children who gain weight rapidly after birth (Friedrich *et al.* 2006; Lucas *et al.* 2004). This is an interesting finding in the context of the predictive adaptive responses theory; rapid postnatal weight gain following lower birthweight may occur as a consequence of mismatch between pre and postnatal nutrient supply. This suggests an *in utero* environment less conducive to somatic growth also impairs lung development.

However, epidemiological evidence addressing the relationship between poor fetal growth and later wheeze or asthma is less consistent. A relationship between lower birthweight and subsequent wheeze has been found by many investigators (Braback & Hedberg 1998; Carrington & Langley-Evans 2006; Lewis *et al.* 1995; Seidman *et al.* 1991; Shaheen *et al.* 1999; Svanes *et al.* 1998), although others have found evidence for an association with higher birthweight (Remes *et al.* 2008; Yuan *et al.* 2002), and some no relationship at all (Kelly *et al.* 1995; Oliveti *et al.* 1996; Rona *et al.* 1993; Sears *et al.* 1996; Strachan *et al.* 1996a; Taveras *et al.* 2006) (Table 1). A recent meta-analysis concluded that an increased risk of future asthma is associated with higher birthweight (Flaherman & Rutherford 2006). It is arguable however, that some misclassification of outcome may have occurred in this analysis as wheeze outcomes measured at different ages were combined; this may not be valid given that the prevalence of the various wheeze phenotypes varies with age. Moreover, this meta-analysis looked specifically for an effect of high birthweight compared to average birthweight and did not consider the effects of the full range of birthweights.

The relationship between birth anthropometry and later atopy has also been studied in many cohorts using a variety of measures of size, proxy measures of growth and several different outcome measures. These studies have produced slightly more consistent results than those relating to wheeze outcomes; most frequently atopy has been found to be associated with high rates of fetal growth or some indication of disproportionate growth. For example, elevated serum total IgE in adulthood has been found to be associated with larger head circumference at birth and higher birthweight (Godfrey *et al.* 1994). It has been proposed that disproportionate head growth may reflect prioritisation of the growth of the brain under conditions where the nutritional needs of the fetus cannot be met. Fetuses adopting a rapid growth trajectory from early pregnancy, possibly due to programming by good maternal nutrition at conception, have higher nutritional requirements and are therefore more vulnerable to reduced nutrient supply. In fetuses with disproportionate growth, the relatively poor nutrition of the body may affect rapidly growing tissues such as the thymus, and other components of the immune system, and subtly alter function. Animal studies have provided some evidence that suggests the cytokines associated with the Th2 bias of normal pregnancy may have additional properties in terms of promoting fetal growth (Whitsett & Wert 2007). These findings are not without controversy, however, as the association between large birth

dimensions and later atopy has not been a consistent finding of all epidemiological studies of either children (Arshad *et al.* 1993) or adults (Laerum *et al.* 2005) and there is evidence to contradict the proposal that larger head size is associated with decreased thymic volume (Benn *et al.* 2001).

A limitation of previous studies has been the use of birth anthropometry as a proxy for fetal growth. Failure to identify an association between size or weight at birth and wheeze or atopy implies not that intrauterine factors are unimportant in the development of these phenotypes, only that relevant intrauterine factors may not strongly affect birth anthropometry. No other study to date has used serial prenatal ultrasound scans to assess fetal growth or its relation to wheeze and atopic outcomes directly. Suitable ultrasound scans can only be collected in a cohort such as the Southampton Women's Survey (SWS) where participants are recruited and followed up from before conception.

Citation	Study participants	Principal findings
Birth anthropometry inversely related to wheeze outcome		
Seidman *et al.* 1991	20,312 Israeli conscripts aged 17 years	Birthweight inversely related to asthma (no correction for gestation)
Lewis *et al.* 1995	1712 males aged 16 years	Birthweight inversely related to transient wheeze (up until 5 years)
	Prospective UK 1970 birth cohort	Birthweight not related to wheeze persisting until 16 years
Braback & Hedberg	149,398 Swedish conscripts	Birthweight inversely related to asthma (corrected for gestation)
1998	aged 17-20 years	Birthweight inversely related to rhinitis
Svanes *et al.* 1998	Birth register and questionnaire data	Birthweight inversely related to asthma
	690 Norwegian adults aged 20-24 years	Weak positive association with birth length
Shaheen *et al.* 1999	1970 British birth cohort	Birthweight inversely related to doctor-diagnosed asthma and wheezing in
	prospective data from 8960 aged 26 years	adults but not to hayfever (corrected for gestation)
Carrington & Langley-	Historical birth data and questionnaire data 256	Head circumference at 10-15 days (but not birthweight) inversely associated
Evans 2006	UK children aged 7 years	with parent-reported current wheeze at age 7 years
No association between birth anthropometry and wheeze outcome		
Rona *et al.* 1993	UK retrospective data 5573 aged 5-11 years	No relationship between gestational age adjusted birthweight and asthma
Kelly *et al.* 1995	UK retrospective data 5618 aged 5-11 years	No relationship between being small for gestational age and asthma
Sears *et al.* 1996	New Zealand birth cohort	No relationship between birthweight and asthma, bronchial
	1037 children aged 18 years	hyperresponsiveness or sensitisation
Oliveti *et al.* 1996	US retrospective data	Birthweight inversely related to asthma until corrected for social confounders
	269 children aged 4-9 years	
Strachan *et al.* 1996a	British 1958 cohort 18,559 aged 33 years	No relationship between birthweight and asthma
Taveras *et al.* 2006	Project Viva prospective birth cohort	No relationship between gestation-standardised birthweight and asthma or
	1372 children aged 2 years	wheeze

Table 1 Studies investigating the relationship between birth anthropometry and wheeze and atopic outcomes in childhood (continued on next page)

Birth anthropometry positively related to wheeze outcome		
Fergusson *et al.* 1997	Prospective New Zealand birth cohort	Head circumference at birth positively related to asthma in childhood
	1265 adolescents aged 16 years	No relationship between ponderal index and asthma
Leadbitter *et al.* 1999	Prospective New Zealand birth cohort	Head circumference at birth positively related to IgE at 11 years
	734 children aged 13 years	Birth length positively related to asthma symptoms at age 13
Yuan *et al.* 2002	Danish birth cohort 10,440 aged 12 years	Birthweight and ponderal index positively related to asthma hospitalisation
	Registry based hospitalisation data	(corrected for gestation)
Remes *et al.* 2008	Northern Finland prospective birth cohort	Birthweight positively related to asthma in atopic children
	5995 children aged 16 years	
Birth anthropometry inversely related to atopic outcome		
Arshad *et al.* 1993	UK prospective cohort	Birthweight inversely related to asthma, dust mite sensitisation and bronchial
	1174 children at age 2 years	hyperresponsiveness
Birth anthropometry unrelated to atopic outcome		
Laerum *et al.* 2005	European Community Respiratory Health Survey	No association between birthweight or head circumference and IgE, rhinitis or
	1683 adult men and women	eczema
Birth anthropometry positively related to atopic outcome		
Godfrey *et al.* 1994	UK birth records data	Head circumference and birthweight positively related to total serum IgE
	280 adults aged 47-55 years	Only head circumference remained significant in simultaneous analysis
Gregory *et al.* 1999	Hospital records of birth data	Head circumference at birth positively related to total serum IgE
	239 UK children aged 6-23 years	Birthweight weakly positively related to asthma, IgE and skin sensitisation
Katz *et al.* 2003	Sheffield child development study	Birthweight & one month head circumference positively related to rhinitis
	10,809 adolescents aged 11-16 years	Head circumference:birthweight inversely related to rhinitis
		No measures associated with asthma
Bolte *et al.* 2004	Birth record and questionnaire data	Birthweight positively related to atopic sensitisation and serum IgE
	1138 German children aged 5-7 years	Head circumference:birthweight ratio positively related to sensitisation

Table 1 (continued from previous page) Studies investigating the relationship between birth anthropometry and wheeze and atopic outcomes in childhood

1.4.2 Maternal nutrition and childhood respiratory health

In addition to the evidence provided by birth anthropometry studies which link maternal nutrition to the risk of wheeze and atopy in childhood, temporal trends in diet have been identified which parallel recent changes in prevalence of asthma and atopic disease. In the early 1990s Seaton and colleagues in Aberdeen observed that a decline in antioxidant intake had occurred over the previous 50 years (DEFRA 1940). They went on to propose that the increase in asthma observed over this period may be attributable to reduced anti-oxidant defences (Seaton *et al.* 1994). Epidemiological support for an association between maternal micronutrient intake and childhood wheeze has been found subsequently for several micronutrients (Camargo, Jr. *et al.* 2007; Devereux *et al.* 2006; Devereux *et al.* 2007). As not all micronutrients thought to have a protective effect have anti-oxidant properties, it is now thought likely that protective effects arise due to effects upon the early development of the airways or immune system.

1.4.2.1 Maternal body composition

Increases in both obesity (Heslehurst *et al.* 2007) and atopic diseases (Anderson *et al.* 2004) have been observed over the same time course. These changes have occurred too rapidly to be accounted for by common genetic influences alone and causal links between obesity and both asthma and atopy have been proposed (Castro-Rodriguez *et al.* 2001; Fredberg *et al.* 1997; Gunnbjornsdottir *et al.* 2004; Hallstrand *et al.* 2005; Thomsen *et al.* 2007; Unterborn 2001; Weisberg *et al.* 2003; Zerah *et al.* 1993). It is possible that maternal body composition may affect respiratory health in childhood via an effect upon fetal growth or via alteration of the *in utero* environment in a manner which predisposes the fetus to atopy. Aspects of a woman's nutritional status, such as high fat mass, may alter fetal concentrations of growth factors, such as IGFs, TGF-β and EGF. This may in turn promote both fetal growth and the development of atopy (Kawano *et al.* 2005). Certainly, there is evidence from the Norwegian Mother and Child population-based cohort study (MoBa) that wheeze risk at 18 months increases linearly with maternal pre-pregnancy BMI (Haberg *et al.* 2009). Moreover, low maternal fat and muscle mass prior to conception are known to be associated with impaired fetal growth (Sanin Aguirre *et al.* 2004) and harmful effects of perinatal undernutrition upon lung development have been demonstrated in animal studies. In rats, for example, early

postnatal undernutrition has been shown to affect cell division in the bronchial epithelium (Massaro *et al.* 1988).

1.4.2.2 Maternal dietary patterns

Public health dietary interventions based upon whole foods are particularly valuable as they are easier to understand and disseminate than guidance based upon individual nutrients. Therefore it is important when considering health outcomes, to evaluate associations with intakes of whole foods and the patterns in which these foods are combined. Dietary patterns and the frequency of consumption of specific foods are important because food items contain a combination of micronutrients that might contribute more to childhood outcomes than the sum of their parts. In addition, associations that may be currently unrecognised, or not easily quantifiable, can be examined using dietary pattern analysis.

Recent evidence suggests that following a predefined 'Mediterranean' dietary pattern during pregnancy might protect against wheeze and allergy in the offspring (Chatzi *et al.* 2008). Dietary patterns can also be investigated using principal component analysis. Principal component analysis is a data-driven approach which has the advantage of reducing large numbers of correlated nutrients to a smaller number of overall dietary dimensions which are uncorrelated. Principal component analysis of dietary patterns within the Avon Longitudinal Study of Parents and Children (ALSPAC) found atopy, asthma, early wheezing and Forced expiratory flow in one second (FEV_1) to be positively associated with a 'health conscious' dietary pattern but concluded that dietary patterns, after controlling for confounders, did not predict asthma and related childhood outcomes (Shaheen *et al.* 2009).

1.4.2.3 Maternal intake of specific foods

Many studies of specific food intakes during pregnancy have concentrated upon avoidance of foods perceived to be allergenic. However, there is little evidence to suggest avoiding potential allergens is beneficial (Salvatore *et al.* 2005). More recent studies have focused upon the possibility that certain foods modulate the immune system and are thereby able to protect against the development of an atopic predisposition. One such study from Aberdeen assessed maternal dietary intake according to 150 food items divided into 20 food groups and found beneficial effects associated with maternal apple and fish intake during pregnancy. Maternal apple intake

was found to be inversely associated with 'ever wheezing', 'ever asthma' and 'doctor-confirmed wheezing' in the first five years of life', maternal fish consumption was found to be inversely associated with 'doctor-confirmed eczema' and 'doctor-confirmed hayfever' (Willers *et al.* 2007). On the strength of these findings it was hypothesised that, rather than acting as a marker of a generally healthy lifestyle, the specific association between childhood atopic disease and maternal apple consumption might reflect a property specific to apples and an effect related to phytochemical content was proposed. Whilst the effect of apple consumption requires confirmation by other studies, beneficial effects of maternal fish consumption during pregnancy have been reported elsewhere (Salam *et al.* 2005).

In the LISA study from Germany (Influences of Lifestyle-related factors on the immune system and development of Allergies in childhood) a semi-quantitative food-frequency questionnaire (FFQ) administered in the last four weeks of pregnancy was used to explore the influence of diet upon eczema and allergic sensitisation in the offspring at 2 years of age. This study found sensitisation to food allergens was associated with high citrus fruit or celery intakes and sensitisation to inhalant allergens was associated with high intakes of n-6 polyunsaturated fatty acids (PUFAs), raw sweet pepper and citrus fruit. Eczema was found to be associated with high n-6 PUFA and low fish intake (Sausenthaler *et al.* 2007).

Although the Aberdeen study was able to detect associations between particular foods and childhood atopic and wheeze outcomes, only a small proportion of the cohort underwent fractional exhaled nitric oxide (FENO) measurement, lung function and skin prick testing and this precluded detailed investigation of the effects of specific foods upon either airway or immune development, or the aetiology of specific wheeze phenotypes (Willers *et al.* 2007).

1.4.2.4 Maternal nutrient intakes and nutrient status during pregnancy

A number of studies have investigated the relationship between maternal nutrient intakes during pregnancy and later respiratory and atopic disease in the offspring in childhood. In addition to the present study, two epidemiological studies have investigated maternal vitamin intake in an unselected birth cohort, the Study of Eczema and Asthma To Observe the effects of Nutrition (SEATON) in Aberdeen and the US

Project Viva study. Both studies used similar FFQs to that used in the SWS and the Aberdeen group also collected measures of nutrient status and lung function. The wheeze outcome measures for each study were questionnaire based and atopy was measured in the Aberdeen study by skin sensitisation. Data relating to outcomes up to and including 3 years have been published from Project Viva, whilst the Aberdeen study has published the results of follow up until 5 years. The demographics of these cohorts are compared in Table 2 to those of the SWS. Of note are differences in latitude, smoking and vitamin D intakes (Gillman *et al.* 2004; Litonjua *et al.* 2006; Martindale *et al.* 2005). Smoking during pregnancy was most prevalent in the Aberdeen cohort, whilst vitamin D intake was greatest in Project Viva due to higher rates of supplementation and fortification of dairy products. The American women were also likely to have greater synthesis of vitamin D in the skin due to greater sun exposure.

SWS Cohort (Inskip *et al.* 2006)	Aberdeen Cohort (Devereux *et al.* 2006; Martindale *et al.* 2005)	Project Viva Cohort (Camargo, Jr. *et al.* 2007; Gillman *et al.* 2004; Litonjua *et al.* 2006)
12,583 non-pregnant women in Southampton 1998-2002 6-year follow-up babies born 2000-2002	2000 healthy women in Aberdeen from antenatal clinics at 12 weeks' gestation 1997-1999	2670 women in Massachusetts from first prenatal clinical visit 1999-2002
Factors related to sun exposure		
Latitude 51° N	Latitude 57° N	Latitude 42° N
Representativeness		
Compared to non-participants, participating women were slightly older, less likely to smoke in pregnancy and more likely to be primiparous, participating children had less smoke exposure. 16% of mothers smoked in pregnancy.	Compared to non-participants, participating women were less likely to smoke or to have asthma but older, of higher socioeconomic status, and had higher ascorbate and tocopherol levels. Children were larger at birth, more often a caesarian delivery or breast fed. 23% of mothers smoked in pregnancy.	Compared to non-participants, participating women were more likely to be white of higher socioeconomic status. 10% of mothers smoked in pregnancy.
Dietary information		
11 and 34 week FFQ (data from last 3 months) (n=469) Energy-adjusted and non-adjusted continuous intakes	32 week FFQ (data from last 3 months) (n=1751) Energy-adjusted intakes in quintiles	Mean intakes from 12 & 26 week FFQs (data from last 3 months); Vitamin D non-energy adjusted analysed in quartiles; Vitamin E energy adjusted in quartiles (n=1194)
Nutritional status		
34 week blood sample (n=370) Retinol by high-performance liquid chromatography Ascorbate by enzymatic colorimetric assay 25(OH) vitamin D by radioimmunoassay α-tocopherol by high-performance liquid chromatography	12 week blood sample (n=1089) ß-carotene by high-performance liquid chromatography Ascorbate by enzymatic colorimetric assay α-tocopherol by high-performance liquid chromatography	No vitamin status data presented
Confounders		
Excluded gestation <35 weeks Corrected for maternal age, education, smoking during pregnancy, atopy, history of asthma, eczema or rhinitis, paternal history of asthma, eczema or rhinitis, child's gender, birthweight, gestation, birth order, breast feeding, pet and smoke exposure	Corrected for maternal age, maternal atopy, maternal smoking, maternal vitamin C intake, maternal vitamin E intake, maternal zinc intake, father's social class, maternal age on leaving education, deprivation index, birthweight, head circumference, birth crown-heel length, gender, birth order, breast feeding, early antibiotic use	Excluded gestation <34 weeks Corrected for maternal or paternal history of asthma, number of children in household, income, maternal age, calcium and retinol intake, pre-pregnancy BMI and child's gender, birthweight, passive smoking and breast feeding
Outcomes		
Questionnaire at 3 (n=1548) and 6 (n=469) years FEV_1 by Spirometry (n=310), BDR (n=143), Skin sensitisation (n=374), FENO (n=248), Methacholine challenge (n=107)	Questionnaire at 2 (n=1300) and 5 (n=1253) years FEV_1 by Spirometry (n=478), BDR (n=269), Skin sensitisation (n=700), FENO (n=167)	Questionnaire at 2 (n=1290) and 3 years (n=1194) No measures of lung function

Table 2 Comparison of the demographic characteristics of the SWS, Aberdeen and Project Viva birth cohorts

1.4.2.4.1 *Vitamin A*

It is recommended that pregnant women avoid foods rich in vitamin A because high levels are believed to be teratogenic (Rothman *et al.* 1995). However, the requirement for vitamin A increases during pregnancy (Ortega *et al.* 1997) and it is likely that a diet very low in vitamin A is associated with adverse effects upon fetal development. Animal studies demonstrate that vitamin A is involved in normal embryonic lung development, including alveolarisation, (Massaro & Massaro 1996; Pierce & Michael 2000) and in maintenance of lung function (Maden & Hind 2004). It is only possible to investigate the effects of mild deficiency as rats fed a vitamin A-free diet suffer high rates of spontaneous abortion, fetal malformation and late neonatal death (Takahashi *et al.* 1975). However, such experiments have proven the offspring of vitamin A deficient rats to develop respiratory problems in early life (Antipatis *et al.* 2000; Downie *et al.* 2005). For example, a reduction of between 30 and 60 per cent of blood retinol levels in rats leads to reduced surfactant phospholipid production (Chailley-Heu *et al.* 1999).

Clinical evidence suggests vitamin A may also have significant effects upon human respiratory health. Vitamin A appears to influence surfactant protein gene expression in human development, for example (Metzler & Snyder 1993). Moreover, low birthweight, premature neonates have low levels of plasma vitamin A (Shenai *et al.* 1981) and lung histology consistent with animal models of vitamin A deficiency (Chytil 1992). Although, supplementation studies have produced conflicting results, there is some evidence, however, of a beneficial effect of Vitamin A supplementation in premature infants at risk of bronchopulmonary dysplasia (Shenai *et al.* 1987; Tyson *et al.* 1999).

In contrast to these findings suggesting beneficial effects of vitamin A intake during pregnancy, data from the Third National Health and Nutrition Examination Survey suggest higher vitamin A status in postnatal life may promote allergic sensitisation (McKeever *et al.* 2004). In a cross-sectional logistic regression analysis of 30 serum nutrients, skin sensitisation was found to be positively associated with current vitamin A status. This association was found only in young participants and was not seen in adults. The cross-sectional design provides no information regarding causation and, although the absence of an effect in adults provides some suggestion that sensitivity to high levels of vitamin A may be greatest in early life; no data regarding *in utero* exposure were collected.

1.4.2.4.2 *Vitamin C*

Animal studies suggest adequate vitamin C levels are required for lung development and maturation. For example, vitamin C supplementation of pregnant rhesus monkeys can prevent nicotine-induced impairment of infant lung function (Proskocil *et al.* 2005). However, a recent study of antioxidant intake during human pregnancy has uncovered an unexpected association between high maternal vitamin C intake and wheeze during the second year of life (Martindale *et al.* 2005). This study assessed children from the Aberdeen cohort at an age where it is difficult to accurately characterise wheeze phenotypes, it employed a semi-quantitative FFQ and serum biomarkers of antioxidant vitamin status but no objective measures of lung function. The mechanism by which high vitamin C intakes during pregnancy might predispose to wheeze is unclear and it is uncertain whether this represents a true biological effect. For example, cord blood mononuclear cell (CBMC) responses to *in vitro* allergen stimulation showed no association with vitamin C intake (Devereux *et al.* 2002). It is possible nevertheless that a positive association between maternal vitamin C intake and childhood wheeze may be explained by the pro-oxidant effect of the vitamin at high concentrations, by the promotion of wheezing by other constituents of foods containing high levels of vitamin C, or by some other confounding factor.

1.4.2.4.3 *Vitamin D*

Epidemiological evidence suggests that many women of child bearing age may be vitamin D deficient (Dawodu *et al.* 2003; Nesby-O'Dell *et al.* 2002). The major source of vitamin D is not dietary (Holick 2003), although the vitamin is found in a few foods such as oily fish and margarine. Most vitamin D is obtained from a photosynthetic process in the skin. Non-dietary factors, including season, latitude, skin pigmentation, use of sunscreen and clothing, therefore influence women's serum levels of vitamin D. A woman's serum concentration of vitamin D is known to be strongly predictive of her child's vitamin D status (Hollis & Pittard 1984).

Many immune cells possess receptors for vitamin D and vitamin D biases the immune system towards a Th2 phenotype (Jirapongsananuruk *et al.* 2000; Matheu *et al.* 2003). Furthermore, the gene for the vitamin D receptor has been linked to asthma in two separate studies (Poon *et al.* 2004; Raby *et al.* 2004) (although other studies have failed to find a genetic association (Vollmert *et al.* 2004; Wjst 2005)). In the late 1990s, Wjst suggested high maternal levels of vitamin D in early pregnancy might be of significance

in the development of atopy and atopic disorders in the offspring (Wjst & Dold 1999). This theory was founded upon the observations that the rise in asthma prevalence parallels temporal (Heinrich *et al.* 1998a), geographic (Jarvis & Burney 1998) and social (Smith 1976) trends in the consumption of vitamin D fortified foods and that seasonal variation in allergic disorders occurs which may be related to sun exposure derived vitamin D (Wjst *et al.* 1992). In contrast, Litonjua and Weiss (2007) argued that the epidemiology of vitamin D deficiency appears to mirror that of asthma and atopy. In particular, deficiency of vitamin D has been linked to obesity (Wortsman *et al.* 2000), African American race (Nesby-O'Dell *et al.* 2002), inner city populations (Lee *et al.* 2007) and recent immigration to westernised countries (Skull *et al.* 2007).

Supplementation studies support the hypothesis that high intakes of vitamin D may predispose to atopy (Hypponen *et al.* 2004). Furthermore, a local study from the Princess Anne Hospital Study Group, which investigated the relationship between atopic disorders in childhood and serum measures of maternal vitamin D status during pregnancy, found children whose mothers had the highest intakes of vitamin D during pregnancy to have increased eczema on clinical examination at 9 months and increased reported asthma at 9 years compared to those children whose mothers had lower intakes (Gale *et al.* 2008). It has been argued, however, that the loss to follow up rate of more than 60% detracts from this result. Although animal studies suggest vitamin D induces a shift in the balance between Th subsets towards Th2 dominance (Cantorna *et al.* 2004), *in vitro* work on human tissues have not confirmed a similar effect in humans (Pichler *et al.* 2002). Epidemiological data is now accruing which casts doubt on Wjst's hypothesis that vitamin D intake is positively related to risk of allergic disorders. Follow up in Project Viva and the comparable unselected Aberdeen birth cohort found higher maternal vitamin D intakes were inversely associated with wheeze prevalence at ages 3 and 5 years (Camargo, Jr. *et al.* 2007; Devereux *et al.* 2007). This inverse relationship has also been confirmed in a Finnish cohort of children with HLA-DQB1-conferred susceptibility for diabetes; energy-adjusted total maternal vitamin D intake and vitamin D from food were found to be inversely related to asthma at 5 years of age (Erkkola *et al.* 2009). Serum measures of vitamin D were not available within these cohorts and measures of lung function and airway inflammation were available for only a subset of the children in the Aberdeen study.

1.4.2.4.4 *Vitamin E*

There is evidence that vitamin E reduces IgE production in a rat animal model (Yamada *et al.* 1996) and that adults with higher vitamin E intakes also have lower serum IgE and demonstrate lower levels of cutaneous sensitisation (Fogarty *et al.* 2000). Moreover, preliminary evidence has linked low maternal vitamin E intake with a predisposition to atopy in the offspring. Vitamin E is an important antioxidant and may also affect the developing immune system. For example, the Aberdeen group have shown low maternal vitamin E intake during pregnancy to be linked with elevated CBMCs' responsiveness to allergens (Devereux *et al.* 2002), a property believed to be predictive of childhood atopic disease (Warner *et al.* 1994). This group also found low levels of vitamin E intake during pregnancy appear to be associated with childhood wheeze (Martindale *et al.* 2005). Total maternal vitamin E intake during pregnancy was inversely associated with 'wheeze in the absence of a cold' during the first 2 years of life. The authors suggested that, rather than influencing lung development or the immune response to infection, low rates of consumption of vitamin E during pregnancy 'Th2 biased' immune development and thereby increased the likelihood of developing atopy and asthma. Total vitamin E intake during pregnancy was also found to be inversely associated with eczema in the first 2 years of life, although this was only significant in the children of atopic mothers.

Review of the Aberdeen cohort at 5 years of age revealed that the proposed association between wheeze in 2-year-old children and maternal vitamin E intake during pregnancy appears to persist into later childhood. Lung function and FENO measures were attempted in the 5-year-old children to further define the asthmatic phenotype. Measures of lung function were positively correlated with maternal plasma levels of vitamin E measured at 12 weeks gestation, whilst, in children of atopic mothers, measures of vitamin E at delivery correlated inversely with FENO measures (Devereux *et al.* 2006). Vitamin E was suggested to have a dual effect on lung function and airway inflammation, acting possibly at early and late phases of pregnancy respectively. Maternal vitamin E intake was not associated with eczema or atopic sensitisation in children aged 5 years.

1.4.2.4.5 *Polyunsaturated fatty acids*

Essential fatty acids (EFAs) and their long chain polyenes (LCPs) are indispensable for human development and health. Humans cannot synthesise EFAs and can only ineffectively synthesise LCPs, so EFAs need to be consumed as part of the diet and the EFA status of the fetus is dependent upon that of its mother. EFAs can be elongated and desaturated to form LCPs. There are two essential polyunsaturated fatty acid (PUFA) families, n-3 PUFAs derived from α-linolenic acid and n-6 PUFAs derived from linoleic acid. Arachidonic acid derived from linoleic acid is an important structural fatty acid in the brain and is a precursor of prostanoids with inflammatory properties, docosahexaenoic (DHA) and eicosapentanoic acid (EPA) are derived from α-linolenic acid and give rise to prostanoids with anti-inflammatory properties.

In 1997 Black and Sharp (1997) highlighted changes in dietary fat intake that preceded and paralleled recent increases in asthma and atopy. Dietary intake of saturated fats, present in butter and lard, has decreased and consumption of n-6 PUFAs, present in margarine and vegetable oils has increased, probably as a response to public health messages regarding coronary heart disease. Dietary intake of oily fish containing n-3 PUFAs has also decreased (Calder 2003). It has been proposed that an increase of the n-6:n-3 PUFA ratio leads to increased inflammatory cell membrane concentrations of arachidonic acid and thus increased synthesis of prostaglandin E_2. *In vitro* prostaglandin E_2 is known to suppress Th1 differentiation (Miles *et al.* 2003) and this, along with related changes in cytokine expression, may be responsible for an increased atopic propensity.

A review of trials of n-3 fatty acid supplements in children with established asthma found little evidence to recommend supplementation or to increase dietary intake (Woods *et al.* 2002). However, low n-3 intakes during critical periods of lung and immune development could have long-term respiratory consequences (Mihrshahi *et al.* 2004). There is also evidence to suggest that individuals with asthma differ in their response to n-3 fatty acid supplementation and that this might reflect genetic differences (Okamoto *et al.* 2000b). Results from supplementation during infancy or pregnancy support the conjecture that EFA exposure during early life may be particularly important. In the Asthma Prevention Study, children receiving an n-3 PUFA supplement from 6 months of age displayed a 9.8% reduction in wheeze before

18 months compared to control subjects (Mihrshahi *et al.* 2004). Moreover, a large population-based intervention study has found n-3 PUFA supplementation in late pregnancy to be beneficial in relation to childhood asthma (Olsen *et al.* 2008). In cord blood erythrocyte samples from the population-based ALSPAC study the linoleic:α-linolenic ratio was found to be positively associated with late onset wheeze and the Arachidonic acid:EPA ratio was positively associated with eczema (Newson *et al.* 2004). The authors of this study concluded that fetal exposure to LCPs is unlikely to be an important determinant of early childhood wheezing or atopic disease as, after adjusting for multiple comparisons, these associations were no longer significant. It is interesting to note, however, that these findings were consistent with the *a priori* hypothesis relating high arachidonic acid levels to allergic sensitisation. There are also methodological difficulties associated with erythrocyte phospholipid measurements due to sample instability. Further clarification of the role of EFA status in immune development is needed.

1.4.3 Further early life influences upon childhood respiratory health

This thesis explores early life risk factors for childhood asthma and wheeze; it focuses primarily upon the influences of maternal nutrition during pregnancy and early life growth. However, it is likely that dietary and other factors acting both prenatally and in early postnatal life might also significantly influence respiratory health.

There is evidence that, in addition to the nutrients discussed in this thesis, a number of minerals and trace elements may influence the risk of asthma and allergy in childhood. For example, many pregnant women in the UK are believed to receive inadequate intakes of selenium in their diet (Rayman 1997). Data from the ALSPAC birth cohort suggest that fetal selenium status may be inversely correlated with persistent wheeze (Shaheen *et al.* 2004). Corroborating evidence from animal experiments demonstrates maternal selenium deficiency during pregnancy impairs lung development in the offspring (Kim *et al.* 1991). Data from the ALSPAC cohort also suggest an inverse association exists between umbilical cord iron levels and eczema and wheeze (Shaheen *et al.* 2004). Evidence from Aberdeen exists which suggests maternal zinc intake may be associated with the risk of childhood wheeze and atopy. In the Aberdeen cohort asthma and eczema at 5 years of age were found to be inversely related to maternal zinc intake during pregnancy and it has been speculated that the zinc-dependent metalloproteinase

ADAM33, which has been identified as a potential asthma susceptibility gene, may underlie this relationship (Devereux *et al.* 2006). The number of nutrients considered in this thesis was limited in order to avoid chance findings due to multiple comparisons. Selenium in particular, was not investigated because determining selenium intake from questionnaire data is complicated by the significant influence of soil selenium levels upon crop selenium content (Rayman 1997).

Although this thesis concentrated upon early developmental influences in prenatal life, evidence can also be found to support dietary manipulation in postnatal life. A childhood diet rich in fruit and vegetables, for example, appears to confer a protective effect upon respiratory health (Cook *et al.* 1997). Protective effects have been proposed for increased childhood intake of dietary antioxidants (vitamins C, E, carotenoids and selenium) (Harik-Khan *et al.* 2004) and childhood intakes of vitamins A and D are thought to be important for lung development (Gilliland *et al.* 2003). High intakes of these vitamins, however, may increase sensitisation to allergens (McKeever *et al.* 2004; Wjst & Dold 1999). Low vitamin C intake in childhood has been related to impaired lung function and asthma (Romieu *et al.* 2004a). Finally, there is limited evidence for beneficial effects of childhood intake of long chain n-3 PUFAs in fish, and of butter and whole milk intake, and for harmful effects of n-6 PUFAs and margarine intakes (Black & Sharpe 1997).

1.5 GENE-ENVIRONMENT INTERACTIONS

Multiple genes have been found to be associated with asthma. Many of these associations may be chance findings, nevertheless a number of these associations have been replicated in several separate studies. Genetic association studies have not been consistent across all populations, indicating that genes which confer susceptibility to asthma may only cause disease when they are combined with specific environmental exposures, and otherwise may not be risk factors for asthma (Martinez 2007). An example of modification of genetic susceptibility by environmental exposure can be found in the interaction between glutathione S-transferase M1 genotype and ozone exposure. Asthmatic children with a homozygous deletion polymorphism of the glutathione S-transferase M1 gene appear more susceptible to the deleterious effects of ozone upon small airway function and derive greater benefit from antioxidant supplementation (Romieu *et al.* 2004b).

Environmental factors may also interact with genetic risk by influencing the transcription of asthma susceptibility genes. Vitamin E, for example, is known to exert a direct effect upon the IL-4 promoter region (Li-Weber *et al.* 2002). Alternatively, the environmental factors may interact with the genome via epigenetic mechanisms, heritable modification of gene expression by mechanisms other than changes in the underlying DNA sequence. Several basic mechanisms of epigenetic modification of gene expression have been discovered (Reik *et al.* 2001). Firstly, gene regulatory regions may be methylated or demethylated; this process may alter gene expression in a graded fashion. Secondly, the structure of the protein-DNA complex may be modified by histone chromatin acetylation. There is widespread demethylation and remethylation during gametogenesis and also during early embryogenesis just prior to blastocyst development; any modifications are then stable through subsequent somatic differentiation. Epigenetic mechanisms have been shown to influence expression of transcription factors that control T cell lineage (Fields *et al.* 2002; Kim & Leonard 2004; Lee *et al.* 2002). Hypomethylation of naïve T cells enhances the expression of FoxP3 (Kim & Leonard 2004), resulting in skewing towards a Th1 phenotype. A recent study indicates that in a mouse model *in utero*, dietary methyl donors can increase the severity of allergic airway disease through epigenetic mechanisms (Hollingsworth *et al.* 2008).

1.6 DIFFICULTIES ASSOCIATED WITH THIS RESEARCH

The research described in this thesis and similar epidemiological studies provides useful information regarding association but is unable to prove causation. Epidemiological findings can be difficult to interpret and the direction of causality may not be correctly identified. It can also be difficult to identify a significant exposure when many factors vary in a collinear fashion. For example, individuals following a healthy diet are also likely to take exercise, refrain from smoking, and possess a generally healthy lifestyle. Where the nature of the mechanism linking exposure and outcome is unclear, such studies are particularly vulnerable to unidentified confounding factors. It is often not possible to adjust for confounding factors completely, particularly where subtle differences in lifestyle or behaviour may significantly affect outcome.

The need to collect detailed information must be balanced against the burden placed upon participants. For example, whilst it was important to determine mothers' atopic status for this study, it was acknowledged that not all women would wish to undergo

skin prick testing. The accuracy with which exposure and outcome variables are characterised must similarly be balanced against feasibility in a large study. It is not feasible to obtain doctor-confirmed wheeze and asthma status in a large epidemiological study. Moreover, those participants willing to attend an assessment by a doctor are likely to be unrepresentative of the cohort as a whole. Changes in the characteristics of the cohort over time are also a problem; those individuals remaining under follow up in longitudinal studies may differ in terms of factors relevant to health, for example wealth or mobility, compared to individuals who are lost to follow up. This is a particular problem in studies such as this when the outcome is separated in time from exposures of likely importance. Finally, epidemiological studies of childhood asthma are associated with specific problems related to the absence of a gold standard for this diagnosis and the technical difficulty associated with obtaining valid measures of lung function in young children.

1.7 UNANSWERED QUESTIONS

Evidence from several cohort studies suggests that maternal nutrient intake during pregnancy influences childhood wheeze and atopic disease. These data are intriguing, but previous studies lack the preconception data of the Southampton Women's Survey (SWS), and also the depth of characterisation of childhood lung function. Previous cohorts have generally had a short follow up period and have not been able to assess the effects of maternal nutrition upon asthma, specifically, rather than wheeze in early childhood. Exploring these issues in the SWS will allow replication of previous findings, investigation of the effects of pre-pregnancy diet and of fetal growth, and finally information concerning the interplay between environmental influences upon atopy and upon lung function in the development of childhood wheeze phenotypes.

Specific areas for clarification are:

- The relationship between childhood wheeze phenotypes and growth in early life
- Whether any maternal nutrient intake is significantly associated with asthma, specifically, rather than the more heterogeneous diagnosis of childhood wheeze
- Whether the associations seen between childhood wheeze phenotypes and maternal nutrient intakes can be replicated using measures of maternal nutrient status

- Whether the associations seen between childhood wheeze phenotypes and maternal nutrient intakes reflect an influence upon the development of atopy or lung function or a combination of such effects.

This thesis draws upon data collected from a unique cohort of women of childbearing age in Southampton, UK (Inskip *et al.* 2006). The cohort characteristics are described in detail in the next chapter; in brief, women were recruited before they became pregnant, enabling detailed characterisation of lifestyle, anthropometry, diet, and blood parameters both before pregnancy and during early and late pregnancy. The children were followed up regularly during the first few years of life and similarly characterised. Comprehensive data is potentially available for approaching 1000 appropriately aged mother-child pairs; the SWS is, therefore, an ideal cohort in which to attempt to answer the questions raised by previous research.

1.8 SUMMARY OF THE SPECIFIC AIMS OF THIS STUDY

This thesis seeks to explore the early life origins of childhood wheeze; its focus is maternal nutrition during pregnancy and growth during fetal and early infant life. Study participants were members of an unselected, prospectively recruited birth cohort. The study was designed to first establish whether particular patterns of fetal growth are associated with paediatric wheeze. To address this issue, data collected at the 3-year follow-up was used in order to maximise the number of mother-child pairs contributing data. The main body of this study uses more detailed data collected from a subset of these children at the 6-year follow-up and seeks to assess the influence of maternal nutrition upon lung function and atopy and specific wheeze phenotypes.

The specific objectives were:

- To relate prenatal and early postnatal growth patterns to wheeze and atopy risk at age 3 years in order to explore the hypothesis that factors which promote adaptive change in the relative growth of body tissues might have functional consequences for the respiratory and immune systems in later life.
- To test the hypothesis that the development of atopy at 6 years of age is associated with aspects of maternal body composition and maternal diet immediately prior to and during pregnancy.

(Considering specifically the effects of high maternal fat mass, high vitamin D status, low vitamin E intake and low omega 3 fatty acid status.)

- To examine the hypothesis that impaired maternal nutrition immediately prior to and during pregnancy is associated with impaired lung function at 6 years of age.

  (Considering specifically the effects of low maternal fat mass, low maternal muscle mass, low vitamin A intake and low vitamin C intake.)

- To test the hypothesis that maternal nutrition and faltering of fetal growth in late gestation have differential effects on the development of each of the childhood wheeze phenotypes.

Chapter Two
Methods

2.1 THE SOUTHAMPTON WOMEN'S SURVEY

The Southampton Women's Survey (Inskip *et al.* 2006) was founded in 1998 and is one of the largest surveys of women's health and lifestyle ever carried out in the UK. A principal aim of the survey was to identify maternal influences acting before and during pregnancy that determine pre- and postnatal growth and development. A population sample of 12,583 women was recruited. These women were aged 20 to 34 years and resident in the city of Southampton; they were broadly representative of the British population in terms of ethnicity and deprivation (Inskip *et al.* 2006). Over 3000 women within the SWS became pregnant and delivered live born infants. These women were invited to participate in the pregnancy and childhood follow-up phases of the study. The SWS has generated a number of studies focusing upon particular areas of interest at different stages of pregnancy and childhood (Harvey *et al.* 2007; Harvey *et al.* 2008; Lucas *et al.* 2004; Marriott *et al.* 2008; Martin *et al.* 2007). This research draws upon existing SWS data in combination with new data with a respiratory focus collected from children followed up at between 6 and 7 years of age (Figure 2). Follow up at this age provides an excellent opportunity to investigate early life influences upon asthma and wheeze as by this age the majority of children that will receive a diagnosis of asthma in childhood can be identified and their wheeze phenotype characterised (Martinez *et al.* 1995; Yunginger *et al.* 1992).

Ethical approval for the main SWS study was granted by the Southampton and South West Hampshire Local Research Ethics Committee (LREC Number 276/97). The same local committee also approved the collection of nutritional data during pregnancy

(LREC Number 307/97), the infant follow-up study (LREC Number 089/99) and the study of respiratory health in 6-year-olds (LREC Number 06/Q1702/104).

2.2 DATA COLLECTED WITHIN THE SOUTHAMPTON WOMEN'S SURVEY

2.2.1 Pre-pregnancy

The primary method of recruitment was via general practitioners (GPs): each woman received a letter from her GP's surgery and then a follow up telephone call from a member of the SWS team. A local advertising campaign was used to encourage self-referrals from those women not registered with a GP, or whose contact details were out of date. Approximately 75% of women contacted agreed to participate. Participation could not be quantified exactly as some of the contact details held by GPs were out of date and this precluded calculation of the total number of potential participants.

After enrollment, women were interviewed by a trained research nurse. The information recorded at the initial visit is summarised in Table 3. At this visit, the nurse administered the FFQ and made detailed body composition measurements. Information was also recorded about smoking, family background, education, ethnicity, housing, household composition, childcare arrangements, general health and the woman's occupation, and that of her partner.

Demographics	Health	Lifestyle	Anthropometry
Age	Illness	Smoking status	Height
Employment		Alcohol use	Weight
Social class		Diet	Body mass index
Education			Skinfold thicknesses
Parity			Arm muscle area

Table 3 Pre-pregnancy data recorded at the initial interview

2.2.2 During pregnancy

Enrolled women were asked to inform the study coordinators immediately if they became pregnant: written consent was also requested for their GP or hospital doctor to communicate this information. Women who became pregnant were invited to attend interviews at 11 and 34 weeks of gestation. During the pregnancy visits, a questionnaire

similar to that administered at the initial visit was used to assess diet and lifestyle factors. More detailed information about both parents' health was collected, including whether either parent had a history of asthma or atopic disorders. The anthropometry measures were repeated, and at the 34 week visit venous blood was collected and plasma and serum samples stored at −80°C. Fetal dimensions were recorded from ultrasound scans (USS) at 11, 19 and 34 weeks of gestation (Table 4).

Maternal Data	**Paternal data**	**Fetal Data**
11 & 34 weeks	**11 weeks**	**11, 19 & 34 week USS**
Diet	Smoking	Crown-rump length (11 week only)
Smoking	Asthma	Femur length (19 & 34 week only)
Anthropometry	Eczema	Head circumference
Asthma	Rhinitis	Abdominal circumference
Eczema		
Rhinitis		

Table 4 Data recorded in early and late pregnancy

2.2.3 Childhood Follow-up

At birth, the babies' lengths, weights, and head and abdominal circumferences were measured and skinfold thicknesses were recorded. At 6 months, 1 year, 2 years and 3 years a nurse visited the children at home, to record data relating to body size, feeding, diet, general health and living conditions. Atopic status was assessed in infancy by skin prick testing at the 1 and 3 year visits, mothers also underwent skin prick testing at the 1 year follow-up. A questionnaire was administered to the mothers during the 3-year follow-up which enabled their children to be classified according to wheeze history. Information about potential confounders including respiratory illness in early life and other exposures relevant to later respiratory health was also gathered by questionnaire (Table 5).

In addition, objective lung function measurements were recorded in early infancy from a small subset of the cohort. This data will be available for future analysis but was not used in this thesis as only a small proportion of this subset had attended follow-up at the time of writing.

Maternal Data	Children's Data		
	Health	**Environment**	**Anthropometry**
Atopy	Asthma	Smoke exposure	Height
Employment	Eczema	Diet	Weight
Social class	Rhinitis		Skinfold thicknesses
Education	Atopy		

Table 5 Data recorded at the childhood follow-up interview

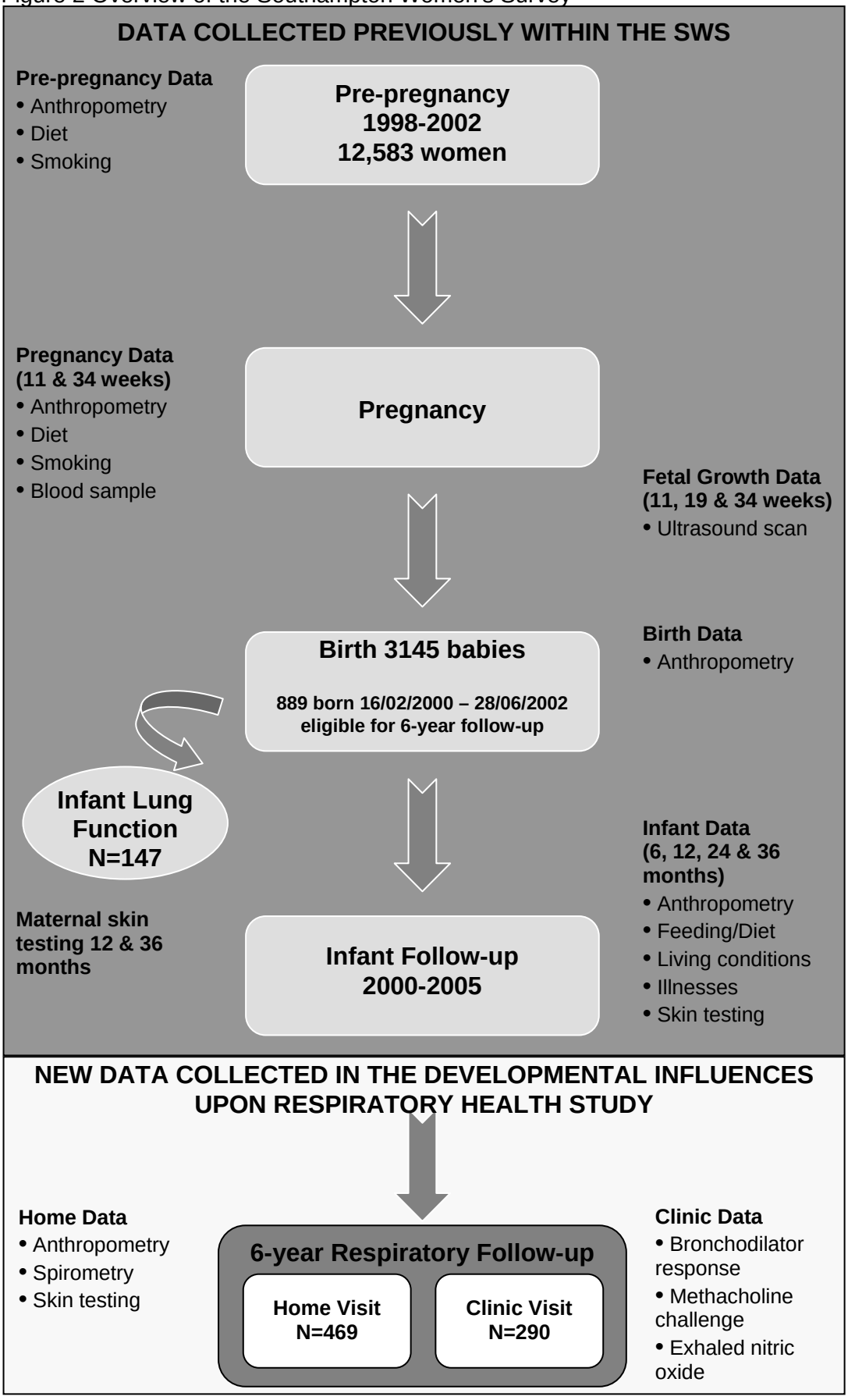
2.3 THE DEVELOPMENTAL INFLUENCES UPON RESPIRATORY HEALTH 6-YEAR FOLLOW-UP STUDY

The Developmental Influences upon Childhood Respiratory Health Study began in January 2007 and is planned to continue until at least January 2010. Children between their sixth and seventh birthdays are invited to participate. In total, 1852 children will meet the aged-based eligibility criteria during this period. This thesis reports on data collected between 1st January 2007 and 12th September 2008 (1058 children eligible in principle); it includes data from the first 469 children interviewed within the Developmental Influences upon Childhood Respiratory Health Study. The full protocol for this study is included within Appendix 2.

Recruitment was managed by experienced SWS staff. Study information was posted to mothers of appropriately aged children who were then invited to participate via telephone. Contact was possible for approximately 80 per cent of eligible children and of those contacted 75 per cent agreed to participate (overall follow up rate of 60%). The number of children included in this thesis is less than 60% of the total eligible in principle as, due to the ongoing nature of the Developmental Influences upon Childhood Respiratory Health Study, not all of the eligible children were contacted within the time frame for data collection for this thesis. The 469 children seen had dates of birth between 16/02/2000 and 28/06/2002. A further 420 children were born between these dates but, for various reasons, could not be seen at this stage of the study and these children were used as a comparison group to assess the representativeness of the sample. Additional attempts were made to contact subjects who had moved away or lost contact with the study: in such instances, health records and the electoral role were accessed with the aim of finding parental contact details.

The Developmental Influences upon Childhood Respiratory Health Study collected a detailed dataset combining information about atopy and respiratory health. Preliminary spirometry and questionnaire data were collected during a visit to participants' homes (Appendix 3). This was followed by an invitation to attend the Wellcome Trust Clinical Research Facility for more extensive lung function testing including measurement of FENO and bronchodilator response (BDR) or bronchial hyperresponsiveness (BHR) (Appendix 4).

Figure 2 Overview of the Southampton Women's Survey



2.3.1 Exposure variables

2.3.1.1 Maternal body composition

Each woman's pre-pregnancy height and weight was measured using portable equipment and according to a standardised protocol (Appendix 6). Weight was measured on Seca 835 scales (Seca Ltd., Birmingham, UK) and height with a Leicester stadiometer (Invicta Plastics Ltd., Leicester, UK); both were calibrated according to the manufacturers' directions. Mid upper arm circumference was measured with rigid tape and biceps, triceps, suprailiac and subscapular skinfold thicknesses were measured with Harpenden 'John Bull' skinfold calipers (British Indicates Ltd., St Albans, UK), (calibrated twice a year). Skinfold measurements were taken from each site following a standard protocol until three readings within 10% of each other were recorded. Body mass index, body fat percentage and arm muscle area were derived from these simple anthropometric measurements according to the formulae below (Figure 3). The nurses were carefully trained and regular intra- and inter-observer variability studies were performed to ensure measurements were as accurate as possible.

Figure 3 Measures of body composition derived from anthropometry

$$\text{Body mass index} = \frac{\text{weight (kg)}}{\text{height}^2\ (\text{m}^2)}$$

$$\text{Body fat percentage} = (4.95/\text{density} - 4.5) \times 100$$

$$\text{where density} = c - (m \times \log.\ \text{sum skinfolds at 4 measured sites})$$

c and m being constants which vary with age (Durnin & Womersley 1974)

Arm muscle area

$$= [(\text{mid-upper arm circumference (cm)} - \pi \times \text{triceps skinfold (cm)})^2/4\pi] - 6.5$$

the correction of 6.5 cm is included to allow for mean female humerus area

Although epidemiological evidence suggests asthma and obesity are associated the mechanisms linking obesity with wheeze and atopic outcomes are unclear. As it is not known whether absolute or relevant fatness represents the greatest risk both absolute and percentage body fat estimates were calculated. The ratio of subscapular to triceps skinfold thickness was calculated to explore whether the relative distribution of fat between central and peripheral sites influenced outcome. Arm muscle area was

calculated to explore the influence of lean body mass. Finally, weight gain during pregnancy was also estimated.

Total and percentage body fat and arm muscle area were calculated from measurements of body weight, skinfold thicknesses and mid upper arm circumference made during the early and late pregnancy visits (11 and 34 weeks' gestation). Body composition equations are rarely validated in pregnant women so an identical equation was used to calculated body fat percentage from skinfolds both before and during pregnancy. A pragmatic measure of pregnancy weight gain was derived from the pre-pregnancy weight and that at 34 weeks' gestation; the validity of this measure was affected by the variable duration between the pre-pregnancy interview and conception. The median (interquartile range (IQR)) time between the initial interview and the 34 week interview was 1.5 years (1.0-2.2 years) and the longest duration was 3.8 years.

2.3.1.2 Dietary intake

Diet was assessed at the initial interview and during both the early and late pregnancy interviews using a 100-item FFQ to record the average frequency of consumption over the preceding 3 months (Appendix 5). Frequencies of foods not listed on the FFQ were also recorded if consumed once per week or more. Standard portion sizes were allocated to each food apart from milk and sugar for which daily quantities consumed were recorded. Women often make dietary changes upon learning that they are pregnant, although diet during pregnancy can be expected to be correlated with that eaten before conception. Pre-pregnancy diet was considered separately from diet during pregnancy in order to determine whether health outcomes are associated specifically with the diet followed during pregnancy. Moreover it was also considered important to consider pre-pregnancy diet given that maternal stores of important nutrients, particularly fat-soluble vitamins such as vitamin D, may be accrued in the months preceding pregnancy.

2.3.1.3 Nutrient intake

The dietary data was run against food composition data to provide estimates of nutrient intake. The food composition database was based upon the UK Royal Society of Chemistry data (McCance & Widdowson 5th Edition plus all additional supplements) and supplemented with composition information from manufacturers, recipes and other sources including the US Department of Agriculture, and Food Standards Australia

New Zealand. The database included more than 4000 foods. Nutrient intakes were calculated by multiplying the frequency of consumption of a portion of each food by its nutrient content. Validation using 4-day food diaries and measurement of maternal micronutrient concentrations has indicated that this questionnaire can be used to rank the nutrient intakes of individuals (Robinson *et al.* 1996).

2.3.1.4 Dietary patterns

Principal component analysis was used to summarise the data obtained from the food frequency data and to provide a picture of the diet as a whole. Principal components are designed to indicate the independent axes along which participants vary the most. In common with other studies, the score derived from the first principal component provides a broad summary of the degree to which each woman complies with current dietary recommendations. This score is termed the prudent diet score. The score is standardised so has a mean of zero and a standard deviation of one (Crozier *et al.* 2006).

2.3.1.5 Maternal micronutrient status

A limited number of micronutrients were assayed in the maternal blood samples obtained at 34 weeks' gestation.

2.3.1.5.1 *25(OH) vitamin D*

Maternal 25(OH) vitamin D concentrations were measured in the Chemical Pathology department at St. Thomas' Hospital, London.

Maternal 25(OH) vitamin D concentrations in late pregnancy serum samples were measured using a radio-immunoassay (DiaSorin, Stillwater, USA) which has a coefficient of variation (CV) of less than 10%. This assay is a two-step procedure. First, 25(OH) vitamin D was rapidly extracted from the samples with acetonitrile. Then an equilibrium radio-immunoassay procedure using an antibody specific to 25(OH) vitamin D was used to assay the samples. The sample, antibody and tracer were incubated for 90 minutes at 20 - 25ºC. Phase separation was accomplished after 20 minutes incubation at 20 - 25ºC with a second antibody-precipitating complex. Non-specific binding/Addition buffer was added after this incubation prior to centrifugation to reduce non-specific binding.

2.3.1.5.2 *Dietary antioxidants*

Dietary antioxidants were measured in the University of Southampton, Institute of Human Nutrition.

Total antioxidant status

Total antioxidant status was measured using a total antioxidant status reagent kit (Randox Laboratories, Crumlin, UK) on a Konelab 20 Autoanalyser (Labmedics, Salford, UK) according to the manufacturer's protocol. The chromogen ABTS (2,2'-Azino-di-[3-ethylbenzthiazoline sulphonate]) was reacted with a peroxidase and hydrogen peroxide to produce a radical cation with a relatively stable absorption at 600 nm. The antioxidant content of the maternal serum samples decreased the peroxidise-mediated colour formation to a degree proportional to the antioxidant concentration in the sample. The antioxidant concentration was calculated by comparing the decrease in absorption at 600 nm to that produced by a (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) standard. The interbatch CV for this assay was 4.4%.

Vitamin C

Serum ascorbic acid concentration was measured using the Konelab 20 Autoanalyser (Labmedics, Salford, UK). Dehydroascorbic acid (oxidised vitamin C) reacts with o-phenylenediamine to give a product with absorbance at 340 nm. Serum was added to a reaction mixture containing o-phenylenediamine which couples with any pre-formed dehydroascorbic acid and other non-specific components. The absorbance was measured at 340 nm as the sample blank. The enzyme ascorbate oxidase (EC 1.10.3.3), which quantitatively converts ascorbic acid to dehydroascorbic acid, was then added to the mixture and the increase in absorbance at 340 nm was used to quantify the serum ascorbic acid concentration by comparison with the response of a standard of known concentration. The assay had an interbatch CV of 7%.

Vitamins A and E

Retinol, ß-carotene and α-tocopherol were analysed by high pressure liquid chromatography (HPLC) using a combination of fluorescence and spectrophotometric detection. The sera were extracted into hexane, dried and reconstituted in ethanol containing 0.1% butlyated hydroxyl toluene. α-tocopherol acetate was added to the sample extraction mixture as an internal standard. Beckman 32 Karat software was used

to control the chromatography system and for data collection. The HPLC equipment comprised a Beckman System Gold 125 Solvent System and 508 Autosampler (Beckman Coulter Ltd., High Wycombe, UK) connected to a Supelcosil C18 reverse phase column (25 x 0.46 cm, 5 microns particle size) (Sigma-Aldrich Chemical Co., Gillingham, UK). The mobile phase was a gradient running from 100% methanol to methanol: iso-propanol in ratio 6:4 vol:vol at 1 ml/min. For detection, an Agilent 205 and a Spectraphysics spectrophotometer were connected in series with a Perkin Elmer LC250 fluorimeter.

Retinol was measured by absorbance at 325 nm, ß-carotene by absorbance at 460 nm and α-tocopherol and tocopherol acetate by fluorescence with absorption at 290 nm and emission at 330 nm. Working standards of retinol, α-tocopherol and ß-carotene were prepared daily from stock solutions stored at -80°C and concentrations were determined by spectrophotometry using molar extinction coefficients. The peak area ratios of each analyte (retinol, α-tocopherol and ß-carotene) to the internal standard (α-tocopherol acetate) were calculated and the concentration of the samples calculated by comparison to the standards.

Interbatch precision

Retinol	CV = 5.9 % at level of 1.70 μmol/L, N=10
α-tocopherol	CV = 7.0 % at level of 17.7 μmol/L, N=10
ß-carotene	CV = 8.5 % at level of 0.15 μmol/L, N=10

Total cholesterol concentration was measured to enable the α-tocopherol / total cholesterol concentration ratio to be calculated. Serum tocopherol is bound to lipoproteins, the concentration of tocopherol is thus affected by both cholesterol concentration and the acute phase response. Correcting for cholesterol allows tissue stores to be assessed independently of the acute response. Total serum cholesterol concentration was measured using the Konelab Cholesterol reagent kit on a Konelab 20 Autoanalyser (Labmedics, Salford, UK) according to the method supplied by the kit manufacturer. Cholesterol esters were enzymatically hydrolysed to free cholesterol and non-esterified fatty acids. Free cholesterol, including any endogenous free cholesterol was oxidised by cholesterol oxidase with the liberation of hydrogen peroxide which, in presence of peroxidase, reacts with 4-aminoantipyrine and hydroxybenzoic acid to form

a quinoneimine chromophore with absorbance in the wavelength region of 500–550 nm. The absorbance was linearly related to the total cholesterol concentration of the sample. The CV of this assay was 2.9%.

2.3.1.5.3 *Fatty acid profiling*

The fatty acid composition of plasma phospholipid phosphatidylcholine was measured in the University of Southampton, Institute of Human Nutrition.

Solid phase extraction was used for lipid extraction and purification (aminopropylsilica cartridge, Varian Inc., California). This was followed by reaction with methanol, in the presence of a sulphuric acid catalyst. The methylation reaction releases fatty acids from phospholipids, triacylglycerol and cholesteryl esters and creates fatty acid methyl esters (FAMEs) of low boiling points. FAMEs were separated by a Gas Chromatograph (Series 6890, Hewlett Packard, BPX 70 column SGE Europe Ltd.) according to difference in chain length and number of double bonds which affect the temperature at which they become volatile. The end of the column was located within a flame ionisation detector. The hydrogen flame combusts the FAMEs and generates an ion current proportional to the amount of FAME in the sample. The resulting chromatogram contains a series of peaks, each corresponding to a FAME. The area under each peak is proportional to the mass of the FAME injected onto the column. The CVs for each fatty acid were less than 10%.

In order to limit the number of analyses conducted, it was decided to analyse the fatty acid data according to percentage fatty acid concentration only. Percentage concentrations provide information relevant to the likely phospholipid composition of immune and other cell membranes. This was thought to be of greater functional consequence than the information provided by measures of absolute concentration which would be of greater relevance if the total fatty acid delivery in maternal blood to the placenta were considered of prime mechanistic importance.

2.3.1.6 Fetal growth

Ultrasound measurements of fetal size were recorded at 11, 19 and 34 weeks' gestation (Appendix 7). Measurements were recorded by three operators using Acuson 128 XP, Aspen & Sequioa ultrasound machines, calibrated to 1540 m/s and following a standardised protocol. The method of Royston (Royston 1995) was used to generate Z-

scores (adjusted for duration of gestation) for linear size, head and abdominal circumferences in early, mid and late pregnancy and at birth. The Royston method was also used to calculate velocities of growth conditional upon the initial measurement of size in order to account for regression to the mean.

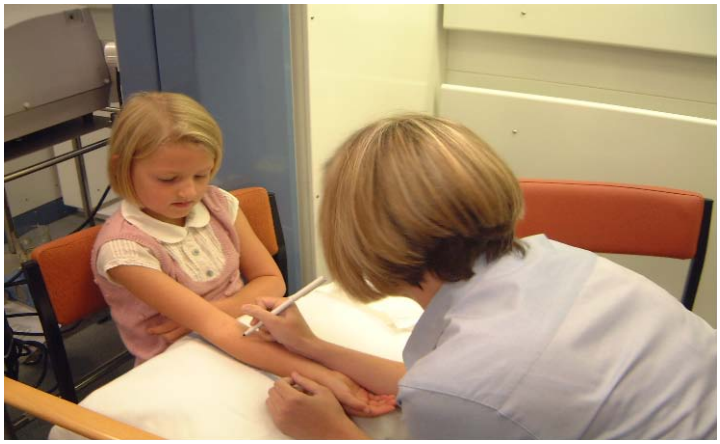
2.3.1.7 Infant growth

Research nurses measured the infants after delivery. The recorded measurements were infant length (Harpenden infantometer, Britsih Inidcates Ltd., St. Albans, UK), weight (Seca scales, calibrated twice a year, Seca Ltd., Brimingham, UK), occipito-frontal circumference, abdominal circumference, and triceps and subscapular skinfold thicknesses (Holtain skinfold calipers, calibrated twice a year, Holtain Ltd., Dyfed, UK). Skinfolds measurements were conducted according to a protocol similar to that used for the maternal measurements. The same anthropometric measurements were also recorded at 6 and 12 months of age.

2.3.2 Outcome variables

2.3.2.1 Atopy

Figure 4 Skin sensitisation testing



Skin prick testing was conducted according to local protocol (Appendix 9 & Figure 4) Written consent for skin testing was obtained from parents and verbal assent from children. Most children were skin tested during the home visit; those children with a history of allergic reactions were tested in the clinic. The children were asked not to use any anti-histamine medications for at least seven days before their skin test.

Testing to cat[1], dog[1], grass pollens[1], house dust mite[1], milk[1], egg allergens[2] and tree pollens[3] ([1]Hollister-Stier, Spokane, WA, [2]Alyostal, Antony, France, [3]ALK Abelló Hørsholm, Denmark) was undertaken with a single headed lancet. Average wheal

diameters for each allergen were measured and a positive result defined as one that was at least 3 mm in diameter in the presence of valid controls. The controls were deemed valid if the negative (50% glycerin[1]) control was zero and positive control (histamine 10 mg/ml[3]) was at least 3 mm. Presence of at least one positive result was considered evidence of atopy.

2.3.2.2 Exhaled nitric oxide

Figure 5 NIOX® measurement of FENO

Exhaled nitric oxide was measured as a non-invasive marker of airway inflammation (Figure 5). Where possible, three exhaled nitric oxide measurements were recorded using a NIOX® chemiluminescence analyser (Aerocrine AB). This machine requires regular calibration and servicing; on occasions when it was not available, two exhaled nitric oxide readings were measured using an alternative analyser, the portable NIOX MINO® (Aerocrine AB). Both analysers were operated according to European Respiratory Society/American Thoracic Society (ERS/ATS) recommendations and measured exhaled nitric oxide at a flow rate of 50 ml/s (Appendix 11 & Appendix 12) (Wilson *et al.* 2001).

2.3.2.3 Questionnaire-derived outcomes

The home visit questionnaire was administered to each child's primary carer by a trained interviewer (Appendix 3). The following questions from the ISAAC core questionnaire wheezing module were asked (Asher *et al.* 1995):

- 'Has your child ever had wheezing or whistling in the chest at any time in the past?'
- 'Has your child ever had wheezing or whistling in the chest in the last twelve months?'
- 'Has your child ever had asthma?'

In addition the parents were asked:

- 'Was the asthma diagnosed by a doctor?'

If needed, the parents were offered the following prompts:

Asthma – wheeze or whistling in the chest with exercise or other triggers that is rapidly relieved with a reliever inhaler.

Wheeze – whistling in the chest when breathing out.

These data were combined where necessary to form the following primary outcomes:

- Doctor-diagnosed asthma – 'yes' to 'has your child ever had asthma' and 'yes' to 'was the asthma diagnosed by a doctor'
- Wheeze in the last 12 months – 'yes' to 'has your child ever had wheezing or whistling in the in the last twelve months'

Secondary outcomes included:

- Recent asthma – 'yes' to 'has your child ever had asthma', 'yes' to 'was the asthma diagnosed by a doctor' and 'yes' to 'has your child ever had wheezing or whistling in the in the last twelve months'
- Childhood wheeze phenotypes – outcomes categorising the child participants according to presence or absence of atopy or time course of wheeze are described later in the text (Section 5.2.2).

2.3.2.4 Lung function

Written consent for lung function testing was obtained from parents and verbal assent from the children.

2.3.2.4.1 *Spirometry*

Flow volume loops were measured at the home visit using a Koko spirometer with volume-driven incentive software (KoKo version 4; PDS Instrumentation; Louisville, USA) (Appendix 10). Spirometry was conducted following ERS/ATS recommendations (Beydon *et al.* 2007; Miller *et al.* 2005), modified to account for the age and maturity of the subjects (Section 3.3.1). Spirometry efforts were ranked by the sum of FVC and FEV_1; the effort having the highest sum was considered the 'best effort'. Spirometry loops were visually assessed by a study doctor and deemed acceptable if the expiratory limb demonstrated a rapid rise to peak, a smooth descent and no evidence of sub-maximal effort or premature termination (Section 3.3.1). FEV_1, FVC and $FEF_{25-75\%}$ values were recorded from the best acceptable spirometry loop. Research nurses were

instructed to encourage the children to exhale maximally and to obtain two FEV_1 values within 200 ml of the best value, although measurements were not excluded on the grounds of reproducibility alone. Nose clips were not worn because the likelihood of their increasing reproducibility was judged insufficient to warrant the potential discomfort associated with their use (Chavasse *et al.* 2003).

2.3.2.4.2 *Methacholine challenge*

BHR was measured by bronchial provocation challenge (Appendix 13). Children with a history of wheeze were preferentially invited to participate in provocation testing with the aim of enriching the sample with children possessing risk factors for BHR. Participants were eligible for provocation testing if they had a FEV_1 of at least 70%, had been free from respiratory infection for at least two weeks and were not receiving oral steroids. It was also checked that they had not consumed caffeine-containing food or drink or received a ß-agonist in the preceding 12 hours.

Figure 6 Methacholine provocation challenge testing

Methacholine was administered using a dosimeter (Koko; PDS Instrumentation; Louisville, USA) with a compressed air source at 2 bar and nebuliser output of approximately 0.37 ml/min (Sidestream® disposable nebuliser Respironics, UK).

Under these operating conditions 80% of nebuliser output will be of a suitable particle size to reach the lower airways (less than 5 microns). At the start of the challenges baseline spirometry was performed until three consistent flow-volume loops were achieved (FEV_1 within 5% of maximal FEV_1). The participants were then instructed to perform five deep breaths from the dosimeter to receive an initial inhalation of normal saline solution. This was followed one minute later by spirometry recording to obtain a reference value. Incremental concentrations of methacholine (0.06 mg/ml to 16 mg/ml) were then administered. Challenges were terminated following a fall of 20% in the FEV_1 or, if this did not occur, after administration of the 16 mg/ml dose. Upon completion of the challenge, all participants

were given 600 mcg salbutamol via a volumatic spacer. The participants were assessed before discharge by remeasuring FEV_1 15 minutes after salbutamol administration.

2.3.2.4.3 *Bronchodilator response*

Figure 7 Salbutamol reversibility testing

BDR was measured in those children attending the clinic visit who did not undergo provocation testing. Flow-volume loops were recorded on the KoKo spirometer both before and after administration of 600 mcg salbutamol via a volumatic spacer (Appendix 14). Spirometry was conducted in a similar manner to that at the home visit. The children were encouraged to perform both pre- and post-salbutamol spirometry until three consistent flow-volume loops were recorded (FEV_1 within 200 ml of maximal FEV_1). The 'best effort' (that with the greatest sum of FEV_1 and FVC) was used to calculate the BDR. BDR was calculated as the change in the percentage predicted FEV_1 for each child (as described in section 3.3.3.2).

2.4 ANALYSIS

All data were double entered. Cleaning programs were run to check for outlying or missing data and to ensure consistency between doubled-entered records. Inconsistencies were identified and either corrected, accepted or set to missing as appropriate after review of the original records. All statistical analyses were performed using Stata™ 8.2 (StataCorp LP, Texas, USA). Appropriate transformations were used, where necessary, to convert variables to a normal distribution. This was usually by natural logarithm, although the methacholine challenge data required a more complicated transformation. Where predictor variables were transformed they were also standardised. Outcomes in these cases were expressed in units of change in outcome per SD change in predictor. Non-standardised variables were used as outcomes in some

regression models, for example FENO, where the outcome was easily understood in terms of the non-standardised units.

To compare differences between those children followed-up and those who were not, Student's t-test was used on normally distributed and transformed variables. To assess relationships between exposures and outcome variables, regression techniques were used. The binary outcomes defined in this research were common and this made the use of logistic regression inappropriate as odds ratios relating to common outcomes are hard to interpret; poisson regression with robust variance was therefore used for all binary outcomes (Barros & Hirakata 2003). Throughout, a 5% level of statistical significance was used, with no correction for multiple testing, as the associations investigated were based on *a priori* hypotheses.

Power calculations were based on the likely prevalence of wheeze and atopy in a general population cohort of children of comparable age. Prevalence estimates were based upon data collected from UK children in the ISAAC study and locally from a birth cohort on the Isle of Wight. To have 85% power at the 5% significance level, to detect a difference of 0.30 SD in FEV_1 between the top half and the bottom half of each exposure variable would require 200 children in each group and 400 overall. A 20% drop out rate was anticipated due to unusable data; therefore it was estimated that 500 children would need to perform spirometry.

Assuming a sample size of 500, for any normally distributed exposure measurement there was 85% power to detect a difference of 0.35 SDs between the exposure of those with recent wheeze and those without, assuming the prevalence of wheezing at six years is 20% (Kurukulaaratchy *et al.* 2002; Stewart *et al.* 2001). Similarly there would be 85% power to detect a difference of 0.35 SDs between the exposure of those with atopy and those without (assuming a 20% prevalence of atopy at age 6 years (Arshad *et al.* 2001; Weinmayr *et al.* 2007)).

Chapter Three

Validation of six-year outcomes

3.1 MEASURES OF ATOPY

3.1.1 Cutaneous sensitisation

The skin prick test (SPT) is a commonly used simple assessment of allergic sensitisation. Allergen solutions are applied to the skin and the skin is pricked by a lancet. The IgE-mediated reaction of the skin is a dermal response, marked by a wheal and flare reaction and dependent upon rapid mast cell degranulation. The result is read at 15 minutes as the size of any wheal produced. Skin prick testing is popular in epidemiological studies because it is easy to perform and adverse reactions are uncommon (Turkeltaub & Gergen 1989). It is important to standardise the SPT technique, however, and to express the results in a robust manner.

3.1.1.1 Published guidelines

SPTs were conducted following the European Academy of Allergy and Clinical Immunology (EAACI) 1989 position paper on skin tests in allergy testing (EAACI 1989). The paper's recommendations were relaxed regarding recent antihistamine use to prevent preferential exclusion of atopic subjects.

3.1.1.2 Reproducibility and validity

Significant variation in histamine wheal size can occur depending upon the operator performing or reading the test (Meinert *et al.* 1994). These effects were minimised by limiting skin prick testing to a small number of trained research nurses. Wheal size is also known to be affected by the depth of allergen penetration within the skin. Lancets with a 1 mm point were used as these have been shown to be more reliable than 25

gauge needles (Brown *et al.* 1981). The lancets were pressed through the test solution at a 90° angle to the skin without jabbing so that the same penetration of the skin was achieved on each occasion (Dreborg 1989).

A histamine solution of 10 mg/ml was used as a positive control. This concentration is recommended internationally (Malling 1984) and has been proven to be more reproducible than lower concentrations (Taudorf *et al.* 1985). Allergens were selected to include aeroallergens of known clinical significance. Duplicate testing was not required as skin prick testing has been shown to have good short term repeatability. Published coefficients of repeatability in school-age children are 0.38 – 0.72 mm depending upon the allergen used (Johnston *et al.* 1992). The nurses in this study monitored the reproducibility of their technique annually by performing five positive controls on the same subject on the same occasion. The least consistent operator produced positive controls which varied by 1.8 mm from the mean and the most consistent produced five identically sized wheals. The mean coefficient of variability was 8.2% (range 0-27%). Skin prick test interpretation requires skill and experience as a positive test is not synonymous with allergic disease. However, the larger the reaction, the more likely it is to be clinically significant (Bernstein & Storms 1995). Wheal size is positively associated with frequency of symptoms in allergic rhinitis (Burrows *et al.* 1976), the risk of bronchial hyperresponsiveness (Peat *et al.* 1987) and serum levels of specific IgE (Krilis *et al.* 1985). The magnitude of SPT reactions are also known to be dependent upon allergen dose, concurrent use of antihistamines (Purohit *et al.* 2002) and site of testing (Galant & Maibach 1973).

3.1.1.3 Criteria for assessing sensitisation

A wheal of at least 3 mm diameter is generally required for a reaction to be classed as positive. Rarely, wheals of 1-2 mm diameter can be induced by pricking the skin even with a dry needle (Dreborg 1987). It is recommended, therefore, that if wheal diameters of less than 3 mm are regarded as positive the response to the negative control must be carefully considered and documented (EAACI 1989). It has also been suggested that, as wheal sizes may depend upon the age of the participant, positive responses should be assessed according to the ratio of the allergen wheal to that produced by histamine (Barbee *et al.* 1976). Ratio criteria are viewed as superior to absolute criteria by some who believe they are less affected by age and observer effects (Meinert *et al.* 1994).

Due to the narrow age range of the subjects and high standard of training of the observers in this study it was not believed necessary to adjust allergen responses for the size of the histamine wheal. Moreover, as significant dermographism was rare, it was not necessary to routinely subtract the size of the negative control from the allergen wheal. Wheal size was recorded by measuring the widest diameter and the diameter at right angles to this and then calculating the mean of the two readings. Subjects were defined as atopic if they had a response of 3 mm or more to one or more allergens, with a histamine response of 3 mm or more, and a negative control response of 0 mm. Although instructed not to, three children had taken antihistamines within the last 7 days. Of these three children, one had an invalid positive control reaction; the remaining two were included in order to avoid preferential exclusion of atopic children. Those children that had taken antihistamines were more likely at the (10% level) to be atopic than children who hadn't taken antihistamine medication (Fisher's exact p = 0.079).

3.1.2 Fractional exhaled nitric oxide

FENO has been studied by a number of groups as a non-invasive measure of eosinophilic airway inflammation (Baraldi *et al.* 1999; Brussee *et al.* 2005; Roberts *et al.* 2004; Sippel *et al.* 2000). It is considered a better measure of eosinophilic airway inflammation than symptoms or lung function (Wilson *et al.* 2001) and is less time consuming and better tolerated than sputum induction (Vignola *et al.* 2002).

3.1.2.1 Guidelines

The ATS and ERS published guidelines for the measurement of exhaled nitric oxide in children in 2001 (Baraldi *et al.* 2002); measurements from children are also mentioned in the 2005 adult guideline (American Thoracic Society 2005). FENO measurement in this study followed recommendations from these guidelines, although strict reproducibility criteria were not applied as this would have limited the number of participants from whom data could be collected.

3.1.2.2 Reproducibility

The guidelines recommend that repeated exhalations (2–3 that agree within 10%, or 2 within 5%) are performed with at least 30-second intervals, and that mean FENO is recorded. Only 141 children (56.9% of those contributing FENO data) recorded more than one reading. Sixty nine of these children (48.9%) achieved two readings within 5% and only 13 (9.2%) three readings within 10%. However, the median reading (IQR) for

all 248 children contributing FENO data was 9 ppb (6 to 13 ppb), at these low ranges the manufacturers of the NIOX analyser quote a precision of 2.5 ppb and recommend that readings within 2.5 ppb may be considered consistent (Aerocrine 2009). Using this criterion, 95% of the children from whom more than one reading was recorded were able to produce consistent readings.

Of those participants that recorded three FENO readings the median (IQR) values for the first, second and third readings were respectively 7.8 ppb (5.4 to 11.8 ppb), 7.8 ppb (5.8 to 11.4 ppb) and 7.7 ppb (5.3 to 12.1 ppb). These values did not differ significantly (paired t-tests $p>0.3$ for each comparison) and published data also supports the suggestion that FENO levels are unaffected by multiple measurement attempts (Deykin *et al.* 2000). As systematic bias would not be introduced by including a variable number of sequential FENO readings, a mean value of FENO was calculated, where possible from a maximum of three readings. Participants with only one FENO reading were not excluded.

3.1.2.3 Feasibility and reproducibility

The NIOX® analyser has been tested for ease of use and reproducibility. Despite initial concerns regarding children's abilities to use the NIOX® (Jobsis *et al.* 1999), it has been estimated recently that 95% of children aged between 6 and 18 years are able to achieve three acceptable readings with this device within their first six attempts (Alving *et al.* 2006). The accuracy of FENO measurements is sensitive to contamination of exhaled breath by nasal nitric oxide. Contamination was avoided by asking participants to exhale against a positive mouthpiece pressure which ensures velum closure. Changes in exhalation flow rate can also cause considerable variation in FENO values as this alters the relative contribution of different sites in the lung and the time for diffusion of nitric oxide into the airway. Due to this flow dependence, expiratory flow rate must be standardised. Low flow rates are more sensitive but result in longer exhalation times and may be uncomfortable to sustain (Silkoff *et al.* 1997).

Both discrimination and reproducibility are found to be maximal at a flow rate of 0.05 l/s (Pedroletti *et al.* 2002). At this flow rate the intraclass correlation coefficient (ICC) has been demonstrated to be 0.99 in both children and adults (Kharitonov *et al.* 2003). The analysers used in this study measure FENO at the ATS/ERS recommended flow

rate of 0.05 l/s. *In vitro* testing at this flow rate has characterised the equipment's detection range as 2 - 200 ppb with a precision expressed as SD of 2.5 ppb for measurements < 50 ppb and < 5% of the measured value for measurements > 50 ppb.

3.1.2.4 Clinical Relevance

Exhaled nitric oxide is a useful non-invasive measure of eosinophilic inflammation within the lungs, although not specific to any particular disease or site of inflammation. Many physiological and pathological conditions affect FENO levels (Franklin *et al.* 1999; Kharitonov *et al.* 1995; Paredi *et al.* 1999), but asthmatic subjects have been demonstrated to have higher FENO concentrations as a group than non-asthmatic controls (Frank *et al.* 1998). Exhaled nitric oxide is thought to be particularly sensitive index of atopic asthma (Franklin *et al.* 2003). It is known that longitudinal measurements of FENO within individuals with grass pollen-induced asthma increase with increasing exposure to grass pollen (Roberts *et al.* 2004). This suggests an individual's FENO level varies with the severity of their airway inflammation and, indeed, FENO levels have been shown to correlate with markers of inflammation within the lungs (Baraldi *et al.* 2002).

3.1.2.5 Comparison of measurements from the NIOX MINO® and NIOX® analysers

Chemiluminescence analysers, such as the NIOX® manufactured by Aerocrine, are regarded as the gold standard for FENO measurement. However, the portable NIOX MINO® device, which measures FENO with an electrochemical sensor, is believed to produce readings of similar accuracy and precision (Alving *et al.* 2006; Hemmingsson *et al.* 2004). The NIOX MINO® was used in this study when the NIOX® analyser was undergoing repair or maintenance. A small study was conducted to compare the performance of the two analysers. The devices were also compared in terms of ease of use.

COMPARISON OF NIOX® AND NIOX MINO® ANALYSERS

Aim

To compare the performance of the NIOX MINO® analyser with that of the NIOX® in terms of accuracy, precision and ease of use.

Method

Eleven subjects performed triplets of readings on each analyser. All readings were taken on the same day. Each subject was assigned at random to test either the NIOX® or NIOX MINO® first. The subjects, three males and eight females, ranged in age from 23 - 43 years and included two with atopy and two with asthma.

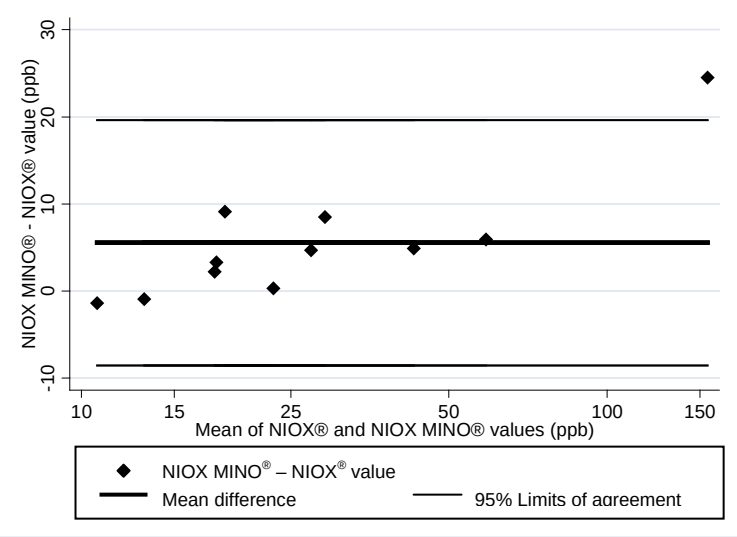
Results

Excellent agreement was found between the NIOX® and the NIOX MINO®. The Pearson correlation coefficient between mean NIOX® and mean NIOX MINO® values from each subject was 0.98, ($p < 0.001$). Agreement was greatest over the normal healthy range. The NIOX MINO® on average recorded a higher reading than the NIOX®. The mean (±SD) difference between the analysers' readings was 5.55 ± 7.18 ppb. The NIOX® had a lower within subject variability (SD 2.1) compared with the NIOX MINO® (SD 2.9). These values for within subject variability were small compared with the between subjects variability (SD 41.2).

Exhaled nitric oxide readings can be used to form diagnostic categories; 0 - 5 ppb being considered low in adults, 5 - 50 normal and > 50 ppb high. The diagnostic category was not dependent upon the device used for any subject and the kappa statistic for agreement was 1.000, ($p < 0.001$).

Both devices proved easy to use; the mean number of attempts required to achieve three valid FENO measurements was 3.7 for NIOX® and 3.8 for NIOX MINO®.

Figure 8 Bland Altman plot comparing NIOX® and NIOX MINO® FENO values



Conclusion

Strong agreement exists between the NIOX® and the NIOX MINO®. Agreement is good across the normal range and remains acceptable at higher levels of FENO. Both analysers demonstrate clinically acceptable repeatability.

Upon completion of data collection it was possible to compare the geometric mean readings of children using each of the analysers. In total 112 children used the NIOX MINO®, of these children, 90 recorded one reading only and 22 recorded two readings. One hundred and thirty six children recorded FENO readings on the NIOX® analyser and of these 115 (85%) recorded three readings. The median (IQR) reading for the NIOX MINO® and NIOX® analyser were respectively 10.75 ppb (7.00 to 14.00 ppb) and 7.48 ppb (5.33 to 11.48 ppb). Although small this difference was statistically significant (t-test p=0.002), therefore a sensitivity analysis of analyses based upon FENO data pooled from the two analysers was conducted.

3.2 QUESTIONNAIRE DERIVED OUTCOMES

Questionnaires are frequently used in epidemiological studies as they are relatively economical compared to clinical examination of multiple subjects. It is difficult to design a completely reliable questionnaire due to both variation in the medical knowledge of the general public and the wide range of asthma presentations and severity. Questionnaires may lack precision due to the subjective nature of asthma symptoms and, as a large proportion of children will experience respiratory symptoms at some point, questions relating to symptom history cannot discriminate between trivial symptoms and clinically significant asthma. Questionnaires can be validated by three methods: comparing questionnaire response to an objective clinical measure such as BHR (Jenkins *et al.* 1996), comparing questionnaire responses with responses to another questionnaire, and comparing questionnaire response with previous diagnoses validated by a doctor (Jenkins *et al.* 1996). Doctor-validated diagnosis may be considered the best standard for comparison, although this too may vary according to geographical or temporal trends.

Symptom-based questions, asking about 'wheeze' or 'chest tightness', are believed to be most sensitive as they are not reliant upon a lay understanding of asthma. The

questionnaire outcomes in this study were based upon the ISAAC core questionnaire wheezing module (Asher *et al.* 1995). ISAAC, the International Study of Asthma and Allergies in Childhood, was founded to maximise the value of epidemiological research into asthma and allergic disease by establishing a standardised methodology and facilitating international collaboration. The ISAAC core questionnaire wheezing module was designed for use in phase one of ISAAC and has been administered in 156 collaborating centres in 56 countries with a total of 721,601 children participating. Questions are ordered within the questionnaire such that enquiry proceeds from relatively mild to relatively severe symptoms and enquiries about symptoms precede those about diagnosis. Several studies indicate good repeatability for questionnaires of this type (Burney *et al.* 1989; Clifford *et al.* 1989; Salome *et al.* 1987). The ISAAC wheezing module, has been found to be 85% sensitive and 81% specific when compared to a diagnosis of current asthma from a doctor and to have a positive predictive value of 61% (Jenkins *et al.* 1996). Positive responses to questionnaire enquiries concerning diagnosed asthma or recent wheeze are in the region of 80% specific for a positive bronchial challenge, although BHR itself is not an appropriate gold standard for asthma diagnosis (Peat *et al.* 1992a). A definition of asthma requiring both a positive questionnaire response and BHR yields an index that is 94% specific for asthma but only 47% sensitive (Jenkins *et al.* 1996).

Responses may be influenced by a variety of social, cultural and psychological factors (Burney & Chinn 1987). When questionnaires are repeatedly administered to the same subjects roughly equal numbers change from positive to negative and negative to positive responses (Peat *et al.* 1992a). Although prevalence estimates may be unaffected by instability of responses, this has obvious implications for longitudinal studies. Questions relating to night time cough or to symptoms occurring over 12 months ago are less repeatable than questions relating to recent wheeze or doctor-diagnosed asthma (Peat *et al.* 1992a). For this study, questions were selected which maximise both sensitivity and specificity. Parents were asked whether their children had 'ever wheezed' in order to include as many children as possible with clinically relevant symptomatology, whilst the inclusion of 'doctor-diagnosed asthma' identified a more homogeneous outcome group. A third outcome, that of 'doctor-diagnosed asthma and wheeze within the last 12 months' has been used successfully in epidemiological studies on the Isle of

Wight (Arshad *et al.* 2005; Kurukulaaratchy *et al.* 2002) and was adopted to refine the outcome to those with a validated diagnosis and recent relevant symptoms.

3.3 MEASURES OF LUNG FUNCTION

3.3.1 Spirometry

Spirometry is the most frequently used measure of lung function. The feasibility of spirometry varies with age and size (Nystad *et al.* 2002). Recent publications suggest that spirometry is both feasible (Nystad *et al.* 2002; Turner *et al.* 2007) and reproducible (Crenesse *et al.* 2001; Turner *et al.* 2007) even in preschool-age children. Spirometry, therefore, can be reasonably expected to produce reliable measures of lung function in even the youngest and smallest children in this study.

3.3.1.1 Published guidelines

The ATS and ERS recommend spirometry in school-age children should be conducted according to adult guidelines (Miller *et al.* 2005). Spirometry was performed in this study according to these guidelines; although, to avoid participant discomfort, nose-clips were not used. Data analysis was guided, in addition, by recommendations from the guidelines for preschool children which state that adult reproducibility criteria may need to be relaxed for young children (Beydon *et al.* 2007).

3.3.1.2 Feasibility

Quality-control criteria for adult subjects specify that the expiratory manoeuvre should be of at least six seconds duration. The ATS/ERS spirometry guidelines for preschool children suggest these acceptability criteria are unsuitable for very young children and this has been confirmed in recent appraisals of children's spirometry technique (Aurora *et al.* 2004; Turner *et al.* 2007). Expiration is completed rapidly in young children because they have small absolute lung volumes and large airway size relative to lung volume. Children often complete exhalation in less than six seconds and sometimes do so in less than one second. However, the proportion of children able to generate an exhalation of sufficient length increases with age and approaches 80% in those aged 5 years (Aurora *et al.* 2004). The current study provides further evidence that spirometry is feasible in young school-age children. Although the majority of the children participating in the home visit stage of this study had no previous experience of spirometry, over 80% were able to generated measures of FEV_1 considered valid according to the criteria in Table 6.

3.3.1.3 Reproducibility and validity

The reproducibility of spirometry is dependent upon expiratory flow limitation. Once this is achieved, airflow is effort independent and solely reflects airway diameter. Most adults can be coached to achieve flow limitation such that successive FEV_1 measurements differ by less than 5%. Recently, a technique in which brief periods of negative pressure are applied during forced exhalation has been used to demonstrate that expiratory airflow is flow limited in young children (Jones *et al.* 1999). Whilst it is likely that highly reproducible values of FEV_1 are theoretically achievable, reproducibility is clearly affected by both age and experience. For example, it has been reported that only 58% of inexperienced 4-year-old children are able to produce repeat FEV_1 values within 5% of their best effort (Nystad *et al.* 2002), whilst, in contrast, a study of 5 – 8 year-old children experienced in performing maximal expiratory flow manoeuvres found 91% were able to achieve this level of reproducibility (Arets *et al.* 2001).

The ATS recommendations state that failure to meet repeatability criteria need not necessarily invalidate a manoeuvre and it has been argued that, for children in particular, reproducibility criteria should be relaxed (Aurora *et al.* 2004). For this reason, it was decided not to exclude any data on the basis of poor reproducibility alone. The children in this study were encouraged to achieve two readings consistent to within 200 ml of the best effort although this was not possible in all cases. Limitations in the spirometry software dictated that data was exported only from the loop with the highest sum of FEV_1 and FVC values. Therefore, it was not possible to formally assess the reproducibility of spirometric indices collected at the home visits. A detailed record of spirometry attempts was collected, however, from a consecutive subset of 40 participants that attended the clinic visit; 37 of these children (93%) were able to produce FEV_1 values reproducible to within 5%. Spirometry loops were reviewed on a monthly basis and classified as valid or invalid according to the criteria in Table 6.

Criteria	Exclude if
Start	Rise to peak flow not rapid
Artefacts	Early cough or one which appears to have affected FEV_1 or $FEF_{25-75\%}$
	Glottis closure that influences measurement
	Effort that is not maximal throughout
Duration	Obvious premature termination

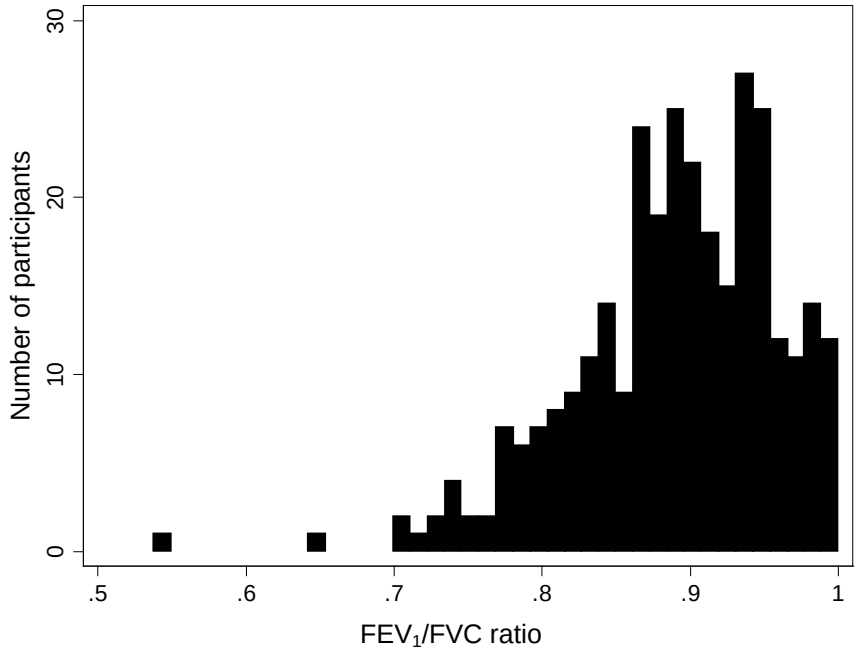
Table 6 Validity criteria for spirometry

3.3.1.4 Clinical relevance

Spirometric performance is related to age, sex and height. The variability of individual measurements around the median is not uniform across the age/height range and there is skewness in the distributions such that multiple regression analysis cannot adequately model reference data. This study employed new reference ranges for lung function from the 'growing lungs' project. These reference ranges are designed to account for non-uniform dispersion and skewness (Stanojevic *et al.* 2008). Using these reference ranges it is possible to express spirometric indices as z-scores which allow clinical thresholds of normal to be defined which account for the between subject variability of each index.

At present these reference ranges have only been collated for FEV_1 and their use is limited by the issue of whether this is the most appropriate outcome measure of respiratory function during childhood. FEV_1 is thought to provide information regarding large and central airway function. The underlying physiology of this parameter is complex; FEV_1 depends upon factors that determine maximum expiratory flow at a given lung volume, the change in maximum expiratory flow with lung volume and the change in intrapulmonary resistance with change in lung volume. As a consequence of young children's small absolute lung volumes and large airway size relative to lung volume, the FEV_1, although measurable in young children, is often approximately equal to the FVC. This may prevent the FEV_1/FVC from being an informative index of bronchial obstruction (Aurora *et al.* 2004; Vilozni *et al.* 2005).

Figure 9 Distribution of FEV_1/FVC data within the study cohort

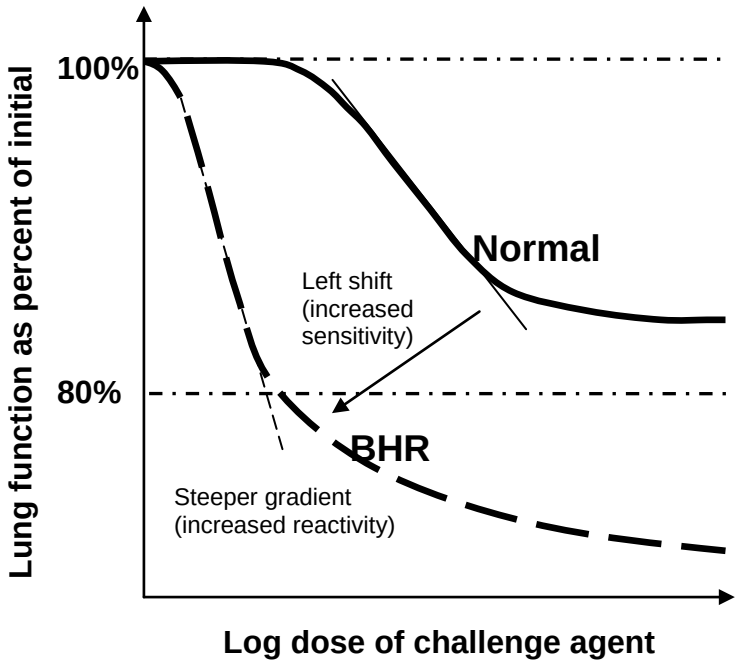


Fortunately, data from the 6-year follow-up participants demonstrate a wide range of FEV_1/FVC values. The median value was 0.90 and the IQR 0.84 to 094, only 60 participants (18.9%) had a value greater than 0.95 (Figure 9).

3.3.2 Bronchial hyperresponsiveness

Bronchial hyperresponsiveness is a non-specific tendency of the bronchial smooth muscle to contract excessively in response to a variety of stimuli. When lung function is plotted against the log. dose of bronchoconstrictor a sigmoid curve is obtained in normal subjects; lung function decreases with increasing agonist dose and at high doses the maximal response is seen to plateau. Dose-response curves from subjects with BHR in contrast are left shifted with a steeper curve demonstrating greater sensitivity to lower doses of agonist (Figure 10).

Figure 10 Bronchial responsiveness of normal subjects and those with BHR



A distinction has been made, although not consistently, between hyperresponsiveness as a general term, hypersensitivity as a leftward shift in the dose-response curve and hyperreactivity as an increase in the curve's slope. The mechanisms underlying the displacement of the dose-response curve are incompletely characterised but are thought to include: airway inflammation (Bradley *et al.* 1991), abnormalities of smooth muscle (Solway & Fredberg 1997) and neural control (Lommatzsch *et al.* 2005), and alteration of the elastic loads against which the airway smooth muscle contracts (Lambert & Pare 1997). Together these factors enhance the airway luminal resistance caused by a given degree of smooth muscle shortening. Different mechanisms may be of proportionately greater or lesser importance in different asthma phenotypes.

3.3.2.1 Significance of BHR in childhood wheezing disorders

There is a strong relationship between BHR and childhood asthma but not all children with BHR have asthma and not all asthmatic subjects exhibit BHR. Childhood BHR has been found to be positively associated with atopy (Sears *et al.* 1991; Van Asperen *et al.* 1990), sex (male sex in infancy (Peat *et al.* 1987) and female sex in the teenage years (Forastiere *et al.* 1996)), and environmental factors, including tobacco exposure (Forastiere *et al.* 1994) and lower respiratory tract infections (Peat *et al.* 1992b). In some individuals BHR is present asymptomatically. Studies of the natural history of BHR

suggest individuals with asymptomatic BHR may later develop asthma symptoms (Laprise & Boulet 1997).

Bronchial provocation testing can be used clinically to separate asthmatics from normal subjects (Cockcroft *et al.* 1977), to quantify asthma severity (Murray *et al.* 1981) and to follow the course of individual patients (Cartier *et al.* 1982). Unsurprisingly, given the complex relationship between BHR and clinically symptomatic asthma, the positive predictive value of measures of provocation testing is low. The positive predictive value depends upon the proportion of asthmatics in the population. In unselected children, the positive predictive value of methacholine challenge has been estimated to be between 20 and 60% depending upon the prevalence of asthma in the population under study and the cut-off value used to define a positive test (Godfrey *et al.* 1999).

3.3.2.2 Provocation testing

Bronchoprovocation tests measure the dose of bronchoconstrictor required to cause a predetermined decrease in lung function. A bronchoconstrictor is administered in increasing concentrations by one of two widely used techniques. The first, described by Cockcroft *et al.* (1977), uses the Wright nebuliser and tidal breathing, whilst the second, a technique described by Chai *et al.* (1975), uses a De Vilbiss No. 42 nebuliser attached to a dose metering device.

3.3.2.2.1 *Published guidelines*

Provocation testing in this study was based upon the ATS Guidelines for Methacholine and Exercise Challenge Testing (Crapo *et al.* 2000). Once more, for reasons of participant comfort, nose-clips were not worn. A second minor deviation from the guideline was that, for reasons of safety, participants were asked to withhold bronchodilator medication for 12 hours only, rather than 48 hours before the test. Some children found it difficult to achieve acceptable flow-volume curves within the recommended 3 - 4 manoeuvres, further attempts were permitted so long as this did not prolong the period between dose administrations beyond 5 minutes. If one acceptable flow-volume curve was achieved within 5 minutes the challenge was permitted to continue but if no acceptable curves were achieved in this time the challenge was terminated. The ATS guidelines recommend nebulisers with an output of 0.9 ml/min for use in dosimeter protocols. The manufacturers of the sidestream® nebuliser used in this study state its output to be 0.37 – 0.46 ml/min at a driving pressure of 0.8-1.24 bar.

The low output of the sidestream nebuliser® may potentially affect the comparability of the results contained in this thesis with those of other studies.

3.3.2.2.2 *Methacholine*

Methacholine was chosen largely due to local experience with this agonist and also because extensive data exist regarding its effects and tolerability. Recently it has been suggested that indirect agonists such as exercise or adenosine, which act by mast cell degranulation, may more reliably distinguish asthmatic children from controls and children with non-asthmatic obstructive airways disease (Avital *et al.* 1995). However, fewer comparative studies exist using adenosine and the protocols for adenosine administration are less standardised. Moreover, methacholine provocation tests are relatively insensitive to the effects of inhaled steroids and thus testing can be conducted in asthmatic participants without stipulating that maintenance medications should be withheld for long periods.

Methacholine is a parasympathomimetic synthetic analogue of the neurotransmitter acetylcholine. It acts as a non-specific direct stimulant of muscarinic, postganglionic parasympathetic receptors, resulting in airway smooth muscle contraction and increased tracheobronchial secretions. When inhaled in concentrations up to 25 mg/ml, methacholine is without significant side-effects. The bronchoconstriction is rapid in onset and reaches a peak within 1 – 4 minutes. Peak effects last for approximately 75 minutes (range 17- 150 minutes) and there is a spontaneous return to baseline over 2 hours (Cartier *et al.* 1983).

3.3.2.3 Abbreviated dosimeter technique

An abbreviated dosimeter protocol was used to minimise challenge duration. In contrast to the conventional practice of doubling agonist concentration, concentration was increased fourfold at each stage from 0.06 to 16 mg/ml. Shortening the challenge maximised the children's engagement with the process and ensured consecutive doses of methacholine had a cumulative effect. If a participant's FEV_1 dropped by more than 20 % of the reference value, the challenged was terminated; if the FEV_1 declined by 15 - 20% the methacholine concentration was not quadrupled before the next stage, merely doubled. These precautions were particularly important for child participants because children are likely to receive a higher dose per body weight than adults. The entire

dosimeter output is delivered to the participant, regardless of size as inspiratory flow greatly exceeds the driving flow to the nebuliser (Le Souëf *et al.* 1995).

The effect of a given concentration of nebulised methacholine upon lung function reflects the absolute dose of agonist delivered and its distribution within the airways (Brain & Valberg 1979). Technical factors that determine the amount of methacholine reaching the lower airways include the inspiratory flow rate and breath-hold time and the fraction of methacholine particles of respirable size (less than 5 microns). These factors were standardised within the protocol. The amount of methacholine available to subjects depends on the driving pressure applied to the nebuliser, the duration of nebulisation and whether or not the subject dilutes the aerosol delivery by nose-breathing. Standardisation aids internal comparison; however, in order to compare results between studies, it is desirable to measure nebuliser output so that the exact amount of methacholine delivered can be estimated.

STUDY TO ESTIMATE NEBULISER OUTPUT

Aim

To estimate the output of the sidestream® nebuliser.

Method

A variable volume pipette was used to add 3 ml of normal saline to the nebuliser and the nebuliser weighed. The nebuliser was reweighed following six blocks of five nebuliser actuations to the same subject. The subject avoided exhaling with each breath into the nebuliser to minimise the confounding effect of water vapour condensation. A small exhalation into the nebuliser unit was, however, necessary to trigger the dosimeter controlling nebuliser administration. The change in weight of the nebuliser unit was plotted against actuation time. Nebuliser output was calculated from the regression slope of this line.

Two separate nebuliser units were calibrated to determine whether output varied significantly between units. A single unit was also trialled at three different pressures. Within the study protocol the nebuliser was operated at 2.00 bar as, due to the scale

on the pressure meter, this was the minimum pressure possible. It is possible that, due to operator error, the driving pressure may have varied slightly between participants, although the apparatus is unlikely to have been operated at a pressure greater than 4 bar. For this reason pressures of 2 bar, 4.5 bar and 7 bar were trialled.

Results

Figure 11 Variation in output between nebuliser units operated at 2 bar

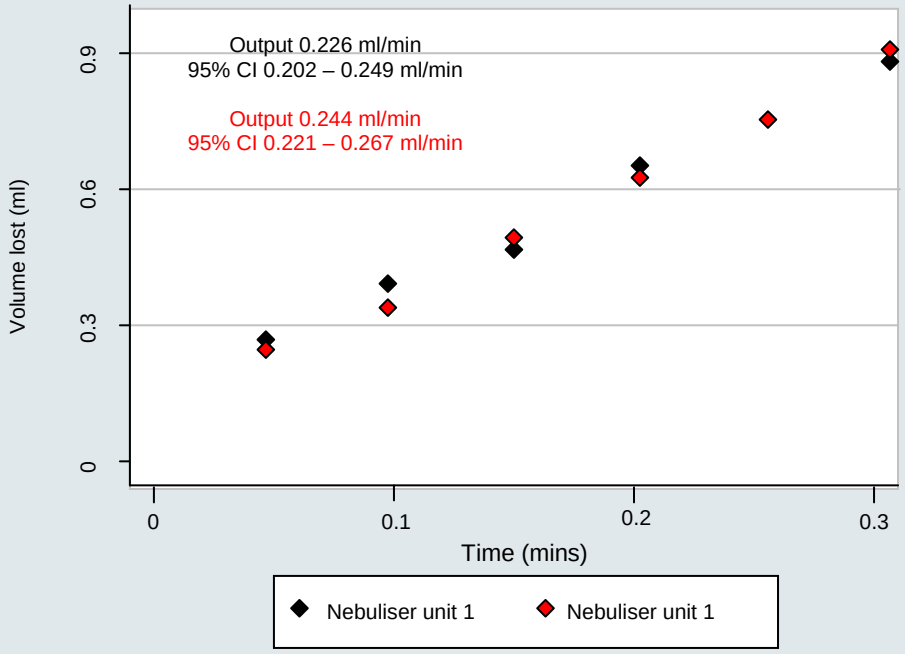


Figure 12 Nebuliser output at 4.5 bar driving pressure

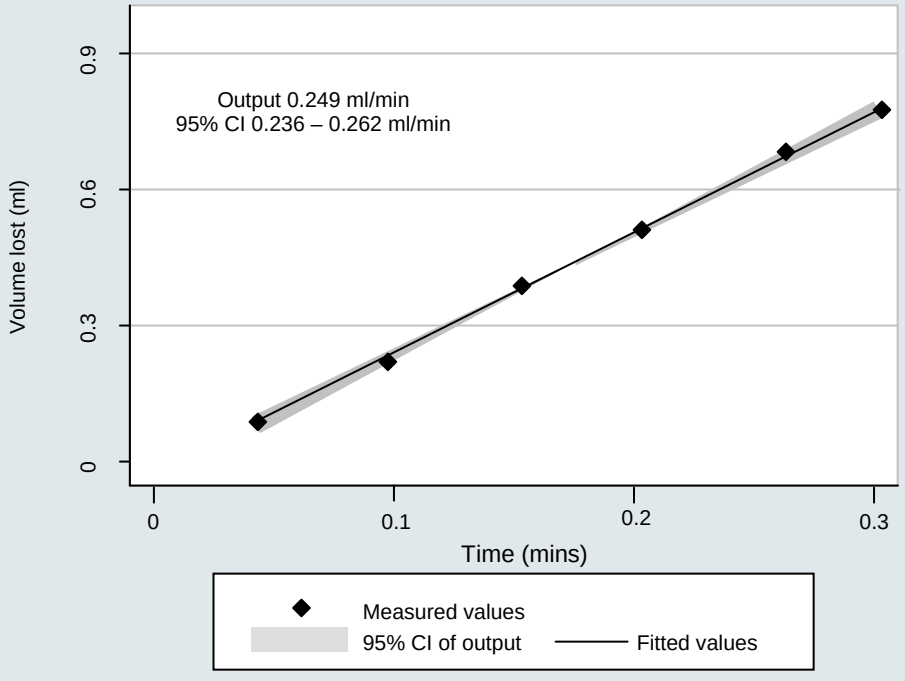
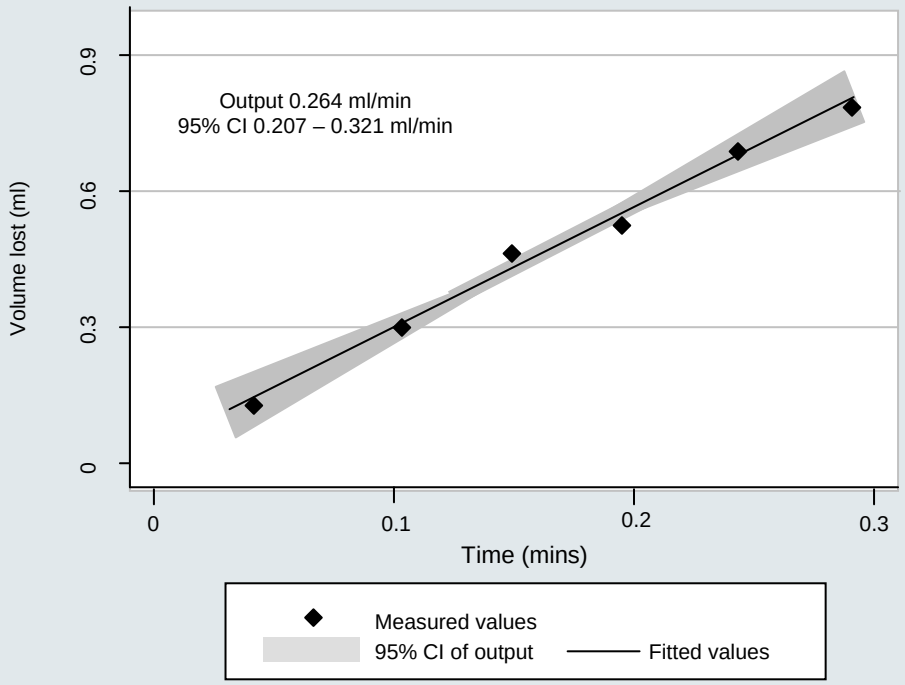


Figure 13 Nebuliser output at 7 bar driving pressure



Conclusion

The outputs of the two nebuliser units tested were very consistent, although at 0.23 ml/min and 0.24 ml/min both were lower than the range quoted by Respironics (0.37 – 0.46 ml/min at 0.80 - 1.24 bar). It is possible that output was underestimated because it was not possible to completely avoid condensation of water vapour from exhaled air within the nebuliser. The output appears reasonably robust to variation in driving pressure within the range likely to be encountered in standard operation, although output per actuation increased slightly and appeared to become more variable at the very high driving pressure of 7 bar.

3.3.2.4 Suitability of FEV_1 for provocation testing

Lung function indices used in provocation testing should be easy to measure, sensitive and reproducible measures of bronchoconstriction. FEV_1 is the measure most frequently used. The main disadvantage of FEV_1 is that it is a less sensitive measure of airway obstruction than measures of flow, such as $FEF_{50\%.}$ Furthermore, in small children FEV_1 might remain effort dependent as they may never achieve flow limitation. However, alternative measures, including $FEF_{50\%}$ and measures of airway resistance, display greater baseline variability which, in turn, limits their suitability (Cochrane *et al.* 1977).

There are several advantages associated with FEV_1. Forced vital capacity manoeuvres may induce bronchodilatation in healthy subjects and bronchoconstriction in hyperresponsive individuals (Pellegrino *et al.* 1998); this may serve to increase the discriminative capacity of FEV_1. Furthermore, not only is FEV_1 more precise and more easily measured than airways resistance, it is not affected to the same extent by other pathologies such as allergic rhinitis (Marseglia *et al.* 2007).

Some investigators record the lowest FEV_1 at any given dose stage to avoid falsely low BHR values as the methacholine effect wears off. However, in this study all manoeuvres were completed within 3 minutes of nebulisation and this problem was less likely to be an issue than poor effort during forced exhalation. Therefore, the highest FEV_1 achieved within 3 minutes of methacholine administration was recorded. It is likely that, even in the absence of consistent values for confirmation, the highest FEV_1 value best reflects maximal expiratory effort.

Once more, it was considered inappropriate to discard FEV_1 data solely on the basis of poor repeatability, particularly as bronchconstrictor agents are known to make FEV_1 less reproducible (Scott & Kung 1985). Nevertheless, if the pre-agonist reference FEV_1 were underestimated, challenges might continue below 80% of the true maximal FEV_1. At the reference stage, therefore, spirometry was repeated until two FEV_1 values within 5% of the best effort were achieved to ensure that the reference FEV_1 was an accurate measure of the participant's maximal lung function. At subsequent stages one further value within 5% of the best FEV_1 for that stage was considered acceptable

reproducibility, although this was not an absolute requirement. Reproducibility was explored in a random sample of ten challenges; the reproducibility standard of two values within 5% was found to be met on 98% of challenge stages.

3.3.2.5 Provocation testing data analysis

Various summary measures have been proposed to describe the dose-response curve. Threshold measures record the agonist dose causing a predetermined fall in lung function from the mean (Habib *et al.* 1979). The PC_{20}, that is the concentration of agonist which provokes a 20% fall in FEV_1, is the preferred measure for many and there is evidence to suggest that it is more reproducible and discriminative than other threshold measures (Cockcroft *et al.* 1983a). The PC_{20} can also be expressed as PD_{20}, the cumulative dose of agonist required to provoke a 20% drop in lung function. Although popular clinically, the PC_{20}, or PD_{20}, is a poor index of BHR for epidemiological studies, because very few subjects in general community samples will experience a 20% drop with the concentrations of agonist that are generally used. Moreover, PC_{20} is an oversimplification as it indicates only the position, not the shape, of the dose-response curve. Arguably, the notion that PD_{20} corresponds to a particular provocation dose is misleading and implies an unwarranted level of precision as, even if the individual aerosol output of each nebuliser is known, the amount of methacholine delivered to the lung depends on many unquantifiable characteristics of the participant and nebuliser.

The problem of censored data can be overcome by fitting simple (Bellia *et al.* 1983) or more complicated (Woolcock *et al.* 1984) mathematical models to individual subjects' data to determine a threshold value. There is no evidence, however, that such models express the dose-response relationship more effectively than simpler threshold measures. The main alternative measures to PD_{20} are measures of the dose-response slope. The simplest measure of slope is that described by O'Connor *et al.* (1987) as percentage decline in FEV_1 at last dose / cumulative methacholine dose in micromol. As the percent decline in FEV_1 is approximately linear over the range of dosages commonly used (Cockcroft & Berscheid 1983), the slope of this line is highly correlated with PD_{20}.

The dose-response slope (DRS) has the important advantage of being a continuous measure that can be reported for all subjects. An alternative measure of slope based

upon linear regression was proposed by Abramson *et al.* (1990); this bears the advantage of using information from all the challenge stages and thereby is less affected by measurement error associated with any one stage.

Slope measures are superior to the categorical PD_{20} on statistical grounds. Analysis of the percentage of participants below an arbitrary cut-off by logistic regression is misleading as BHR is unimodally distributed within the general population (Cockcroft *et al.* 1983b; Niggemann *et al.* 2001). Clinically significant differences in asthma symptoms have been shown to be associated with variation in BHR below the conventional PD_{20} threshold (de Meer *et al.* 2005). This information is lost when using a categorical measure of BHR. Although censored regression or survival analysis can be used to maximise the power contained in the PD_{20} or PC_{20} measure, these require assumptions to be made regarding the underlying censored distribution. Greater power is potentially available with continuous measures and this is reinforced by the finding of greater ICCs for slope measures than the PC_{20} (Chinn & Schouten 2005). Slope measures are also more amenable to meta-analysis as effect sizes can easily be calculated for comparison between studies by dividing the mean slope by the within group standard deviation (Chinn 1998)

Continuous measures are appealing but they are, unfortunately, associated with their own limitations. Firstly, the simple DRS is not sufficiently reproducible when measurement responses are limited to less than 20% decline because the measurement error and within subject error at this level are high when compared to between subject variation (Chinn *et al.* 1993). Secondly, an inverse or log. function is required to achieve normality and a constant must first be added to remove the negative values that can occur in subjects with low BHR (Chinn *et al.* 1993).

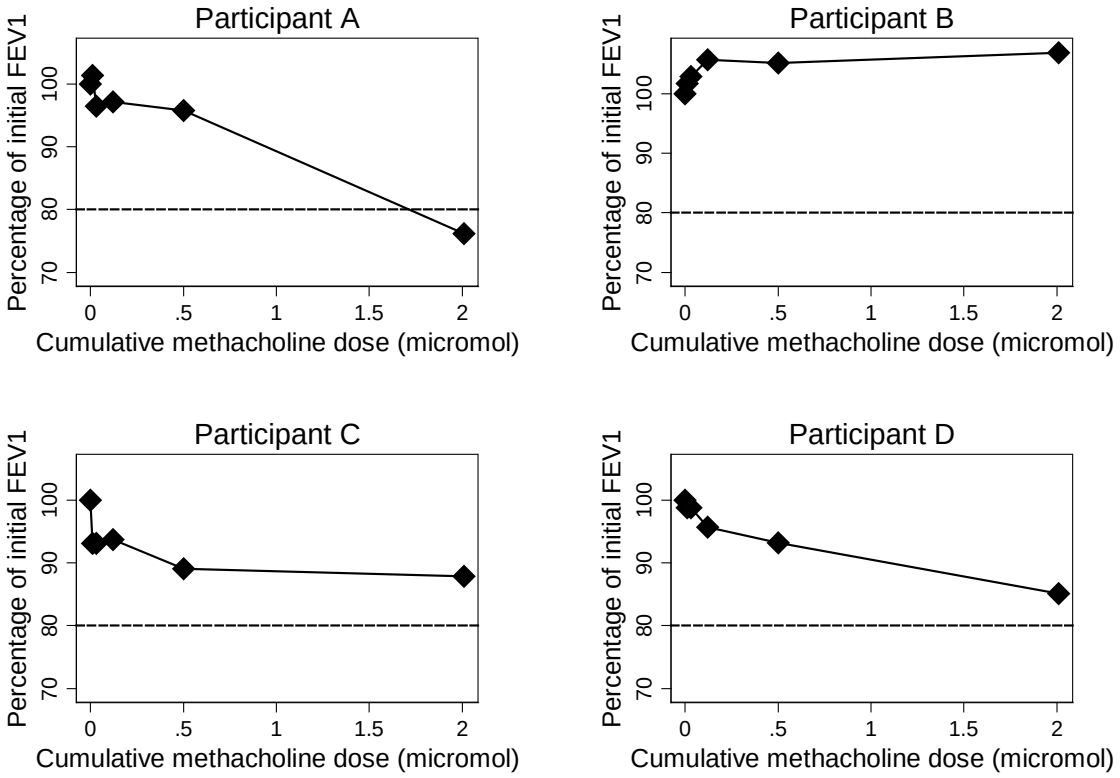
Unfortunately, as summary measures of BHR are a proxy measure of the biological processes which underlie BHR, attempts to identify the summary value which best represents a biologically significant property are limited by incomplete understanding of these processes. The measure that best summarises the dose-response is, to some extent, dependent upon context. For example, studies which employ bronchoprovocation challenges as a diagnostic tool may favour the categorical PD_{20}.

Conversely, epidemiological studies considering the distribution of BHR within a given population might prefer a continuous variable derived from the DRS.

3.3.2.6 Critique of methods of analysing bronchial challenge data

Examples of participant dose-response curves are displayed in Figure 14. The dose-response curves of the majority of participants were similar to those of Participants A or D, FEV_1 declining with successive methacholine doses. Twenty nine participants (27%) clearly reached a PC_{20} and a further 33 (31%) displayed definitive decreases of less than 20%. However, at the doses tested, FEV_1 was broadly unaffected in 25 participants (23%). Amongst those participants who were relatively unaffected by methacholine a response similar to that of Participant C was occasionally recorded where an initial small decline in FEV_1 appeared to plateau. A further 20 participants (19%) including participant B showed an increase in FEV_1 with increasing methacholine concentration. This effect has been reported previously (Chinn *et al.* 1987) and may be an effect of practise or of a slight stiffening of the central airways which causes the equal pressure point to move downstream. Similarly, a declining FEV_1 may not arise purely due to bronchoconstriction but may include the effects of tiring or boredom.

Figure 14 Dose-response curves displaying representative changes in FEV_1 (Dotted lines show PD_{20})



3.3.2.7 Modification of traditional measures of BHR

3.3.2.7.1 *Modification of PD_{20}*

The amount of censored data can be decreased by measuring the dose of methacholine responsible for a lesser decline in FEV_1 than 20%. Unfortunately, measures such as PD_{15} and PD_{10} are of limited value because within subject FEV_1 variation is sufficiently large to obscure the effects of bronchoconstriction upon FEV_1. An alternative means of decreasing censored data is to calculate PD_{20} values for participants who experienced a decline in FEV_1 of less than 20% using extrapolation. Extrapolation would only yield PD_{20} values for participants such as D where the FEV_1 was clearly declining with increasing methacholine dose. Generally, extrapolation is regarded as inadvisable because of uncertainty about the underlying relationship, the possible existence of a plateau in the dose-response curve and measurement error.

3.3.2.7.2 *Modification of dose-response slope*

Slope measures do not completely solve the problem of quantifying BHR in subjects with low reactivity. No data is censored by the DRS but the gradient of shallow slopes tends to be poorly reproducible and occasional negative values occur which are difficult to interpret. In many cases when the dose-response curves were examined visually it appears that, FEV_1 remained relatively constant for several doses and then declined precipitously. Such a pattern may merely be a consequence of the quadrupling dose protocol but a variable training effect may also counteract the effect of the bronchoconstrictor upon FEV_1. Summary measures which focus upon the end of the dose-response curve have been trialled (Ownby *et al.* 2000) but these measures generally contain no more information than the standard DRS. Moreover, it is possible that greater error may be introduced by relying on later data points because the FEV_1 becomes more variable after successive doses of methacholine.

COMPARISON OF METHODS OF METHACHOLINE CHALLENGE ANALYSIS

Aim

Stage One

To consider possible methods of analysing methacholine challenges and to assess these according to:

1) amount of censored data
2) evidence of training effects

Stage Two

To compare the ability of PD_{20} and DRS to discriminate children with wheeze from those without wheeze.

Method

Stage One

The dose-response curves of the first 70 methacholine challenges were analysed. Challenge data were summarised as the cumulative methacholine dose provoking a 20% drop from the saline FEV_1 (PD_{20}) and as the dose-response curve's slope (DRS). The data were then re-analysed according to modified versions of these measures.

1) Modifications to reduce censored data.

Firstly an alternative cut-off was considered; $PD_{15,}$ the dose of methacholine producing a fall in FEV_1 of 15%.

Secondly, an extrapolated $PD_{20,}$ PD_{20}ex, was calculated by linear extrapolation for those patients whose FEV_1 did not drop by more than 20%, even at the maximal dose. PD_{20}ex, was recorded in those subjects whose extrapolated value fell within one dose stage of the final concentration (that is PD_{20}ex less than 8.06 micromol, the cumulative concentration that would have been administered following a sixth stage of 64 mg/ml concentration based upon a nebuliser output of 0.37 ml/min – this value was chosen because estimates of nebuliser output, calculated under the conditions

used in this study, suggested the nebuliser's output was likely to be at the lower end of the manufacturers' quoted range).

2) Modifications to minimise 'training effect'

Firstly, an alternative PC_{20} measure, PC_{20}peak, was calculated based upon a 20% drop in FEV_1 from the peak in place of the saline reference value. Secondly, DRS was modified to increase the weight of the values attained later in the challenge after greater training.

DRSpen, was generated from:

$$\frac{(\text{final } FEV_1 - \text{penultimate } FEV_1)}{(\text{final methacholine concentration - penultimate methacholine concentration})}$$

Stage Two

Once the final data set of 107 challenges was collected and questionnaire identification of asthmatic status was available, the ability of PD_{20} and DRS to discriminate children who had received a diagnosis of asthma from those who had not was assessed. The BHR measures were also tested according to ability to distinguish between those with recent wheeze and those without.

Results

Stage One

1) Censored data

	Number of censored values (%)
PD_{20} (from saline)	56 (80%)
PD_{20}ex	33 (47%)
PD_{15}	52 (74%)
PD_{20} (from peak)	54 (77%)
DRS (saline and last value)	0 (0%)
DRSpen (saline and penultimate value)	0 (0%)

Table 7 Proportion of BHR values censored according to measure used

DRS was calculable for every participant but the PD_{20} was censored in 80% of cases. The alternative threshold measures PD_{20}peak and PD_{15} censor only marginally fewer data than PD_{20}. In contrast, the PD_{20}ex censored data for 47% of participants.

2) Training effects

The majority of participants (77%) demonstrated FEV_1 values after methacholine administration greater than the reference FEV_1 measured after saline inhalation. The mean increase was 4.6 ±5.5%. In 23% of participants FEV_1 at stage 5 (2.02 micromol cumulative methacholine administration) was greater than that at the saline stage; when this occurred the DRS (expressed as fall in FEV_1 / change in concentration) was negative. DRSpen was negative for 19% of children.

Stage Two

The sensitivity and specificity of PD_{20} to discriminate those children with a diagnosis of asthma from those without is shown in Figure 15. Censored values of PD_{20} > 2.01 micromol were included as negative test results. PD_{20} had maximal sensitivity and specificity at a cut point of 1.91 micromol. For log. slope the greatest sensitivity and specificity was achieved at a cut point of 5.22.

The area under the receiver operator characteristic (ROC) curves (95% CI) for PD_{20} and log slope were similar at 0.584 (0.468 to 0.700) and 0.588 (0.454 to 0.723) respectively. Substituting extrapolated values of PD_{20} for censored data produced an area under the ROC curve of 0.556 (0.417 to 0.694).

The 95% CIs for the area under each ROC curve suggest neither test performed significantly better than chance. However, both tests performed better at discriminating those children with wheeze in the last year from those without; areas under the ROC curve (95% CI) were 0.634 (0.518 to 0.750) for PD_{20} and 0.580 (0.428 to 0.732) for log. slope.

Figure 15 ROC curve for asthma diagnosis using PD_{20}

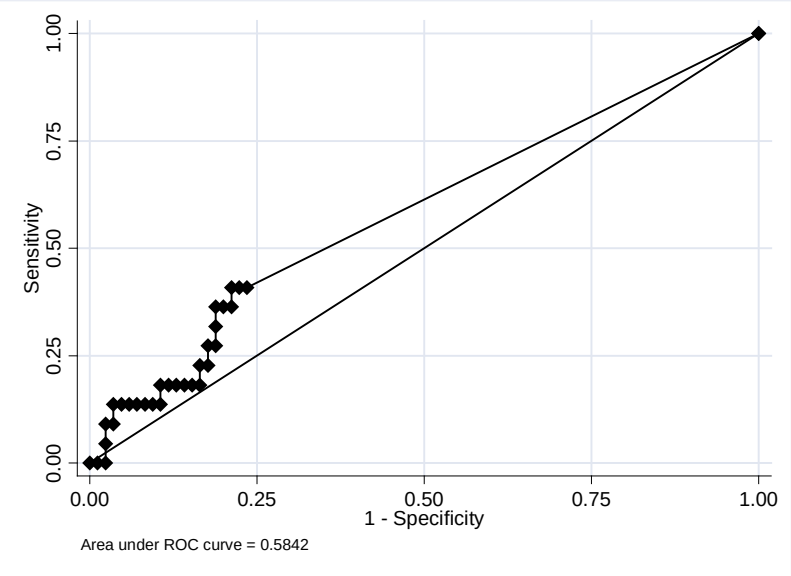
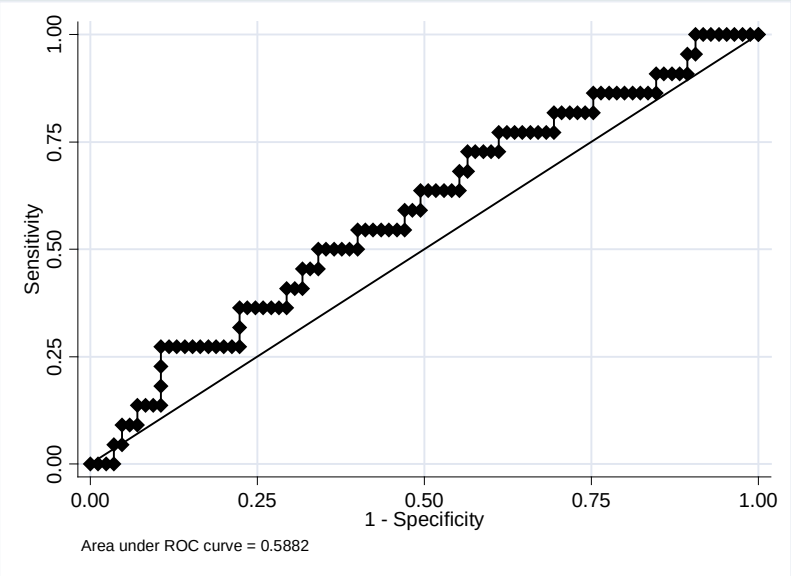


Figure 16 ROC curve for asthma diagnosis using log. slope



Conclusion

Stage One

1) The DRS censors fewer data than the PD_{20} and therefore is of particular use in epidemiological studies. The alternative threshold measures PD_{20}peak and PD_{15} censor only marginally fewer data and do not appear superior to PD_{20}. Extrapolating PD_{20} reduces data censoring even if extrapolation is restricted to only one further dose stage.

2) The FEV_1 values of many children increased during the early challenge stages; an effect which may be due to training. The influence of training is difficult to quantify and varies between children. DRSpen does not avoid the problem of negative values more effectively than DRS.

Stage Two

The areas under the ROC curves for PD_{20} and log. slope were similar and neither test performed significantly better than chance as the 95% CI for both ROC curves included 0.5. This may reflect an underestimation of the area under the curve associated with non-parametric estimation or may reflect the imprecision of the correlation between BHR, however measured, and a diagnosis of asthma. Replacing censored data with extrapolated values of PD_{20} did not improve discrimination.

3.3.2.8 Summary measures of provocation testing

3.3.2.8.1 *Methacholine concentration or cumulative dose*

The ATS guidelines quotes concentration thresholds of 16 mg/ml, 8 mg/ml and 1 mg/ml for borderline, mild and severe BHR respectively; these are based upon a standardised tidal breathing technique and may not be appropriate for dosimeter methods. Following the protocols commonly used, (tidal breathing for two minutes versus five dosimeter actuations), at any given concentration, a greater mass of agonist is delivered in the tidal breathing protocol. However, differences in nebuliser calibration, aerosol particle size and aerosol deposition between protocols may counteract any difference in total cumulative dose such that results from different protocols may not differ as much as may be expected (Cockcroft *et al.* 2005).

Comparison between studies is limited by the fact that cumulative methacholine dose is rarely specified. There is some evidence that the ability of provocation tests to discriminate between healthy individuals and those with BHR is increased when a cumulative dose of more than 8 micromol is administered (Agalliu *et al.* 2003). Following standard protocols this is usually achieved following the 8 mg/ml concentration stage. Whilst the cumulative dose received in this study is likely to be in the region of 2 micromol, internal comparisons remain valid so long as the protocol is standardised between participants.

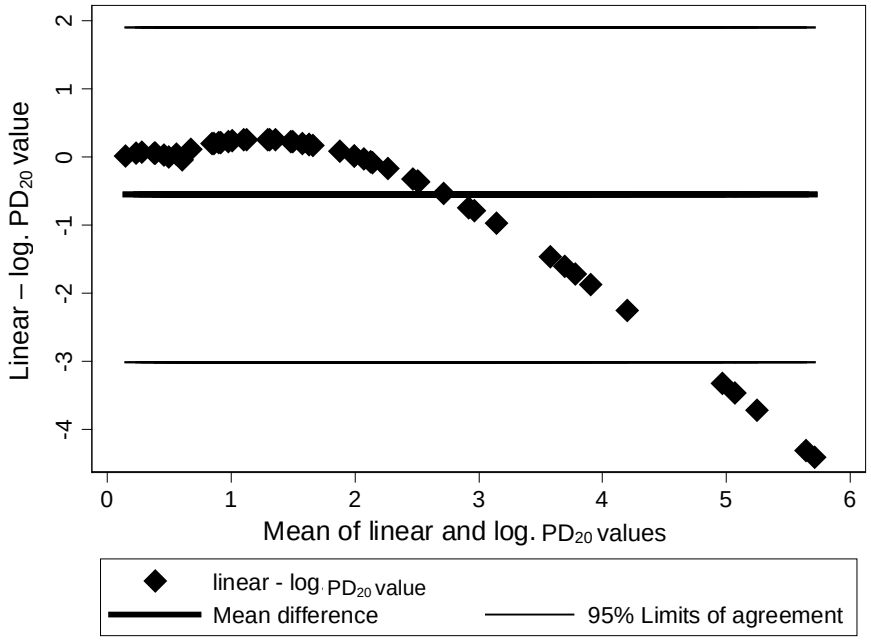
3.3.2.8.2 *Continuous or categorical measure*

Censored data and loss of power due to a categorical outcome are the main disadvantages of the PD_{20}. It was decided that the PD_{20} should be calculated however to ease comparison with the many previous studies which have presented results using the PD_{20} and because, due to clinical familiarity with this variable, results expressed in this way will be widely understood. Given the strong statistical argument for a continuous outcome it was decided to record a measure of dose-response slope as well as the PD_{20}.

3.3.2.8.3 *Linear or logarithmic Scale*

Cockcroft and Berscheid presented data to suggest a linear model fits histamine dose-response better than a logarithmic one and that PC_{20} best correlates with DRS measured from a linear dose-response curve (Cockcroft & Berscheid 1983). In this study, participants' data did not appear on visual inspection to correlate well with either a linear or a log.-linear model. Due to the fourfold increases in methacholine concentration it was not possible to compare the fit of participants' data using the coefficient of determination, R^2, as the non-uniform spacing of the data points effectively increased the correlation with a linear model. It is reassuring, however, that measures of PD_{20} calculated by interpolation on either a linear or logarithmic scale appear to correlate well over the range of doses administered in this study (maximal dose administered, hence recorded maximal PD_{20}, 2.01 micromol) (Figure 17).

Figure 17 Agreement between PD_{20} calculated from a linear scale and that calculated from a logarithmic dose scale



Published evidence confirms that the differences observed between the two methods of interpolation are negligible compared with variability over short or long periods in the measurement of FEV_1 using conventional challenge doses (Juniper *et al.* 1978). As there was no evidence to suggest a better correlation between log. dose and response than that between untransformed dose and response, PD_{20} was calculated on a linear scale. Where a 20% drop in FEV_1 occurred within the dose range studied, linear interpolation was used to determine the exact PD_{20}.

3.3.2.8.4 *Extrapolated values*

The dashed red lines in Figure 18 show an example of linear extrapolation from the last two points to estimate PD_{20}. Alternatively, PD_{20} can be linearly extrapolated from the least squares regression line of all experimental points. Each of the points on the dose-response curve is associated with error, therefore extrapolations based upon these points should be made with caution (Verlato *et al.* 1996). It is likely that extrapolation based upon the last two points alone is particularly vulnerable to chance variation associated with individual values. Indeed values of PD_{20} estimated by linear extrapolation from the penultimate pair of data points have been found to correlate poorly with the actual value measured by interpolation between the last pair of data points (Jokic *et al.* 1998).

Extrapolation is particularly difficult when the underlying shape of the dose-response curve is unknown. This can be seen in Figure 17 where extrapolated values based upon linear and logarithmic dose scales are compared; beyond the maximal administered dose of 2.01 micromol dose, the two values are no longer linearly correlated and agreement is poor.

Extrapolated PD_{20} values were not used in this study due to validity concerns. However, due to their mathematical derivation shallow dose-response slopes are essentially similar to extrapolated PD_{20} values and can be expected to have a similar level of reproducibility.

3.3.2.9 Measures of dose-response slope

Figures 18 & 19 display in black the dose-response curves of a single participant. Three separate techniques were used to derive measures of dose-response slope from the provocation data.

These measures were calculated as follows:

1) **Simple slope** (blue Figure 18)

$$\frac{\text{Drop in } FEV_1 \text{ as a percentage of the post-saline } FEV_1}{\text{Cumulative methacholine dose (micromol)}}$$

2) **Least squares slope** (green Figure 18) - calculated as the gradient of the regression line of all points on the dose response curve and expressed in units of % per micromol.

3) **Log. slope** (black dashed Figure 19) - a transformation of the slope of a regression line through FEV_1 drop and methacholine concentration where the concentration is plotted on a logarithmic scale.

$$\frac{100}{[\text{regression slope between } FEV_1 \text{ drop and } \log_{10}(\text{cumulative methacholine dose}) + 10]}$$

This measure includes a constant to remove negative values and an inverse transformation so that it is normally distributed and can be used in parametric analyses. This measure was developed for use in multi-centre trials and has the advantage of being less affected by variation in nebuliser output between centres. Its drawbacks are its unfamiliarity and its dependence on the dose schedule, as the relationship between percentage fall and log. dose is not strictly linear (Chinn *et al.* 1997). For the simple and least squares slope a higher score would be assigned to subjects with greater BHR; for the inverse log. slope measure, increased BHR would be associated with lower values.

Figure 18 Derivation of summary measures from provocation test data

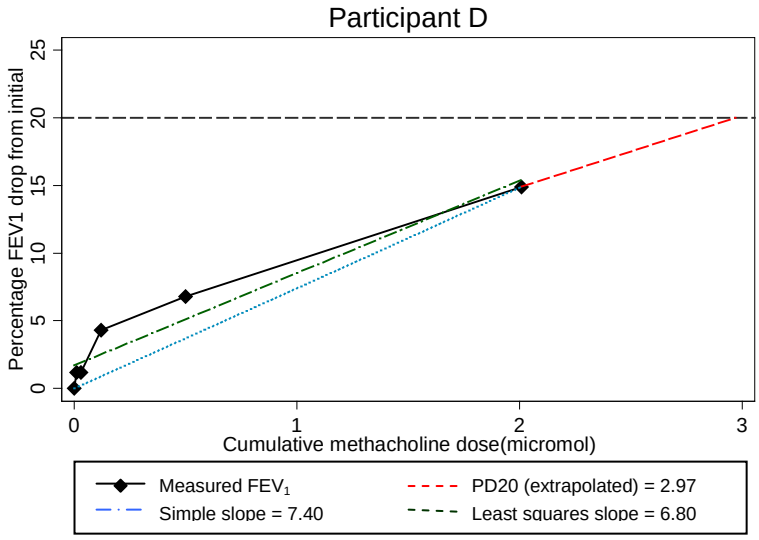
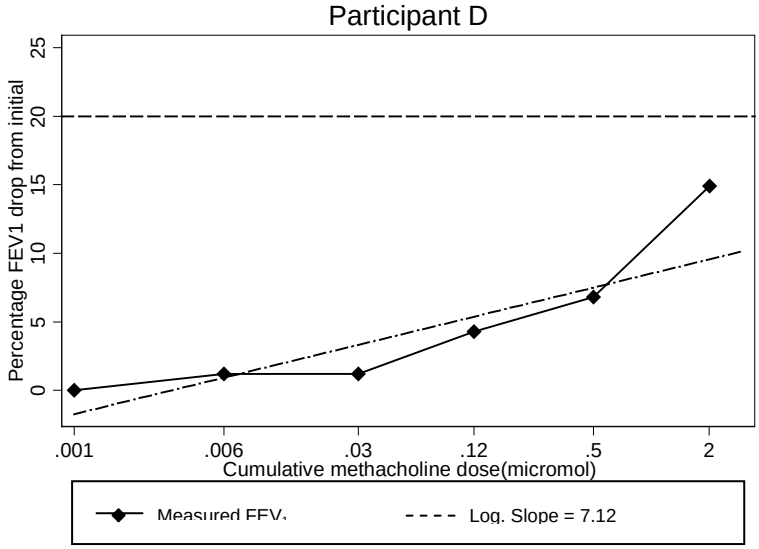


Figure 19 Derivation of the log. slope measure from methacholine challenge data

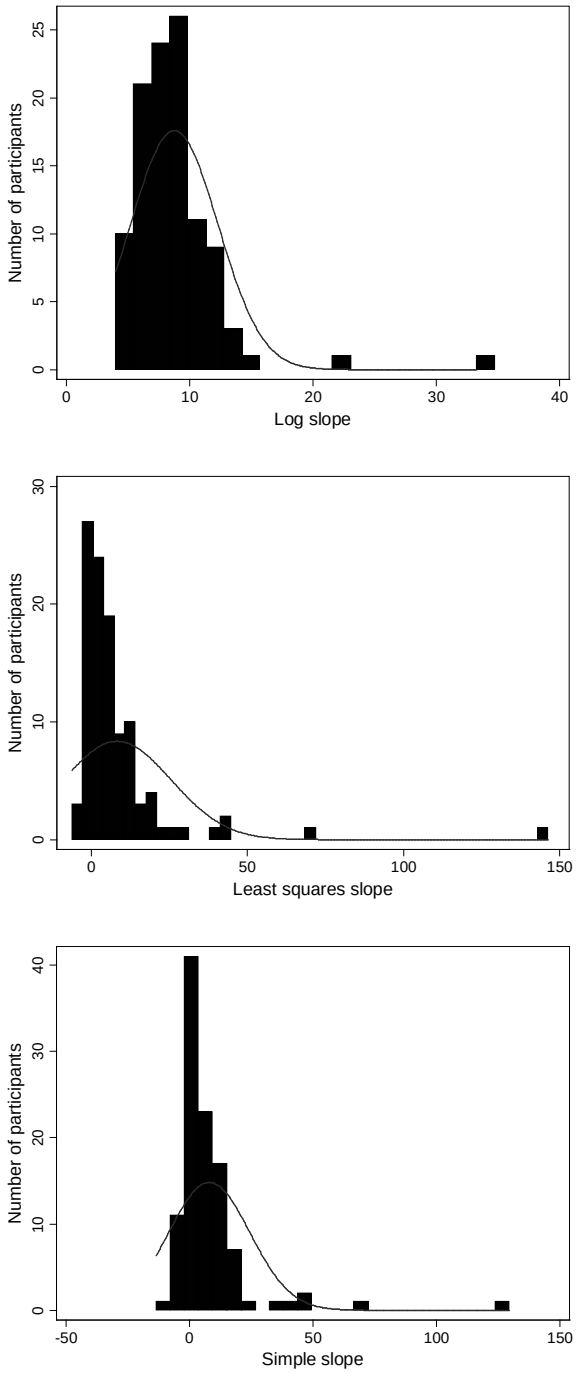


Following collection of data it was possible to assess the distributions of the various slope measures (Figure 20). The log. slope demonstrated a reasonably normal distribution, particularly considering the small numbers of participants contributing provocation data. There were outlying values for all three measures of slope, although these were more numerous and extreme for the simple slope and least squares slopes.

3.3.2.10 Correction of data

BHR has been demonstrated to decrease with age (Forastiere *et al.* 1996). It has been argued that, as nebuliser output is not corrected for size, any age-dependency of BHR need not necessarily arise from inherent biological variability (Le Souëf *et al.* 1995). Younger children are likely to be both shorter than older children and to have smaller lungs; Le Souëf suggested that younger children receive a proportionately larger dose of agonist but that this can be accounted for by correcting for height. Other workers have suggested BHR is not related to height but to baseline measures of lung function. For example, Ownby *et al.* (2000) concluded that methacholine responsiveness in children is significantly influenced by the percent predicted FEF_{25-75}, percent predicted FVC, atopy and sex. Provocation challenge data from this study were not corrected for age as the cohort included children from a narrow age range only. Amongst the participants in this study, there were no significant correlations between BHR, measured as either PC_{20} or log. slope and either height or age. Furthermore, measures of size or baseline lung function were not corrected for as these factors may be situated on the casual pathway between exposures, such as maternal nutrition, and respiratory outcomes.

Figure 20 Comparison of the dose-response slope measures' distributions



3.3.3 Bronchodilator response

The BDR measures the change in lung function which occurs after bronchodilator administration. It is not clear whether the BDR measures the same phenotypic characteristic as the provocation challenge (Benson 1975). Although only weak correlations have been found in children between reversibility and provocation studies (Bibi *et al.* 1991; Waalkens *et al.* 1993), some adult studies suggest a stronger correlation (Benson 1978). Observational and pathophysiological evidence suggests these tests are

not interchangeable. Severe asthmatics with irreversible airflow obstruction may exhibit limited bronchoconstrictor responses (Loren *et al.* 1978) and healthy subjects with negative provocation challenges may demonstrate considerable BDR (Casan *et al.* 1919). Moreover, bronchodilator and provocation studies are differentially affected by pharmaceutical agents: FEV_1 is similarly increased after inhalation of anticholinergics or beta-agonists, whilst the protective effect of anticholinergics against bronchoconstriction during challenge is less than that of beta-agonists (Woolcock *et al.* 1984).

3.3.3.1 Suitability of FEV_1 for BDR measurement

Pulmonary mechanics measures such as Raw and sGaw (measured via plethysmography) or airway resistance and reactance (measured with the forced oscillation technique) demonstrate large changes following bronchodilator administration. However, spirometric measures, such as FEV_1, are more frequently used as they are reproducible and easy to perform. As stated previously, there is evidence to support both ease of measurement and reproducibility of FEV_1 in young school-age children. As many of the children in this study had no prior experience of inhaling from a metered dose inhaler (MDI), 600 mcg of salbutamol was administered to ensure maximal effect was nevertheless achieved. Administration of an adequate dose was further aided by using a large volume spacer.

3.3.3.2 Expression of bronchodilator response

The change in FEV_1 with bronchodilator administration can be expressed in several ways: as an absolute difference (ΔFEV_1(l)), as a percentage of the predicted value of the variable (ΔFEV_1%pred) or initial value of the variable (Δ FEV_1%init), or as a percentage of the deficit in the variable (from predicted) at baseline (FEV_1 (pred-init)). A worked example of these alternatives is shown (Table 8).

Δ FEV_1%init tends to preferentially select as responders subjects with low initial values, whilst ΔFEV_1(pred-init) will preferentially select those with high initial values.ΔFEV_1 selects preferentially taller subjects and, although this size effect is eliminated by using ΔFEV_1%pred, this conveys no information regarding the clinically relevant post-bronchodilator value.

	Participant 1 (high predicted but low initial value) FEV_1 predicted 2 l	**Participant 2 (low predicted but high initial value)** FEV_1 predicted 1 l
Initial value	400 ml 20% predicted	800 ml 80% predicted
Post bronchodilator value	600 ml 30% predicted	900 ml 90% predicted
ΔFEV_1 (ml)	+200 ml	+100 ml
ΔFEV_1%pred	+10%	+10%
ΔFEV_1%init	+50%	+12.5%
ΔFEV_1(pred-init)	+12.5%	+50%

Table 8 Worked examples demonstrating the dependence of different methods of calculating BDR upon absolute and predicted initial value of FEV_1

To compare children of different airway caliber it is necessary to find an index of BDR that is not confounded by stature or age. Furthermore, as asthma is characterised by variable airways obstruction, another desirable feature when attempting to distinguish children with this condition from healthy controls is that the index is independent of initial airway calibre. Waalkens *et al.* (1993) recommend ΔFEV_1%pred for use in children and suggested that this value is unrelated to age and stature and also permits a clinically significant response to be assessed independently of initial FEV_1. Data from this study support Waalken's assertions. There were no significant associations between ΔFEV_1%pred and either height or age. Moreover, whilstΔ FEV_1%init was significantly associated with baseline FEV_1 ($p=0.04$), ΔFEV_1%pred was not significantly associated with the initial FEV_1.

3.3.3.3 Threshold of positive response

BDR measures in normal children overlap those of children with asthma. Specificity and sensitivity vary in opposition, and the proportion of children with asthma over- or underdiagnosed on the basis of their BDR depends upon the threshold. As BDR is unimodally distributed, separation into normal and abnormal values is arbitrary. It is particularly difficult to calculate an appropriate cut-off for diseases, such as asthma, where no gold standard diagnostic tool is available for comparison.

A BDR threshold of 12-15% of initial FEV_1 is recommended by the adult ATS/ERS guidelines (Pellegrino *et al.* 2005). No recommended threshold for change in either initial or percentage predicted FEV_1 exists for use in the paediatric age range. Thresholds have been calculated based upon maximising sensitivity and specificity. For

example, Dundas *et al.* (2005) studied 142 children, aged 5 to 9 years, and found that a threshold of 9% ΔFEV_1%pred best distinguished asthmatic from non-asthmatic individuals. An alternative approach is to determine threshold by estimating the variability expected between two readings in the absence of true change. The CV of FEV_1 measurements can be used to calculate the minimum difference significant at the 95% probability level according to the equation, minimum significant difference = 1.64 x √2 x CV. Strachan (1989b) reported the CV of FEV_1 to be 4.3% in a population study of 7-year-old children. This estimate of CV provides a threshold value of 10.0% change in initial FEV_1. This shows reasonable agreement with the value of 10.3% of percentage predicted FEV_1 calculated after placebo administration (Bussamra *et al.* 2005) and that of 10.5% of initial FEV_1 based upon the 95th centile of bronchodilator responses in the normal paediatric population (Casan *et al.* 1919). However, to avoid the difficulties associated with an arbitrary threshold BDR was treated as a continuous variable in this study in order to maximise power.

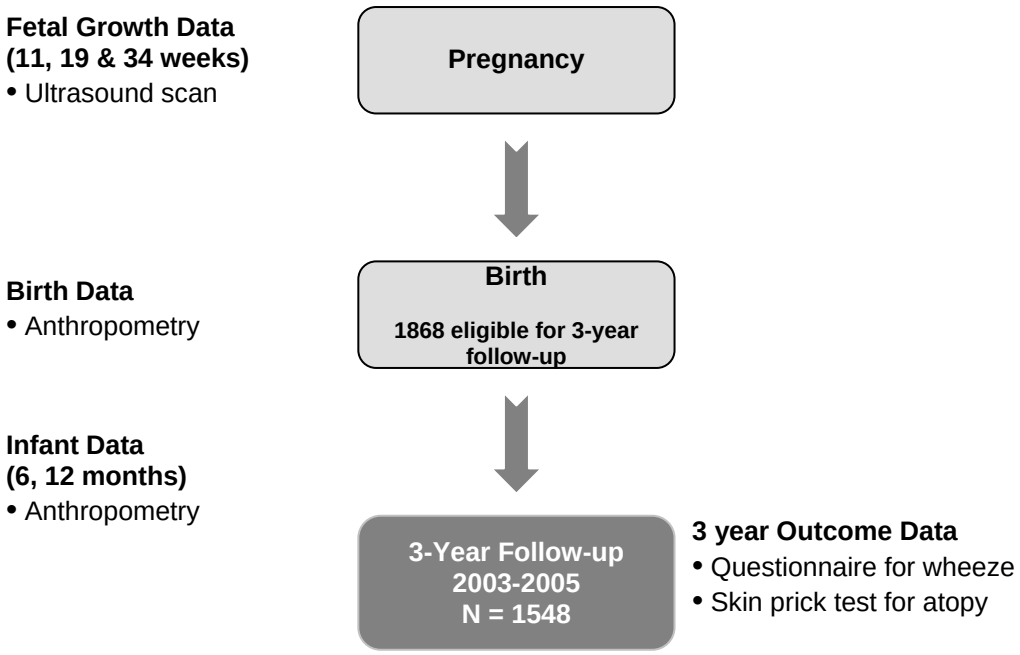
Chapter Four

Predictors of wheeze and atopy at 3 Years

4.1 RATIONALE AND OBJECTIVE

Little is known about whether patterns of early growth are associated with altered respiratory or immune development, as reflected in respiratory symptoms and atopy. This study aimed to relate prenatal and early postnatal growth patterns to wheeze and atopy risk at age 3 years and to explore the hypothesis that factors which promote adaptive change in the relative growth of body tissues might have functional consequences for the respiratory or immune systems in later life. Following on from previous work in the Medical Research Council Epidemiology Resource Centre, it was hypothesised that a rapid early fetal growth trajectory followed by late gestation growth faltering and postnatal adiposity gain might be associated with later respiratory ill health (Lucas *et al.* 2004).

Figure 21 Data collected to assess the relationship between fetal and early infant growth and wheeze and atopy in 3-year-old children



4.2 SUMMARY OF METHODS

A detailed explanation of the methods used to collect exposure and outcome variables may be found in chapter two and the cohort is defined in relation to the phases of the SWS in Figure 21. Mothers were invited to receive a visit from a research nurse to follow up their child's development. Wheeze was identified from questionnaire response and atopic status was determined by skin prick testing. Measures of size and conditional growth velocity were calculated from antenatal ultrasounds and infant anthropometry. Information relating to likely confounding variables was obtained from previous SWS questionnaires.

4.3 ANALYSIS

A cohort of eligible children was identified based upon date of birth. Children born at less than 37 weeks' gestation were excluded to avoid confounding by the effects of lung disease of prematurity. Children were classified according to whether they were reported to have ever wheezed and whether or not they were atopic. Relative risks were calculated for atopy compared to no atopy, and for atopic and non-atopic wheeze, where never-wheezing, non-atopic children served as the comparator. Relative risks

(RR) were also calculated for secondary outcomes of ever wheezing and doctor-diagnosed asthma using the absence of each condition as the comparator.

All outcome variables were binary, but the outcomes were common; therefore Poisson regression with robust variance was used to derive relative risks for each outcome. Logistic regression was not used as odds ratios relating to common outcomes are hard to interpret (Barros & Hirakata 2003). Exposure variables were transformed by natural logarithms where necessary to achieve normality and transformed variables were then standardised. The method of Royston was used to calculate conditional measures of fetal growth in order to account for both gestation and regression to the mean (Royston 1995). Conditional measurements of infant growth in length and weight which accounted for age were also calculated using this method. For subscapular skinfold change, the method of Royston proved unsuitable as adiposity does not increase monotonically with age. Instead, subscapular skinfold growth velocity conditional upon initial size was calculated using a regression method. Specific analyses are described in the course of the results. The analyses were adjusted for exposures identified as likely confounders.

4.4 RESULTS

4.4.1 Summary of study population characteristics

By the end of 2003, 1987 babies had been born to women enrolled in the SWS. Of these, 119 babies were born at a gestation of less than 37 weeks and were excluded from the analyses. Of the remaining 1868 children, 1548 (83%) were seen at 3 years of age. The mean age (10^{th} – 90^{th} centiles) at the 3-year follow-up was 3 years, 1 month (2 years, 361 days – 3 years, 70 days). 1512 of the 1548 children (98%) were seen at all four time points: 6 months, 1, 2 and 3 years. The characteristics of the children seen at 3 years are presented in Table 9 and compared with the remaining 320 children who were not premature but who could not be seen at this time, either because they had moved away, lost contact with the SWS or had asked to withdraw from follow-up.

Data were available for all 1548 mothers from the initial pre-pregnancy interview. The majority of these women also had data in early pregnancy (n=1535) and in late pregnancy (n=1521). Skin prick results were available for 1342 mothers (87%); of these 628 were atopic (47%). The mean (SD) age of the mothers was 30.2 years (3.8 years)

and 46% were in their first pregnancy. Those children seen at 3 years of age had a higher mean birthweight, and tended to have mothers who were older, less likely to smoke, more likely to have tried breastfeeding, and who had higher educational attainment. The differences between the children followed up and those not seen at 3 years may bias prevalence estimates, however, the variation in exposure variables seen between the children seen at 3 years is likely to be sufficient to sustain internal comparison and to support investigation of associations between exposure and outcome variables.

		Children seen at 3 years (n=1548)		Children not seen at 3 years (n=320)		P-value
Parental characteristics						
Mother's age at child's birth (years), mean (SD)		30.2 (3.8)	1548	29.4 (3.8)	308	<0.001
Mother's educational qualifications (%)	None or CSE	12.8	1544	18.8	319	<0.001
	O Level	10.0		34.2		
	A Level	28..9		21.0		
	HND	7.3		7.2		
	Degree	21.4		18.8		
Maternal smoking during pregnancy, (%)		16.3	1504	26.9	298	<0.001
Maternal asthma, (%)		22.4	1534	23.8	311	0.599
Maternal eczema in childhood, (%)		17.9	1533	16.1	311	0.432
Maternal rhinitis, (%)		41.7	1534	37.9	311	0.225
Paternal asthma, (%)		17.5	1511	19.8	303	0.334
Paternal eczema in childhood, (%)		10.7	1459	10.4	297	0.897
Paternal rhinitis, (%)		34.3	1474	33.9	311	0.883
Birth characteristics						
Birthweight (g), mean (SD)		3525 (475)	1531	3456 (467)	306	0.020
Gestational age (weeks), mean (SD)		40.1 (1.2)	1548	40.1 (1.2)	320	0.909
Primiparous, (%)		45.5	1547	35.6	320	0.001
Completed months of breastfeeding, n (%)*	None	17.4	1536	30.2	232	<0.001
	< 1	20.4		20.7		
	1 - 3	19.5		17.7		
	4 - 6	16.8		13.8		
	7 - 11	15.7		10.8		
	12 or more	10.2		6.9		
Characteristics at 6 month follow-up*						
Maternal smoking, (%)		18.5	1537	29.7	246	<0.001
Other smokers in the home, (%)		30.7	1501	35.4	243	0.145
Ever wheezed, (%)		26.2	1537	33.7	246	0.013
Cat or dog in home, (%)		45.5	1537	42.5	247	0.384
Characteristics at 1 year follow-up*						
Wheezed in past 6 months, (%)		30.2	1539	40.8	196	0.002
Cat or dog in home, (%)		44.0	1536	41.8	196	0.575
Characteristics at 2 year follow-up*						
Wheezed in past year, (%)		27	1523	27	104	0.954

***Of the 320 children not seen at 3 years, 247 were seen at 6 months, 196 at 1 year and 104 at 2 years**
Binary outcomes were compared by χ^2 tests and categorical outcomes by Mann Whitney tests.
Continuous variables were compared by t-tests for using geometric means for skewed variables

Table 9 Comparison of the characteristics of those children seen in the 3-year follow-up with those children who could not be seen

4.4.1.1 Categorisation of illness data

Data on childhood illnesses were collected at 6, 12, 24 and 36 months of age; the mothers of the children were asked whether their child had 'experienced any episodes of chestiness associated with wheezing or whistling in his/her chest' since the child was last seen within the Survey. At the 3-year visit, each mother was asked whether a doctor had ever diagnosed her child as having asthma. The answers to these questions were used to define variables relating to wheeze outcome. Of the 36 children not seen at all four time points, 13 children had wheezed at least once. These children were included in the analysis in order to maximise the power to detect potential risk factors associated with wheeze. Inclusion of these children should not introduce bias towards risk factors for early wheeze as there was no bias in the pattern of missing data, (early visits being missed as frequently as later visits). Including these children may, however, cause the prevalence of wheeze to be very slightly overestimated. Data were available for the derivation of the doctor-diagnosed asthma variable for 1545 children (99.8%), and for the 'ever wheezed' variable data for 1522 children (98%).

At age three years, skin prick testing was performed on the children, and at the one-year visit the mothers of the children were also tested. Mothers and children were defined as atopic if they developed a wheal to any of these allergens that was at least 3mm in diameter. It was possible to determine atopic status for 1184 children (76%). Although 54 children had taken antihistamine medication within 7 days of skin prick testing, this resulted in an invalid histamine control in only seven children; there was also one child who had an invalid negative control. The remaining 46 children were found to be significantly more likely to be classed as atopic by skin prick testing than those who had not taken antihistamines (χ^2 $p<0.001$). These children were not excluded in order to maximise power to detect significant exposures.

Table 10 shows the percentages of children in each outcome group and the numbers in the comparator groups. At age 3 years, 106 children (7%) had received a diagnosis of asthma from a doctor in the last year and 890 (58%) had ever experienced wheeze; 199 children (17%) were atopic. 1164 children could be classified according to both wheeze and atopic status; of these 127 (11%) had wheezed and were atopic and 555 (48%) had wheezed but were non-atopic. Skin prick testing had been carried out in 74 of the children diagnosed with asthma: 22 were atopic (30%) and 55 were non-atopic (70%).

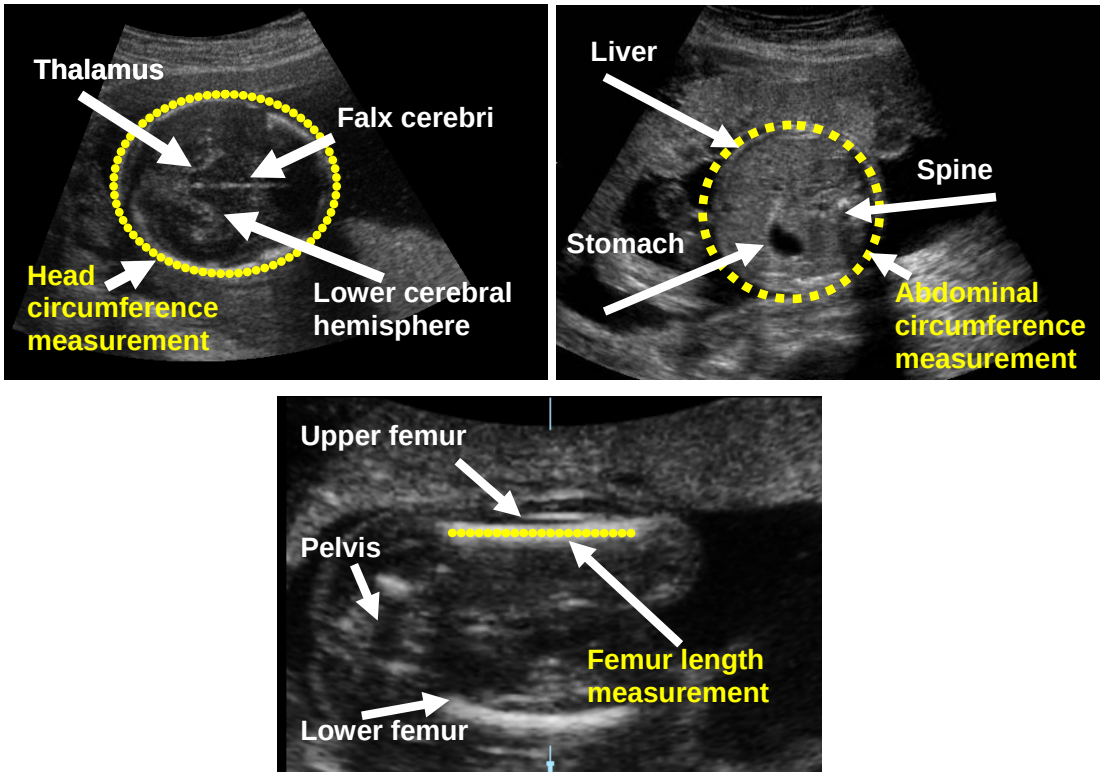
Outcomes	Frequency (%)
Doctor-diagnosed asthma	106 (7%)
Atopy at 3 years	199 (17%)
Ever wheeze	890 (58%)
Never wheezed, non-atopic	415 (36%)
Ever wheezed, non-atopic	555 (48%)
Never wheezed, atopic	67 (6%)
Ever wheezed, atopic	127 (11%)

Table 10 3-year-old participants classified according to wheeze and atopy outcomes

4.4.1.2 Fetal size and growth measurement

Gestational age was determined using an algorithm based upon last menstrual period and early ultrasound data. Last menstrual period data was used in preference to ultrasound data if the two measurements agreed to within 14 days. Where there was a greater discrepancy, ultrasound measurements were used to date the pregnancy.

Figure 22 Ultrasound measurement of head circumference, abdominal circumference and femur length



The mean (10^{th} – 90^{th} centile) gestational ages at the 11, 19 and 34 week USS were 11.8 weeks (11.1 – 12.6), 19.5 weeks (18.9 – 20.2) and 34.5 (33.9 – 35.1) respectively. Complete fetal measurements were available at all three time points for 769 fetuses. All fetal USS measures were completed by three highly trained ultrasonographers over a period of five years between August 1998 and October 2003. Summary measures of fetal size are shown in Table 11.

USS measure mean (SD, n)	**Gestation**		
	11 weeks	**19 weeks**	**34 weeks**
Head circumference	70.0 mm (9.2 mm, 920)	167.7 mm (8.4 mm, 1479)	316.9 mm (11.0 mm, 1456)
Abdominal circumference	55.9 mm (7.6 mm, 873)	145.8 mm (8.9 mm, 1472)	307.4 mm (15.8 mm, 1526)
Femur length	Cannot be measured	30.6 mm (2.0 mm, 1481)	64.9 mm (2.7 mm, 1527)

Table 11 Fetal ultrasound scan measurements

Sequential measures of fetal size were used to calculate conditional measures of growth. Conditional growth in early pregnancy was not significantly associated with conditional growth in late pregnancy. Individual fetuses did not necessarily remain in the same tertile of head or abdominal circumference growth between early and late pregnancy; Table 12 Table 13 shows that a range of patterns of growth were present in this sample.

		Tertile of head circumference growth 19 – 34 weeks, n (%)			
Tertile of head circumference growth 11 – 19 weeks		Lowest	Middle	Highest	Total
	Lowest	73 (38)	58 (30)	63 (32)	194 (100)
	Middle	57 (29)	72 (37)	68 (34)	197 (100)
	Highest	67 (35)	72 (38)	52 (27)	191 (100)
					(χ^2, p=0.2)

Table 12 Pattern of head circumference growth

		Tertile of abdominal circumference growth 19 – 34 weeks, n (%)			
		Lowest	Middle	Highest	Total
Tertile of abdominal circumference growth 11 – 19 weeks	Lowest	61 (32)	65 (35)	63 (33)	189 (100)
	Middle	67 (35)	58 (31)	64 (34)	189 (100)
	Highest	62 (33)	76 (40)	50 (27)	188 (100)
					(χ^2, p=0.3)

Table 13 Pattern of abdominal circumference growth

4.4.1.3 Infant size and growth measurements

Research nurses measured infant weight, length, and subscapular skinfold thickness at birth and at ages 6 and 12 months. The mean gestational age at birth was 40 weeks and the range was from 37 weeks to 42 weeks and 6 days. The mean ages at the 6 and 12 month visits were 27 weeks (SD 4.3 weeks) and 54 weeks (SD 5.7 weeks) respectively. Summary measures of infant size are shown in Table 14.

Size measure	**Age**		
mean (SD, n)	**birth**	**6 months**	**12 months**
Weight	3.525 kg (0.475kg, 1531)	8.0 kg (1.0 kg, 1524)	10.1 kg (1.2 kg, 1519)
Subscapular skinfold thickness	5.1 mm (1.1 mm, 1525)	7.4 mm (1.7 mm, 1522)	7.1 mm (1.7 mm, 1499)
Length	50.1 cm (1.9 cm, 1502)	67.4 cm (2.5 cm, 1514)	75.9 cm (2.7 cm, 1495)

Table 14 Infant size measurements

4.4.2 Poisson regression analyses

Potential confounders are listed in Table 15. The analyses were adjusted for key factors which evidence suggests are associated with the outcome. These variables were maternal education (Heinrich *et al.* 1998b), maternal atopy (Arshad *et al.* 1993; Kjellman 1977) and child's birth order (Matricardi *et al.* 1998; Strachan 1989a; Strachan *et al.* 1996b) for atopic outcomes. For wheeze outcomes the following were considered key factors: maternal education (Ernst *et al.* 1995; Lawlor *et al.* 2004), maternal history of asthma (Litonjua *et al.* 1998), smoking in pregnancy (Dezateux *et al.* 2001) and paternal history of asthma (Dold *et al.* 1992). Additional confounders were identified using univariate tests of association. Factors associated with the outcome at a significance level of 10%

or less were included in a stepwise forwards selection. Regression models were built including all confounders significantly associated with each outcome in a mutually adjusted logistic regression model ($p<0.05$). Confounders identified in this manner were then combined with those previously selected on grounds of likely biological importance.

Child's Characteristics	Parental Characteristics	Environmental Characteristics
Gender	Maternal age	Others smoking in the home
Birthweight	Maternal education	(when the child was 6 months old)
Gestational age	Maternal smoking in pregnancy	Ownership of a cat or dog
Birth order	Maternal smoking	(during the child's first year of life)
Mode of infant feeding	(when child aged 6 months)	
	Maternal history of asthma	
	Maternal history of rhinitis	
	Maternal eczema in childhood	
	Maternal atopy	
	Paternal history of asthma	
	Paternal history of rhinitis	
	Paternal eczema in childhood	

Table 15 Potential confounders

4.4.2.1 Atopy

The relative risk of atopy at 3 years increased by 46% per SD increase in abdominal circumference growth velocity between 11-19 weeks' gestation ($p=0.007$), and was greater in children who had a greater abdominal circumference at 19 weeks' gestation (RR = 1.24 per SD increase in abdominal circumference, $p=0.021$). In contrast, each SD increase in abdominal growth velocity from 19-34 weeks' gestation decreased atopy risk by 20% ($p=0.011$) (Table 16). Atopy risk was associated with greater femur length at 19 weeks (RR = 1.26% per SD of femur length, $p=0.008$) and crown-heel length at birth (RR = 1.17 per SD of crown-heel length, $p=0.032$), and not associated with femur growth velocity from 19-34 weeks' gestation. Prenatal head circumference growth, birthweight and measures of postnatal growth were not associated with the risk of atopy (Table 16).

	Unadjusted analyses				Adjusted* analyses			
	RR	**(95% CI)**	**P-value**	**n**	**RR**	**(95% CI)**	**P-value**	**n**
Fetal size variable								
11 weeks' gestation								
Crown-rump length	1.10	0.92-1.31	0.295	635	1.14	0.95-1.37	0.165	563
Head circumference	1.18	0.96-1.46	0.115	467	1.19	0.95-1.49	0.137	408
Abdominal circumference	1.11	0.86-1.43	0.418	433	1.14	0.87-1.48	0.339	380
19 weeks' gestation								
Femur length	1.24	1.06-1.45	0.007	721	1.26	1.06-1.50	0.008	633
Head circumference	1.17	0.99-1.38	0.072	719	1.14	0.94-1.37	0.187	631
Abdominal circumference	1.24	1.06-1.47	0.009	716	1.24	1.03-1.50	0.021	629
34 weeks' gestation								
Femur length	1.16	0.99-1.36	0.071	719	1.15	0.97-1.37	0.115	634
Head circumference	1.03	0.87-1.23	0.705	689	1.08	0.86-1.26	0.676	607
Abdominal circumference	0.91	0.77-1.08	0.278	719	0.89	0.75-1.06	0.207	634
Birth size variables								
Crown-heel length	1.20	1.06-1.36	0.005	1149	1.17	1.01-1.34	0.032	1004
Weight	1.10	0.97-1.25	0.137	1171	1.08	0.95-1.24	0.241	1023
Head circumference	1.06	0.93-1.20	0.408	1167	1.00	0.86-1.15	0.957	1020
Abdominal circumference	1.13	1.00-1.29	0.054	1167	1.13	0.99-1.29	0.068	1020
Subscapular skinfold	1.09	0.96-1.23	0.204	1166	1.06	0.92-1.22	0.401	1020
Conditional fetal growth								
11-19 weeks								
Head circumference	0.93	0.72-1.18	0.534	464	0.80	0.60-1.06	0.123	405
Abdominal circumference	1.44	1.20-1.86	0.005	431	1.46	1.11-1.93	0.007	378
19-34 weeks								
Femur length	1.03	0.89-1.20	0679	712	1.02	0.87-1.20	0.766	629
Head circumference	0.96	0.81-1.13	0.608	682	0.98	0.82-1.17	0.817	601
Abdominal circumference	0.80	0.69-0.94	0.006	707	0.80	0.68-0.95	0.011	625
Conditional Infant growth								
0 – 6 months								
Length	0.93	0.83-1.05	0.244	1131	0.89	0.78-1.02	0.104	991
Weight	1.09	0.97-1.22	0.131	1157	1.06	0.92-1.21	0.416	1013
Subscapular skinfolds	1.09	0.97-1.23	0.157	1153	1.09	0.96-1.24	0.185	1011
6 – 12 months								
Length	1.08	0.93-1.26	0.326	1069	1.02	0.86-1.21	0.843	941
Weight	1.00	0.83-1.19	0.961	1086	1.03	0.84-1.26	0.778	956
Subscapular skinfolds	1.05	0.92-1.20	0.485	1078	1.11	0.97-1.27	0.142	950

N = 1184 *Adjusted for gender, maternal eczema, maternal atopy, maternal education and birth order.

Relative risks were calculated as change in risk per SD change in size or growth measurement.

Table 16 Relative risks for the association between growth and atopy at age 3 years

4.4.2.2 Atopic wheeze

The pattern of risk for atopic wheeze was similar to that for atopy (Table 17). Relative risk increased with a higher 11-19 week abdominal growth velocity (32% per SD, p=0.114), a higher 19 week fetal abdominal circumference (34% increase per SD, p=0.019), and a lower 19-34 week abdominal growth velocity (20% per SD, p=0.046). Atopic wheeze risk was also associated with greater femur length at 19 weeks (34% increase per SD, p=0.022), but not with crown heel length at birth (p=0.31). The risk of atopic wheeze was not associated with prenatal head circumference growth and birthweight, but was associated with greater weight and adiposity increases in the first year of life (Table 17). SD increases in subscapular skinfold gain and conditional weight gain between birth and 6 months were associated with 27% and 22% increases in atopic wheeze risk (p=0.002 and p=0.02, respectively); each SD increase in subscapular skinfold gain between 6 and 12 months was associated with a 20% increase in atopic wheeze risk (p=0.018).

4.4.2.3 Non-atopic wheeze

The associations between fetal growth and risk of non-atopic wheeze differed from those for atopic wheeze. Non-atopic wheeze was not associated with faster early abdominal growth velocity followed by abdominal growth faltering, but instead increases in risk were seen for smaller 11 week crown rump length (RR= 0.92 per SD increase, p=0.048), lower conditional head growth between 11 and 19 weeks and smaller 34-week head circumference (RR = 0.91 per SD of head circumference, p=0.015) (Table 18). Similar to the associations for atopic wheeze, non-atopic wheeze risk increased by 6% per SD increase in adiposity gain during the first 6 months of infancy (p=0.024) and by 8% per SD increase in weight gain between 6 and 12 months (p=0.036) (Table 18).

	Unadjusted analyses				Adjusted* analyses			
	RR	**(95% CI)**	**P-value**	**n**	**RR**	**(95% CI)**	**P-value**	**n**
Fetal size variable								
11 weeks' gestation								
Crown-rump length	1.13	0.89-1.43	0.316	290	1.18	0.92-1.50	0.191	261
Head circumference	1.27	0.95-1.70	0.104	208	1.16	0.88-1.54	0.301	184
Abdominal circumference	1.25	0.90-1.73	0.178	198	1.16	0.84-1.58	0.366	173
19 weeks' gestation								
Femur length	1.29	1.05-1.59	0.015	327	1.34	1.04-1.72	0.022	291
Head circumference	1.28	1.03-1.60	0.025	326	1.16	0.90-1.49	0.257	290
Abdominal circumference	1.40	1.13-1.72	0.002	325	1.34	1.05-1.72	0.019	289
34 weeks' gestation								
Femur length	1.14	0.93-1.40	0.206	326	1.10	0.96-1.36	0.346	293
Head circumference	1.11	0.89-1.38	0.358	311	0.98	0.75-1.27	0.868	279
Abdominal circumference	0.96	0.77-1.20	0.724	326	0.91	0.72-1.15	0.430	293
Birth size variables								
Crown-heel length	1.09	0.94-1.26	0.247	527	1.08	0.93-1.26	0.313	456
Weight	1.06	0.91-1.23	0.461	537	1.02	0.87-1.19	0.810	465
Head circumference	1.05	0.90-1.22	0.546	538	0.93	0.79-1.10	0.411	466
Abdominal circumference	1.11	0.95-1.30	0.190	538	1.10	0.93-1.27	0.282	466
Subscapular skinfold	1.13	0.97-1.31	0.123	538	1.11	0.93-1.31	0.255	466
Conditional fetal growth								
11-19 weeks								
Head circumference	0.96	0.69-1.33	0.818	207	0.79	0.54-1.15	0.216	183
Abdominal circumference	1.42	1.05-1.92	0.024	197	1.32	0.94-1.85	0.114	172
19-34 weeks								
Femur length	1.01	0.83-1.22	0.930	323	0.97	0.82-1.15	0.703	290
Head circumference	0.98	0.80-1.21	0.866	308	0.88	0.69-1.12	0.294	276
Abdominal circumference	0.82	0.66-1.01	0.066	321	0.80	0.65-1.00	0.046	288
Conditional Infant growth								
0 – 6 months								
Length	1.00	0.86-1.16	0.987	518	0.96	0.82-1.12	0.602	499
Weight	1.25	1.09-1.43	0.001	530	1.22	1.03-1.43	0.020	460
Subscapular skinfolds	1.22	1.07-1.40	0.004	533	1.27	1.09-1.49	0.002	463
6 – 12 months								
Length	1.02	0.85-1.22	0.829	497	0.98	0.80-1.20	0.842	432
Weight	1.14	0.92-1.41	0.234	504	1.19	0.94-1.31	0.147	438
Subscapular skinfolds	1.14	0.99-1.32	0.073	502	1.20	1.03-1.39	0.018	436

N = 542 *Adjusted for gender, smoking during pregnancy, maternal asthma and maternal rhinitis, maternal atopy, paternal asthma, maternal education and birth order.

Relative risks were calculated as change in risk per SD change in size or growth measurement.

Table 17 Relative risks for the association between growth and whether the child had ever wheezed by age 3 years and was atopic, compared with children who had never wheezed and were not atopic

	Unadjusted analyses				Adjusted* analyses			
	RR	**(95% CI)**	**P-value**	**n**	**RR**	**(95% CI)**	**P-value**	**n**
Fetal size variable								
11 weeks' gestation								
Crown-rump length	0.92	0.85-0.99	0.032	522	0.92	0.86-1.00	0.048	510
Head circumference	0.95	0.88-1.04	0.252	386	0.96	0.88-1.05	0.375	375
Abdominal circumference	0.94	0.85-1.04	0.241	363	0.96	0.87-1.07	0.481	353
19 weeks' gestation								
Femur length	0.96	0.89-1.03	0.225	591	0.98	0.91-1.05	0.531	578
Head circumference	0.94	0.87-1.01	0.107	589	0.93	0.86-1.01	0.070	576
Abdominal circumference	0.94	0.87-1.01	0.093	586	0.94	0.87-1.02	0.116	573
34 weeks' gestation								
Femur length	0.93	0.87-0.99	0.031	595	0.93	0.87-1.00	0.056	583
Head circumference	0.91	0.85-0.98	0.014	571	0.91	0.84-0.98	0.015	559
Abdominal circumference	1.02	0.95-1.09	0.622	595	1.02	0.95-1.09	0.626	583
Birth size variables								
Crown-heel length	0.97	0.92-1.03	0.282	944	0.97	0.92-1.03	0.317	926
Weight	0.98	0.93-1.04	0.486	960	0.97	0.92-1.03	0.356	942
Head circumference	0.98	0.92-1.03	0.416	957	0.97	0.91-1.02	0.244	939
Abdominal circumference	0.98	0.93-1.04	0.518	957	0.98	0.93-1.03	0.422	939
Subscapular skinfold	1.02	0.97-1.08	0.440	957	1.02	0.96-1.08	0.506	939
Conditional fetal growth								
11-19 weeks								
Head circumference	0.92	0.83-1.01	0.090	383	0.90	0.81-1.00	0.041	373
Abdominal circumference	0.98	0.88-1.09	0.744	361	0.97	0.87-1.09	0.637	352
19-34 weeks								
Femur length	0.94	0.88-1.01	0.073	589	0.94	0.87-1.00	0.068	577
Head circumference	0.93	0.87-1.00	0.058	564	0.94	0.88-1.01	0.096	552
Abdominal circumference	1.06	0.99-1.13	0.117	584	1.05	0.98-1.13	0.136	572
Conditional Infant growth								
0 – 6 months								
Length	1.01	0.96-1.06	0.825	933	0.99	0.94-1.05	0.766	915
Weight	1.05	1.00-1.10	0.044	952	1.04	0.99-1.10	0.093	934
Subscapular skinfolds	1.07	1.02-1.12	0.009	950	1.06	1.00-1.11	0.024	932
6 – 12 months								
Length	0.97	0.91-1.03	0.320	878	0.98	0.92-1.05	0.636	862
Weight	1.04	0.97-1.11	0.289	891	1.08	1.00-1.15	0.036	876
Subscapular skinfolds	1.00	0.95-1.06	0.929	887	1.02	0.96-1.09	0.465	871

N = 970 *Adjusted for gender, maternal age, smoking during pregnancy, maternal asthma, maternal rhinitis, paternal asthma, maternal education and birth order.
Relative risks were calculated as change in risk per SD change in size or growth measurement.

Table 18 Relative risks for the association between growth and whether the child had ever wheezed by age 3 years but was not atopic, compared with children who had never wheezed and were not atopic

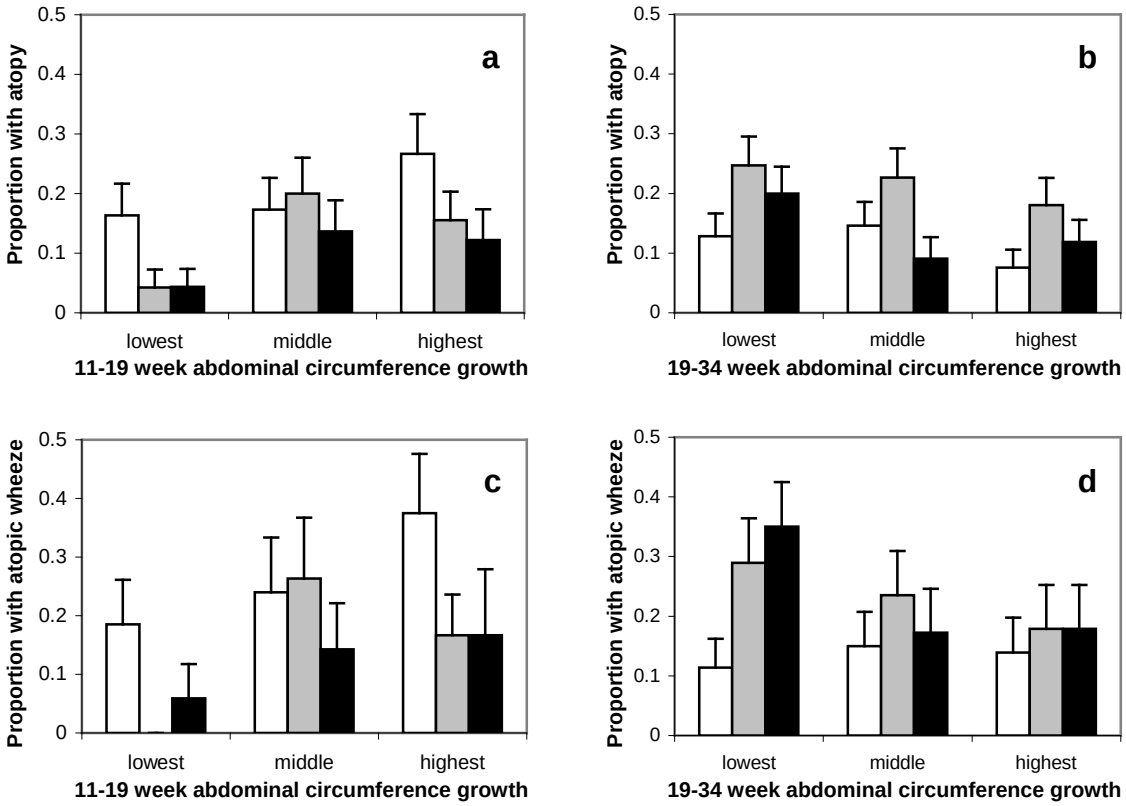
4.4.2.4 Simultaneous analysis and interaction between early and late prenatal and postnatal growth

The conditional measures of growth velocity were calculated to ensure that they were independent of initial size. Perhaps as a result of this, the relative risk ratios for atopy, atopic wheeze and non-atopic wheeze associated with measures of conditional prenatal abdominal growth and postnatal adiposity gain changed little after simultaneous inclusion of these growth measures in a multivariate analysis.

Formal testing showed no significant linear interactions between conditional 11-19 and 19-34 week abdominal growth velocities or between 19-34 week abdominal growth and birth to 6 months adiposity gain in their predictions of outcomes. However, grouping of subjects into tertiles of growth velocity over the different time periods revealed that the prevalences of childhood atopy and atopic wheeze were 27% and 38%, respectively, in those who had above average abdominal growth velocity in early pregnancy followed by growth faltering and below average abdominal growth velocity in late pregnancy (Figure 23 a,c). Comparable prevalences of childhood atopy and atopic wheeze in those who had below average abdominal growth velocity in early pregnancy and above average abdominal growth velocity in late pregnancy were 4% and 6%, respectively (Figure 23 a,c). The association of postnatal adiposity gain with atopic wheeze was strongest in those who had below average late pregnancy abdominal growth (Figure 23 d); the prevalence was 35% in those who had below average late pregnancy abdominal growth velocity and above average adiposity gain from birth to 6 months, as compared with 14% in those who had above average late pregnancy abdominal growth velocity and below average early infancy adiposity gain (Figure 23 d).

The association of postnatal adiposity gain with non-atopic wheeze was strongest in those with below average 34-week head circumference (Figure 24 a); the prevalence was 68% in those who had below average 34-week head circumference and above average adiposity gain from birth to 6 months, as compared with 41% in those who had above average 34 week head circumference and below average early infancy adiposity gain

Figure 23 Interactions between early and late pregnancy abdominal circumference growth and between late pregnancy faltering of abdominal circumference growth and adiposity gain between birth and 6 months

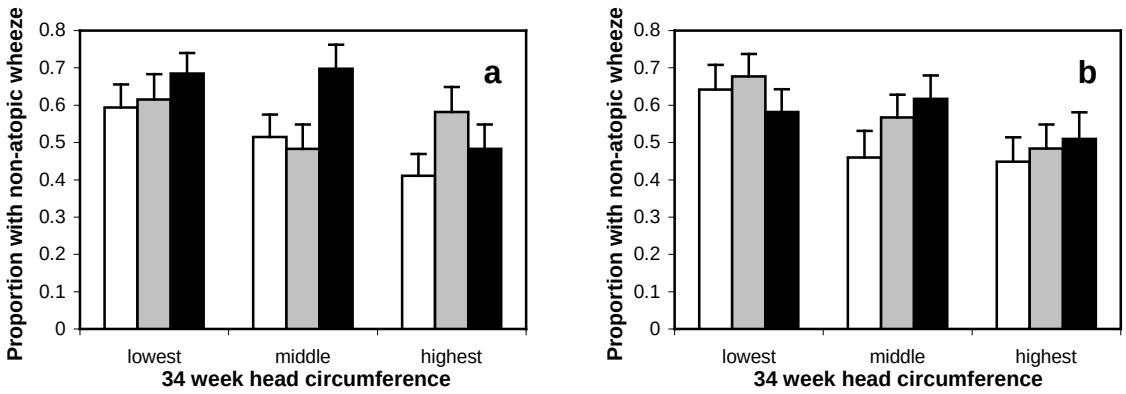


□ Lowest abdominal circumference growth 19-43 weeks
■ Middle abdominal circumference growth 19-43 weeks
■ Highest abdominal circumference growth 19-43 weeks

□ Lowest adiposity gain birth-6 months
■ Middle adiposity gain birth-6 months
■ Highest adiposity gain birth-6 months

Error bars represent standard error for proportion with each outcome

Figure 24 Interactions between 34 week head circumference growth and adiposity gain between birth and 6 months and between 6 and 12 month



□ Lowest adiposity gain birth-6 months
■ Middle adiposity gain birth-6 months
■ Highest adiposity gain birth-6 months

□ Lowest adiposity gain 6-12 months
■ Middle adiposity gain 6-12 months
■ Highest adiposity gain 6-12 months

Error bars represent standard error for proportion with each outcome

4.4.2.5 Atopy in the absence of wheeze

This outcome was not associated with measures of either fetal or birth size (Table 19). The only measure of conditional fetal growth significantly associated with atopy in the absence of wheeze was 11-19 week head circumference growth velocity (47% decrease in risk per SD increase p=0.022), and there were no significant associations with conditional infant growth (Table 19).

4.4.2.6 Wheezing in early childhood and doctor-diagnosed asthma

Seven percent of the children had received a diagnosis of asthma from a doctor and, of these children, 30% were atopic. The risk of doctor-diagnosed asthma was not related to early pregnancy abdominal growth velocity, but, similar to the pattern seen for atopic wheeze, the risk tended to increase with a lower fetal abdominal growth velocity from 19-34 weeks, and with greater infant conditional growth in weight and adiposity (Table 20). Doctor-diagnosed asthma was also associated with smaller late gestation abdominal circumference and birthweight. Grouping together those with atopic or non-atopic wheeze, wheezing in early childhood had no significant associations with fetal and birth measurements (Table 21), but was associated with greater weight and adiposity gain both between birth and 6 months (both 5% per SD increase, p=0.020 and 0.017, respectively) and from 6 to12 months (6% per SD increase in weight, p=0.041 and 7% per SD increase in subscapular skinfold thickness, (p=0.001)).

	Unadjusted analyses				Adjusted* analyses			
	RR	**(95% CI)**	**P-value**	**n**	**RR**	**(95% CI)**	**P-value**	**n**
Fetal size variable								
11 weeks' gestation								
Crown-rump length	0.79	0.59-1.05	0.102	271	0.88	0.64-1.21	0.430	241
Head circumference	0.92	0.62-1.35	0.664	196	1.12	0.72-1.73	0.626	172
Abdominal circumference	0.72	0.48-1.08	0.114	187	0.84	0.54-1.31	0.443	162
19 weeks' gestation								
Femur length	1.03	0.81-1.33	0.793	306	1.02	0.76-1.35	0.918	272
Head circumference	0.84	0.64-1.11	0.225	305	0.89	0.64-1.23	0.478	271
Abdominal circumference	0.90	0.68-1.19	0.456	304	0.86	0.63-1.18	0.354	270
34 weeks' gestation								
Femur length	0.99	0.77-1.27	0.945	304	0.96	0.70-1.32	0.812	272
Head circumference	0.75	0.57-0.99	0.040	293	0.81	0.60-1.11	0.191	261
Abdominal circumference	0.83	0.63-1.11	0.204	304	0.79	0.60-1.07	0.126	272
Birth size variables								
Crown-heel length	1.28	1.03-1.58	0.028	468	1.26	1.00-1.58	0.052	409
Weight	1.10	0.88-1.39	0.399	479	1.13	0.88-1.45	0.349	419
Head circumference	0.99	0.80-1.24	0.931	478	1.05	0.81-1.37	0.690	418
Abdominal circumference	1.10	0.86-1.41	0.441	478	1.14	0.87-1.49	0.354	418
Subscapular skinfold	1.01	0.80-1.28	0.907	477	0.95	0.73-1.23	0.675	418
Conditional fetal growth								
11-19 weeks								
Head circumference	0.66	0.40-1.07	0.90	195	0.53	0.30-0.91	0.022	171
Abdominal circumference	1.29	0.87-1.89	0.203	186	1.26	0.83-1.94	0.280	161
19-34 weeks								
Femur length	0.95	0.74-1.21	0.660	302	0.94	0.70-1.27	0.696	270
Head circumference	0.82	0.63-1.06	0.123	290	0.90	0.67-1.20	0.471	258
Abdominal circumference	0.88	0.69-1.13	0.304	300	0.86	0.65-1.14	0.286	268
Conditional Infant growth								
0 – 6 months								
Length	0.86	0.70-1.06	0.170	464	0.92	0.73-1.15	0.470	405
Weight	1.03	0.84-1.26	0.798	476	1.02	0.82-1.27	0.860	416
Subscapular skinfolds	1.10	0.89-1.37	0.377	475	0.99	0.79-1.24	0.930	416
6 – 12 months								
Length	1.12	0.86-1.44	0.402	444	1.01	0.75-1.37	0.937	388
Weight	0.86	0.58-1.28	0.462	450	0.88	0.56-1.39	0.577	313
Subscapular skinfolds	0.78	0.66-1.01	0.055	447	0.83	0.63-1.00	0.159	391

N = 482 *Adjusted for maternal eczema, maternal atopy, maternal education and birth order.
Relative risks were calculated as change in risk per SD change in size or growth measurement.

Table 19 Relative risks for the association between growth and whether the child had never wheezed by age 3 years but was atopic, compared with children who had never wheezed and were not atopic

	Unadjusted analyses				Adjusted* analyses			
	RR	(95% CI)	P-value	n	RR	(95% CI)	P-value	n
Fetal size variable								
11 weeks' gestation								
Crown-rump length	0.80	0.60-1.07	0.140	827	0.89	0.65-1.21	0.461	802
Head circumference	0.80	0.56-1.15	0.232	611	0.90	0.58-1.39	0.640	588
Abdominal circumference	0.90	0.62-1.30	0.570	573	0.96	0.63-1.48	0.866	553
19 weeks' gestation								
Femur length	0.80	0.62-1.03	0.084	941	0.87	0.67-1.12	0.288	914
Head circumference	0.87	0.67-1.15	0.333	940	0.92	0.69-1.21	0.532	875
Abdominal circumference	0.86	0.65-1.14	0.288	935	0.88	0.64-1.19	0.398	902
34 weeks' gestation								
Femur length	0.88	0.70-1.12	0.314	941	1.01	0.80-1.27	0.940	914
Head circumference	0.83	0.64-1.07	0.141	901	0.83	0.64-1.07	0.148	875
Abdominal circumference	0.76	0.60-0.95	0.016	941	0.74	0.57-0.95	0.018	914
Birth size variables								
Crown-heel length	0.88	0.74-1.04	0.130	1499	0.87	0.74-1.01	0.082	1432
Weight	0.83	0.69-1.01	0.061	1528	0.79	0.66-0.95	0.012	1459
Head circumference	1.01	0.84-1.21	0.942	1523	0.90	0.75-1.07	0.225	1454
Abdominal circumference	0.82	0.69-0.98	0.027	1523	0.80	0.67-0.94	0.009	1454
Subscapular skinfold	0.99	0.80-1.23	0.940	1522	1.00	0.80-1.24	0.972	1453
Conditional fetal growth								
11-19 weeks								
Head circumference	1.09	0.74-1.61	0.665	608	1.10	0.71-1.70	0.670	586
Abdominal circumference	1.20	0.88-1.64	0.249	570	1.11	0.81-1.54	0.511	551
19-34 weeks								
Femur length	1.01	0.80-1.28	0.935	931	1.10	0.87-1.40	0.411	904
Head circumference	0.88	0.70-1.12	0.298	892	0.88	0.68-1.12	0.292	866
Abdominal circumference	0.83	0.66-1.06	0.131	925	0.80	0.62-1.03	0.081	898
Conditional Infant growth								
0 – 6 months								
Length	1.04	0.87-1.25	0.666	1468	0.93	0.78-1.12	0.449	1402
Weight	1.16	0.99-1.37	0.073	1505	1.03	0.88-1.21	0.685	1437
Subscapular skinfolds	1.18	0.98-1.41	0.079	1497	1.14	0.93-1.39	0.199	1429
6 – 12 months								
Length	1.11	0.90-1.37	0.319	1361	1.12	0.89-1.41	0.322	1301
Weight	1.18	0.93-1.50	0.169	1388	1.21	0.97-1.51	0.092	1327
Subscapular skinfolds	1.15	0.96-1.37	0.136	1373	1.14	0.95-1.37	0.152	1311

N = 1545 *Adjusted for gender, maternal age, smoking during pregnancy, maternal asthma, paternal asthma, maternal education and birth order
Relative risks were calculated as change in risk per SD change in size or growth measurement.

Table 20 Relative risks for the association between growth and doctor-diagnosed asthma by age 3 years, compared with not having received such a diagnosis

	Unadjusted analyses				Adjusted* analyses			
	RR	**(95% CI)**	**P-value**	**n**	**RR**	**(95% CI)**	**P-value**	**n**
Fetal size variable								
11 weeks' gestation								
Crown-rump length	0.97	0.92-1.03	0.384	815	0.98	0.92-1.05	0.576	815
Head circumference	0.99	0.92-1.05	0.679	601	0.98	0.91-1.05	0.593	601
Abdominal circumference	1.00	0.93-1.08	0.956	565	1.00	0.93-1.09	0.914	565
19 weeks' gestation								
Femur length	0.98	0.93-1.04	0.585	926	0.99	0.93-1.05	0.722	926
Head circumference	0.99	0.94-1.05	0.842	924	0.97	0.91-1.03	0.356	924
Abdominal circumference	0.99	0.93-1.05	0.687	920	0.97	0.91-1.04	0.419	920
34 weeks' gestation								
Femur length	0.96	0.90-1.01	0.106	927	0.97	0.92-1.03	0.298	927
Head circumference	0.99	0.93-1.04	0.642	887	0.97	0.92-1.03	0.370	887
Abdominal circumference	1.03	0.97-1.09	0.294	927	1.03	0.97-1.09	0.316	927
Birth size variables								
Crown-heel length	0.96	0.92-1.00	0.061	1479	0.96	0.92-1.01	0.088	1479
Weight	0.97	0.93-1.01	0.197	1506	0.97	0.93-1.02	0.206	1506
Head circumference	0.99	0.95-1.04	0.738	1503	0.98	0.93-1.02	0.322	1503
Abdominal circumference	0.98	0.94-1.02	0.337	1503	0.98	0.94-1.02	0.333	1503
Subscapular skinfold	1.01	0.97-1.06	0.498	1502	1.03	0.98-1.07	0.227	1502
Conditional fetal growth								
11-19 weeks								
Head circumference	0.98	0.91-1.06	0.574	597	0.94	0.87-1.02	0.155	597
Abdominal circumference	0.98	0.89-1.07	0.611	562	0.95	0.87-1.04	0.285	562
19-34 weeks								
Femur length	0.96	0.91-1.01	0.144	917	0.97	0.92-1.03	0.347	917
Head circumference	0.98	0.93-1.04	0.598	877	0.98	0.93-1.04	0.572	877
Abdominal circumference	1.04	0.99-1.10	0.129	911	1.04	0.99-1.10	0.092	911
Conditional Infant growth								
0 – 6 months								
Length	1.01	0.97-1.05	0.574	1450	0.98	0.94-1.03	0.409	1450
Weight	1.08	1.03-1.12	<0.001	1485	1.05	1.01-1.09	0.020	1485
Subscapular skinfolds	1.06	1.02-1.10	0.003	1480	1.05	1.01-1.10	0.017	1480
6 – 12 months								
Length	0.97	0.93-1.02	0.307	1346	0.98	0.93-1.04	0.579	1346
Weight	1.04	0.98-1.09	0.190	1372	1.06	1.00-1.12	0.041	1372
Subscapular skinfolds	1.06	1.01-1.10	0.010	1368	1.07	1.03-1.12	0.001	1368

N = 1522 *Adjusted for gender, smoking during pregnancy, age last breastfed, maternal asthma, maternal rhinitis, paternal asthma, maternal education and birth order.
Relative risks were calculated as change in risk per SD change in size or growth measurement.

Table 21 Relative risks for the association between growth and whether the child had ever wheezed by age 3 years, compared with never having wheezed

4.5 SUMMARY DISCUSSION

4.5.1 Principal findings

- Rapid early fetal growth followed by growth faltering was associated with atopy at 3 years of age.
- Lower fetal growth trajectory was associated with non-atopic wheeze.
- Postnatal adiposity gain was associated with both types of wheeze.

Wheezing and atopic disorders have overlapping and mixed phenotypes such that any associations between pre- and postnatal growth and the study outcomes are likely to be complex. Despite this, this analysis has provided data consistent with the initial hypothesis that factors which promote adaptive change in the relative growth of body tissues might have functional consequences for the respiratory and immune systems in later life.

In response to an impaired intrauterine environment, so long as this is not prolonged or extreme, head circumference is preserved at the expense of abdominal growth (Rudolph 1984). If placental nutrient transfer, for example, is impaired in late gestation, the fetus may adapt by sacrificing fat accretion and abdominal visceral growth in order to maintain growth of the brain. This results in a pattern of asymmetrical growth restriction which is particularly marked in those fetuses initially following a rapid growth trajectory (Campbell & Thoms 1977). Serial measurements of head and abdominal circumferences permit cautious identification of those fetuses whose growth trajectories may have been affected by an impaired intrauterine environment and allow investigation of the respiratory and immune outcomes associated with such growth impairment.

4.5.2 Prenatal growth and wheeze and atopic outcomes

Rapid growth in early pregnancy followed by growth faltering in late pregnancy was associated with atopy at 3 years. This suggests atopic development is influenced by the prenatal environment and that it is particularly sensitive to factors causing growth restriction beyond 19 weeks' gestation.

The atopic wheeze outcome was also significantly associated with faltering of abdominal circumference, although non-atopic wheeze was not. This may reflect a greater

sensitivity of immune than respiratory development to late gestation growth faltering. Alternatively, the atopic wheeze phenotype may be more homogeneous and closely linked to common mechanistically important events in early life than non-atopic wheeze which is a prevalent, often transient, condition. As the majority of infants and young children may experience transient wheeze often in association with viral infection this outcome may not discriminate between those with and without a predisposition to wheeze due to prenatal events. The most vulnerable infants are likely to be those with the smallest airways.

Non-atopic wheeze was associated with a pattern of slower head circumference growth between 11 and 19 weeks' gestation. There was a non-significant association between the risk of non-atopic wheeze and smaller fetal and birth measurements. An increased relative risk appeared to be associated with slower growth throughout prenatal life. Although those children who developed non-atopic wheeze were not significantly smaller at birth than non-atopic children who did not wheeze, slowed growth in early gestation may be mechanistically linked to later wheeze susceptibility. For example, slower head circumference growth might indicate the occurrence of early intrauterine stress. This, in turn, may cause disproportionate slowing of lung compared to somatic growth or alterations in respiratory mechanics that predispose to airway narrowing and wheeze during viral respiratory infections.

4.5.3 Postnatal growth and wheeze and atopic outcomes

The relative risk for atopy was not significantly affected by postnatal growth velocity parameters. In contrast, increased weight and adiposity gain in the first year of life were observed to increase the relative risk of wheezing before 3 years of age. Similar associations were seen for both non-atopic and atopic wheeze, although the effect size was greatest with atopic wheeze.

It is possible that above average postnatal weight gain is associated with atopic wheeze risk because increased postnatal weight gain is a consequence of late pregnancy growth faltering which we have demonstrated to be associated with atopy. Unless exposed to severe or prolonged restriction in nutrient supply *in utero*, fetuses exposed to growth restriction late in gestation tend to 'compensate' by increased postnatal weight gain (Healy *et al.* 1956; Tanner 1981). Alternatively, above average postnatal growth, rather

than serving as a marker for intrauterine growth restriction, may itself impair lung development. Indeed, this is supported by the association between non-atopic wheeze and increased postnatal adiposity gain.

4.5.4 Strengths and limitations of this study

A strength of this study is the level of detail regarding patterns of growth afforded by sequential ultrasound scans. Moreover, USS measurements were available for a sample of in excess of 1500 children representative of the general population. The principal limitations of this analysis are due to its observational nature. The influences of pre- and postnatal growth upon respiratory and allergic disease cannot be reliably distinguished in the absence of intervention-based data.

4.5.5 Main conclusions and further work

In conclusion, a pattern of rapid early prenatal growth followed by growth faltering was found to be associated with later atopy. This suggests immune development is sensitive to fetal programming by the prenatal environment. A second association was found between postnatal adiposity gain and wheezing disorders. This association may also exist because of impaired fetal growth which predisposes to 'catch up' growth. It is also possible that postnatal growth exerts an effect independent from that that occurs as a consequence of growth restriction. Smaller fetal size and slower prenatal growth are associated with non-atopic wheeze; this might reflect smaller airway size. This data corroborates previous studies of birth anthropometry which suggested an association can be found between factors exerting an early influence upon growth and later development of wheeze and atopy. The remainder of this thesis will explore whether maternal nutrition might be an important factor in this respect.

Chapter Five

Characterisation of children and mothers in the 6-year follow-up

This chapter characterises in detail the mothers and children who participated in the 6-year follow-up. An overview of the findings can be found in the text, whilst the details are contained in the tables. Data were collected from children who were aged between their sixth and seventh birthdays at the time of invitation to receive a home visit. Data from those children born at a gestation greater than or equal to 35 weeks were included in the analysis and children who were of equivalent gestation and date of birth but were not seen at a home visit were considered as a comparison group. According to these criteria a total of 889 children were eligible for assessment during the study period. Owing to difficulties with contacting the children's mothers, refusals and non-attendance, the number visited at home was 469 (53%), and 290 (62%) of these children attended the clinic for detailed respiratory assessment.

5.1 MATERNAL CHARACTERISTICS

Data on 469 mother-child pairs were available. The mean (SD) age of the mothers was 30.1 years (3.7 years) and 44.8% were in their first pregnancy. All mothers were interviewed at least once during pregnancy; early and late pregnancy interview data were not available for three and ten subjects, respectively. Classification according to education was possible for all mothers and 453 women could be classified according to occupationally defined social class. These data are summarised in Table 22.

Characteristic			**n**
Age, years (mean (SD))		30.1 (3.7)	469
Percentage primiparous		44.8	469
Social Class	I	22 (4.9)	453
(number (%))	II	181 (40.0)	
	IIIn	166 (36.6)	
	IIIm	30 (6.6)	
	IV	48 (10.6)	
	V	6 (1.3)	
Education	None	8 (1.7)	469
(number (%))	CSE	50 (10.7)	
	O Level	147 (31.3)	
	A Level	118 (25.2)	
	HND	36 (7.7)	
	Degree	110 (23.5)	

Table 22 Maternal demographics, social class and education

5.1.1 Anthropometry

Maternal anthropometric measures before, and in early and late pregnancy are summarised in Table 23. Height and pre-pregnancy percentage and total fat mass were normally distributed, whilst weight, BMI, and arm muscle area were positively skewed. Maternal percentage body fat derived from skinfold measurements showed significant increases with each successive time point and arm muscle area also increased from before to early and from early to late pregnancy (paired t-tests, p values <0.05).

Characteristic		n
Height, cm (mean, SD)	163.4 (7.0)	466
Pre-pregnancy		
Weight, kg (median, IQR)	65.3 (58.2 - 73.3)	465
BMI, kg/m^2 (median, IQR)	24.3 (22.0 - 27.5)	465
Total fat mass % (mean, SD)	31.3 (5.9)	466
Arm muscle area, cm^2 (median, IQR)	33.4 (28.2 - 38.8)	465
Early pregnancy		
Total fat mass % (mean, SD)	32.0 (5.5)	381
Arm muscle area,cm^2 (median, IQR)	33.5 (29.3 - 39.8)	380
Late pregnancy		
Total fat mass % (mean, SD)	34.1 (4.9)	459
Arm muscle area, cm^2 (median, IQR)	34.5 (30.4 - 40.7)	459

Table 23 Maternal anthropometry before, and in early and late pregnancy

5.1.2 Smoking and diet

One hundred and nine mothers (23.2%, n=469) reported smoking before pregnancy. In early and late pregnancy 14.5% (n=462) and 13.7% (n=459) reported smoking respectively. There were statistically significant associations between smoking habits and both maternal social class and education (Tables 24 & 25).

Pre-pregnancy smoking	Maternal social class					
	I	II	IIIn	IIIm	IV & V	Total
No	22	153	120	20	35	350
%	100	84.5	72.3	66.7	64.8	77.3
Yes	0	28	46	10	19	103
%	0	15.5	27.7	33.3	35.2	22.7
Total	22	181	166	30	35.2	453
%	100	100	100	100	100	100
					(χ^2 for trend, p< 0.001)	

Table 24 Maternal pre-pregnancy smoking according to social class

Pre-pregnancy smoking	Maternal education					
	Degree	HND	A level	O Level	CSE or None	Total
No	102	31	91	98	38	360
%	92.7	86.1	77.1	66.7	65.5	76.8
Yes	8	5	27	49	20	109
%	7.3	13.9	22.9	33.3	34.5	23.2
Total	110	36	118	147	58	469
%	100	100	100	100	100	100

(χ^2 for trend, p< 0.001)

CSE level educational qualifications and none are combined due low membership of these classes

Table 25 Maternal smoking before pregnancy according to education

Smoking during early pregnancy	Maternal education					
	Degree	HND	A level	O Level	CSE or None	Total
No	105	33	98	109	38	383
%	98.1	94.3	85.2	76.3	69.1	84.2
Yes	2	2	17	34	17	72
%	1.9	5.7	14.8	23.8	30.9	15.8
Total	107	35	115	143	55	455
%	100	100	100	100	100	100

(χ^2 for trend, p< 0.001)

CSE level educational qualifications and none are combined due low membership of these classes

Table 26 Maternal smoking in early pregnancy according to education

At the initial pre-pregnancy interview 35.2% of women in social class IV or V reported current smoking compared to none in social class I and 15.5% in social class II. A similar pattern was seen in early and in late pregnancy. Compared to those women with higher educational qualifications, a greater proportion of women with no educational qualifications smoked before pregnancy and during early and late pregnancy. When the analysis was restricted to those mothers assessed at all time points, of those who were smoking before pregnancy, 41.5% had given up by early pregnancy (Table 27) and 45.3% by late pregnancy (Table 28). Of those mothers who smoked in early pregnancy 87.9% were still smoking in late gestation (Table 29).

Pre-pregnancy smoking	**Early pregnancy smoking**		
	No	Yes	Total
No	342	4	346
%	98.8	1.2	100
Yes	44	62	106
%	41.5	58.5	100
Total	386	66	452
%	85.4	14.6	100

Table 27 Maternal smoking before and in early pregnancy

Pre-pregnancy smoking	**Late pregnancy smoking**		
	No	Yes	Total
No	343	3	346
%	99.1	0.9	100
Yes	48	58	106
%	45.3	54.7	100
Total	391	61	452
%	86.5	13.5	100

Table 28 Maternal smoking before and in late pregnancy

Early pregnancy smoking	**Late pregnancy smoking**		
	No	Yes	Total
No	383	3	386
%	99.2	0.8	100
Yes	8	58	66
%	12.1	87.9	100
Total	391	61	452
%	86.5	13.5	100

Table 29 Maternal smoking in early and in late pregnancy

Maternal FFQ-derived intakes of selected micronutrients are summarised in Table 30. FFQ data are not considered as accurate as data collected from weighed food diaries, but FFQ data can be used to rank individuals according to their relative intake. Nutrient intakes calculated from the FFQ data were comparable with expected ranges, suggesting that this method is reasonably accurate (Robinson *et al.* 1996). For vitamins A, C and E the median and lower quartile values of total intake were above the minimum daily recommendations. Many women did not receive the minimum daily recommended amount of vitamin D from their diet; this is not unexpected as ordinarily with adequate sun exposure 95% of vitamin D is derived from photosynthesis in the skin (Holick 2003). The main determinants of vitamin D synthesis in the skin are duration and extent of skin exposure, latitude and season. In Northern latitudes there is seasonal variation in levels of serum vitamin D, with peaks over the summer months and a trough in the winter. Deficiency is most likely in those with pigmented skin and limited sun exposure during the summer which may prevent adequate synthesis of vitamin D and subsequent storage in fat for the winter months (Holick 2004).

Vitamin		**Recommended intake (Food Standards Agency)**	
		Minimum	**Maximum**
Vitamin A μg/dy retinol equivalents (median, IQR)	1005 (747 - 1416)	600	1500
Vitamin C mg/dy (median, IQR)	130.2 (93.1 - 187.4)	40	1000
Vitamin D μg/dy (median, IQR)	3.6 (2.5 - 5.5)	10*	25
Vitamin E mg/dy(median, IQR)	12.3 (9.2 - 17.5)	3	540

***Vitamin D supplementation recommended if pregnant, dark skinned, rarely exposed to sunlight or eat no meat or oily fish**

Table 30 Maternal diet before pregnancy compared to intakes recommended by the Food Standards Agency

Although the majority of women reported total vitamin intakes within nationally recommended ranges, sufficient variation was demonstrated between individual women to explore the relationship between maternal diet and child respiratory health across a range of values for each nutritional variable.

Dietary habits changed throughout pregnancy in a manner broadly consistent with current dietary recommendations for pregnant women. Pregnant women are advised to consume a variety of fruits and vegetables and to maintain adequate intakes of iron and

folic acid whilst avoiding high intakes of vitamin A (Food Standards Agency 2009). Table 31 illustrates that less women consumed high total intakes of vitamin A during pregnancy than before pregnancy. Tables 32 & 33 show that total intakes of vitamin C and D increased during pregnancy. Table 34 contains data relating to vitamin E intake which suggest that the interindividual variation in total intake of this vitamin reduced during pregnancy.

Total vitamin A intake μg/dy	Pre-pregnancy	Early Pregnancy	Late Pregnancy
		Frequency (%)	
0 - 747	117 (25.0)	148 (38.9)	131 (28.5)
747 -1005	118 (25.2)	107 (28.1)	158 (34.4)
1005 - 1416	116 (24.7)	89 (23.4)	134 (29.2)
> 1416	118 (25.2)	37 (9.7)	36 (7.8)
	(paired t-test comparing geometric mean intake before and in early pregnancy) (p<0.001)		

Table 31 Total maternal vitamin A intake before, and in early and late pregnancy according to quartile of pre-pregnancy intake

Total vitamin C intake mg/dy	Pre-pregnancy	Early Pregnancy	Late Pregnancy
		Frequency (%)	
0 - 93	117 (25.0)	72 (18.9)	84 (18.3)
93 -130	115 (24.5)	78 (20.5)	112 (24.4)
130 - 187	118 (25.2)	107 (28.1)	106 (33.1)
> 187	119 (25.4)	124 (32.6)	157 (34.2)
	(paired t-test comparing geometric mean intake before and in early pregnancy) (p=0.005)		

Table 32 Total maternal vitamin C intake before, and in early and late pregnancy according to quartile of pre-pregnancy intake

Total vitamin D intake μg/dy	Pre-pregnancy	Early Pregnancy	Late Pregnancy
		Frequency (%)	
0 – 2.5	111 (23.7)	84 (22.1)	101 (22.0)
2.5 – 3.6	119 (25.4)	77 (20.2)	110 (24.0)
3.6 – 5.5	122 (26.0)	106 (27.8)	135 (29.4)
> 5.5	117 (25.0)	114 (30.0)	113 (24.6)
	(paired t-test comparing geometric mean intake before and in early pregnancy) (p=0.007)		

Table 33 Total maternal vitamin D intake before, and in early and late pregnancy according to quartile of pre-pregnancy intake

Total vitamin E intake mg/dy	Pre-pregnancy	Early Pregnancy	Late Pregnancy
		Frequency (%)	
0 – 9	113 (24.1)	81 (21.3)	87 (19.0)
9 -12	112 (23.9)	106 (27.8)	130 (28.3)
12 – 17.5	127 (27.1)	106 (27.8)	160 (34.9)
> 17.5	117 (25.0)	88 (23.1)	82 (17.9)
	(paired t-test comparing geometric mean intake before and in early pregnancy) (p=0.5)		

Table 34 Total maternal vitamin E intake before, and in early and late pregnancy according to quartile of pre-pregnancy intake

Pre-pregnancy diet varied according maternal education and social class (Table 36). The most striking effect was that of maternal education upon prudent diet score; maternal education was significantly correlated with prudent diet before pregnancy and in early and late pregnancy ($p<0.001$). Women of higher educational achievement tended to eat more citrus fruit and to have higher intakes of vitamins C and D than women with lower or no education qualifications, both before and during pregnancy.

Vitamin supplements were taken by 19.4% of women before pregnancy and by 16.8% and 6.1% of women in early and late pregnancy respectively. Supplementation with vitamins C and E was more common than vitamin A or D supplementation. For vitamin A, supplemental intakes were small in comparison to food-derived intakes whilst, in contrast relatively similar amounts of food- and supplement-derived intakes of vitamin D contributed to total intake (Table 35).

Supplemental vitamin intake	**Pre-pregnancy**	**Early Pregnancy**	**Late Pregnancy**
	Number of women using supplements (%), median intake (IQR)		
Vitamin A	107 (22.8%),	64 (16.8%),	31 (6.8%),
μg/dy	400 (167-799)	267 (169-649)	300 (156-640)
Vitamin C	155 (33%),	140 (36.7%),	99 (21.6%),
mg/dy	59 (23-100)	58 (22-98)	70 (33-100)
Vitamin D	130 (27.7%),	38 (10.0%),	89 (19.4%),
μg/dy	2.62 (1.11-5.00)	3.42 (1.48-8.33)	5.00 (1.94-12.22)
Vitamin E	134 (28.6%),	119 (45.4%),	83 (18.1%),
mg/dy	6.61 (2.78-10.0)	6.67 (3.11-10.0)	8.89 (5.00-10.00)

Table 35 Pattern of vitamin supplementation amongst women before, and in early and late pregnancy

Intake	Maternal education						p-value
	Degree	HND	A level	O level	CSE	None	
Total energy intake, kcal/dy (median)	2127	2226	2118	2147	2184	2224	0.165
Prudent diet score, (standardised z-score)	0.76	0.27	−0.01	−0.26	−0.61	−0.90	<0.001
Citrus fruit, (median consumption per week)	4.6	4.5	4.5	3	2.3	3.0	0.003
Oily fish, (median consumption per week)	0.25	0.50	0.50	0.50	0.18	0.25	0.662
Vitamin A intake, mcg/dy retinol equivalents (median)	1040	1051	1026	978	968	782	0.102
Vitamin C intake, mg/dy (median)	155	123	133	121	107	125	<0.001
Vitamin D intake, μg/dy (median)	4.0	3.9	3.7	3.4	3.0	3.5	0.037
Vitamin E intake, mg/dy (median)	12.7	14.4	12.0	11.8	11.6	14.2	0.155

***Pearson's correlation coefficient where maternal education is coded as a normally distributed continuous variable**

Table 36 Maternal diet before pregnancy according to education

5.1.3 Serum micronutrients

Serum micronutrients were assayed in late pregnancy. The results of these assays are presented in Table 37. The numbers differ across analyses due to differences between the numbers of individual samples that were suitable for each assay.

Nutrient status	Late pregnancy SWS summary statistic	n	Reference range
Vitamin serum concentration			**Analysing laboratory's normal range**
Retinol (Vitamin A), μmol/l (mean, SD)	1.3 (0.3)	370	1.5 - 4.2
Ascorbic acid (Vitamin C), μmol/l (median, IQR)	32.9 (21.1 - 48.2)	332	15 - 90
25(OH) vitamin D, nmol/l (median, IQR)	62.0 (42.0 - 93.0)	319	40 - 195
α-tocopherol (Vitamin E), μmol/l (mean, SD)	35.1 (7.2)	369	11.6 - 37.1
Fatty acids from plasma phospholipid phosphatidylcholine absolute concentration			**Median (IQR)***
Total fatty acids, μg/ml (median, IQR)	1402 (1097 - 1757)	447	1754.9 (1540.3 - 1953.1)
Total omega-3, μg/ml (median, IQR)	71.5 (54.6 - 92.4)	447	87.4 (71.9 -102.8)
Total omega-6, μg/ml (median, IQR)	500.0 (387.4 - 614.2)	447	601.9 (545.6 - 674.6)
Alpha linolenic acid, μg/ml (median, IQR)	3.90 (2.91 - 5.35)	447	3.7 (2.5 - 5.0)
Linoleic acid, μg/ml (median, IQR)	325.6 (257.3 - 403.5)	447	359.7 (310.9 - 410.8)
Arachadonic acid, μg/ml (median, IQR)	105.0 (81.0 - 131.8)	447	146.6 (122.9 - 196.3)
Docosahexaenoic acid μg/ml (median, IQR)	53.5 (40.7 - 68.0)	447	65.2 (54.2 - 77.7)
Eicosapentaenoic acid***,*** μg/ml (median, IQR)	5.08 (3.54 - 7.74)	447	5.2 (3.8 - 7.1)
Fatty acid composition of plasma phospholipid phosphatidylcholine percentage of total fatty acids			**95% CI of the mean†**
Total omega-3, (% of total fatty acids)	5.04 (4.36 - 5.83)	447	4.8 - 6.5
Total omega-6, (% of total fatty acids)	35.46 (34.15 - 36.88)	447	31.6 - 33.9
Alpha linolenic acid, (% of total fatty acids)	0.29 (0.23 - 0.36)	447	0.1 - 0.2
Linoleic acid, (% of total fatty acids)	23.24 (21.68 - 24.57)	447	19.2 - 22.4
Arachadonic acid, (% of total fatty acids)	7.58 (6.71 - 8.46)	447	7.2 - 8.6
Docosahexaenoic acid, (% of total fatty acids)	3.79 (3.23 - 4.41)	447	3.6 - 4.7
Eicosapentaenoic ***acid,*** (% of total fatty acids)	0.37 (0.27 - 0.49)	447	0.4 - 0.7

***(Rum & Hornstra 2002) data from plasma phospholipid at delivery n= 760. †(De, Vriese Sr. *et al.* 2001) data from plasma phospholipids in women of 29 – 36 weeks' gestation n=16**

Table 37 Comparison of participant women's serum micronutrient status with available normal ranges

5.1.4 Comparison of mothers of those children seen at 6 years of age with the mothers of those not seen

Many dietary variables display positive skew and are therefore best summarised by their median value and interquartile range. All skewed data were logarithmically transformed before using t-tests to compare geometric means. The prudent diet score was not skewed as this is a standardised measure. The mean value of close to zero and standard deviation of close to one suggest that the women included in the 6-year follow-up may be considered to follow diets broadly similar to those of the complete SWS cohort in which the prudent diet score was devised and standardised. Comparison of the mothers of the children seen at 6 years with those mothers whose children were born at greater than 35 weeks gestation and of equivalent age but not seen at age six demonstrated significant differences relating only to maternal age, smoking during pregnancy and parity. Table 38 summarises the pre-pregnancy findings in the two groups. Mothers in the comparison group were younger, more likely to smoke and more likely to have other children. There were no differences in pregnancy body composition or dietary measures between the groups.

The mothers of children seen at the clinic stage did not differ significantly from those that only contributed data to the home visit stage on any of the measures listed in Table 39 except vitamin E intake, this was higher in mothers whose children attended the clinic (median 12.5 mg/day vs. 12.0 mg/day (p=0.041)). The level of vitamin E corrected for serum cholesterol did not differ between groups.

5.2 CHARACTERISATION OF CHILD PARTICIPANTS

In total, 469 children contributed data. Fifty four percent of the children were male; the mean age (SD) at the home visit was 6.56 years (0.21 years) and, at the clinic visit, 6.72 years (0.22 years). Two children had data missing from the 2 year follow up and five had no 3-year follow-up data. Not all parents consented to the clinic visit or to all the tests of respiratory function. Moreover, not all of the 6-year-old children were able to master the techniques involved, therefore the data available from the 6-year follow-up comprised a combination of spirometry, fractional exhaled nitric oxide, salbutamol reversibility and methacholine challenge, as shown in Table 39.

		Children seen at 6 years (n=469)		Children not seen at 6 years (n=420)		P-value
Maternal characteristics						
Age at child's birth, mean (SD)		30.1 (3.7)	469	29.6 (3.7)	420	0.045
Primiparous (%)		44.8	469	37.4	420	0.025
Education qualification, (%)	None or CSE	12.4	469	16.7	419	0.082
	O Level	31.3		28.4		
	A Level	25.2		29.8		
	HND	7.7		6.0		
	Degree	23.5		19.1		
Smoking during pregnancy, (%)		15.8	455	25.6	402	<0.001
Maternal asthma, (%)		20.4	466	24.8	411	0.117
Maternal childhood eczema, (%)		16.7	466	18.8	410	0.429
Maternal rhinitis, (%)		43.8	466	40.2	411	0.277
Height, cm (mean, SD)		163.4 (7.0)	466	162.7 (6.1)	418	0.147
Pre-pregnancy weight,kg (median, IQR)		65.3 (58.2 - 73.3)	465	63.2 (57.8 - 71.8)	418	0.256
Pre-pregnancy BMI, kg/m^2 (median, IQR)		24.3 (22.0 - 27.5)	465	23.9 (21.8 - 26.9)	418	0.559
Pre-pregnancy total fat mass % (mean, SD)		31.3 (5.9)	466	30.8 (6.2)	412	0.278
Pregnancy weight gain, kg (mean, SD)		11.9 (5.6)	455	12.0 (5.8)	400	0.851
Pre-pregnancy arm muscle area, cm^2 (median, IQR)		33.4 (28.2 - 38.8)	465	32.5 (28.0 - 38.8)	411	0.763
Total energy intake, kcal (median, IQR)		2147 (1803 - 2541)	469	2206 (1820 - 2671)	419	0.108
Prudent diet score		0.04 (1.02)		0.00 (1.04)	419	0.515
Citrus fruit, (median/week, IQR)		4.5 (1.5 - 7.0)	469	3.0 (0.6 - 7.0)	419	0.464
Oily fish, (median/week, IQR)		0.5 (0.1 - 1.5)	469	0.5 (0.0 - 1.5)	419	0.580
Vitamin A intake, μg retinol equivalents (median, IQR)		1005 (747 - 1416)	469	1025 (786 - 1340)	419	0.253
Vitamin C intake, mg (median, IQR)		130.2 (93.1 -187.4)	469	132.2 (92.3 - 183.7)	419	0.886
Vitamin D intake, μg/dy (median, IQR)		3.6 (2.5 - 5.5)	469	3.7 (2.4 - 5.2)	419	0.349
Vitamin E intake, mg (median, IQR)		12.3 (9.2 - 17.5)	469	12.8 (9.7 - 17.5)	419	0.359
Vitamin E/cholesterol (median, IQR)		5.0 (4.5 - 5.8)	360	5.1 (4.6 - 5.6)	328	0.386
Total antioxidant capacity mmol/l, (mean, SD)		1.37 (0.19)	430	1.35 (0.21)	381	0.355
Paternal characteristics						
Paternal asthma, (%)		17.4	459	16.4	403	0.523
Paternal eczema in childhood, (%)		11.8	431	9.8	386	0.362
Paternal rhinitis, (%)		34.3	432	31.5	390	0.407

Binary outcomes were compared by χ^2 tests and categorical outcomes by Mann Whitney tests. Continuous variables were compared using t-tests. All of the mothers of the 420 children not seen at 6 years supplied pre-pregnancy data.

Table 38 Comparison of the characteristics of the parents of those children seen in the 6-year follow-up with the parents of those children who could not be seen

Investigation	Number completing (%)
Spirometry	310 (66)
Salbutamol reversibility	143 (30)
Methacholine challenge	107 (23)
Skin prick testing	374 (80)
Fractional exhaled nitric oxide	248 (53)

Table 39 Number of children contributing detailed data at 6 years of age

5.2.1 Questionnaire outcomes

Questionnaire data were available for all 469 children participating in the 6-year follow-up. There were no missing data relating to wheeze status. The number of children in each outcome group for wheeze (as defined in chapter two) is shown in Table 40.

Outcome	Frequency (%) N=469
Doctor-diagnosed asthma	67 (14.3)
Current asthma	33 (7.0)
Current wheeze	67 (14.3)
Ever wheezed	156 (33.3)

Table 40 Number of children in each outcome group according to 6-year questionnaire data

Of the children seen at 6 years, 464 had data recorded at 3 years of age. Those children with data recorded during the infant follow-up stage of the survey are characterised in Table 41 according to the time of onset and persistence of their symptoms. The early infancy data enabled classification of children according to wheeze phenotype. The transient wheeze phenotype comprised those who wheezed before 3 years but not at 6 years, whilst those that wheezed before 3 years and at 6 years constituted the persistent wheeze phenotype. A further outcome of late onset wheeze was identified as those children who were reported to wheeze at 6 years but had not experienced wheeze before 3 years of age.

5.2.2 Atopic phenotypes

Skin prick data were available for 374 children of whom 103 (27.5%) proved to be atopic. Table 42 classifies the children seen at 6 years of age according to atopic outcome and information regarding wheeze status. Of those children who had ever

been diagnosed with asthma 43% were atopic at 6 years, whilst of those with wheeze or a diagnosis of asthma within the last 12 months 57% and 60% were atopic respectively.

Outcome	Frequency (%) N=464
Ever wheezed	292 (63.0)
Transient wheeze	226 (48.7)
Persistent wheeze	57 (12.3)
Late onset wheeze	9 (1.9)

Table 41 Number of children in each outcome group according to 3 and 6-year questionnaire data

Outcome	Atopy		Total
	Yes	No	
Transient wheeze	44 (11.9)	137 (37.1)	181 (49.1)
Persistent wheeze	25 (6.8)	22 (6.0)	47 (12.7)
Late onset wheeze	5 (1.4)	0 (0.0)	5 (1.4)
Never wheeze	29 (7.9)	107 (30.0)	136 (36.9)
Total	103 (27.9)	266 (72.1)	369 (100)
(percentage figures) refer to percentage of total n= 369			

Table 42 Number of children in each outcome group according to atopic status

5.2.3 Comparison of those children seen at 6 years of age with those not seen

Children participating in the 6 year follow-up were more likely to have been breast fed and less likely to have been exposed to parental smoking than those children who were not followed up. The children seen at the clinic stage did not differ significantly from those who only contributed data to the home visit stage on any of the measures listed in Table 43 except atopic status at age 3 years. Of the children seen in clinic 21.5% were atopic, compared to 13.0% of those seen only at home (χ^2, p=0.043). It was anticipated that atopy might be more prevalent amongst those seen at the clinic stage as many parents were keen to know the results of skin testing, and for children with a history of significant allergic reactions, this was recommended to be carried out under clinical supervision.

		Children seen at 6 years (n=469)		Children not seen at 6 years (n=420)		P-value
Birth characteristics						
Birthweight (g), mean (SD)		3480 (513)	463	3455 (512)	416	0.473
Gestational age (weeks), mean (SD)		39.9 (1.5)	468	39.9 (1.5)	420	0.652
Completed months of breastfeeding, n (%)*	None	15.6	468	26.2	420	0.002
	< 1	19.9		16.9		
	1 – 3	18.2		18.2		
	4 – 6	17.3		14.9		
	7 – 11	15.2		13.8		
	12 or more	13.9		10.0		
Characteristics at 6 month follow-up*						
Maternal smoking, (%)		17.5	464	26.2	397	0.002
Other smokers in the home, (%)		26.1	459	33.8	394	0.015
Ever wheezed, (%)		24.5	469	26.4	398	0.530
Cat or dog in home, (%)		45.3	468	40.7	398	0.174
Characteristics at 1 year follow-up*						
Wheezed in past 6 months, (%)		32.4	469	32.9	377	0.882
Cat or dog in home, (%)		44.1	469	39.3	377	0.145
Atopy, (%)		11.0	420	12.3	309	0.316
Characteristics at 2 year follow-up*						
Wheezed in past year, (%)		29.4	466	27.6	333	0.585
Characteristics at 3 year follow-up*						
Wheezed in past year, (%)		26.1	464	24.3	280	0.586
Atopy, (%)		18.5	373	17.0	200	0.656
Asthma diagnosed by a doctor, (%)		6.0	464	7.6	278	0.420

Binary outcomes were compared by χ^2 tests and categorical outcomes by Mann Whitney tests. Continuous variables were compared by t-tests. *Of the 420 children not seen at 6 years, 399 were seen at 6 months, 377 at 1 year, 333 at 2 years, and 280 at 3 years.

Table 43 Comparison of the characteristics of those children seen in the 6-year follow-up with those children who could not be seen

5.3 SUMMARY DISCUSSION

The mothers of the 6-year-old children followed up for this thesis were similar to the mothers of those children not followed up at this stage; they were, however, slightly older, less likely to smoke in pregnancy and more likely to be primiparous. The children followed up at 6 years of age did not differ significantly from those not followed up other than in terms of smoke exposure and breastfeeding. The dietary intake and

nutritional status measures of the children's mothers agreed with published norms and displayed sufficient variation to support internal comparison over a range of exposures. Some of the variation in maternal diet and body composition can be explained by education and social class.

Chapter Six

Early life determinants of atopy at age 6 years

6.1 AIM

To test the hypothesis that the development of atopy at 6 years of age is associated with aspects of maternal body composition and maternal diet immediately prior to and during pregnancy.

Specifically, associations were sought between atopy and:-

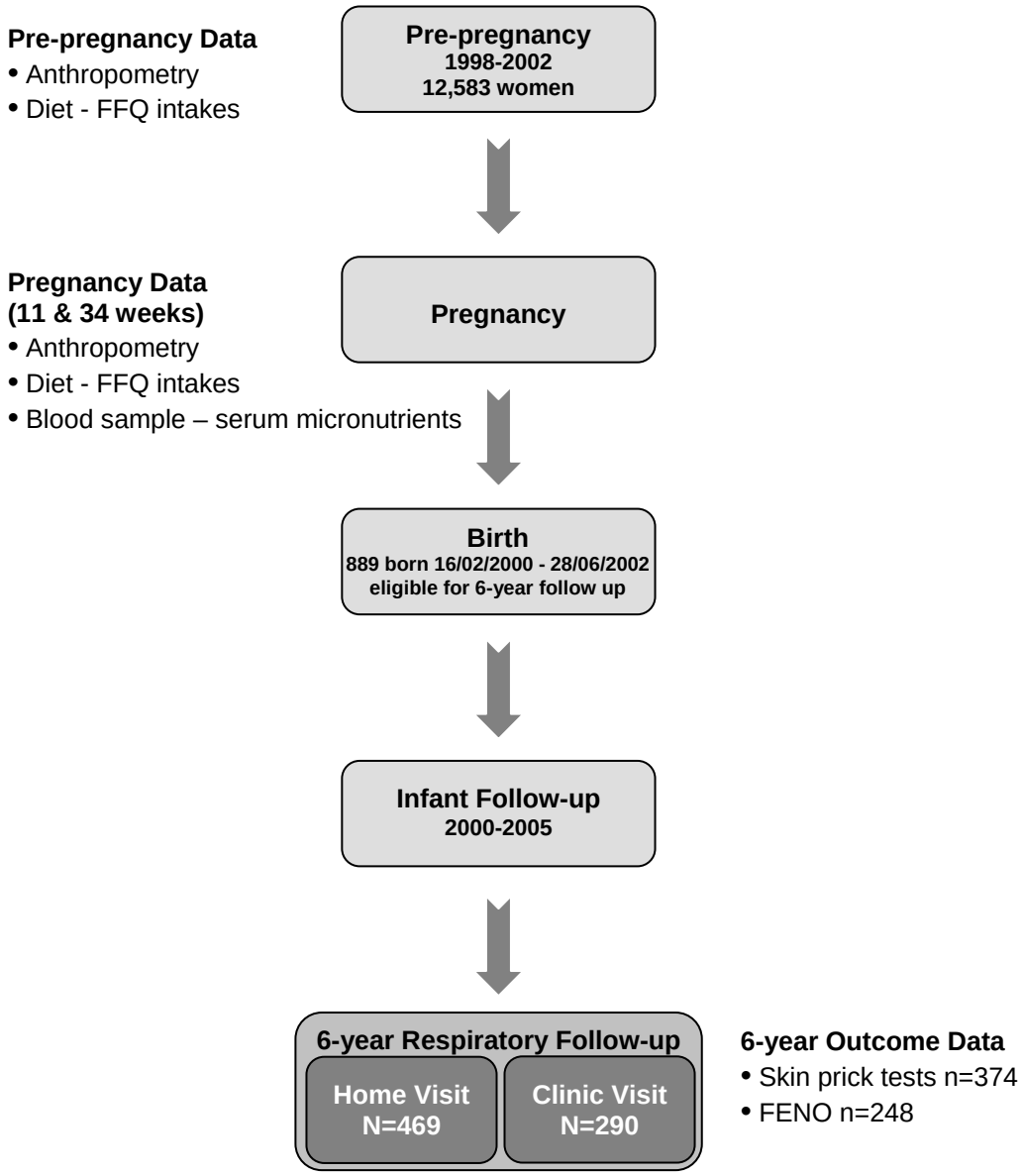
- high maternal fat mass
- high maternal vitamin D status
- low maternal vitamin E intake
- low maternal n-3 PUFA status

6.2 INTRODUCTION

This chapter investigates the hypothesis that maternal nutrition plays a significant role in the development of atopy in childhood. There is evidence that sensitisation may occur early in life and that exposures acting *in utero* may promote sensitisation (Jones *et al.* 1996; Kondo *et al.* 1992). Dietary factors are believed to be important early life exposures (Devereux *et al.* 2002; Martindale *et al.* 2005; Willers *et al.* 2007). Changes in diet at a population level (Department for Environment 2009; Seaton *et al.* 1994) have been observed over a similar time course to changes in the prevalence and incidence of atopy (Anderson *et al.* 2004; Eder *et al.* 2006) and plausible biological mechanisms have been proposed to explain how specific nutrients might influence the developing

immune system (Calder 2006; Cantorna *et al.* 2004; Li-Weber *et al.* 2002). Obesity has also increased in prevalence in recent years and is known to be associated with changes in inflammatory cytokines (Fantuzzi 2005; Madan *et al.* 2009). It is possible that maternal body composition may influence the *in utero* environment in a manner that predisposes to atopy. The hypotheses in this chapter were designed to examine biologically plausible mechanisms and to test the findings of previous studies where associations between nutritional factors and atopic status have been described.

Figure 25 Data collected to explore the relationship between maternal nutrition and atopy in 6-year-old children



6.3 SUMMARY OF METHODS

A detailed explanation of the methods used to collect exposure and outcome data is given in chapter two and the cohort is defined in Figure 25. Mothers of those children who were between their sixth and seventh birthdays during the study period were invited to receive a visit from a research nurse and to attend the clinic. Skin prick testing to common aeroallergens was used to determine the children's atopic status and FENO was measured at the clinic to provide additional information concerning eosinophilic inflammation in the lower airways. The mothers' body composition and nutrition data were drawn from the pre-pregnancy and pregnancy phases of the SWS.

6.4 ANALYSIS

A cohort of eligible children was identified based upon date of birth. It was planned that children born at less than 35 weeks gestation were excluded from tests of association between lung function and maternal nutrition in order to avoid confounding of the respiratory outcomes by the effects of lung disease of prematurity; so, for consistency, children born at less than 35 weeks gestation were also excluded from analyses of atopic outcomes.

Cutaneous sensitivity to any of the allergens tested was considered primary evidence of atopy. Poisson regression was used to test for associations between cutaneous sensitisation and measures of maternal body composition, nutritional intake and serum biomarkers of nutritional status. The relationship between these maternal factors and childhood allergic airway inflammation was also tested using FENO as a continuous outcome in linear regression analysis. FENO and continuous exposure variables were transformed by natural logarithm where necessary to achieve normality. Standard deviation (z-) scores were derived for all continuous exposure variables.

The analyses were adjusted for exposures identified as likely confounders of each outcome. Potential confounders considered are listed in Table 44. The categorical variables of maternal education and parity were converted to approximately normally distributed continuous variables by assigning integer values to the data (Table 45).

The analyses were adjusted for key factors which evidence suggests are associated with the outcome. These variables were maternal education (Heinrich *et al.* 1998b), maternal

atopy (Arshad *et al.* 1993; Kjellman 1977) and child's birth order (Matricardi *et al.* 1998; Strachan 1989a; Strachan *et al.* 1996b). Additional confounders were identified using univariate tests of association. Factors associated with the outcome at a significance level of 10% or less were included in a stepwise forwards selection. Regression models were built including all confounders significantly associated with each outcome in a mutually adjusted logistic regression model ($p<0.05$). Confounders identified in this manner were then combined with those previously selected on grounds of likely biological importance.

Child's Characteristics	Parental Characteristics	Environmental Characteristics
Gender	Maternal age	Others smoking in the home
Birthweight	Maternal education	(when the child was 6 months old)
Gestational age	Maternal smoking in pregnancy	Ownership of a cat or dog
Birth order	Maternal smoking	(during the child's first year of life)
Mode of infant feeding	(when child aged 6 months)	
	Maternal history of asthma	
	Maternal history of rhinitis	
	Maternal eczema in childhood	
	Maternal atopy	
	Paternal history of asthma	
	Paternal history of rhinitis	
	Paternal eczema in childhood	

(These confounders were included in a stepwise selection to create a regression model if associated with the outcome with p<0.10)

Table 44 Potential confounders

Maternal education	Value	Parity	Value
No qualifications	1	Primiparous	0
CSE	2	Mother to one child	1
O Level	3	Mother to two children	2
A Level	4	Mother to three or more children	3
HND	5		
Degree	6		

Table 45 Coding of categorical variables to achieve normally distributed continuous variables

Throughout, a 5% level of statistical significance was used, with no correction for multiple testing, as the associations investigated were based upon *a priori* hypotheses.

6.5 RESULTS

Skin sensitisation to one or more allergens was present in 27.5% of participants. The pattern of sensitisation to specific antigens is shown in Table 46. Fifty-six children were sensitised to a single allergen, 31 to two, 11 to three and 5 to four allergens. Patterns of sensitisation vary geographically and with age; suitable comparison data include those from the UK cohort in the ISAAC asthma and allergy prevalence study and the Isle of Wight 1989 birth cohort. The prevalence of skin sensitisation in these studies was 17.5% amongst 8 to 12-year-olds in the ISAAC study (Weinmayr *et al.* 2007) and 19.6% amongst 4-year-olds in the Isle of Wight cohort (Arshad *et al.* 2001). Both results are broadly consistent with the prevalence of atopy determined in this study. The pattern of sensitisation supports the view that sensitisation to food allergens declines with age and that to aeroallergens increases (Kulig *et al.* 1999) (Table 46). Sensitisation to food allergens, at 3.5%, was greater in the younger Isle of Wight cohort than in this study, whilst sensitisation to aeroallergens, at 19.2%, was less (Arshad *et al.* 2001).

Allergen	Children with positive response N=374, (%)
Cat	24, (6.4)
Dog	6, (1.6)
Grass pollen	64, (17.1)
House dust mite	62, (16.6)
Milk	3, (0.8)
Egg	2, (0.5)
Tree pollen	11, (2.9)

Table 46 Pattern of skin sensitisation in children at age 6 years

Skin sensitisation at 3 years	Skin sensitisation at 6 years		
	Yes	No	Total
Yes	43	14	57
%	75	25	100
No	40	204	244
%	16	84	100
Total	83	218	301
%	28	72	100

Table 47 Relationship between atopic status at 3 and 6 years of age

Skin sensitisation was associated, on univariate analysis at the 10% significance level, with child's gender, smoke exposure during the first 6 months of life, month of birth, and mother's education level, parity and history of atopy, asthma and rhinitis. The associations between atopy and month of birth, smoking exposure and maternal rhinitis became insignificant upon multivariate analysis. The final multivariate model, combined factors identified as potential confounders *a priori* with those selected by stepwise forward selection and included the variables listed in Table 48. The children undergoing skin testing did not differ significantly according to any of the confounding variables from those children who were seen at 6 years of age but not skin prick tested.

Factors identified as potential confounders of the relationship between maternal nutrition and FENO are listed in Table 49. The children who recorded FENO measures did not differ significantly according to any of these confounding variables from those children who were seen at 6 years but in whom FENO was not measured.

Binary variables (χ^2 test)	**Atopic**	**Non-atopic**	**Univariate P-value**	**Final model P-value**
Male gender	63.7%	50.6%	0.023	0.011
Maternal asthma	29.7%	16.7%	0.005	0.027
Maternal atopy	56.7%	44.2%	0.037	0.069
Continuous variables (t-test)	**Atopic**	**Non-atopic**	**Univariate P-value**	**Final model P-value**
Maternal education	4.20	3.89	0.043	0.050
Parity	0.62	0.80	0.060	0.188

Table 48 Univariate analysis of factors considered confounders of the relationship between maternal nutrition and skin sensitisation at age 6 years

Binary variables (t-test)				**Univariate P-value**	**Final P-value**
Male gender	0.125	Female gender	−0.142	0.036	0.014
Maternal eczema	0.317	No maternal eczema	−0.084	0.013	0.001
Maternal atopy	0.099	No maternal atopy	−0.090	0.143	0.200
Categorical variable (oneway ANOVA)				**Univariate P-value**	**Final P-value**
Month of birth				0.059	0.003
Continuous variables Pearson's correlation coefficient				**Univariate P-value**	**Final P-value**
Maternal education			0.041	0.520	0.560
Parity			−0.004	0.948	0.572

Table 49 Univariate analysis of factors considered potential confounders of the relationship between maternal nutritional status and nutrient intake and standardised FENO at 6 years of age

The questionnaire data demonstrated that symptoms potentially associated with atopic disease were highly prevalent in this cohort. It was possible to determine which children were reported to wheeze within the last 12 months and which of these had also received a diagnosis of asthma from a doctor. The questionnaire data did not contain information which could confirm a recent diagnosis of rhinitis, however, although the following question was asked:

- 'In the past 12 months, has your child had a problem with sneezing, or a runny, or blocked nose when he/she did not have a cold or the 'flu?'

Parents were also asked:

- 'In the past twelve months, has your child suffered from an itchy skin condition? Has this skin condition affected the cheeks, the outer arms or legs, or the skin creases?'

The question concerning itchy skin conditions is not wholly specific for atopic eczema and a positive response to this question was not strongly associated with skin sensitisation.

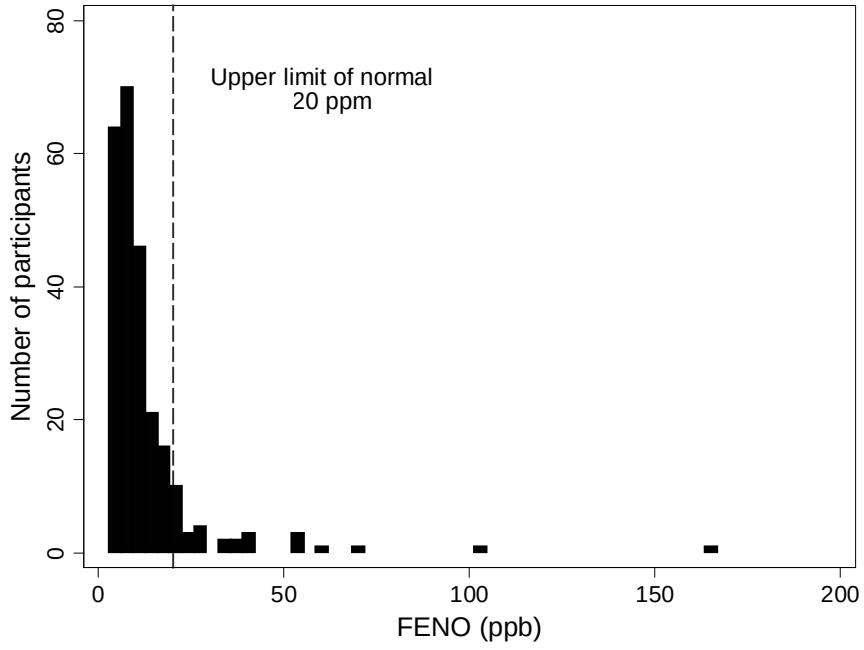
Parents of 205 children returned at least one positive response to any of the questions asking about wheeze, nasal symptoms or itchy skin conditions. Those children who were reported to wheeze or have nasal symptoms were significantly more likely to be cutaneously sensitised than those who were not reported to have symptoms of wheeze or nasal problems (Table 50).

Atopic disease	Frequency, n (%)		χ^2 p-value
	With skin sensitisation	No skin sensitisation	
Wheeze within last year	30/103 (29.1)	23/271 (8.5)	<0.001
Asthma within last year	18/103 (17.5)	12/271 (4.4)	<0.001
Nasal symptoms within last year	49/103 (47.6)	49/271 (18.1)	<0.001
Itchy skin disorder affecting cheeks, outer arms or legs, or skin creases	33/69 (47.8)	43/99 (43.4)	0.574

Table 50 Atopic disease reported at age 6 years

The range of FENO concentrations measured from participants in this study is depicted in Figure 26.

Figure 26 Distribution of FENO values in study cohort



In total FENO readings were recorded from 276 children in this study. The geometric mean (95% CI) FENO reading was 9.6 ppb (8.9 – 10.4 ppb); this is comparable to that

of 6.5 ppb (5.9 – 7.0 ppb) recorded from 167 5-year-old children from the Aberdeen cohort (Devereux *et al.* 2006). Raised FENO is known to be associated with atopic conditions (Malmberg *et al.* 2006). Of the 248 children providing NIOX®/NIOX MINO® data for this thesis, 26 produced values greater than the suggested the upper range of normal for children younger than 12 years of age, (20 ppb) (Aerocrine 2009). These children were significantly more likely than those with FENO less than or equal to 20 ppb to be skin sensitised (χ^2, $p<0.001$), to have wheeze within the last 12 months (χ^2, $p<0.001$), or to have nasal symptoms within the last 12 months (χ^2, $p<0.012$).

6.5.1 Maternal body composition and atopy in the offspring

Primary hypothesis: maternal body fat is positively associated with atopy in the offspring at age 6 years

Rationale: children of obese women are more likely themselves to be obese than children of non-obese women (Whitaker *et al.* 1997). Obesity and atopy may share genetic or environmental links (Weiss 2005). Moreover, maternal obesity may directly increase the risk of atopy in the offspring as endocrine changes associated with obesity may influence the developing fetal immune system (Kawano *et al.* 2005; Vieira *et al.* 2005).

Secondary hypothesis: atopy may be more closely related to a measure of relative fatness such as BMI or percentage body fat, or weight gain in pregnancy or, indeed, atopy may be associated, possibly inversely, with a measure of lean mass.

Rationale: under the predictive adaptive response theory it is proposed that fetal development is sensitive to modification according to the likely postnatal environment. Maternal body composition may serve to transmit a signal to the developing fetus regarding the expected postnatal environment but it is not known exactly which aspect of maternal body composition is important. Maternal body composition may also be a marker for the overall quality of the diet and may be associated with atopy as a consequence of nutritional influences upon immune development (Guo *et al.* 2004).

Results: no significant associations were found between mothers' body composition and atopic outcomes in their children at 6 years of age.

Maternal pre-pregnancy total body fat did not significantly affect the relative risk for atopy defined as at least one positive skin test. Table 51 shows that the relative risk for atopy, so defined, was also unaffected by pre-pregnancy body fat percentage, subscapular to triceps skinfold ratio, BMI or arm muscle area.

There were no significant associations between pregnancy weight gain or any measure of maternal body composition in early or late pregnancy and skin sensitisation, either adjusted or unadjusted (Table 51).

No significant associations were found between any measure of maternal body composition before or during pregnancy and FENO measured at 6 years of age (Table 52).

Maternal body composition z-score	**Unadjusted analyses**			**Adjusted* analyses**		
	RR (95% CI)	**P value**	**n**	**RR (95% CI)**	**P value**	**n**
Pre-pregnancy						
Total body fat (kg)	1.03 (0.86-1.23)	0.751	371	1.05 (0.87-1.25)	0.616	343
Body fat percentage	1.03 (0.86-1.22)	0.779	372	1.04 (0.87-1.23)	0.682	344
Subscapular:triceps	0.93 (0.80-1.09)	0.371	371	0.95 (0.81-1.11)	0.524	343
BMI (kg/m^2)	1.01 (0.84-1.20)	0.942	370	1.03 (0.86-1.24)	0.751	342
Arm muscle area	0.96 (0.81-1.13)	0.592	371	0.99 (0.84-1.17)	0.886	343
During pregnancy						
11 week body fat (kg)	1.05 (0.86-1.29)	0.648	301	1.09 (0.88-1.34)	0.439	282
34 week body fat (kg)	1.03 (0.85-1.24)	0.773	365	1.05 (0.86-1.28)	0.623	340
11 week arm muscle	0.92 (0.75-1.14)	0.436	301	0.94 (0.76-1.16)	0.574	281
34 week arm muscle	0.92 (0.75-1.10)	0.371	365	0.93 (0.78-1.12)	0.446	340
Pregnancy weight gain	0.99 (0.85-1.17)	0.948	362	0.98 (0.84-1.15)	0.806	337

***Adjusted for child's gender, and maternal education, history of asthma, atopic status and parity**
Relative risks were calculated as change in risk per SD change in body composition measurement.

Table 51 Relative risk of skin sensitisation according to maternal pre-pregnancy and pregnancy body composition, and weight gain during pregnancy.

Maternal body composition z-score	Unadjusted analyses Exp Beta (95% CI)	P value	n	Adjusted* analyses ExpBeta (95% CI)	P value	n
Pre-pregnancy						
Total body fat (kg)	0.98 (0.90-1.06)	0.628	246	1.01 (0.93-1.09)	0.887	232
Body fat percentage	0.96 (0.89-1.05)	0.388	246	0.98 (0.90-1.06	0.531	232
Subscapular:triceps	0.94 (0.86-1.02)	0.142	245	0.94 (0.86-1.02)	0.159	231
BMI (kg/m^2)	0.98 (0.90-1.06)	0.548	247	1.02 (0.94-1.10)	0.685	233
Arm muscle area	0.95 (0.88-1.03)	0.206	245	0.99 (0.92-1.07)	0.886	231
During pregnancy						
11 week body fat (kg)	0.99 (0.90-1.09)	0.859	198	1.03 (0.94-1.12)	0.501	188
34 week body fat (kg)	0.98 (0.91-1.07)	0.685	240	1.01 (0.94-1.10)	0.762	233
11 week arm muscle	1.00 (0.91-1.10)	0.942	197	1.03 (0.94-1.12)	0.536	187
34 week arm muscle	0.98 (0.90-1.07)	0.653	246	1.04 (0.96-1.12)	0.381	232
Pregnancy weight gain	0.95 (0.87-1.03)	0.214	245	0.95 (0.88-1.02)	0.158	231

***Adjusted for child's gender and month of birth and maternal education, history of eczema, atopic status and parity**
Regression coefficients were calculated as change in FENO per SD change in body composition measurement.

Table 52 FENO according to maternal pre-pregnancy and pregnancy body composition, and weight gain during pregnancy

6.5.2 Maternal diet and atopy in the offspring

6.5.2.1 Maternal micronutrient intake

Primary hypothesis: low maternal vitamin E intake during pregnancy is associated with atopy in the offspring at 6 years of age.

Rationale: low maternal vitamin E intake has been found to be associated with elevated CBMC responsiveness to allergens (Devereux *et al.* 2002) and to elevated FENO in the offspring at 5 years of age (Devereux *et al.* 2006).

Secondary hypothesis: high vitamin D intake during pregnancy is associated with atopy in the offspring at the age of 6 years.

Rationale: research conducted locally suggests that children born to women with high serum levels of 25(OH) vitamin D in pregnancy are at risk of atopic disease (Gale *et al.* 2008). Although vitamin D status is largely influenced by sun exposure, maternal dietary vitamin D intake may also be important. However, whilst it has been proposed that

high childhood vitamin D intake is associated with an increased risk of atopy in adulthood (Hypponen *et al.* 2004), conflicting evidence exists regarding the role of maternal intake. Several groups have published epidemiological evidence which suggests higher maternal vitamin D intake during pregnancy might exert a protective influence against childhood wheeze. It is unclear whether this protective effect is specific for atopic wheezing disorders.

Results: the hypothesised association between low intakes of vitamins D and E during pregnancy and atopic outcome was not confirmed in this cohort.

Neither total early nor late pregnancy vitamin E intake was significantly associated with skin sensitisation. The data presented in Table 53 relate to non-energy-adjusted intakes; no significant association between total vitamin E intake and skin sensitisation was found when the data were re-analysed using energy-adjusted intakes (Appendix 1, Table 113).

The risk of skin sensitisation was not clearly elevated by high vitamin D intake. There was a 15% increase in risk of skin sensitisation per SD increase in total vitamin D intake in late pregnancy but this effect was not statistically significant (p=0.081) and became less so upon adjustment for confounders (p=0.185) (Table 53). Neither pre-pregnancy nor early pregnancy vitamin D intake significantly elevated the risk of atopy. The risk of skin sensitisation was not significantly associated with energy-adjusted vitamin D intake (Appendix 1, Table 113).

Neither vitamin E nor D intake, before or during pregnancy, was significantly associated with FENO (Table 54). The lack of association between total maternal intakes of vitamin D and E and FENO remained when the data was re-analysed using energy-adjusted vitamin intakes (Appendix 1, Table 114).

Standardised total intake	Unadjusted analyses RR (95% CI)	P value	n	Adjusted* analyses RR (95% CI)	P value	n
Vitamin E						
Pre-pregnancy	1.04 (0.88-1.22)	0.672	374	0.94 (0.79-1.12)	0.483	346
11 weeks	1.01 (0.86-1.19)	0.901	302	0.97 (0.82-1.16)	0.753	282
34 weeks	1.08 (0.93-1.25)	0.296	365	1.04 (0.89-1.21)	0.631	340
Vitamin D						
Pre-pregnancy	0.94 (0.79-1.11)	0.438	374	0.91 (0.77-1.07)	0.260	346
11 weeks	1.06 (0.87-1.28)	0.579	302	1.01 (0.83-1.22)	0.920	282
34 weeks	1.15 (0.98-1.35)	0.081	365	1.12 (0.95-1.32)	0.185	340

***Adjusted for child's gender and maternal education, history of asthma, atopic status and parity**
Relative risks were calculated as change in risk per SD change vitamin intake.

Table 53 Relative risk of skin sensitisation according to maternal intakes of vitamins D and E before and during pregnancy

Standardised total intake	Unadjusted analyses Exp Beta (95% CI)	P value	n	Adjusted* analyses Exp Beta (95% CI)	P value	n
Vitamin E						
Pre-pregnancy	1.01 (0.93-1.10)	0.846	248	0.98 (0.91-1.06)	0.626	234
11 weeks	0.99 (0.90-1.09)	0.863	198	0.97 (0.88-1.07)	0.506	188
34 weeks	1.03 (0.95-1.12)	0.499	246	1.01 (0.94-1.10)	0.713	232
Vitamin D						
Pre-pregnancy	0.97 (0.90-1.05)	0.490	248	0.98 (0.90-1.05)	0.537	234
11 weeks	0.98 (0.90-1.08)	0.743	198	0.99 (0.91-1.08)	0.870	188
34 weeks	1.00 (0.92-1.08)	0.919	246	0.98 (0.91-1.06)	0.631	232

***Adjusted for child's gender and month of birth and maternal education, history of eczema, atopic status and parity**
Regression coefficients were calculated as change in FENO per SD change in vitamin intake.

Table 54 Multiplicative increase in FENO according to maternal intakes of vitamin D and E before and during pregnancy

6.5.2.2 Influence of vitamin supplementation upon atopic outcomes

When vitamin intakes from foods were considered no significant association was seen between food-derived vitamin D and skin sensitisation. The direction of effect for food derived-vitamin D intakes was similar to that for total vitamin D intake (Table 55). No significant associations were found between total vitamin D intake and skin sensitisation when women using supplements were considered separately from those women who did not supplement their diet with vitamin D (Table 56).

Standardised intake food-derived Vitamin D	Unadjusted analyses RR (95% CI)	P value	n	Adjusted* analyses RR (95% CI)	P value	n
Pre-pregnancy	0.96 (0.82-1.14)	0.656	374	0.93 (0.78-1.10)	0.405	346
11 weeks	1.04 (0.84-1.27)	0.727	302	0.99 (0.82-1.21)	0.955	282
34 weeks	1.03 (0.87-1.23)	0.693	365	1.00 (0.83-1.21)	0.973	340
Standardised total vitamin D intake						
Pre-pregnancy	0.94 (0.79-1.11)	0.438	374	0.91 (0.77-1.07)	0.260	346
11 weeks	1.06 (0.87-1.28)	0.579	302	1.01 (0.83-1.22)	0.920	282
34 weeks	1.15 (0.98-1.35)	0.081	365	1.12 (0.95-1.32)	0.185	340

***Adjusted for child's gender and maternal education, history of asthma, atopic status and parity**
Relative risks were calculated as change in risk per SD change in vitamin intake.

Table 55 Relative risk of skin sensitisation according to maternal vitamin D intake from food

Standardised total vitamin D intake	Unadjusted analyses RR (95% CI)	P value	n	Adjusted* analyses RR (95% CI)	P value	n
Women using supplements						
Pre-pregnancy	0.88 (0.68-1.13)	0.303	268	0.87 (0.67-1.13)	0.297	251
11 weeks	0.93 (0.62-1.40)	0.734	193	0.93 (0.63-1.35)	0.691	178
34 weeks	1.09 (0.34–1.42)	0.513	292	1.02 (0.76-1.36)	0.914	269
Women not using supplements						
Pre-pregnancy	0.87 (0.62-1.22)	0.413	106	0.71 (0.45-1.13)	0.153	95
11 weeks	0.85 (0.64-1.15)	0.299	109	0.87 (0.65-1.17)	0.366	104
34 weeks	1.17 (0.83-1.65)	0.360	73	1.19 (0.83-1.69)	0.347	71

***Adjusted for child's gender and maternal education, history of asthma, atopic status and parity**
Relative risks were calculated as change in risk per SD change in vitamin intake.

Table 56 Relative risk of skin sensitisation according to total maternal vitamin D intake, comparing women using vitamin D supplements with those not using supplements

Vitamin E intake and skin sensitisation were not significantly associated regardless of the source of vitamin E. Directions of effects were similar for food and total intake (Table 57). No significant associations were found between total vitamin E intake and skin sensitisation when women using supplements were considered separately from those women who did not supplement their diet with vitamin E (Table 58).

Standardised intake food-derived vitamin E	**Unadjusted analyses**			**Adjusted* analyses**		
	RR (95% CI)	**P value**	**n**	**RR (95% CI)**	**P value**	**n**
Pre-pregnancy	1.02 (0.86-1.20)	0.847	374	0.93 (0.78-1.11)	0.425	346
11 weeks	1.02 (0.85-1.23)	0.835	302	0.98 (0.81-1.19)	0.834	282
34 weeks	1.02 (0.86-1.20)	0.854	365	0.96 (0.80-1.14)	0.617	340
Standardised total vitamin E intake						
Pre-pregnancy	1.04 (0.88-1.22)	0.672	374	0.94 (0.79-1.12)	0.483	346
11 weeks	1.01 (0.86-1.19)	0.901	302	0.97 (0.82-1.16)	0.753	282
34 weeks	1.08 (0.93-1.25)	0.296	365	1.04 (0.89-1.21)	0.631	340

***Adjusted for child's gender and maternal education, history of asthma, atopic status and parity**
Relative risks were calculated as change in risk per SD change in vitamin intake.

Table 57 Relative risk of skin sensitisation according to maternal vitamin E intake from food

Standardised total vitamin E intake	**Unadjusted analyses**			**Adjusted* analyses**		
	RR (95% CI)	**P value**	**n**	**RR (95% CI)**	**P value**	**n**
Women using supplements						
Pre-pregnancy	1.05 (0.79-1.40)	0.738	262	0.95 (0.69-1.30)	0.744	244
11 weeks	1.11 (0.81-1.50)	0.519	209	1.08 (0.77-1.52)	0.644	193
34 weeks	1.00 (0.79-1.27)	0.970	297	0.92 (0.70-1.19)	0.511	274
Women not using supplements						
Pre-pregnancy	0.94 (0.67-1.310	0.705	112	0.83 (0.53-1.29)	0.412	102
11 weeks	0.87 (0.67-1.13)	0.299	93	0.85 (0.64-1.14)	0.273	89
34 weeks	1.01 (0.80-1.29)	0.922	68	0.98 (0.76-1.25)	0.843	66

***Adjusted for child's gender and maternal education, history of asthma, atopic status and parity**
Relative risks were calculated as change in risk per SD change in vitamin intake.

Table 58 Relative risk of skin sensitisation according to total maternal vitamin E intake, comparing women using vitamin E supplements with those not using supplements

When vitamin intakes from foods were considered no significant association was seen between food-derived vitamin D and FENO. The direction of effect for food derived-vitamin D intakes was similar to that for total vitamin D intake (Table 59). No significant associations were found between total vitamin D intake and skin sensitisation when women using supplements were considered separately from those women who did not supplement their diet with vitamin D (Table 60).

Standardised intake food-derived vitamin D	**Unadjusted analyses**			**Adjusted* analyses**		
	Exp Beta (95% CI)	**P value**	**n**	**Exp Beta (95% CI)**	**P value**	**n**
Pre-pregnancy	0.99 (0.91-1.08)	0.815	248	0.99 (0.91-1.07)	0.803	234
11 weeks	1.03 (0.94-1.12)	0.496	198	1.06 (0.97-1.15)	0.219	188
34 weeks	1.07 (0.99-1.16)	0.105	246	1.06 (0.98-1.15)	0.153	232
Standardised total vitamin D intake						
Pre-pregnancy	0.97 (0.90-1.05)	0.490	248	0.98 (0.90-1.05)	0.537	234
11 weeks	0.98 (0.90-1.08)	0.743	198	0.99 (0.91-1.08)	0.870	188
34 weeks	1.00 (0.92-1.08)	0.919	246	0.98 (0.91-1.06)	0.631	232

***Adjusted for child's gender, month of birth, maternal education, history of eczema, atopy and parity**
Regression coefficients were calculated as change in FENO per SD change in vitamin intake.

Table 59 Multiplicative increase in FENO according to maternal vitamin D intake from food

Standardised total vitamin D intake	**Unadjusted analyses**			**Adjusted* analyses**		
	RR (95% CI)	**P value**	**n**	**RR (95% CI)**	**P value**	**n**
Women using supplements						
Pre-pregnancy	1.00 (0.88-1.14)	0.991	171	0.98 (0.87-1.11)	0.779	163
11 weeks	1.02 (0.89-1.18)	0.734	122	1.10 (0.94-1.27)	0.225	116
34 weeks	1.06 (0.93-1.20)	0.401	191	1.03 (0.91-1.16)	0.669	179
Women not using supplements						
Pre-pregnancy	0.90 (0.75-1.08)	0.250	77	0.96 (0.75-1.23)	0.748	71
11 weeks	0.89 (0.73-1.08)	0.224	76	0.90 (0.75-1.08)	0.247	72
34 weeks	0.89 (0.69-1.09)	0.211	55	0.84 (0.66-1.07)	0.151	53

***Adjusted for child's gender, month of birth, maternal education, history of eczema, atopy and parity**
Relative risks were calculated as change in risk per SD change in vitamin intake.

Table 60 Multiplicative increase in FENO according to total maternal vitamin D intake, comparing women using vitamin D supplements with those not using supplements

Vitamin E from food in late pregnancy was significantly associated with FENO level. FENO level increased by 9% per SD increase in vitamin intake from food (p=0.035). Early pregnancy vitamin E intake from food was not significantly associated with FENO (Table 61). No significant associations were found between total vitamin E intake and FENO when women using supplements were considered separately from those women who did not supplement their diet with vitamin E (Table 62).

Standardised intake food-derived vitamin E	**Unadjusted analyses**			**Adjusted* analyses**		
	Exp Beta (95% CI)	**P value**	**n**	**Exp Beta (95% CI)**	**P value**	**n**
Pre-pregnancy	1.04 (0.96-1.13)	0.368	248	0.98 (0.91-1.06)	0.641	234
11 weeks	1.01 (0.92-1.10)	0.898	198	0.99 (0.90-1.08)	0.834	188
34 weeks	1.10 (1.01-1.20)	0.028	246	1.09 (1.01-1.19)	0.035	232
Standardised total vitamin E intake						
Pre-pregnancy	1.01 (0.93-1.10)	0.846	248	0.98 (0.91-1.06)	0.626	234
11 weeks	0.99 (0.90-1.09)	0.863	198	0.97 (0.88-1.07)	0.506	188
34 weeks	1.03 (0.95-1.12)	0.499	246	1.01 (0.94-1.10)	0.713	232

***Adjusted for child's gender and month of birth and maternal education, history of eczema, atopic status and parity**
Regression coefficients were calculated as change in FENO per SD change in vitamin intake.

Table 61 Multiplicative increase in FENO according to vitamin E intake from food

Standardised total vitamin E intake	**Unadjusted analyses**			**Adjusted* analyses**		
	RR (95% CI)	**P value**	**n**	**RR (95% CI)**	**P value**	**n**
Women using supplements						
Pre-pregnancy	1.06 (0.92-1.22)	0.410	171	0.93 (0.81-1.07)	0.294	160
11 weeks	1.01 (0.88-1.17)	0.850	136	0.99 (0.85-1.14)	0.865	130
34 weeks	1.07 (0.94-1.21)	0.288	194	1.04 (0.92-1.18)	0.492	182
Women not using supplements						
Pre-pregnancy	0.96 (0.82-1.13)	0.636	77	1.05 (0.88-1.26)	0.588	74
11 weeks	0.95 (0.76-1.18)	0.621	62	0.85 (0.65-1.11)	0.218	58
34 weeks	1.04 (0.85-1.27)	0.680	52	1.10 (0.88-1.38)	0.372	50

***Adjusted for child's gender and month of birth and maternal education, history of eczema, atopic status and parity**
Relative risks were calculated as change in risk per SD change in vitamin intake.

Table 62 Multiplicative increase in FENO according to total maternal vitamin E intake, comparing women using vitamin E supplements with those not using supplements

6.5.2.3 Maternal nutrient status and atopy in the offspring

6.5.2.3.1 *Serum vitamin concentration at 34 weeks' gestation*

Primary hypothesis: high serum levels of 25(OH) vitamin D are associated with atopy in the offspring at age 6 years.

Rationale: an association between high vitamin D status in pregnancy and atopy in the offspring has been found in a previous study (Gale *et al.* 2008). There is also evidence that high levels of vitamin D bias the immune system towards an atopic phenotype in animal experiments (Matheu *et al.* 2003) and that supplementation of vitamin D in childhood is associated with and increased risk of atopy in adulthood (Hypponen *et al.* 2004).

Secondary hypothesis: maternal serum α-tocopherol at 34 weeks' gestation is associated with increased atopy in the offspring at 6 years of age.

Rationale: previous work suggests an association exists between low maternal vitamin E intake during pregnancy and atopy in the offspring, the effect of low intakes of vitamin E may be mediated by an effect upon maternal vitamin E status which may in turn influence fetal immune development.

Results: neither maternal serum 25(OH)-vitamin D nor serum α-tocopherol, measured at 34 weeks' gestation, was significantly associated with atopy in the offspring at 6 years of age.

Skin sensitisation was not significantly associated with maternal 25(OH)-vitamin D status either before or after adjusting for confounders. In contradiction to the hypothesised harmful effect of vitamin D, a 10% decrease in FENO was found to be associated with each SD increase in serum 25(OH)-vitamin D concentration ($p=0.051$). This effect became non-significant, however, upon adjusting for confounders ($p=0.75$) (Table 63).

There were no significant associations between serum α-tocopherol (corrected for cholesterol) and either skin sensitisation or FENO level (Table 64 & Table 65).

It was not possible to analyse the risk of skin sensitisation according to whether maternal serum 25(OH)-vitamin D was deficient (< 25 nmol/l) or replete (> 25 nmol/l), because less than 1% of the population studied were 25(OH)-vitamin D deficient by this definition therefore the power of the analysis was low.

Standardised serum concentration	**Unadjusted analyses**			**Adjusted* analyses**		
	RR (95% CI)	**P value**	**n**	**RR (95% CI)**	**P value**	**n**
Cutaneous sensitisation						
25(OH)-vitamin D	0.94 (0.78-1.14)	0.537	270	0.98 (0.81-1.18)	0.821	254
α-tocopherol†	1.10 (0.91-1.32)	0.310	281	1.06 (0.87-1.30)	0.551	262
FENO	**Exp Beta (95% CI)**	**P value**	**n**	**Exp Beta (95% CI)**	**P value**	**n**
25(OH)-vitamin D	0.90 (0.82-1.00)	0.051	186	0.98 (0.86-1.11)	0.748	178
α-tocopherol †	1.05 (0.96-1.16)	0.261	187	1.00 (0.91-1.10)	0.980	176

***Adjusted for child's gender and maternal education, history of asthma, atopic status and parity for cutaneous sensitisation and for child's gender month of birth and maternal education, history of eczema, atopic status and parity. †corrected for cholesterol**
Relative risks were calculated as change in risk per SD change in vitamin status.

Table 63 Relative risk of skin sensitisation according to maternal serum vitamin concentrations at 34 weeks' gestation

6.5.2.3.2 *Plasma phospholipid fatty acid composition at 34 weeks' gestation*

Primary hypothesis: low maternal n-3 PUFA status at 34 weeks' gestation is associated with an increased risk of atopy in the offspring at 6 years of age.

Rationale: intake of n-3 PUFAs has declined over the same time period during which atopy has become more prevalent (Calder 2003). Retrospective (Salam *et al.* 2005) and prospective (Sausenthaler *et al.* 2007; Willers *et al.* 2007) studies of fish intake in pregnancy suggest fish oil exposure may protect against atopy and a study of n-3 PUFA supplementation during pregnancy has also demonstrated a protective effect (Olsen *et al.* 2008).

Secondary hypothesis: high n-6 PUFA status or a high proportion of n-6 PUFAs compared to n-3 PUFAs during pregnancy increases the risk of atopy in the offspring.

Rationale: encorporation of the n-3 PUFAs DHA and EPA into cell membranes leads to decreased availability of arachidonic acid and increased competition for the enzymes

responsible for prostaglandin and leukotriene synthesis (Calder 2006; Prescott *et al.* 2007) this, in turn, may alter the balance of Th1 and Th2 cytokines in a manner which promotes atopy.

Results: there was no evidence of a protective effect upon skin sensitisation for either individual or total n-3 PUFA status or for the ratio of n-3 to n-6 PUFAs. However, upon adjusting for confounders a positive association was found between a high percentage of arachidonic acid and skin sensitisation.

For each SD increase in arachidonic acid percentage composition of maternal plasma phospholipids, an increase of 20% was seen in the risk of skin sensitisation (p=0.041). α-Linolenic acid was inversely associated with skin sensitisation in the adjusted analysis (Table 64), a 16% decrease in risk was associated with each SD increase in percentage composition (p=0.035). The percentage of arachidonic acid in plasma phospholipid phosphatidylcholine was strongly positively associated with FENO levels. This association became stronger upon adjusting for confounders. FENO increased by 12% per SD increase in the percentage of arachidonic acid in plasma phospholipids (p=0.007).

There was unexpected evidence of a protective effect for the n-6 PUFA, linoleic acid. FENO decreased by 13% per SD increase in percentage linoleic acid (p=0.001). Also unexpected was an apparent association between higher total n-3 PUFA percentage and DHA and EPA percentage and increased FENO, although these associations were not significant (Table 65).

Percentage of total fatty acids	Unadjusted analyses			Adjusted* analyses		
	RR (95% CI)	P value	n	RR (95% CI)	P value	n
n-3 PUFAs	1.07 (0.90-1.28	0.460	355	1.04 (0.87-1.24)	0.663	331
n-6 PUFAs	0.96 (0.82-1.13)	0.639	355	0.96 (0.82-1.12)	0.612	331
n-3:6 ratio	1.07 (0.90-1.27)	0.462	355	1.04 (0.88-1.24)	0.633	331
α-Linolenic acid	0.87 (0.74-1.02)	0.090	335	0.84 (0.71-0.99)	0.035	311
Linoleic acid	0.91 (0.78-1.06)	0.209	355	0.88 (0.76-1.03)	0.115	331
DHA	1.08 (0.91-1.29)	0.368	355	1.07 (0.89-1.28)	0.465	331
EPA	1.04 (0.88-1.23)	0.670	346	0.99 (0.83-1.18)	0.945	322
Arachidonic acid	1.14 (0.96-1.34)	0.136	355	1.20 (1.01-1.42)	0.041	331

***Adjusted for child's gender and maternal education, history of asthma, atopic status and parity**
Relative risks were calculated as change in risk per SD change in plasma phospholipids fatty acid composition.

Table 64 Relative risk of skin sensitisation according to fatty acid composition of maternal plasma phospholipid phosphatidylcholine at 34 weeks' gestation

Percentage of total fatty acids	Unadjusted analyses			Adjusted* analyses		
	Exp beta (95% CI)	P value	n	Exp beta (95% CI)	P value	n
n-3 PUFAs	1.04 (0.95-1.14)	0.415	237	1.07 (0.98-1.17)	0.133	224
n-6 PUFAs	1.00 (0.91-1.09)	0.966	237	0.94 (0.86-1.02)	0.131	224
n-3:6 ratio	1.03 (0.94-1.13)	0.483	237	1.08 (0.99-1.17)	0.101	224
α-Linolenic acid	0.96 (0.88-1.05)	0.393	225	0.97 (0.90-1.06)	0.508	212
Linoleic acid	0.93 (0.86-1.02)	0.124	237	0.87 (0.80-0.94)	0.001	224
DHA	1.06 (0.97-1.16)	0.177	237	1.08 (0.99-1.17)	0.086	224
EPA	1.05 (0.96-1.15)	0.286	232	1.08 (0.99-1.18)	0.070	220
Arachidonic acid	1.11 (1.02-1.21)	0.019	237	1.12 (1.03-1.21)	0.007	224

***Adjusted for child's gender and month of birth and maternal education, history of eczema, atopic status and parity**
Regression coefficients were calculated as change in FENO per SD change in plasma phospholipids fatty acid composition.

Table 65 Multiplicative increase in FENO according to fatty acid composition of maternal plasma phospholipid phosphatidylcholine at 34 weeks' gestation

6.5.2.4 Maternal dietary patterns

Exploratory analysis: to explore the relationship between maternal dietary patterns and the risk of atopy in the offspring at 6 years.

Rationale: previous studies have identified that eating according to a 'Mediterranean' dietary pattern during pregnancy serves to protect against atopy in the offspring (Chatzi *et al.* 2008). The diets of the women in the SWS have previously been analysed to determine how well they conform to a 'prudent' pattern of eating, the data can be used to assess the association between this 'prudent' dietary pattern and childhood atopy.

Results: in unadjusted univariate analysis, higher mothers' prudent diet scores at the initial, and early and late pregnancy interviews were all associated with increased relative risk of atopy in their children at age 6 years. However, although the direction of this effect was unchanged, these associations became non-significant at the 5% significance level following adjustment for maternal confounders, including maternal education (Table 66). In neither the adjusted nor unadjusted analyses were prudent diet scores, before or during pregnancy, significantly associated with FENO (Table 67).

Prudent diet score	Unadjusted analyses			Adjusted* analyses		
	RR (95% CI)	P value	n	RR (95% CI)	P value	n
Pre-pregnancy	1.24 (1.05-1.46)	0.010	374	1.13 (0.95-1.35)	0.160	346
11 weeks' gestation	1.22 (1.01-1.48)	0.041	302	1.13 (0.91-1.41)	0.264	282
34 weeks' gestation	1.24 (1.05-1.46)	0.013	365	1.15 (0.95-1.39)	0.166	340

***Adjusted for child's gender and maternal education, history of asthma, atopic status and parity**
Relative risks were calculated as change in risk per SD change in prudent diet score.

Table 66 Relative risk of skin sensitisation according to maternal prudent diet score

Prudent diet score	Unadjusted analyses			Adjusted* analyses		
	Exp Beta (95% CI)	P value	n	Exp Beta (95% CI)	P value	n
Pre-pregnancy	1.03 (0.95-1.10)	0.485	248	1.04 (0.96-1.12)	0.352	234
11 weeks' gestation	1.01 (0.92-1.11)	0.828	198	1.05 (0.95-1.16)	0.344	188
34 weeks' gestation	1.05 (0.97-1.14)	0.193	246	1.06 (0.97-1.15)	0.211	232

***Adjusted for child's gender and month of birth and maternal education, history of eczema, atopic status and parity**
Regression coefficients were calculated as change in FENO per SD change in prudent diet score.

Table 67 Multiplicative increase in FENO according to maternal prudent diet score

6.5.2.5 Maternal intake of specific food groups

Exploratory analysis: to explore the associations between maternal intake of specific foods during pregnancy and risk of atopy in the offspring at age 6 years.

Rationale: previous studies have found maternal intake of oily fish during pregnancy to protect against atopy (Sausenthaler *et al.* 2007; Willers *et al.* 2007) and for citrus fruits and sweet peppers to increase the risk (Sausenthaler *et al.* 2007). The FFQ was not precise enough to enable analysis of associations between atopy and specific foods but it was possible to explore the associations between atopy and the broad food groups of oily fish and citrus fruits.

Results: no significant associations were found between citrus fruit intake before or during pregnancy and atopy. There was no evidence of a protective effect for oily fish intake, rather an association between higher oily fish intake during early and late pregnancy and increased atopy risk approached significance in the unadjusted analysis. After correcting for confounding variables these associations with early pregnancy oily fish intake weakened (Table 68).

Standardised intake	Unadjusted analyses			Adjusted* analyses		
	RR (95% CI)	P value	n	RR (95% CI)	P value	n
Citrus fruit intake						
Pre-pregnancy	0.98 0.83-1.15)	0.775	352	0.94 (0.80-1.10)	0.418	327
11 weeks	1.05 (0.88-1.27)	0.587	349	0.98 (0.82-1.18)	0.865	326
34 weeks	1.07 (0.89-1.31)	0.467	288	0.93 (0.75-1.15)	0.479	269
Oily fish intake						
Pre-pregnancy	1.07 (0.89-1.29)	0.459	291	2.04 (0.85-1.27)	0.701	267
11 weeks	1.19 (0.95-1.49)	0.137	242	1.09 (0.87-1.38)	0.448	227
34 weeks	1.15 (0.95-1.38)	0.153	293	1.07 (0.98-1.17)	0.128	180

***Adjusted for child's gender and maternal education, history of asthma, atopic status and parity**
Relative risks were calculated as change in risk per SD change in citrus fruit or oily fish intake.

Table 68 Relative risk of skin sensitisation according to maternal citrus fruit and oily fish intake before and during pregnancy

Raised FENO was found to be associated with increased citrus fruit consumption in late pregnancy and increased oily fish consumption in late pregnancy, although neither of these associations remained significant after adjusting for confounders (Table 69).

Standardised intake	Unadjusted analyses			Adjusted* analyses		
	Exp Beta (95% CI)	P value	n	Exp Beta (95% CI)	P value	n
Citrus intake						
Pre-pregnancy	1.01 (0.93-1.10)	0.752	237	1.04 (0.96-1.12)	0.354	224
11 weeks	1.06 (0.97-1.16)	0.187	231	1.03 (0.94-1.12)	0.569	219
34 weeks	1.10 (1.00-1.21)	0.039	187	1.03 (0.94-1.13)	0.508	178
Oily fish intake						
Pre-pregnancy	1.00 (0.91-1.10)	0.987	189	0.99 (0.91-1.09)	0.906	177
11 weeks	1.04 (0.94-1.15)	0.408	149	1.07 (0.97-1.18)	0.182	141
34 weeks	1.11 (1.01-1.21)	0.025	192	1.07 (0.98-1.17)	0.128	180

***Adjusted for child's gender and month of birth and maternal education, history of eczema, atopic status and parity**
Regression coefficients were calculated as change in FENO per SD change in citrus fruit or oily fish intake.

Table 69 Multiplicative increase in FENO according to maternal citrus fruit and oily fish intake before and during pregnancy

6.6 SUMMARY DISCUSSION

This chapter explored the role played by maternal nutrition in the development of childhood atopy. Analyses were undertaken to confirm or refute the findings of previous studies where associations between nutritional factors and atopic status have been described and to explore additional biologically plausible associations.

Summary of main findings:

- Cutaneous sensitisation to allergens was present at a level consistent with that reported previously in the UK and the pattern of sensitisation to specific allergens was consistent with that expected in 6-year-old children.

- Cutaneous sensitisation and elevated FENO were more commonly found in children exhibiting symptoms of atopic disease than in those who did not.

- There was no evidence that maternal fat mass, or any other measure of maternal body composition, was associated with atopy in the offspring at age 6-years.

- There was no evidence that higher total vitamin D intake during pregnancy was associated with an increased risk of atopy in the offspring and no evidence for increased risk associated with vitamin D supplementation.

- There was no evidence that lower total vitamin E intake during pregnancy is associated with an increased risk of atopy in the offspring. In contrast, lower vitamin E from food in late pregnancy was significantly associated with a reduced risk of atopic airway inflammation, as measured by FENO.

- There was no evidence that atopy was significantly associated with serum 25(OH) vitamin D or α-tocopherol level at 34 weeks' gestation.

- The risks of both skin sensitisation and elevated FENO were significantly positively associated with percentage arachidonic acid in plasma phospholipid phosphatidylcholine.

- The positive association between a prudent maternal dietary pattern and skin sensitisation was no longer apparent after adjustment for confounders. A positive association was also seen between FENO and both citrus fruit and oily fish intake, but these associations were also not significant after correcting for confounders.

Chapter Seven

Early life determinants of lung function at 6 years

7.1 AIM

To examine the hypothesis that impaired maternal nutrition immediately prior to and during pregnancy is associated with impaired lung function at 6 years of age.

Specifically, associations were sought between poorer lung function and:-

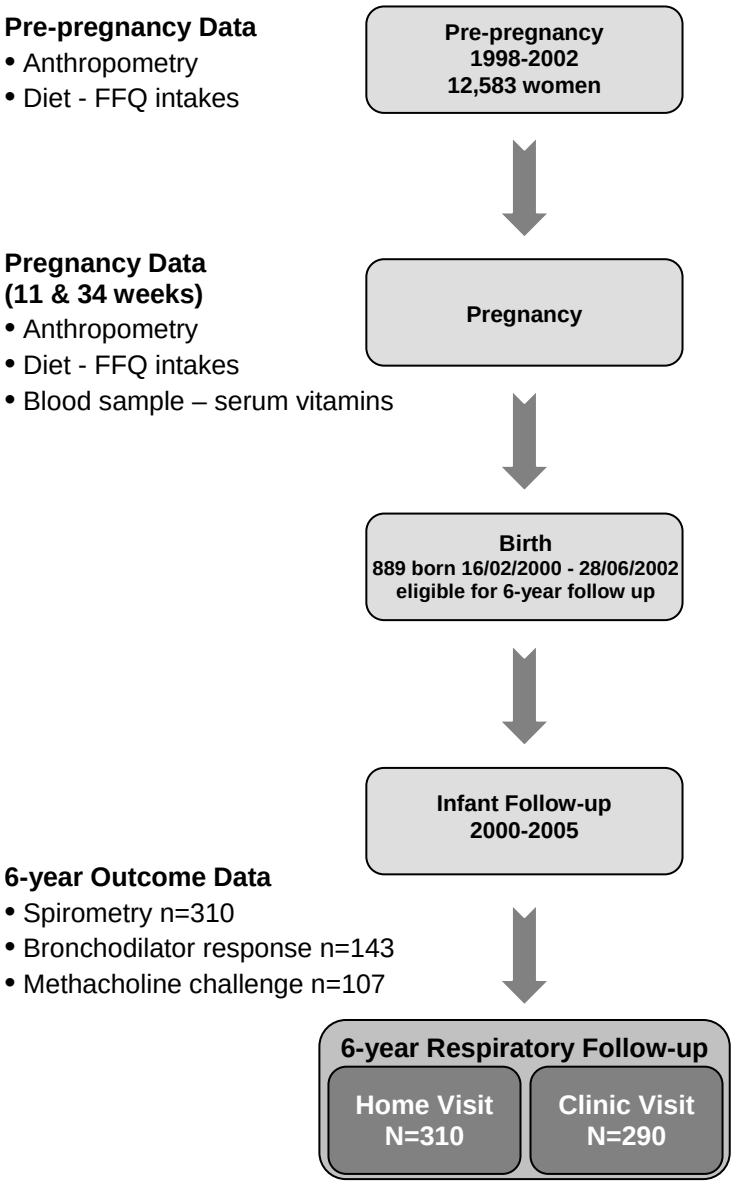
- low maternal fat mass
- low maternal muscle mass
- low vitamin A intake
- low vitamin C intake

7.2 INTRODUCTION

This chapter investigates the hypothesis that maternal nutrition may influence respiratory function in childhood. As discussed in section 1.4, there is evidence that wheezing disorders are associated with impaired lung function (Martinez *et al.* 1995) and that these impairments may occur early in life (Martinez *et al.* 1991) and track throughout infancy and childhood (Turner *et al.* 2004) into adult life (Sears *et al.* 2003). Early life exposures, including the *in utero* environment, are therefore believed important for later respiratory health. Maternal diet could be an important early life exposure (Devereux 2006; Seaton *et al.* 1994) and epidemiological (Devereux *et al.* 2006; Ness *et al.* 1996) and animal studies (Proskocil *et al.* 2005) suggest possible associations exist

between the intakes of specific nutrients and measures of lung function. Moreover, maternal body composition is known to be associated with birthweight (Sanin Aguirre *et al.* 2004) and birthweight, in turn, is associated with lung function in infancy (Friedrich *et al.* 2006; Lucas *et al.* 2004). This raises the possibility that maternal body composition may influence the developing respiratory system. There is sufficient evidence to hypothesise that both the composition of women's diets and the overall adequacy of their nutrition may influence the respiratory health of their children. The hypotheses in this chapter were designed to test biologically plausible mechanisms and to examine the findings of previous studies where associations between nutritional factors and childhood lung function have been described.

Figure 27 Data collected to assess the relationship between maternal nutrition and lung function in 6-year-old children



7.3 SUMMARY OF METHODS

A detailed explanation of the methods used to collect exposure and outcome data is given in chapter two and the cohort is defined in Figure 27. Mothers of those children who were between their sixth and seventh birthdays during the study period were invited to receive a visit from a research nurse and to attend the clinic. Portable spirometry equipment was used to determine the children's lung function. Bronchodilator response or methacholine challenge was conducted at the clinic visit. The mothers' body composition and nutrition data were drawn from the pre-pregnancy and pregnancy phases of the SWS.

7.4 ANALYSIS

A cohort of eligible children was identified based upon date of birth. Children born at less than 35 weeks gestation were excluded to avoid confounding by the effects of lung disease of prematurity. This minimum gestation was slightly lower than that used in the 3-year analysis. A cut-off of 35 weeks was chosen on the grounds that it is rare for infants of 35 weeks' gestation or greater to have significant lung disease of prematurity (Bancalari *et al.* 2003) and because reducing the minimum gestation for inclusion served to maximise the number of participants contributing respiratory data. FEV_1 was selected as the primary outcome measure of lung function for purposes of comparison with previous studies and because this measure is believed to be reasonably reproducible in this age group. The children's FEV_1 measurements were expressed using a standardised z-score from the growing lungs reference ranges (Stanojevic *et al.* 2008). Measurements were expressed relative to the mean FEV_1 value predicted for each child's gender, height and age. Regression analyses were used to determine the effect of maternal body composition and nutrition upon this continuous outcome measure.

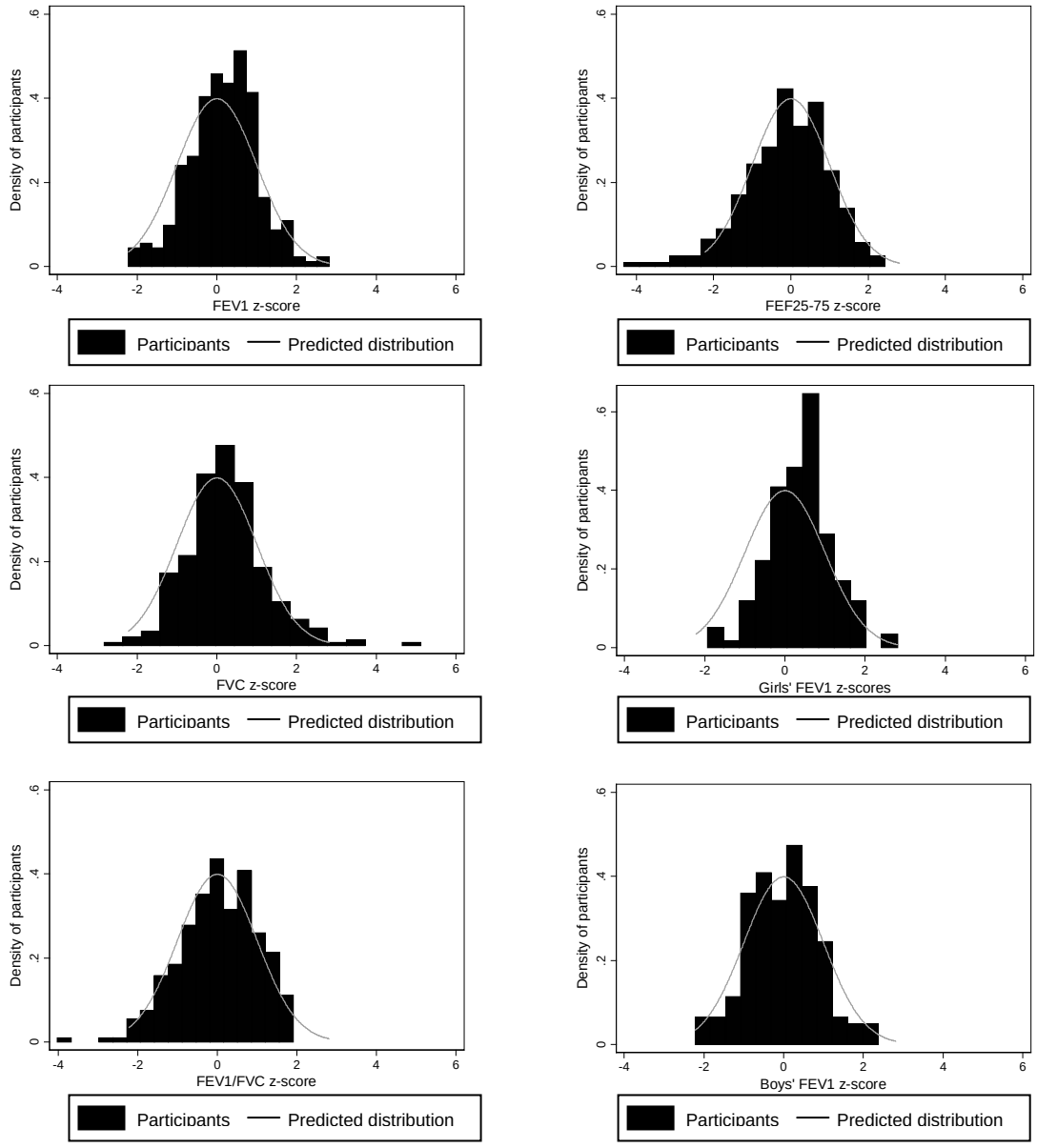
Secondary regression analyses were performed using measures of $FEF_{25-75\%}$ and FEV_1/FVC as these spirometry indices, although less reproducible than FEV_1, may be more sensitive to small airways disease. All spirometric indices were expressed as an age, height and gender adjusted z-score using the growing lungs reference ranges (Stanojevic *et al.* 2008). Analyses were also conducted using continuous BDR and BHR outcomes to further characterise the participants' lung function. BDR values were transformed by natural logarithms to achieve normality and then standardised. The primary measure of

BHR was the inverse log. slope this was selected on the basis of having an approximately normal distribution and therefore did not require further transformation prior to standardisation. Standard deviation (z-) scores were derived for continuous exposure variables, after log. transformation, if necessary, to achieve normality. The analyses were adjusted for exposures identified as likely confounders using the forward selection method described in chapter six. Additional variables were added to the final model based upon previously published associations with lung function. These variables were maternal education (Ernst *et al.* 1995; Lawlor *et al.* 2004), maternal history of asthma (Litonjua *et al.* 1998), smoking in pregnancy (Dezateux *et al.* 2001), paternal history of asthma (Dold *et al.* 1992), and child's gestation (Rona *et al.* 1993).

7.5 RESULTS

As expected for a general population sample, spirometric measures in this cohort were approximately normally distributed about a mean predicted z-score of zero with a standard deviation of one (Figure 28).

Figure 28 Distributions of standardised spirometric values in the SWS cohort



Forced expiratory volume in one second was associated in univariate analyses with child's gender, age at last breast feed and birth order. The potential confounders included in the final multivariate model are listed in Table 71. Of these confounders, only gestation differed significantly between the children who participated in spirometric testing and those who did not perform spirometry. The mean gestation of children who performed spirometry was 40 weeks, whilst that of the non-tested children was slightly less at 39.6 weeks (p=0.007).

Binary variables (t-test)				**Univariate P-value**	**Final P-value**
Male gender	−0.02	Female gender	0.37	<0.001	<0.001
Maternal asthma	0.26	No maternal asthma	0.14	0.31	0.42
Paternal asthma	0.29	No paternal asthma	0.15	0.27	0.32
Smoking in pregnancy	0.21	No smoking in pregnancy	0.17	0.75	0.31
Continuous variables Pearson's correlation coefficient				**Univariate P-value**	**Final P-value**
Gestation		0.09		0.10	0.16
Age last breast fed		0.13		0.02	0.01
Maternal education		0.06		0.29	0.90
Parity		−0.10		0.09	0.16

Table 70 Factors associated with FEV_1 z-score on univariate analysis

The variables selected as confounders of the measures of lung function other than FEV_1 are listed in Table 71. The children from who spirometric values were recorded did not differ according to any of these factors, other than that of than gestation, from those children who did not record spirometry.

FEV_1/FVC	**$FEF_{25-75\%}$**	**BDR**	**BHR**
Maternal asthma	Maternal asthma	Maternal asthma	Maternal asthma
Maternal rhinitis	Maternal rhinitis	Maternal rhinitis	Paternal asthma
Maternal education	Maternal education	Maternal education	Maternal education
Smoking in pregnancy	Smoking in pregnancy	Smoking in pregnancy	Smoking in pregnancy
Paternal asthma	Paternal asthma	Paternal asthma	Parity
Parity	Parity	Parity	Gestation
Gestation	Gestation	Gestation	
	Gender		

Table 71 Potential confounders of the lung function outcomes

Those children who had received a diagnosis of asthma had lower FEV_1 values than those that had not (Figure 29 & Table 72). Similarly, children who were reported to wheeze had lower FEV_1 values than those who did not (Table 72). However, in both cases there was a great deal of overlap between children with and without a wheezing condition and the difference between means was not statistically significant.

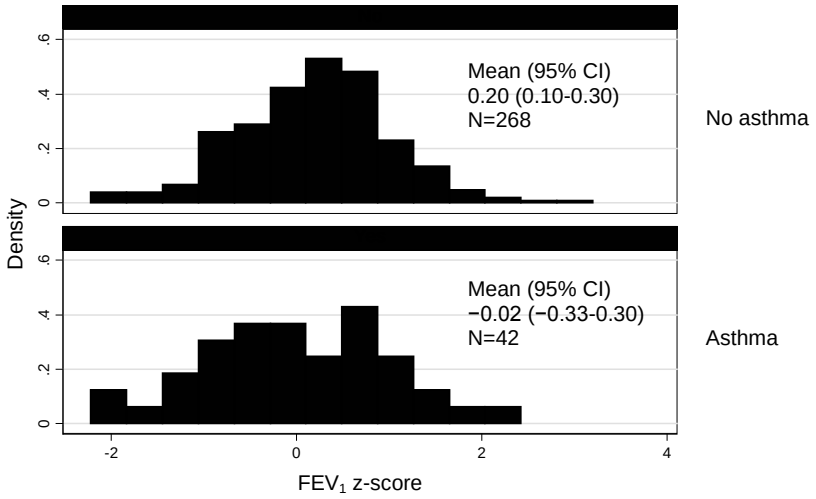


Figure 29 FEV_1 z-score according to presence or absence of asthma

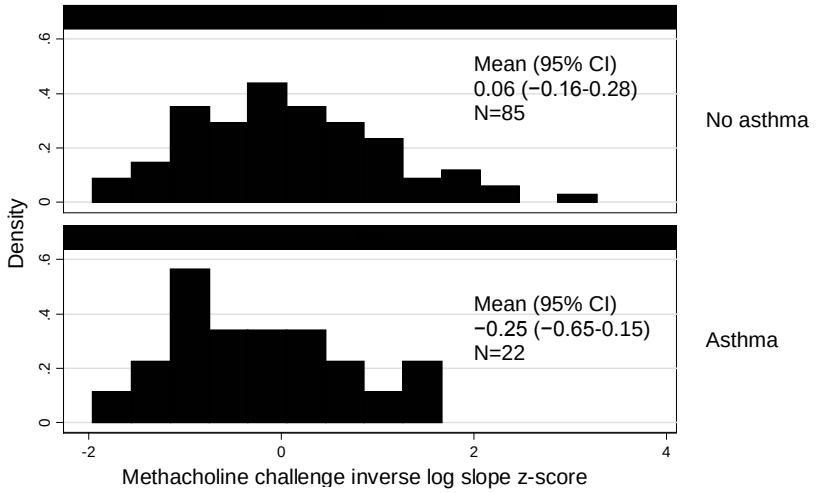


Figure 30 BHR z-score according to presence or absence of asthma

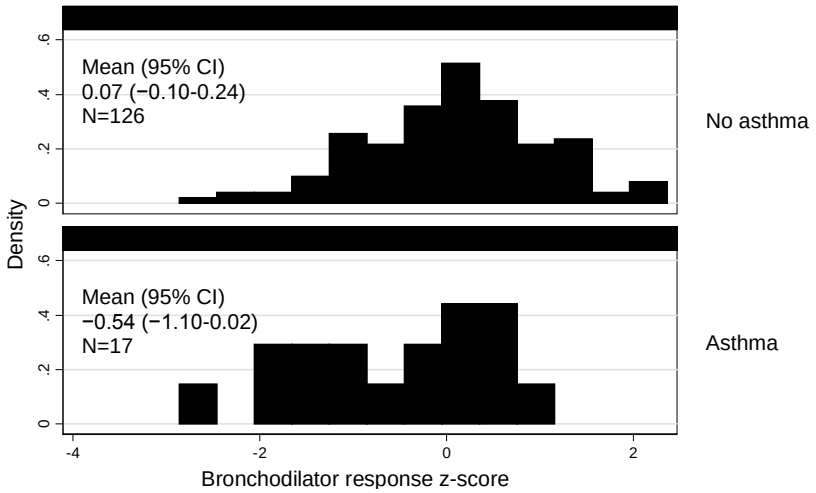


Figure 31 BDR z-score according to presence or absence of asthma

As expected, children with asthma or wheeze were more sensitive to methacholine than children without wheeze, but this difference was non significant at the 5% significance level (Table 72 & Figure 30). The bronchodilator response was lower in children with reported wheeze than in those without, and lower in those with asthma diagnosed by a doctor than those without such a diagnosis (Table 72 & Figure 31). It is unclear why this seemingly counterintuitive result occurred, a potential explanation may be that the population studied contained some relatively mild and well-controlled asthmatics (with low BDR) and a number of undiagnosed asthmatic children (with high BDR).

Lung function measure	**Mean z-score (n)**		**P-value**
	Wheeze	**No wheeze**	
FEV_1	0.07 (37)	0.18 (273)	0.45
FEV_1/FVC	−0.13 (37)	0.05 (273)	0.31
$FEF_{25-75\%}$	−0.32 (37)	−0.08 (273)	0.19
BDR (expressed as percent change in FEV_1)	−0.20 (22)	0.04 (121)	0.30
BHR (expressed as inverse log. slope)	−0.14 (23)	0.04 (84)	0.46
	Mean z-score (n)		**P-value**
	Asthma	**No asthma**	
FEV_1	−0.02 (42)	0.20 (268)	0.12
FEV_1/FVC	−0.03 (42)	0.03 (268)	0.71
$FEF_{25-75\%}$	−0.36 (42)	−0.07 (268)	0.10
BDR (expressed as percent change in FEV_1)	−0.54 (17)	0.07 (126)	0.02
BHR (expressed as inverse log. slope)	−0.25 (22)	0.06 (85)	0.190

Table 72 Lung function measures according to reported wheeze and asthma diagnosis

7.5.1 Maternal body composition and FEV_1 in the offspring

Primary hypothesis: either maternal pre-pregnancy total body fat, or arm muscle area, or both are positively associated with FEV_1 in the offspring at age 6 years

Rationale: maternal body composition is known to be associated with birthweight (Sanin Aguirre *et al.* 2004) and low birthweight is known to be associated with poorer infant lung function (Lucas *et al.* 2004). Factors associated with faltering of fetal growth may also be associated with poor lung development sufficient to affect lung function.

Secondary hypothesis: lung function at 6 years may be more closely related body composition measured during pregnancy or to pregnancy weight gain.

Rationale: maternal body composition may serve to transmit a signal to the developing fetus regarding the expected postnatal environment but it is not known exactly which aspect of maternal body composition is important (Haberg *et al.* 2009). Maternal body composition may also serve as a marker for an effect of a particular type of diet upon lung development (Guo *et al.* 2004).

Results: maternal body composition was not associated with offspring lung function.

Maternal pre-pregnancy total body fat was not associated with children's lung function at 6 years, as measured by FEV_1. Table 73 shows that pre-pregnancy body fat percentage, BMI and arm muscle area were not significantly associated with FEV_1. There were no significant associations between any measure of maternal body composition in early or late pregnancy and FEV_1, either adjusted or unadjusted. Pregnancy weight gain was not significantly associated with FEV_1 (Table 73).

Maternal body composition	Unadjusted analyses			Adjusted* analyses		
	Beta (95% CI)	P value	n	Beta (95% CI)	P value	n
Pre-pregnancy						
Total body fat	0.00 (−0.09-0.10)	0.926	306	0.01 (−0.08-0.10)	0.827	292
Body fat percentage	0.00 (−0.09-0.09)	0.989	307	0.01 (−0.08-0.10)	0.874	293
Subscapular:triceps	0.02 (−0.08-0.12)	0.634	306	0.02 (−0.08-0.12)	0.719	292
BMI	0.02 (−0.07-0.11)	0.640	306	0.03 (−0.07-0.12)	0.560	292
Arm muscle area	0.03 (−0.06-0.13)	0.478	307	0.02 (−0.07-0.11)	0.670	293
During pregnancy						
11 week body fat	0.03 (−0.07-0.14)	0.552	244	0.02 (−0.08-0.13)	0.640	238
34 week body fat	0.02 (−0.07-0.12)	0.616	305	0.03 (−0.07-0.12)	0.564	295
11 week arm muscle	0.04 (−0.06-0.15)	0.402	245	0.04 (−0.07-0.14)	0.482	239
34 week arm muscle	0.03 (−0.06-0.12)	0.548	305	0.03 (−0.06-0.12)	0.502	295
Pregnancy weight gain	0.04 (−0.06-0.13)	0.471	302	0.03 (−0.07-0.12)	0.554	292

***Adjusted for child's gender, gestation, age at last breast feed and birth order, mother's education, smoking in pregnancy and history of asthma and father's history of asthma**
Regression coefficients were calculated as change in FEV_1 per SD change in body composition measurement.

Table 73 Regression analysis of FEV_1 z-score according to maternal pre-pregnancy body composition, body composition during pregnancy and weight gain in pregnancy

7.5.2 Maternal diet and FEV_1 in the offspring

7.5.2.1 Maternal micronutrient intake

Primary hypothesis: low maternal intake of vitamins A and C during pregnancy is associated with reduced FEV_1 in the offspring at 6 years of age.

Rationale: epidemiological evidence suggests that intake of fresh fruits and vegetables and other sources of dietary antioxidants has declined over the same time period that wheezing disorders have increased (Department for Environment 2009). There is also evidence from animal experiments that deficiency of vitamins A (Antipatis *et al.* 2000; Downie *et al.* 2005). and C (Proskocil *et al.* 2005) may negatively impact upon lung development.

Results: vitamin A and C intake either before or during pregnancy were not found in either the adjusted or unadjusted analysis to be significantly associated with FEV_1 (Table 74). There was, however, a weak trend for lower late pregnancy vitamin A intake to be

associated with lower FEV_1. When the data were re-analysed using energy-adjusted intakes, the main findings were unchanged, except the positive association with vitamin A intake at 34 weeks' gestation became significant after adjusting for confounders (p=0.021) (Table 115).

Standardised intake	Unadjusted analyses			Adjusted* analyses		
	Beta (95% CI)	P value	n	Beta (95% CI)	P value	n
Vitamin A						
Pre-pregnancy	0.02 (−0.08-0.11)	0.713	310	0.05 (−0.04-0.15)	0.275	296
11 weeks	−0.05 (−0.16-0.05)	0.329	245	−0.06 (−0.17-0.05)	0.299	239
34 weeks	0.06 (−0.03-0.15)	0.168	305	0.08 (−0.01-0.17)	0.076	295
Vitamin C						
Pre-pregnancy	0.03 (−0.07-0.12)	0.552	310	0.03 (−0.07-0.12)	0.540	295
11 weeks	−0.01 (−0.12-0.10)	0.855	245	−0.03 (−0.14-0.08)	0.631	239
34 weeks	0.02 (−0.08-0.12)	0.644	305	0.04 (−0.06-0.14)	0.459	295

***Adjusted for child's gender, gestation, age at last breast feed and birth order, mother's education, smoking in pregnancy and history of asthma and father's history of asthma**
Regression coefficients were calculated as change in FEV_1 per SD change in vitamin intake.

Table 74 Regression analysis of FEV_1 z-scores according to maternal vitamin A and C intakes before and during pregnancy

7.5.2.2 Maternal nutrient status

7.5.2.2.1 *Serum vitamin concentration at 34 weeks' gestation*

Primary hypothesis: serum levels of retinol (vitamin A) and ascorbic acid (vitamin C) are positively associated with FEV_1 in the offspring at age 6 years.

Rationale: animal studies support a putative protective role for vitamins A (Antipatis *et al.* 2000; Downie *et al.* 2005) and C (Proskocil *et al.* 2005) in lung development. There is also epidemiological evidence that suggests low vitamin A and C status in childhood increases the risk of developing asthma (Harik-Khan *et al.* 2004). In adults, vitamin C status has been found to be positively associated with lung function (Ness *et al.* 1996).

Secondary hypothesis: maternal serum antioxidant status at 34 weeks' gestation is positively associated with FEV_1 in the offspring at 6 years of age.

Rationale: it has been proposed that the protective effects of vitamins A and C upon lung development that are observed in animal studies may be due to antioxidant properties of these vitamins (Seaton *et al.* 1994).

Results: there was no evidence that maternal vitamin A or C status was significantly associated with FEV_1 in either the adjusted or unadjusted analysis. There was also no evidence for a significant association between mothers' total anti-oxidant status at 34 weeks' gestation and FEV_1 measured in their children at age 6 years (Table 75).

Standardised status	Unadjusted analyses			Adjusted* analyses		
	Beta (95% CI)	P value	n	Beta (95% CI)	P value	n
Retinol	−0.01 (−0.12-0.09)	0.798	243	−0.04 (−0.15-0.07)	0.493	234
Ascorbic acid	−0.01 (−0.12-0.10)	0.896	227	−0.03 (−0.15-0.09)	0.640	219
Total anti-oxidants	0.07 (−0.02-0.17)	0.136	287	0.06 (−0.04-0.15)	0.262	276

***Adjusted for child's gender, gestation, age at last breast feed and birth order, mother's education, smoking in pregnancy and history of asthma and father's history of asthma**
Regression coefficients were calculated as change in FEV_1 per SD change vitamin status.

Table 75 Regression analysis of FEV_1 z-scores according to maternal serum vitamin and anti-oxidant concentrations at 34 weeks' gestation

7.5.2.2.2 *Plasma phospholipid fatty acid composition at 34 weeks' gestation*

Exploratory analysis: to examine whether maternal n-3 PUFA status or the ratio of n-3 PUFAs to n-6 PUFAs in plasma phospholipids are associated with FEV_1 measured at 6 years of age.

Rationale: epidemiological studies have found an inverse association between maternal fish consumption and asthma in childhood (Hodge *et al.* 1996; Peat *et al.* 1992b). There is evidence that n-3 PUFA supplementation may have a protective effect in adults including a positive effect upon lung function (Okamoto *et al.* 2000a).

Results: no measure of plasma phospholipid phosphatidylcholine composition was significantly associated with FEV_1 (Table 76).

Percentage intake	Unadjusted analyses			Adjusted* analyses		
	Beta (95% CI)	P value	n	Beta (95% CI)	P value	n
n-3 PUFAs	0.06 (−0.04-0.16)	0.233	296	0.06 (−0.03-0.16)	0.208	285
n-6 PUFAs	−0.03 (−0.13-0.06)	0.508	296	−0.05 (−0.14-0.05)	0.332	285
n-3:6 ratio	0.06 (−0.04-0.15)	0.243	296	0.06 (−0.03-0.16)	0.197	285
α-Linolenic acid	−0.01 (−0.10-0.09)	0.912	283	−0.04 (−0.13-0.06)	0.416	272
Linoleic acid	−0.07 (−0.17-0.03)	0.196	296	−0.08 (−0.18-0.02)	0.112	285
DHA	0.07 (−0.02-0.17)	0.143	296	0.07 (−0.02-0.17)	0.141	285
EPA	0.05 (−0.04-0.14)	0.274	290	0.04 (−0.05-0.13)	0.470	279
Arachidonic acid	0.05 (−0.04-0.15)	0.287	296	0.06 (−0.04-0.15)	0.246	285

***Adjusted for child's gender, gestation, age at last breast feed and birth order, mother's education, smoking in pregnancy and history of asthma and father's history of asthma**
Regression coefficients were calculated as change in FEV_1 per SD change in plasma phospholipid fatty acid composition.

Table 76 Regression analysis of FEV_1 according to percentage fatty acid composition of maternal plasma phospholipid phosphatidylcholine at 34 weeks' gestation

7.5.2.3 Maternal dietary patterns

Exploratory analysis: to explore whether the prudent dietary pattern is associated with measures of lung function.

Rationale: a 'health conscious' dietary pattern has been found to be positively associated with FEV_1 in a population based cohort (Shaheen *et al.* 2009). The health conscious pattern is similar to the prudent dietary pattern.

Results: in unadjusted univariate analyses, mothers' prudent diet scores at the initial and late pregnancy interviews were both associated with increased FEV_1 in their children at age 6 years. However, these associations became non-significant at the 5% significance level following adjustment for confounders (Table 77).

Prudent diet score	Unadjusted analyses			Adjusted* analyses		
	Beta (95% CI)	P value	n	Beta (95% CI)	P value	n
Pre-pregnancy	0.12 (0.03-0.21)	0.010	310	0.10 (−0.01-0.20)	0.077	296
11 weeks' gestation	0.01 (−0.09-0.11)	0.870	245	0.00 (−0.12-0.12)	0.970	239
34 weeks' gestation	0.09 (0.00-0.18)	0.045	305	0.09 (−0.02-0.20)	0.109	295

***Adjusted for child's gender, gestation, age at last breast feed and birth order, mother's education, smoking in pregnancy and history of asthma and father's history of asthma**
Regression coefficients were calculated as change in FEV_1 per SD change in prudent diet score.

Table 77 Regression analysis of FEV_1 z-scores according to maternal prudent diet score before and during pregnancy

7.5.3 Maternal nutrition and secondary measures of lung function in the offspring

Exploratory analysis: to explore the relationship between maternal nutrition during pregnancy and measures of lung function more sensitive to airway obstruction than FEV_1 and to test for associations between women's nutrition and BDR and BHR in their children at age 6 years.

Rationale: although FEV_1 is a robust measure of lung function, $FEF_{25-75\%}$ or FEV_1 standardised for lung size by dividing by FVC may be more sensitive to narrowing of the small airways characteristic of asthma. Maternal nutrition may differentially affect static airway caliber and the dynamic tendency of the airways to constrict. Including both BDR and BHR in the analysis provides a more thorough assessment of the relationship between maternal nutrition and respiratory health in childhood than an analysis based upon spirometry alone.

7.5.3.1 Maternal body composition and lung function in the offspring

Results: no measure of maternal body composition either before or during pregnancy was significantly related to FEV_1/FVC. Similarly, no significant associations were found between any measure of maternal body composition and $FEF_{25-75\%}$. An inverse association between FEV_1/FVC and maternal fat mass at 34 weeks' gestation approached significance (p=0.082) but this association weakened upon adjusting for confounders (p=0.105) (Table 78).

Maternal body composition	Unadjusted analyses Beta (95% CI)	P value	n	Adjusted* analyses Beta (95% CI)	P value	n
FEV_1/FVC z-score						
Pre-pregnancy						
Total body fat	−0.05 (−0.16-0.05)	0.344	306	−0.05 (−0.16-0.05)	0.321	293
Body fat percentage	−0.02 (−0.13-0.08)	0.643	307	−0.03 (−0.24-0.08)	0.597	294
Subscapular:triceps	0.01 (−0.10-0.13)	0.799	306	−0.01 (−0.13-0.11)	0.885	294
BMI	−0.05 (−0.15-0.06)	0.377	306	−0.07 (−0.18-0.04)	0.220	293
Arm muscle area	−0.07 (−0.18-0.04)	0.206	307	−0.10 (−0.21-0.01)	0.086	294
During pregnancy						
11 week body fat	−0.05 (−0.16-0.07)	0.403	244	−0.06 (−0.18-0.06)	0.325	239
34 week body fat	−0.09 (−0.20-0.01)	0.082	305	−0.09 (−0.20-0.02)	0.105	296
11 week arm muscle	0.03 (−0.08-0.15)	0.576	245	0.01 (−0.11-0.12)	0.926	240
34 week arm muscle	−0.05 (−0.16-0.05)	0.322	305	−0.05 (−0.16-0.06)	0.374	296
Pregnancy weight gain	−0.08 (−0.19-0.03)	0.171	302	−0.05 (−0.17-0.06)	0.383	293
$FEF_{25-75\%}$ z-score						
Pre-pregnancy						
Total body fat	−0.04 (−0.16-0.08)	0.500	306	−0.05 (−0.17-0.07)	0.393	293
Body fat percentage	−0.02 (−0.14-0.09)	0.680	307	−0.03 (−0.15-0.08)	0.576	294
Subscapular:triceps	0.05 (−0.08-0.17)	0.484	306	0.00 (−0.13-0.13)	0.950	294
BMI	−0.01 (−0.13-0.10)	0.835	306	−0.04 (−0.16-0.08)	0.513	293
Arm muscle area	−0.01 (−0.13-0.11)	0.833	307	−0.06 (−0.18-0.06)	0.332	294
During pregnancy						
11 week body fat	−0.03 (−0.16-0.10)	0.682	244	−0.05 (−0.18-0.08)	0.413	239
34 week body fat	−0.08 (−0.19-0.04)	0.190	305	−0.08 (−0.20-0.04)	0.194	296
11 week arm muscle	0.04 (−0.09-0.16)	0.565	245	0.00 (−0.13-0.13)	0.980	240
34 week arm muscle	−0.03 (−0.15-0.08)	0.557	305	−0.04 (−0.16-0.08)	0.546	296
Pregnancy weight gain	−0.07 (−0.19-0.05)	0.270	302	−0.05 (−0.17-0.07)	0.438	293

***FEV_1/FVC z-score analysis adjusted for child's gestation, mother's education, smoking in pregnancy and history of asthma and rhinitis and father's history of asthma. $FEF_{25-75\%}$ z-score analysis adjusted for child's gender, gestation and birth order, mother's education, smoking in pregnancy and history of asthma and rhinitis and father's history of asthma**
Regression coefficients were calculated as change in lung function measurement per SD change in body composition measurement.

Table 78 Regression analysis of childhood lung function according to maternal body composition before and during pregnancy, and weight gain in pregnancy

Neither the bronchodilator response nor bronchial hyperreactiveness, as quantified by inverse log. slope, were significantly associated with any measure of maternal body composition (Table 79).

Maternal body composition	Unadjusted analyses Beta (95% CI)	P value	n	Adjusted* analyses Beta (95% CI)	P value	n
BDR z-score						
Pre-pregnancy						
Total body fat	0.02 (−0.15-0.19)	0.834	141	−0.01 (−0.19-0.16)	0.901	129
Body fat percentage	0.01 (−0.16-0.18)	0.899	141	−0.02 (−0.20-0.16)	0.864	129
Subscapular:triceps	−0.08 (−0.26-0.11)	0.415	140	−0.11 (−0.30-0.08)	0.262	129
BMI	0.06 (−0.10-0.23)	0.447	141	0.02 (−0.15-0.20)	0.805	129
Arm muscle area	0.09 (−0.07-0.25)	0.271	140	0.05 (−0.13-0.22)	0.605	128
During pregnancy						
11 week body fat	0.09 (−0.08-0.26)	0.306	116	0.03 (−0.15-0.22)	0.713	109
34 week body fat	−0.01 (−0.17-0.15)	0.904	137	−0.03 (−0.20-0.13)	0.682	130
11 week arm muscle	0.05 (−0.12-0.23)	0.558	115	0.04 (−0.15-0.22)	0.701	108
34 week arm muscle	0.00 (−0.16-0.16)	0.989	138	−0.05 (−0.21-0.11)	0.556	131
Pregnancy weight gain	−0.13 (−0.30-0.04)	0.141	136	−0.11 (−0.30-0.07)	0.211	129
BHR z-score						
Pre-pregnancy						
Total body fat	0.11 (−0.09-0.31)	0.277	107	0.11 (−0.11-0.33)	0.338	102
Body fat percentage	0.14 (−0.06-0.34)	0.172	107	0.14 (−0.07-0.35)	0.188	102
Subscapular:triceps	0.01 (−0.20-0.21)	0.930	107	0.00 (−0.22-0.22)	0.991	102
BMI	0.08 (−0.12-0.28)	0.440	107	0.04 (−0.18-0.26)	0.722	102
Arm muscle area	0.05 (−0.16-0.26)	0.649	107	0.03 (−0.19-0.25)	0.801	102
During pregnancy						
11 week body fat	0.10 (−0.18-0.37)	0.488	82	0.08 (−0.21-0.37)	0.579	81
34 week body fat	0.04 (−0.17-0.25)	0.709	107	0.04 (−0.20-0.27)	0.763	102
11 week arm muscle	0.05 (−0.24-0.34)	0.745	82	0.03 (−0.27-0.33)	0.856	81
34 week arm muscle	0.04 (−0.19-0.26)	0.746	107	0.02 (−0.22-0.26)	0.865	102
Pregnancy weight gain	−0.10 (−0.29-0.08)	0.280	107	−0.09 (−0.28-0.11)	0.379	102

***BDR z-score analysis adjusted for child's gestation and birth order, mother's education, smoking in pregnancy and history of asthma and rhinitis and father's history of asthma. Inverse log. slope z-score analysis adjusted for child's gestation and birth order, mother's education, smoking in pregnancy and history of asthma and father's history of asthma**
Regression coefficients were calculated as change in lung function measurement per SD change in body composition measurement.

Table 79 Regression analysis of childhood BDR and BHR according to maternal body composition before and during pregnancy, and weight gain in pregnancy

7.5.3.2 Maternal diet and lung function in the offspring

7.5.3.2.1 *Maternal micronutrient intake*

Neither FEV_1/FVC nor $FEF_{25-75\%}$ were positively associated with maternal vitamin A intake. Similarly, the data for vitamin C intake did not confirm the hypothesis that maternal intake is positively associated with childhood lung function (Table 80).

Standardised intake	Unadjusted analyses			Adjusted* analyses		
	Beta (95% CI)	P value	n	Beta (95% CI)	P value	n
FEV_1/FVC z-score						
Vitamin A						
Pre-pregnancy	0.07 (0.04-0.18)	0.227	310	0.05 (−0.06-0.17)	0.335	297
11 weeks	−0.01 (−0.13-0.11)	0.827	245	0.01 (−0.11-0.14)	0.856	240
34 weeks	0.06 (−0.04-0.16)	0.260	305	0.07 (−0.03-0.18)	0.166	296
Vitamin C						
Pre-pregnancy	0.01 (−0.10-0.12)	0.858	310	0.00 (−0.11-0.12)	0.935	297
11 weeks	0.00 (−0.12-0.12)	0.994	245	0.00 (−0.13-0.13)	0.997	240
34 weeks	0.07 (−0.04-0.19)	0.205	305	0.07 (−0.05-0.19)	0.247	296
$FEF_{25-75\%}$ z-score						
Vitamin A						
Pre-pregnancy	0.04 (−0.08-0.16)	0.535	310	0.05 (−0.07-0.17)	0.408	297
11 weeks	−0.05 (−0.18-0.09)	0.499	245	0.00 (−0.14-00.13)	0.959	240
34 weeks	0.05 (−0.06-0.17)	0.352	305	0.68 (−0.03-0.20)	0.148	296
Vitamin C						
Pre-pregnancy	−0.01 (−0.13-0.11)	0.827	310	−0.01 (−0.14-0.11)	0.817	297
11 weeks	0.00 (−0.13-0.14)	0.942	245	−0.01 (−0.15-0.13)	0.883	240
34 weeks	0.07 (−0.06-0.19)	0.287	305	0.07 (−0.06-0.20)	0.282	296

***FEV_1/FVC z-score analysis adjusted for child's gestation, mother's education, smoking in pregnancy and history of asthma and rhinitis and father's history of asthma. $FEF_{25-75\%}$ z-score analysis adjusted for child's gender, gestation and birth order, mother's education, smoking in pregnancy and history of asthma and rhinitis and father's history of asthma**
Regression coefficients were calculated as change in lung function measurement per SD change in vitamin intake.

Table 80 Regression analysis of childhood spirometry according to maternal vitamin intake before and during pregnancy

No significant associations were found between maternal vitamin A or C intake and either BDR (measured as percentage change in predicted FEV_1) or BHR (measured as inverse log. slope). There was a weak inverse association between vitamin A at 11 weeks' gestation and BDR and vitamin C intake at 34 weeks' gestation showed a weak

inverse association with inverse log. slope. Both associations failed to reach significance at the 5% level and their significance was further reduced by adjusting for confounders (Table 81).

Standardised intake	Unadjusted analyses			Adjusted* analyses		
	Beta (95% CI)	P value	n	Beta (95% CI)	P value	n
BDR z-score						
Vitamin A						
Pre-pregnancy	0.05 (−0.11-0.22)	0.531	143	0.06 (−0.11-0.23)	0.488	131
11 weeks	−0.14 (−0.30-0.02)	0.081	116	−0.14 (−0.31-0.03)	0.109	109
34 weeks	−0.07 (−0.23-0.09)	0.376	138	−0.05 (−0.22-0.11)	0.532	131
Vitamin C						
Pre-pregnancy	0.01 (−0.13-0.16)	0.847	143	−0.02 (−0.18-0.13)	0.784	131
11 weeks	−0.03 (−0.21-0.14)	0.719	116	−0.13 (−0.31-0.06)	0.188	109
34 weeks	0.00 (−0.16-0.16)	0.995	138	−0.03 (−0.20-0.13)	0.685	131
BHR z-score						
Vitamin A						
Pre-pregnancy	−0.15 (−0.35-0.05)	0.139	107	−0.13 (−0.34-0.08)	0.214	102
11 weeks	−0.11 (−0.38-0.16)	0.423	82	−0.15 (−0.43-0.13)	0.284	81
34 weeks	−0.08 (−0.26-0.11)	0.414	107	−0.11 (−0.31-0.08)	0.229	102
Vitamin C						
Pre-pregnancy	−0.02 (−0.25-0.22)	0.894	107	0.02 (−0.23-0.27)	0.867	102
11 weeks	−0.09 (−0.34-0.16)	0.489	82	−0.08 (−0.35-0.19)	0.566	81
34 weeks	−0.17 (−0.34-0.01)	0.061	107	−0.13 (−0.32-0.05)	0.159	102

***BDR z-score analysis adjusted for child's gestation and birth order, mother's education, smoking in pregnancy and history of asthma and rhinitis and father's history of asthma. Inverse log. slope z-score analysis adjusted for child's gestation and birth order, mother's education, smoking in pregnancy and history of asthma and father's history of asthma**
Regression coefficients were calculated as change in lung function measurement per SD change in vitamin intake.

Table 81 Regression analysis of BDR and BHR according to maternal vitamin intake before and during pregnancy

Vitamin C intake remained unrelated to any measure of lung function when the data were re-analysed using energy-adjusted vitamin intakes (Appendix 1, Tables 115-117). As no significant associations were found between mothers' total vitamin C intake and their children's lung function, further analysis of the effects of supplementation were not investigated. After adjusting for the same confounders used in the analysis described in Table 70 & Table 71, energy-adjusted vitamin A intake at 34 weeks'

gestation, however, was found to be significantly positively associated with $FEF_{25-75\%}$ z-score (p=0.023) and inversely associated with BHR as measured by the inverse log. slope (p=0.039) (Table 116 & Table 117).

The inverse association between BHR score and 34-week energy-adjusted vitamin A intake remained significant regardless of the method of BHR expression. That is higher maternal vitamin A intake was associated with lower BH (inverse log. slope) score, thus a greater degree of bronchial hyperresponsiveness. When BHR was considered as a binary variable with a cut-off PC_{20} of 16 mg/ml the relative risk of a positive methacholine challenge (20% drop in FEV_1 at or before reaching the 16 mg /ml concentration) increased by 40% per SD change in energy-adjusted 34-week vitamin A intake, (p=0.02).

Furthermore, these significant associations did not appear dependent upon the source of vitamin A. The positive associations between late pregnancy energy-adjusted vitamin A intake and the z-scores for FEV_1 and $FEF_{25-75\%}$ remained significant when only food-derived vitamin A was considered (beta 0.10, p=0.032 and beta 0.13, p=0.028). These associations also remained significant when total vitamin A intake was analysed including data only from women who were not taking supplements (beta 0.12, p=0.015 and beta 0.16, p=0.014) (Appendix 1 Tables 124 & 125). Similarly, the inverse association between late pregnancy energy-adjusted vitamin A intake and BHR remained significant when only food sources of this vitamin were considered or when total vitamin A intake in non-supplemented women was analysed separately from that of women taking supplemental vitamin A (beta -0.17, p=0.020 and beta -0.22, p=0.020) (Appendix 1). The study was not powered to detect significant associations between measures of lung function and total vitamin A intake in women using supplements because few women used supplements and fewer still supplemented with vitamin A during pregnancy.

7.5.3.2.2 *Maternal nutrient status*

Neither FEV_1/FVC nor $FEF_{25-75\%}$ were significantly associated with maternal vitamin A or C status or with maternal total anti-oxidant status at 34 weeks' gestation (Table 82).

Standardised status	Unadjusted analyses			Adjusted* analyses		
	Beta (95% CI)	P value	n	Beta (95% CI)	P value	n
FEV_1/FVC z-score						
Retinol	0.08 (−0.05-0.20)	0.218	243	0.05 (−0.09-0.18)	0.498	235
Ascorbic acid	−0.01 (−0.12-0.11)	0.907	227	−0.07 (−0.19-0.06)	0.295	220
Total anti-oxidants	−0.02 (−0.13-0.09)	0.721	287	−0.03 (−0.14-0.09)	0.657	277
$FEF_{25-75\%}$ z-score						
Retinol	0.03 (−0.10-0.17)	0.646	243	−0.01 (−0.15-0.13)	0.867	235
Ascorbic acid	−0.01 (−0.15-0.13)	0.885	227	−0.07 (−0.21-0.08)	0.377	220
Total anti-oxidants	0.03 (−0.09-0.15)	0.652	287	0.02 (−0.11-0.14)	0.789	277

***FEV_1/FVC z-score analysis adjusted for child's gestation, mother's education, smoking in pregnancy and history of asthma and rhinitis and father's history of asthma. $FEF_{25-75\%}$ z-score analysis adjusted for child's gender, gestation and birth order, mother's education, smoking in pregnancy and history of asthma and rhinitis and father's history of asthma**
Regression coefficients were calculated as change in lung function measurement per SD change in vitamin status.

Table 82 Regression analysis of spirometry according to maternal serum vitamin and anti-oxidant concentrations at 34 weeks' gestation

After adjusting for relevant confounders neither BDR nor inverse log. slope was significantly associated with maternal vitamin A or C status or with maternal total anti-oxidant status at 34 weeks' gestation (Table 83). In the unadjusted analysis an inverse relationship between serum retinol and BDR approached significance. The significance of this association was greatly changed by inclusion of maternal rhinitis and maternal and paternal asthma in the multivariate model.

Standardised status	**Unadjusted analyses**			**Adjusted* analyses**		
	Beta (95% CI)	**P value**	**n**	**Beta (95% CI)**	**P value**	**n**
BDR z-score						
Retinol	−0.16 (−0.33-0.02)	0.074	106	−0.03 (−0.22-0.15)	0.716	101
Ascorbic acid	0.09 (−0.12-0.29)	0.411	101	0.03 (−0.19-0.26)	0.762	96
Total anti-oxidants	0.14 (−0.02-0.31)	0.092	131	0.15 (−0.03-0.34)	0.109	121
BHR z-score						
Retinol	−0.07 (−0.30-1.17)	0.578	88	−0.08 (−0.35-0.18)	0.528	83
Ascorbic acid	−0.17 (−0.36-0.03)	0.095	79	−0.12 (−0.33-0.09)	0.261	75
Total anti-oxidants	0.09 (−0.15-0.32)	0.455	96	0.04 (−0.20-0.28)	0.736	91

***BDR z-score analysis adjusted for child's gestation and birth order, mother's education, smoking in pregnancy and history of asthma and rhinitis and father's history of asthma. Inverse log. slope z-score analysis adjusted for child's gestation and birth order, mother's education, smoking in pregnancy and history of asthma and father's history of asthma**
Regression coefficients were calculated as change in lung function measurement per SD change in vitamin status.

Table 83 Regression analysis of BDR and BHR according to maternal serum vitamin and anti-oxidant concentrations at 34 weeks' gestation

There were no significant associations between maternal plasma phospholipid phosphatidylcholine composition at 34 weeks' gestation and FEV_1/FVC, although a weak inverse association with the n-3 precursor, α-linolenic acid approached significance. There were no significant associations between $FEF_{25-75\%}$ and maternal fatty acid status. (Table 84).

Percentage intake	Unadjusted analyses			Adjusted* analyses		
	Beta (95% CI)	P value	n	Beta (95% CI)	P value	n
FEV_1/FVC z-score						
n-3 PUFAs	0.01 (0.10-0.12)	0.856	296	0.00 (−0.11-0.12)	0.964	286
n-6 PUFAs	0.03 (−0.07-0.14)	0.528	296	0.01 (−0.10-0.13)	0.842	286
n-3:6 ratio	0.00 (−0.11-0.11)	0.993	296	0.00 (−0.12-0.11)	0.994	286
α-Linolenic acid	−0.10 (−0.21-0.01)	0.081	283	−0.11 (−0.22-0.01)	0.061	273
Linoleic acid	0.03 (−0.08-0.14)	0.601	296	0.02 (−0.10-0.14)	0.752	286
DHA	0.03 (−0.08-0.14)	0.583	296	0.02 (−0.10-0.14)	0.749	286
EPA	−0.04 (−0.14-0.07)	0.502	290	−0.04 (−0.15-0.06)	0.431	280
Arachidonic acid	0.00 (−0.11-0.11)	0.944	296	−0.02 (−0.13-0.09)	0.739	286
$FEF_{25-75\%}$ z-score						
n-3 PUFAs	0.05 (−0.07-0.17)	0.398	296	0.05 (−0.08-0.17)	0.464	286
n-6 PUFAs	0.00 (−0.12-0.12)	0.976	296	−0.03 (−0.16-0.09)	0.590	286
n-3:6 ratio	0.01 (−0.12-0.13)	0.933	296	0.04 (−0.08-0.16)	0.544	286
α-Linolenic acid	−0.06 (−0.18-0.07)	0.383	283	−0.08 (−0.20-0.04)	0.202	273
Linoleic acid	0.01 (−0.11-0.14)	0.817	296	−0.01 (−0.14-0.12)	0.879	286
DHA	0.07 (−0.05-0.19)	0.257	296	0.06 (−0.07-0.18)	0.380	286
EPA	0.01 (−0.11-0.13)	0.866	290	0.00 (−0.12-0.11)	0.939	280
Arachidonic acid	−0.02 (−0.14-0.10)	0.743	296	−0.03 (−0.15-0.09)	0.647	286

***FEV_1/FVC z-score analysis adjusted for child's gestation, mother's education, smoking in pregnancy and history of asthma and rhinitis and father's history of asthma. $FEF_{25-75\%}$ z-score analysis adjusted for child's gender, gestation and birth order, mother's education, smoking in pregnancy and history of asthma and rhinitis and father's history of asthma**
Regression coefficients were calculated as change in lung function measurement per SD change in plasma phospholipids fatty acid composition.

Table 84 Regression analysis of childhood spirometry according to percentage fatty acid composition of maternal plasma phospholipid phosphatidylcholine at 34 weeks' gestation

Neither BDR nor BHR was significantly associated with any measure of maternal plasma phospholipid composition. After adjusting for confounders a weak positive association between α-linolenic acid status and BDR was found (Table 85).

Percentage intake	Unadjusted analyses			Adjusted* analyses		
	Beta (95% CI)	P value	n	Beta (95% CI)	P value	n
BDR z-score						
n-3 PUFAs	0.08 (−0.10-0.26)	0.361	136	0.10 (−0.08-0.29)	0.273	126
n-6 PUFAs	−0.03 (−0.20-0.14)	0.742	136	0.00 (−0.17-0.18)	0.977	126
n-3:6 ratio	0.08 (−0.10-0.25)	0.397	136	0.08 (−0.10-0.26)	0.365	126
α-Linolenic acid	0.12 (−0.07-0.30)	0.213	132	0.16 (−0.03-0.35)	0.097	122
Linoleic acid	−0.02 (−0.19-0.15)	0.827	136	0.02 (−0.16-0.19)	0.845	126
DHA	0.09 (−0.08-0.27)	0.294	136	0.09 (−0.09-0.28)	0.318	126
EPA	0.04 (−0.14-0.22)	0.671	135	0.04 (−0.15-0.23)	0.703	125
Arachidonic acid	−0.02 (−0.22-0.17)	0.811	136	−0.09 (−0.29-0.12)	0.412	126
BHR z-score						
n-3 PUFAs	−0.03 (−0.25-0.20)	0.808	103	0.04 (−0.19-0.27	0.758	98
n-6 PUFAs	0.02 (−0.22-0.25)	0.869	103	0.00 (−0.24-0.25)	0.968	98
n-3:6 ratio	−0.03 (−0.26-0.20)	0.805	103	0.03(−0.20-0.26)	0.798	98
α-Linolenic acid	0.06 (−0.12-0.25)	0.488	96	0.06 (−0.13-0.24)	0.529	91
Linoleic acid	0.03 (−0.19-0.26)	0.763	103	0.06 (−0.18-0.30)	0.619	98
DHA	−0.05 (−0.27-0.16)	0.618	103	0.00 (−0.23-0.22)	0.966	98
EPA	−0.03 (−0.26-0.19)	0.762	99	0.02 (−0.21-0.25)	0.886	94
Arachidonic acid	0.08 (−0.11-0.26)	0.424	103	0.01 (−0.20-0.22)	0.910	98

***BDR z-score analysis adjusted for child's gestation and birth order, mother's education, smoking in pregnancy and history of asthma and rhinitis and father's history of asthma. Inverse log. slope z-score analysis adjusted for child's gestation and birth order, mother's education, smoking in pregnancy and history of asthma and father's history of asthma**
Regression coefficients were calculated as change in lung function measurement per SD change in plasma phospholipids fatty acid composition.

Table 85 Regression analysis of BDR and BHR according to percentage fatty acid composition of maternal plasma phospholipid phosphatidylcholine at 34 weeks' gestation

7.5.3.2.3 *Maternal dietary patterns*

Prudent diet score demonstrated evidence of significant association with both FEV_1/FVC and $FEF_{25-75\%}$. Both measures were positively associated with maternal prudent diet score both before and during pregnancy. The associations between both FEV_1/FVC and $FEF_{25-75\%}$ and the prudent diet score in late pregnancy remained

significant after correcting for confounders (Table 86). BDR and BHR were not significantly associated with prudent diet score either before or during pregnancy (Table 87).

Prudent diet score	Unadjusted analyses			Adjusted* analyses		
	Beta (95% CI)	P value	n	Beta (95% CI)	P value	n
FEV_1/FVC z-score						
Pre-pregnancy	0.10 (−0.01-0.20)	0.064	310	0.10 (−0.03-0.22)	0.135	297
11 weeks' gestation	0.12 (0.00-0.23)	0.043	245	0.13 (0.00-0.26)	0.059	240
34 weeks' gestation	0.14 (0.03-0.24)	0.009	305	0.14 (0.02-0.27)	0.028	296
$FEF_{25-75\%}$ z-score						
Pre-pregnancy	0.14 (0.03-0.26)	0.014	310	0.12 (−0.02-0.25)	0.087	297
11 weeks' gestation	0.10 (−0.02-0.23)	0.107	245	0.11 (−0.04-0.25)	0.148	240
34 weeks' gestation	0.17 (0.06-0.29)	0.003	305	0.17 (0.03-0.30)	0.016	296

***FEV_1/FVC z-score analysis adjusted for child's gestation, mother's education, smoking in pregnancy and history of asthma and rhinitis and father's history of asthma. $FEF_{25-75\%}$ z-score analysis adjusted for child's gender, gestation and birth order, mother's education, smoking in pregnancy and history of asthma and rhinitis and father's history of asthma**
Regression coefficients were calculated as change in lung function measurement per SD change in prudent diet score.

Table 86 Regression analysis of childhood spirometry according to maternal prudent diet score before and during pregnancy

Prudent diet score	Unadjusted analyses			Adjusted* analyses		
	Beta (95% CI)	P value	n	Beta (95% CI)	P value	n
BDR z-score						
Pre-pregnancy	−0.06 (−0.22-0.09)	0.405	143	−0.13 (−0.31-0.06)	0.171	131
11 weeks' gestation	−0.02 (−0.20-0.16)	0.832	116	−0.05 (−0.26-0.17)	0.663	109
34 weeks' gestation	0.02 (−0.15-0.20)	0.775	138	−0.01 (−0.22-0.21)	0.946	131
BHR z-score						
Pre-pregnancy	−0.16 (−0.34-0.02)	0.081	107	−0.13 (−0.35-0.10)	0.258	102
11 weeks' gestation	−0.19 (−0.43-0.05)	0.124	82	−0.16 (−0.46-0.15)	0.312	81
34 weeks' gestation	−0.16 (−0.33-0.03)	0.091	107	−0.10 (−0.33-0.13)	0.395	102

***BDR z-score analysis adjusted for child's gestation and birth order, mother's education, smoking in pregnancy and history of asthma and rhinitis and father's history of asthma. Inverse log. slope z-score analysis adjusted for child's gestation and birth order, mother's education, smoking in pregnancy and history of asthma and father's history of asthma**
Regression coefficients were calculated as change in lung function measurement per SD change in prudent diet score.

Table 87 Regression analysis of BDR and BHR according to maternal prudent diet score before and during pregnancy

7.6 SUMMARY DISCUSSION

This chapter explored the role played by maternal nutrition in the development of lung function in childhood. Analyses were undertaken to confirm or refute the findings of previous studies where associations between nutritional factors and childhood lung function have been described, and to explore additional biologically plausible associations.

Summary of main findings:

- The spirometry values recorded in this study were consistent with those expected in 6-year-old children according to published normal values.

- Lower FEV_1, FEV_1/FVC, $FEF_{25-75\%}$ and inverse log. slope values were recorded more commonly from children who had received a diagnosis of asthma or who had experienced wheeze within the last year than from those who had not; these differences were non-significant, however. Unexpectedly, the bronchodilator response was significantly lower in asthmatic children than in non-asthmatic children.

- There was no evidence that maternal fat or muscle mass, or any other measure of maternal body composition, was associated with lung function in the offspring at age 6-years.

- There was little evidence that higher total vitamin A or C intake during pregnancy was associated with better lung function in the offspring as measured by spirometry, the bronchodilator response or bronchial challenge. Greater energy-adjusted vitamin A intake in late pregnancy was significantly associated with greater FEV_1, greater $FEF_{25-75\%}$ and greater BHR (lower inverse log. slope).

- There was no evidence that any measure of lung function was significantly associated with maternal serum ascorbic acid or retinol concentration at 34 weeks' gestation. Weak inverse associations between anti-oxidant vitamin status and BDR and BHR were found which did not reach significance.

- No measure of lung function at age 6 years was significantly associated with maternal plasma phospholipid status at 34 weeks' gestation. Weak inverse associations between maternal α-linolenic acid status and spirometric measures were found. BDR was weakly positively associated with α-linolenic acid status.

- Maternal dietary patterns were positively associated with spirometric measures. This association was significant in late pregnancy for both FEV_1/FVC and $FEF_{25-75\%}$ and remained so when adjusted for confounders.

Chapter Eight

Maternal nutrition and faltering of fetal growth and childhood wheeze phenotypes

8.1 AIM

To test the hypothesis that maternal nutrition and patterns of fetal growth are differentially associated with the development of each of the childhood wheeze phenotypes.

Wheeze phenotypes were defined according to the presence or absence of atopy and separately according to persistence of wheeze between 3 and 6 years of age. Specific associations were sought between each wheeze phenotype and:-

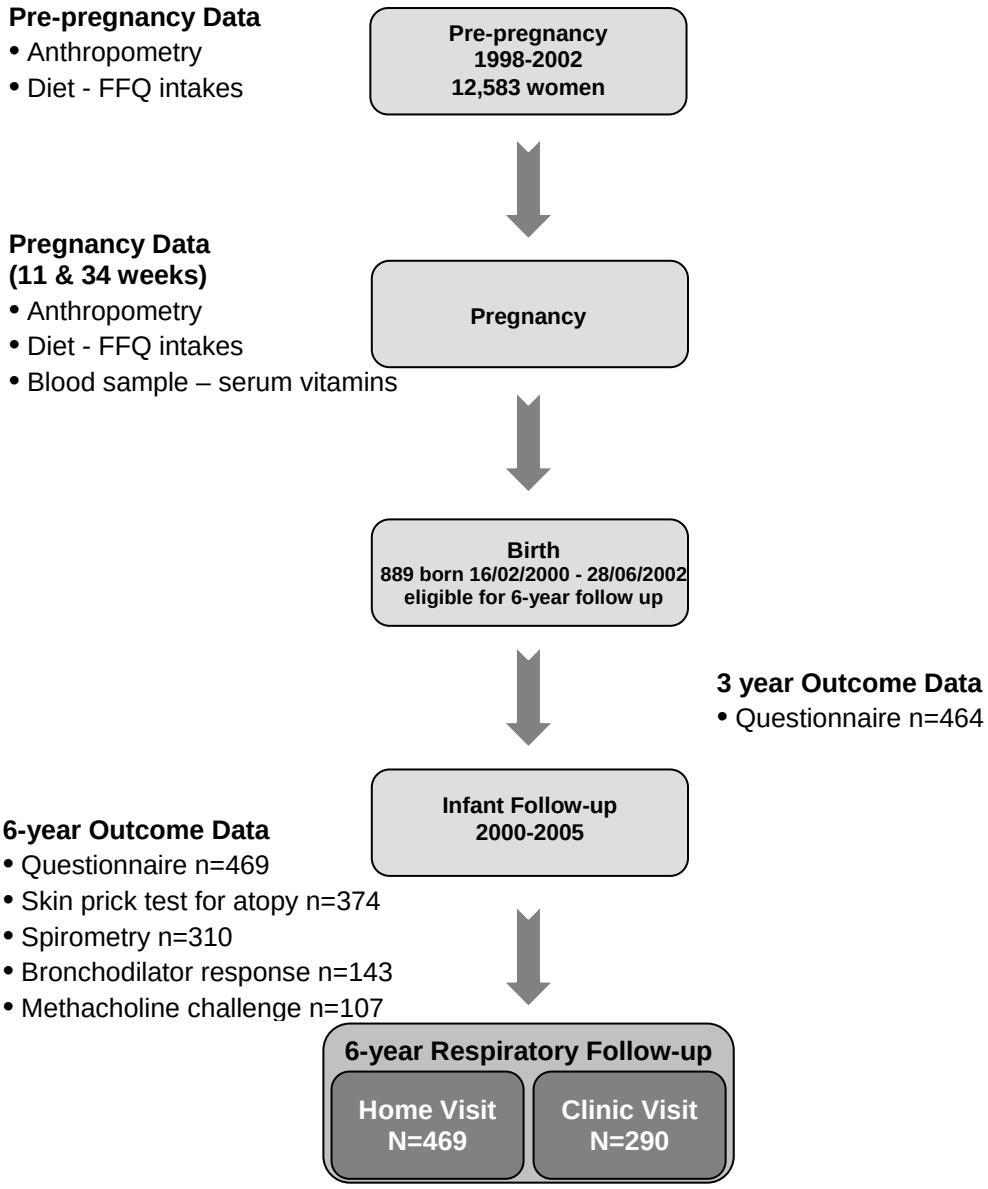
- maternal body composition before and during pregnancy
- maternal vitamin intake before and during pregnancy
- maternal serum vitamin and anti-oxidant status, and plasma phospolipid fatty acid composition in late pregnancy
- fetal and infant growth patterns

8.2 INTRODUCTION

This chapter explores the relationships between both maternal nutrition and fetal growth and the childhood wheeze phenotypes. This chapter builds on the findings of published studies (Camargo, Jr. *et al.* 2007; Devereux *et al.* 2006; Devereux *et al.* 2007)

and the data presented in previous chapters which suggest early life exposures may later influence respiratory health. Maternal nutrition is likely to be an important early life exposure (Devereux 2006; Seaton *et al.* 1994). This chapter considers how both the composition of women's diets and the overall adequacy of their nutrition may influence the respiratory health of their children. The hypotheses in this chapter were designed to test whether the different childhood wheeze phenotypes reflect the effects of different early life factors upon immune and respiratory development.

Figure 32 Data collected to assess the relationship between early life factors and wheeze phenotypes in 6-year-old children



8.3 SUMMARY OF METHODS

A detailed explanation of the methods used to collect exposure and outcome variables is given in chapter two and the cohort is defined in Figure 32. Mothers of those children who were between their sixth and seventh birthdays during the study period were invited to receive a visit from a research nurse and to attend the clinic. Childhood wheeze phenotypes were identified by combining questionnaire data from the 3-year and 6-year follow-up visits and 6-year follow-up clinic data. Information detailing the mothers' body composition and nutrition was drawn from the pre-pregnancy and pregnancy phases of the SWS. Fetal growth data were collected from serial ultrasound scans.

8.4 ANALYSIS

A cohort of eligible children was identified based upon date of birth. Children born at less than 35 weeks gestation were excluded to avoid confounding by the effects of lung disease of prematurity. Exposure variables were transformed by natural logarithm where necessary to achieve normality. Standard deviation (z-) scores were derived for continuous exposure variables. The method of Royston was used to calculate conditional measures of fetal growth in order to account for both gestation and regression to the mean (Royston 1995). Poisson regression was used to explore the relationship between maternal exposure variables and wheeze phenotypes.

The primary outcomes were doctor-diagnosed asthma and wheeze within the last year at age six. Those children that had experienced wheeze were characterised according to wheeze phenotype. Wheeze phenotypes were defined separately according to presence or absence of atopy and according to persistence or resolution of wheeze between the ages of 3 and 6 years. Information concerning atopic status and persistence of symptoms was not combined when defining wheeze phenotypes as the low membership of outcome groups so defined would limit the power of the study. Relative risks were calculated for transient and persistent wheeze using never wheezed as the comparator group; for atopic and non-atopic wheeze the comparator group was those children who had never wheezed and were not atopic at age 6 years.

The analyses were adjusted for exposures identified as likely confounders using the forward selection method described in chapter six. Additional variables were added to the final model for each phenotype based upon previously published associations with that phenotype. Atopic phenotypes were adjusted for the following additional variables maternal education (Heinrich *et al.* 1998b), maternal atopy (Arshad *et al.* 1993; Kjellman 1977) and child's birth order (Matricardi *et al.* 1998; Strachan 1989a; Strachan *et al.* 1996b). Additional variables for wheeze phenotypes were maternal education (Ernst *et al.* 1995; Lawlor *et al.* 2004), maternal history of asthma (Litonjua *et al.* 1998), smoking in pregnancy (Dezateux *et al.* 2001) and paternal history of asthma (Dold *et al.* 1992).

Doctor-diagnosed asthma	Wheeze at 6 years	Transient wheeze	Persistent wheeze	Atopic wheeze	Non-atopic wheeze
Maternal asthma	Maternal asthma	Maternal asthma	Maternal asthma	Maternal asthma	Maternal asthma
Maternal education	Maternal education	Maternal education	Maternal education	Maternal education	Maternal education
Maternal height	Pregnancy smoking	Maternal rhinitis	Maternal age	Pregnancy smoking	Maternal age
Pregnancy smoking	Paternal asthma	Maternal height	Pregnancy smoking	Paternal asthma	Pregnancy smoking
Paternal asthma	Paternal rhinitis	Pregnancy smoking	Paternal asthma	Parity	Paternal asthma
Parity	Parity	Paternal asthma	Parity	Maternal atopy	Parity
Gestation	Gestation	Parity	Gestation	Child's gender	Gestation
	Pet exposure	Gestation	Pet exposure		Pet exposure

Table 88 Potential confounders of the wheeze phenotypes outcomes

8.5 COMPARISON WITH PREVIOUS STUDIES

The primary outcomes of wheeze in the last 12 months and doctor-diagnosed asthma could be determined for all 469 children. Phenotyping by wheeze history was possible for 464 children. Two hundred and ninety two children were reported to wheeze at least once at the 6, 12, 24 or 36 month follow-up or at the 6-year follow up. Of these children, 226 (48.7%) wheezed only at or before 36 months and were classified as having had transient wheeze, 57 (12.3%) wheezed at or before 36 months as well as at 6 years and these children were classed as having persistent wheeze. A minority were reported to wheeze at the 6-year follow-up but had not been reported to wheeze previously, these nine children (1.9%) were classified as having late-onset wheeze. For 374 of those children who had a history of wheeze classification as either atopic or non-atopic wheeze was possible.

The overall prevalence of doctor-diagnosed asthma at 14.3% (95% CI 11.2-17.8%) was comparable to estimates from other UK birth cohorts of similarly aged children. The cohort followed up on the Isle of Wight reported a prevalence of 13% in 10-year-old children (Kurukulaaratchy *et al.* 2002) and the Aberdeen study a prevalence of 11.7% in children of 5 years of age (Devereux *et al.* 2006). The prevalence of wheeze at 6 years (14.3%, 95% CI 11.2-17.8%) was comparable to that found in the Aberdeen study (12.9%) although lower than that in both the Isle of Wight study (18.9%) and that reported for the 6-year-old children in phase I of the ISAAC study (18.4%) (Stewart *et al.* 2001). The relative prevalence of each of the wheeze phenotypes is strongly dependent upon the symptom and age-based criteria used to define particular phenotypes. In the Tucson cohort, for example, Martinez reported that 51.4% of 6-year-old children had never wheezed and the remaining children were classified as experiencing transient wheeze (19.9%), persistent wheeze (13.7%) and late-onset wheeze (15.0%) (Martinez *et al.* 1995). A similar pattern whereby transient and late-onset wheeze were relatively common outcomes and persistent wheeze less so was described in the Italian Studies on Respiratory Disorders in Childhood and the Environment (SIDRIA) (Rusconi *et al.* 1999). However, both wheeze at 6 years and doctor-diagnosed asthma at 8% and 4% respectively were considerably lower in this cohort and hence the percentage of the cohort described by each wheeze phenotype were also lower than those described by Martinez.

In the SWS cohort, 48.7% (95% CI 44.1-53.4%) of children were assigned to the transient wheeze phenotype, 12.3% (95% CI 9.4-15.6%) to persistent wheeze and only 1.9% (95% CI 0.9-3.7%) to the late-onset wheeze phenotype. The comparatively high prevalence of transient wheeze may be a consequence of collecting data from parental report rather than, as in the Tucson cohort, from medical records. The age-based cut-offs used to classify wheeze phenotype according to persistence of symptoms also differ between cohort studies. For example, the SWS cut-off for three year data collection, (mean 3.1 years, SD 0.1 year), was greater than that employed in the SIDRIA study, (2 years); this may also have contributed to the relatively high rate of transient wheeze in the SWS cohort compared with that in other studies.

8.6 RESULTS

8.6.1 Maternal body composition and childhood wheeze phenotypes

Exploratory analysis: to explore potential associations between maternal body composition and childhood wheeze phenotypes.

Rationale: although maternal body composition did not appear strongly predictive of either atopy (section 6.5.1) or lung function (sections 7.5.1 & 7.5.3.1), epidemiological evidence suggests that children of obese mothers are at an increased risk of wheezing in childhood (Haberg *et al.* 2009).

Results: Several measures of maternal body composition both before and during pregnancy were significantly associated with non-atopic wheeze in the offspring at age six years. Maternal body fat during pregnancy was significantly associated with recent doctor-diagnosed asthma. Less significant associations were also seen between higher maternal body fat and atopic wheeze, transient wheeze, wheeze in the last 12 months and doctor-diagnosed asthma ever. Maternal arm muscle area was also significantly associated with transient wheeze.

Maternal body composition	Unadjusted analyses RR (95% CI)	P value	n	Adjusted* analyses RR (95% CI)	P value	n
Doctor-diagnosed asthma						
Pre-pregnancy						
Total body fat	1.13 (0.92-1.39)	0.227	465	1.14 (0.92-1.41)	0.234	441
Body fat percentage	1.10 (0.89-1.36)	0.374	466	1.14 (0.91-1.42)	0.245	442
Subscapular:triceps	1.16 (0.95-1.42)	0.156	465	1.17 (0.93-1.46)	0.173	442
BMI	1.06 (0.87-1.28)	0.582	465	1.14 (0.93-1.39)	0.199	442
Arm muscle area	1.11 (0.91-1.35)	0.323	465	1.13 (0.92-1.39)	0.241	441
During pregnancy						
11 week body fat	1.18 (0.93-1.48)	0.166	380	1.14 (0.89-1.46)	0.297	369
34 week body fat	1.19 (0.98-1.44)	0.079	458	1.20 (0.98-1.48)	0.084	441
11 week arm muscle	1.08 (0.85-1.36)	0.540	380	1.05 (0.83-1.33)	0.677	369
34 week arm muscle	1.02 (0.84-1.24)	0.844	459	1.03 (0.84-1.27)	0.759	442
Pregnancy weight gain	0.90 (0.69-1.16)	0.406	455	0.82 (0.63-1.05)	0.114	440
Wheeze within last 12 months						
Pre-pregnancy						
Total body fat	1.12 (0.90-1.39)	0.300	465	1.13 (0.91-1.42)	0.267	414
Body fat percentage	1.17 (0.95-1.44)	0.138	466	1.19 (0.96-1.49)	0.119	415
Subscapular:triceps	1.08 (0.89-1.31)	0.415	465	1.11 (0.90-1.37)	0.317	415
BMI	1.01 (0.82-1.25)	0.904	465	1.01 (0.81-1.26)	0.929	414
Arm muscle area	1.01 (0.83-1.25)	0.904	465	1.07 (0.87-1.31)	0.533	414
During pregnancy						
11 week body fat	1.31 (1.04-1.65)	0.022	380	1.22 (0.96-1.56)	0.104	343
34 week body fat	1.13 (0.90-1.41)	0.300	458	1.18 (0.95-1.48)	0.137	416
11 week arm muscle	1.10 (0.88-1.38)	0.392	380	1.04 (0.81-1.33)	0.782	343
34 week arm muscle	0.90 (0.73-1.12)	0.340	459	0.91 (0.72-1.14)	0.420	417
Pregnancy weight gain	1.02 (0.78-1.32)	0.895	455	1.00 (0.75-1.34)	0.983	413

***Doctor-diagnosed asthma adjusted for child's gestation, and birth order, mother's height, education, smoking in pregnancy and history of asthma and father's history of asthma. Wheeze in the last 12 months analysis adjusted for child's gestation, pet exposure in first year of life and birth order, education, smoking in pregnancy and history of asthma and father's history of asthma**
Relative risks were calculated as change in risk per SD change body composition m easurement.

Table 89 Relative risk of doctor-diagnosed asthma and wheeze within the last 12 months according to maternal body composition before and during pregnancy, and weight gain in pregnancy

Maternal body composition	Unadjusted analyses RR (95% CI)	P value	n	Adjusted* analyses RR (95% CI)	P value	n
Transient wheeze						
Pre-pregnancy						
Total body fat	1.08 (0.99-1.18)	0.072	394	1.08 (0.99-1.17)	0.088	375
Body fat percentage	1.05 (0.97-1.15)	0.231	395	1.06 (0.98-1.16)	0.137	376
Subscapular:triceps	1.05 (0.97-1.14)	0.226	395	1.07 (0.98-1.17)	0.139	376
BMI	1.07 (0.99-1.16)	0.109	394	1.09 (1.01-1.18)	0.032	376
Arm muscle area	1.05 (0.97-1.13)	0.237	394	1.04 (0.96-1.13)	0.316	375
During pregnancy						
11 week body fat	1.09 (0.99-1.19)	0.092	324	1.09 (0.99-1.19)	0.082	315
34 week body fat	1.08 (0.99-1.18)	0.100	388	1.08 (0.98-1.18)	0.108	375
11 week arm muscle	1.10 (1.01-1.21)	0.035	325	1.10 (1.01-1.21)	0.027	316
34 week arm muscle	1.11 (1.03-1.21)	0.008	389	1.11 (1.02-1.20)	0.015	376
Pregnancy weight gain	1.02 (0.93-1.11)	0.713	385	1.02 (0.93-1.12)	0.676	374
Persistent wheeze						
Pre-pregnancy						
Total body fat	1.03 (0.86-1.23)	0.751	371	1.02 (0.84-1.23)	0.873	350
Body fat percentage	1.03 (0.86-1.22)	0.779	372	1.01 (0.83-1.23)	0.922	351
Subscapular:triceps	0.93 (0.80-1.09)	0.371	371	0.93 (0.79-1.09)	0.349	351
BMI	1.01 (0.84-1.20)	0.942	370	1.01 (0.83-1.22)	0.949	349
Arm muscle area	0.96 (0.81-1.13)	0.592	371	1.00 (0.84-1.18)	0.976	350
During pregnancy						
11 week body fat	1.05 (0.86-1.29)	0.648	301	1.01 (0.081-1.25)	0.943	292
34 week body fat	1.03 (0.85-1.24)	0.773	365	1.03 (0.84-1.27)	0.764	352
11 week arm muscle	0.92 (0.75-1.14)	0.456	301	0.94 (0.76-1.16)	0.536	292
34 week arm muscle	0.92 (0.78-1.10)	0.371	365	0.95 (0.79-1.13)	0.553	352
Pregnancy weight gain	0.99 (0.85-1.17)	0.948	362	0.95 (0.81-1.13)	0.568	349

***Transient wheeze analysis adjusted for child's gestation, birth order and pet exposure and mother's height, education, smoking in pregnancy and history of asthma and rhinitis, and father's history of asthma. Persistent wheeze analysis adjusted for child's gestation, pet exposure in the first year of life and birth order, mother's age, education, smoking in pregnancy and history of asthma and rhinitis, and father's history of asthma**
Relative risks were calculated as change in risk per SD change in body composition m easurement.

Table 90 Relative risk of transient and persistent wheeze according to maternal body composition before and during pregnancy, and weight gain in pregnancy

Maternal body composition	Unadjusted analyses			Adjusted* analyses		
	RR (95% CI)	P value	n	RR (95% CI)	P value	n
Atopic wheeze						
Pre-pregnancy						
Total body fat	1.12 (0.77-1.62)	0.554	275	1.20 (0.78-1.85)	0.399	240
Body fat percentage	1.09 (0.77-1.54)	0.620	276	1.08 (0.71-1.65)	0.721	241
Subscapular:triceps	0.92 (0.70-1.21)	0.553	275	1.00 (0.74-1.34)	0.988	241
BMI	0.96 (0.65-1.42)	0.833	274	1.04 (0.67-1.01)	0.853	239
Arm muscle area	0.91 (0.66-1.27)	0.587	275	1.14 (0.85-1.53)	0.394	240
During pregnancy						
11 week body fat	1.26 (0.81-1.94)	0.303	224	1.30 (0.81-2.09)	0.272	200
34 week body fat	1.14 (0.77-1.70)	0.504	272	1.46 (0.93-2.29)	0.104	242
11 week arm muscle	0.78 (0.49-1.25)	0.303	224	0.86 (0.52-1.40)	0.543	200
34 week arm muscle	0.75 (0.54-1.04)	0.082	272	0.89 (0.62-1.26)	0.500	242
Weight gain	1.02 (0.71-1.47)	0.928	269	0.95 (0.66-1.36)	0.767	239
Non-atopic wheeze						
Pre-pregnancy						
Total body fat	1.48 (1.02-2.16)	0.041	268	1.71 (1.27-2.29)	<0.001	255
Body fat percentage	1.67 (1.16-2.41)	0.006	269	1.85 (1.34-2.56)	<0.001	256
Subscapular:triceps	1.23 (0.91-1.67)	0.181	269	1.10 (0.78-1.55)	0.586	256
BMI	1.29 (0.94-1.77)	0.119	267	1.45 (1.09-1.94)	0.011	254
Arm muscle area	1.19 (0.89-1.60)	0.230	268	1.32 (0.95-1.85)	0.102	255
During pregnancy						
11 week body fat	1.77 (1.10-2.84)	0.018	219	1.82 (1.18-2.81)	0.007	212
34 week body fat	1.31 (0.83-2.05)	0.243	266	1.62 (1.13-2.31)	0.008	257
11 week arm muscle	1.71 (1.14-2.55)	0.009	218	1.66 (1.12-2.46)	0.012	211
34 week arm muscle	1.16 (0.82-1.64)	0.402	266	1.44 (1.03-2.03)	0.035	257
Pregnancy weight gain	0.84 (0.46-1.54)	0.571	263	0.91 (0.47-1.75)	0.774	254

***Atopic wheeze adjusted for child's sex, gestation, and birth order, mother's education, smoking in pregnancy and history of asthma and father's history of asthma. Non-atopic wheeze adjusted for child's gestation, pet exposure in the first year of life and birth order, mother's education, smoking in pregnancy and history of asthma and father's history of asthma**
Relative risks were calculated as change in risk per SD change in body composition m easurement.

Table 91 Relative risk of atopic wheeze and non-atopic wheeze according to maternal body composition before and during pregnancy, and weight gain in pregnancy

The relative risks of both doctor-diagnosed asthma and wheeze at 6 years were consistently positively associated with measures of maternal body fat both before and during pregnancy. However, none of these associations were significant (Table 89). Although maternal body fat at 11 weeks' gestation was significantly associated with wheeze at six years in the unadjusted analysis, this association did not remain significant upon adjusting for confounders. The positive association between total body fat at 34 weeks' gestation and both doctor-diagnosed asthma and wheeze at 6 years approached but did not reach significance. Arm muscle area and weight gain during pregnancy were not significantly associated with either doctor-diagnosed asthma or wheeze in the last 12 months and neither demonstrated a consistent direction of association with these outcomes (Table 89). Re-analysis of the relationship between maternal body composition and doctor-diagnosed asthma using the more specific outcome of recent doctor-diagnosed asthma revealed a positive association between total body fat late in pregnancy (RR 1.35, p=0.045) but no other significant associations.

All measures of maternal body fat and muscle were positively associated with transient wheeze, as was weight gain during pregnancy. Positive associations significant at the 5% level after adjusting for confounders were found for pre-pregnancy BMI, and arm muscle area at 11 and 34 weeks' gestation (RR 1.09, p=0.032, RR 1.10, p=0.027 and RR 1.11, p=0.015 respectively). Positive associations approaching significance were also found for total body fat before pregnancy and at 11 and 34 weeks' gestation (Table 90).

In contrast, there were no significant associations between any measure of maternal body composition either before or during pregnancy and the persistent wheeze outcome. Maternal weight gain in pregnancy was also not significantly associated with persistent wheeze (Table 90).

An inverse association between maternal arm muscle area at 34 weeks' gestation and atopic wheeze approached significance at the 5% level but became non-significant upon correcting for confounders. Although no measure of maternal body composition was significantly associated with the atopic wheeze outcome, a consistent pattern was evident whereby measures of body fatness appeared positively associated with this outcome and measures of arm muscle area appeared inversely associated (Table 91).

The associations between maternal body composition measures and non-atopic wheeze were the most marked of any outcome. Total and percent pre-pregnancy body fat, total body fat at 11 and 34 weeks of pregnancy and arm muscle area at 11 weeks of pregnancy were all significantly positively associated with the non-atopic wheeze outcome both before and after correcting for confounders. Positive associations were also found between non-atopic wheeze and pre-pregnancy BMI (Figure 33) and 34 week arm muscle area in the adjusted analysis (RR 1.45, p=0.011 and RR 1.44, p=0.035) (Table 91). The association with the greatest effect size was that between non-atopic wheeze and pre-pregnancy body fat percentage, each SD increase in maternal body fat was associated with an 85% increase in non-atopic wheeze risk. This was also the most significant association (p<0.001). Large effects were also seen for pre-pregnancy total body fat (Figure 34) (RR 1.71, p<0.001) and 11 week body fat (Figure 35) and arm muscle area (RR 1.82, p=0.007 and RR 1.66, p=0.012) and for 34 week body fat (Figure 36) (RR 1.62, p=0.008).

The positive associations between non-atopic wheeze and both total body fat and percentage body fat remained significant when child's BMI at 3 years was included in multivariate analysis as a measure of childhood adiposity (RR 1.53, p=0.013 and RR 1.71, p=0.006 respectively).

Figure 33 Proportion of children with non-atopic wheeze according to quartile of maternal pre-pregnancy BMI

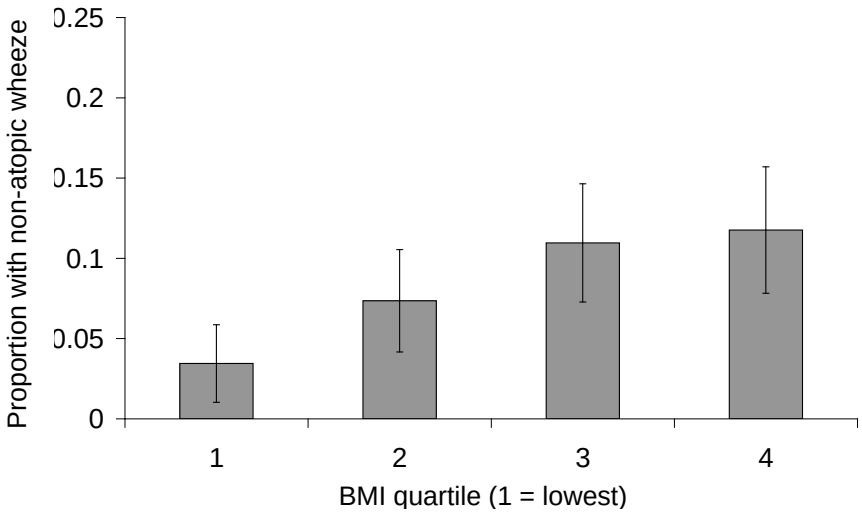


Figure 34 Proportion of children with non-atopic wheeze according to quartile of maternal pre-pregnancy body fat

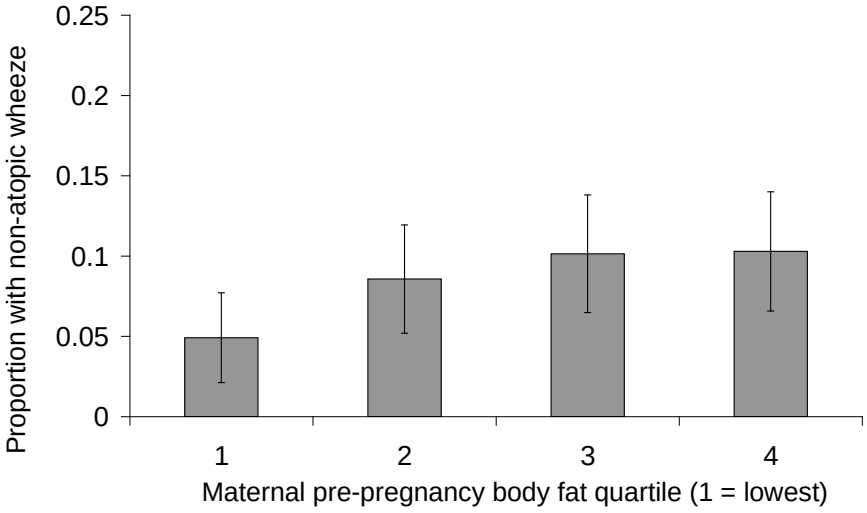


Figure 35 Proportion of children with non-atopic wheeze according to quartile of maternal early pregnancy body fat

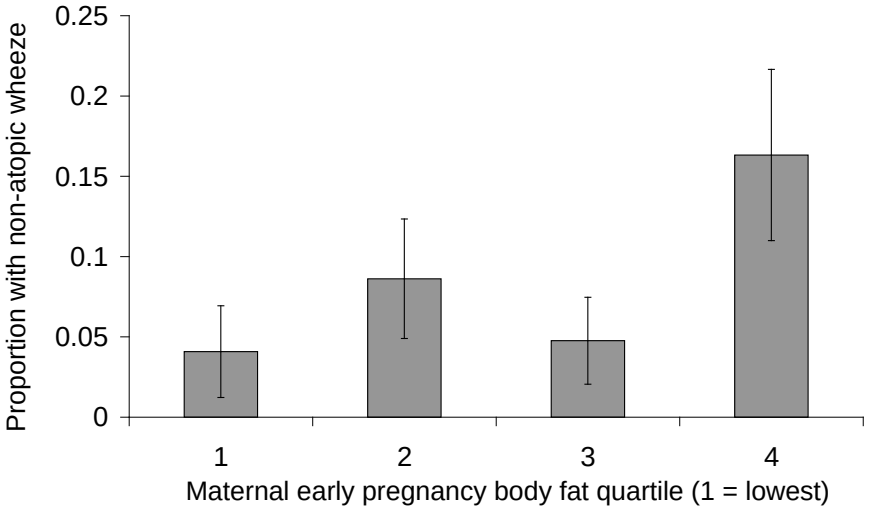
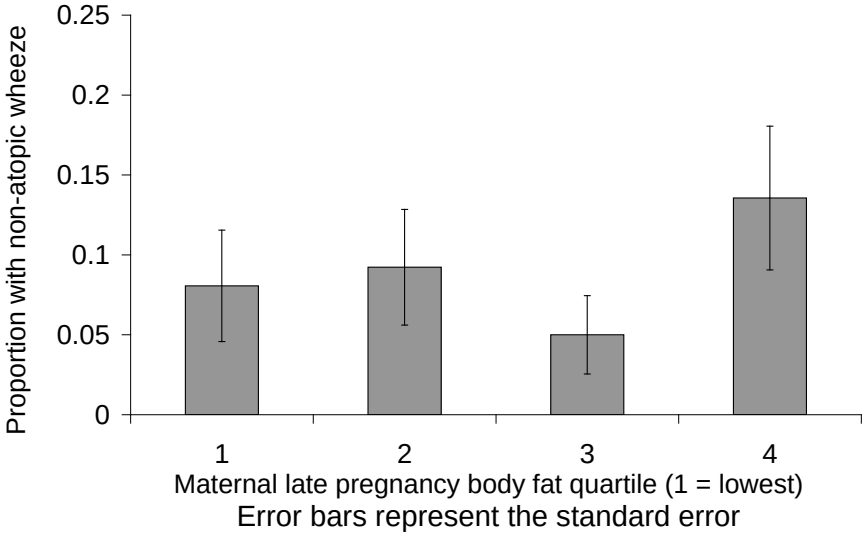


Figure 36 Proportion of children with non-atopic wheeze according to quartile of maternal late pregnancy body fat



8.6.2 Maternal diet and childhood wheeze phenotypes

8.6.2.1 Maternal micronutrient intake

Exploratory analysis: to explore potential associations between maternal diet and the childhood wheeze phenotypes.

Rationale: although neither childhood atopy (section 6.5.2) nor lung function (sections 7.5.2 & 7.5.3.2) was strongly associated with maternal nutrient intake, previous studies suggest associations may exist between childhood wheeze phenotypes and maternal intake of certain nutrients during pregnancy. Specifically there is evidence for:

- An inverse association between vitamin E intake and 'wheeze in the absence of colds' at 2 years (Martindale *et al.* 2005), and inverse associations between vitamin E intake and persistent wheeze, doctor-diagnosed asthma and wheeze at 5 years (Devereux *et al.* 2006).
- An inverse association between vitamin D intake and recurrent wheeze at 3 years (Camargo, Jr. *et al.* 2007) and inverse associations between vitamin D intake and persistent wheeze and wheeze at 5 years (Devereux *et al.* 2007).
- A positive association between vitamin C intake and ever wheezing by the age of 2 years (Martindale *et al.* 2005).

Results: lower maternal pre-pregnancy vitamin D intake was significantly associated with doctor-diagnosed asthma, wheeze in the last 12 months and non-atopic wheeze in the offspring at age 6 years. Significant inverse associations were also found between maternal intakes of vitamins A and E and non-atopic wheeze. Significant positive associations were found between maternal vitamin C intake and doctor-diagnosed asthma, persistent wheeze and atopic wheeze.

Standardised intake	Unadjusted analyses RR (95% CI)	P value	n	Adjusted* analyses RR (95% CI)	P value	n
Vitamin A						
Pre-pregnancy	0.88 (0.70-1.12)	0.298	469	0.89 (0.70-1.11)	0.297	443
11 weeks	1.00 (0.81-1.23)	0.997	381	0.98 (0.81-1.17)	0.787	370
34 weeks	1.05 (0.87-1.27)	0.586	459	1.04 (0.85-1.27)	0.680	442
Vitamin C						
Pre-pregnancy	0.96 (0.78-1.18)	0.709	469	1.02 (0.83-1.24)	0.872	443
11 weeks	1.13 (0.87-1.47)	0.348	381	1.26 (0.97-1.63)	0.080	370
34 weeks	1.16 (0.91-1.48)	0.228	459	1.25 (1.01-1.54)	0.036	442
Vitamin D						
Pre-pregnancy	0.75 (0.58-0.96)	0.021	469	0.75 (0.58-0.96)	0.024	443
11 weeks	0.94 (0.71-1.24)	0.662	381	0.93 (0.70-1.24)	0.631	370
34 weeks	0.86 (0.66-1.10)	0.223	459	0.88 (0.67-1.16)	0.360	442
Vitamin E						
Pre-pregnancy	1.01 (0.81-1.25)	0.958	469	0.96 (0.78-1.19)	0.712	443
11 weeks	0.92 (0.74-1.16)	0.487	381	0.88 (0.69-1.14)	0.332	370
34 weeks	1.14 (0.93-1.39)	0.211	459	1.12 (0.89-1.41)	0.321	442

***Adjusted for child's gestation, and birth order, mother's height, education, smoking in pregnancy and history of asthma and father's history of asthma**
Relative risks were calculated as change in risk per SD change in vitamin intake.

Table 92 Relative risk of doctor-diagnosed asthma according to maternal vitamin intake before and during pregnancy

Standardised intake	Unadjusted analyses RR (95% CI)	P value	n	Adjusted* analyses RR (95% CI)	P value	n
Vitamin A						
Pre-pregnancy	0.92 (0.74-1.15)	0.483	469	0.97 (0.77-1.21)	0.766	418
11 weeks	1.07 (0.85-1.33)	0.571	381	1.13 (0.91-1.39)	0.272	344
34 weeks	1.10 (0.87-1.38)	0.437	459	1.03 (0.80-1.32)	0.846	417
Vitamin C						
Pre-pregnancy	1.10 (0.90-1.36)	0.351	469	1.18 (0.95-1.46)	0.140	418
11 weeks	0.97 (0.73-1.78)	0.807	381	1.08 (0.81-1.45)	0.586	344
34 weeks	1.04 (0.80-1.34)	0.774	459	1.03 (0.77-1.36)	0.858	417
Vitamin D						
Pre-pregnancy	0.77 (0.61-0.96)	0.022	469	0.79 (0.63-1.00)	0.046	418
11 weeks	0.86 (0.64-1.17)	0.340	381	0.88 (0.65-1.19)	0.395	344
34 weeks	0.86 (0.67-1.11)	0.251	459	0.86 (0.67-1.12)	0.280	417
Vitamin E						
Pre-pregnancy	1.07 (0.84-1.36)	0.597	469	1.08 (0.83-1.40)	0.567	418
11 weeks	0.78 (0.60-1.02)	0.070	381	0.79 (0.60-1.03)	0.079	344
34 weeks	0.95 (0.76-1.19)	0.651	459	0.88 (0.69-1.13)	0.324	417

Adjusted for child's gestation, pet exposure in first year of life and birth order, education, smoking in pregnancy and history of asthma and father's history of asthma
Relative risks were calculated as change in risk per SD change in vitamin intake.

Table 93 Relative risk of wheeze in the last 12 months according to maternal vitamin intake before and during pregnancy

Standardised intake	Unadjusted analyses RR (95% CI)	P value	n	Adjusted* analyses RR (95% CI)	P value	n
Vitamin A						
Pre-pregnancy	0.99 (0.91-1.08)	0.774	398	0.98 (0.89-1.07)	0.636	377
11 weeks	1.08 (0.98-1.18)	0.117	325	1.04 (0.95-1.13)	0.429	316
34 weeks	1.03 (0.95-1.13)	0.487	389	1.01 (0.92-1.11)	0.802	376
Vitamin C						
Pre-pregnancy	0.95 (0.87-1.04)	0.242	398	0.96 (0.88-1.04)	0.311	377
11 weeks	1.05 (0.95-1.16)	0.359	325	1.06 (0.96-1.18)	0.233	316
34 weeks	0.99 (0.90-1.08)	0.824	389	1.02 (0.93-1.12)	0.661	376
Vitamin D						
Pre-pregnancy	1.00 (0.92-1.09)	0.965	398	1.00 (0.91-1..09)	0.980	377
11 weeks	1.03 (0.93-1.14)	0.562	325	1.03 (0.93-1.14)	0.588	316
34 weeks	0.93 (0.85-1.02)	0.128	389	0.95 (0.86-1.04)	0.241	376
Vitamin E						
Pre-pregnancy	0.97 (0.89-1.07)	0.561	398	0.95 (0.87-1.04)	0.258	377
11 weeks	1.05 (0.96-1.15)	0.260	325	1.04 (0.95-1.13))	0.429	316
34 weeks	1.01 (0.93-1.10)	0.797	389	1.02 (0.93-1.11)	0.729	376

Adjusted for child's gestation, birth order and pet exposure and mother's height, education, smoking in pregnancy and history of asthma and rhinitis, and father's history of asthma
Relative risks were calculated as change in risk per SD change in vitamin intake.

Table 94 Relative risk of transient wheeze according to maternal vitamin intake before and during pregnancy

Standardised intake	Unadjusted analyses RR (95% CI)	P value	n	Adjusted* analyses RR (95% CI)	P value	n
Vitamin A						
Pre-pregnancy	0.94 (0.75-1.18)	0.595	229	1.08 (0.87-1.34)	0.472	223
11 weeks	1.19 (0.91-1.55)	0.203	186	1.15 (0.90-1.46)	0.264	183
34 weeks	1.05 (0.83-1.33)	0.685	226	0.97 (0.74-1.27)	0.805	223
Vitamin C						
Pre-pregnancy	1.06 (0.87-1.30)	0.554	229	1.22 (1.02-1.48)	0.035	223
11 weeks	0.98 (0.77-1.26)	0.888	186	1.07 (0.86-1.34)	0.537	183
34 weeks	1.00 (0.78-1.27)	0.981	226	1.10 (0.87-1.39)	0.438	223
Vitamin D						
Pre-pregnancy	0.77 (0.61-0.99)	0.040	229	0.84 (0.66-1.07)	0.151	223
11 weeks	0.92 (0.69-1.22)	0.550	186	0.87 (0.67-1.15)	0.333	183
34 weeks	0.80 (0.63-1.03)	0.084	226	0.79 (0.61-1.02)	0.071	223
Vitamin E						
Pre-pregnancy	1.06 (0.86-1.32)	0.580	229	1.21 (0.97-1.55)	0.094	223
11 weeks	0.89 (0.68-1.17)	0.404	186	0.87 (0.66-1.14)	0.320	183
34 weeks	0.93 (0.75-1.15)	0.481	226	0.89 (0.70-1.13)	0.350	223

Adjusted for child's gestation, pet exposure in the first year of life and birth order, mother's age, education, smoking in pregnancy and history of asthma and rhinitis, and father's history of asthma
Relative risks were calculated as change in risk per SD change in vitamin intake.

Table 95 Relative risk of persistent wheeze according to maternal vitamin intake before and during pregnancy

Standardised intake	Unadjusted analyses RR (95% CI)	P value	n	Adjusted* analyses RR (95% CI)	P value	n
Vitamin A						
Pre-pregnancy	1.03 (0.75-1.41)	0.853	278	0.99 (0.69-1.43)	0.972	243
11 weeks	0.98 (0.74-1.31)	0.907	224	1.07 (0.78-1.48)	0.668	200
34 weeks	1.16 (0.84-1.62)	0.368	272	1.38 (0.94-2.04)	0.101	242
Vitamin C						
Pre-pregnancy	1.28 (0.95-1.71)	0.104	278	1.04 (0.69-1.54)	0.865	243
11 weeks	1.06 (0.70-1.59)	0.789	224	0.98 (0.51-1.88)	0.946	200
34 weeks	1.49 (1.12-1.98)	0.006	272	1.46 (1.01-2.11)	0.047	242
Vitamin D						
Pre-pregnancy	0.96 (0.69-1.33)	0.794	278	0.95 (0.65-1.38)	0.775	243
11 weeks	1.00 (0.60-1.65)	0.986	224	0.98 (0.67-1.43)	0.914	200
34 weeks	1.19 (0.83-1.69)	0.338	272	1.15 (0.081-1.63)	0.433	242
Vitamin E						
Pre-pregnancy	1.39 (1.11-1.74)	0.004	278	1.25 (0.93-1.70)	0.144	243
11 weeks	0.76 (0.51-1.15)	0.194	224	0.76 (0.54-1.07)	0.119	200
34 weeks	1.19 (0.95-1.49)	0.133	272	1.12 (0.85-1.47)	0.432	242

***Adjusted for child's sex, gestation, and birth order, mother's education, smoking in pregnancy and history of asthma and father's history of asthma**
Relative risks were calculated as change in risk per SD change in vitamin intake.

Table 96 Relative risk of atopic wheeze according to maternal vitamin intake before and during pregnancy

Standardised intake	Unadjusted analyses RR (95% CI)	P value	n	Adjusted* analyses RR (95% CI)	P value	n
Vitamin A						
Pre-pregnancy	0.73 (0.51-1.06)	0.095	271	0.73 (0.53-1.00)	0.047	258
11 weeks	0.97 (0.71-1.33)	0.845	219	1.01 (0.71-1.44)	0.956	212
34 weeks	0.75 (0.48-1.16)	0.190	266	0.67 (0.44-1.01)	0.055	257
Vitamin C						
Pre-pregnancy	0.83 (0.59-1.18)	0.297	271	0.83 (0.56-1.23)	0.362	258
11 weeks	0.86 (0.58-1.26)	0.427	219	0.95 (0.58-1.56)	0.836	212
34 weeks	0.74 (0.46-1.20)	0.227	266	0.82 (0.50-1.34)	0.419	257
Vitamin D						
Pre-pregnancy	0.47 (0.32-0.68)	<0.001	271	0.46 (0.30-0.71)	<0.001	258
11 weeks	0.68 (0.37-1.26)	0.219	219	0.59 (0.29-1.21)	0.150	212
34 weeks	0.59 (0.35-0.98)	0.042	266	0.59 (0.35-1.02)	0.061	257
Vitamin E						
Pre-pregnancy	0.59 (0.36-0.97)	0.038	271	0.65 (0.45-0.95)	0.027	258
11 weeks	0.85 (0.55-1.32)	0.479	219	0.86 (0.54-1.37)	0.521	212
34 weeks	0.71 (0.45-1.13)	0.150	266	0.59 (0.38-0.93)	0.023	257

***Adjusted for child's gestation, pet exposure in the first year of life and birth order, mother's education, smoking in pregnancy and history of asthma and father's history of asthma**
Relative risks were calculated as change in risk per SD change in vitamin intake.

Table 97 Relative risk of non-atopic wheeze according to maternal vitamin intake before and during pregnancy

The most consistent findings were those related to pre-pregnancy vitamin D intake. Pre-pregnancy vitamin D intake was inversely associated with both doctor-diagnosed asthma and wheeze at 6 years of age, although neither outcome was significantly associated with vitamin D intake recorded at 11 or 34 weeks' gestation (Table 92 & Table 93). For each SD increase in maternal pre-pregnancy vitamin D intake the risk of doctor-diagnosed asthma decreased by 25% (p=0.024). Doctor-diagnosed asthma was also associated with higher vitamin C intake at 34 weeks' gestation (RR 1.25, p=0.036) and a similar positive association approached significance at 11 weeks' gestation (RR 1.26, p=0.080). No further significant associations were found between vitamin intake and doctor-diagnosed asthma (Table 92). The relationship between pre-pregnancy vitamin D intake and recent doctor-diagnosed asthma approached significance (RR 0.71, p=0.058) whilst the relationship between 34-week vitamin C intake and asthma was not confirmed using the specific outcome of recent doctor-diagnosed asthma (RR 1.20, p=0.392).

The lack of association between intakes of vitamins A and E with doctor-diagnosed asthma remained when the data were re-analysed according to energy-adjusted intake (Appendix 1, Table 118) The associations between doctor-diagnosed asthma and energy-adjusted vitamin C intake before and during pregnancy became more significant whilst those between energy-adjusted measures of vitamin D intake and this outcome became less significant. This resulted in the relationship between doctor-diagnosed asthma and energy-adjusted vitamin C intake at 11 weeks' gestation becoming significant (RR 1.32, p=0.030) and that with pre-pregnancy energy adjusted vitamin D intake became marginally non-significant at the 5% significance level (RR 0.76, p=0.052) (Appendix 1, Table 118).

Wheeze in the last 12 months (at 6 years) was not significantly associated with maternal intake of any vitamin other than vitamin D. For each SD increase in pre-pregnancy vitamin D intake the risk of wheeze in the last 12 months was decreased by 21% (p=0.046). A weak inverse association was found between vitamin E intake at 11 weeks of pregnancy but this was not significant either before or after adjusting for confounders (Table 93).

When the data were re-analysed according to energy-adjusted intake, the associations between wheeze in the last 12 months and energy-adjusted intakes of vitamins A and C became strengthened somewhat, whilst those between this outcome and energy-adjusted intakes of vitamins D and E weakened. This resulted in the positive associations between wheeze in the last 12 months and energy-adjusted vitamin A and C intake approaching significance whilst the inverse association with pre-pregnancy energy-adjusted vitamin D intake became non-significant at the 5% significance level (RR 0.81, p=0.078) (Appendix 1, Table 119)

No significant associations were found between maternal intake of vitamins A, C, D or E either before or during pregnancy and transient wheeze (Table 94). This lack of association was unchanged when the data were re-analysed using energy-adjusted vitamin intakes (Appendix 1, Table 120).

A significant positive association was found between persistent wheeze and vitamin C intake before pregnancy after adjusting for confounders (RR 1.22, p=0.035). A significant inverse association was found between pre-pregnancy vitamin D and persistent wheeze, before adjusting for confounders, and inverse associations between persistent wheeze and vitamin D intake before pregnancy and at 34 weeks of gestation, after adjustment, approached but did not reach significance (Table 95).

The positive association between persistent wheeze and pre-pregnancy vitamin C intake remained after replacing total absolute vitamin intakes with energy-adjusted values. Further positive associations were found between persistent wheeze and energy-adjusted vitamin A intake at 11 weeks' gestation and energy-adjusted pre-pregnancy vitamin E intake. The inverse relationship between persistent wheeze and vitamin D intake became less significant following energy adjustment (Appendix 1, Table 121).

Atopic wheeze showed positive associations with vitamin C at 34 weeks' gestation and pre-pregnancy vitamin E intake, only the association with late pregnancy vitamin C intake remained significant after adjusting for confounders (RR 1.46, p=0.047). No significant associations were found between atopic wheeze and intake of either vitamin A or vitamin D (Table 96).

After replacing absolute vitamin intakes with energy-adjusted intakes, significant positive associations were found between atopic wheeze and energy adjusted intake of vitamin A at 34 weeks' gestation (RR 1.44, p=0.045) and energy-adjusted vitamin C intake at 34 weeks' gestation (RR 1.56, p=0.018) (Appendix 1, Table 122).

Non-atopic wheeze was found to be inversely associated with pre-pregnancy vitamin D intake and pre-pregnancy vitamin E intake (RR 0.46, p<0.001 and RR 0.65, p=0.027 respectively). After adjusting for confounders further inverse associations were found between non-atopic wheeze and pre-pregnancy vitamin A intake and intake of vitamin E at 34 weeks' gestation (RR 0.73, p=0.047 and RR 0.59, p=0.023 respectively). Late pregnancy vitamin D intake was also significantly associated with non-atopic wheeze, although only in the unadjusted analysis, and an inverse association between non-atopic wheeze and vitamin D intake at 11 weeks' gestation approached, but did not achieve, significance at the 5% level (Table 97).

After adjusting for energy intake only the inverse association between pre-pregnancy vitamin D intake and non-atopic wheeze remained significant (RR 0.49, p=0.002) (Appendix 1, Table 123).

8.6.2.2 Maternal nutrient status - vitamins

Exploratory analysis: to explore potential associations between markers of maternal nutritional status and the childhood wheeze outcomes.

Rationale: although no strong associations between maternal vitamin status and either childhood atopy or lung function were found in section 6.5.2.3.1, 7.5.2.2 or 7.5.3.2.2, previous studies support associations between childhood wheeze phenotypes and measures of maternal vitamin status. Specifically, there is evidence for an association between high maternal serum 25(OH) vitamin D status during pregnancy and reported asthma at 9 years of age (Gale *et al.* 2008). Moreover studies of vitamin status in childhood suggest early exposure may influence the development of wheeze and atopy later in childhood. Associations have been found between serum ascorbic acid and α-carotene and doctor-diagnosed asthma (Harik-Khan *et al.* 2004) and between serum retinol at 2 months and symptoms of atopic disease at 20 years (Pesonen *et al.* 2007).

Results: maternal serum 25(OH) vitamin D status at 34 weeks' gestation was significantly inversely associated with doctor-diagnosed asthma, atopic wheeze and persistent wheeze in the offspring at age 6 years. A significant positive association was found between maternal serum retinol and atopic wheeze and maternal total anti-oxidant status and persistent wheeze.

Standardised status	Unadjusted analyses			Adjusted* analyses		
	RR (95% CI)	P value	n	RR (95% CI)	P value	n
Doctor-diagnosed asthma						
Retinol	1.20 (0.95-1.52)	0.127	370	1.14 (0.94-1.39)	0.187	354
Ascorbic acid	0.97 (0.76-1.26)	0.842	332	1.09 (0.83-1.43)	0.521	317
α-tocopherol†	0.80 (0.63-1.01)	0.058	360	0.80 (0.63-1.03)	0.082	344
Total anti-oxidants	1.10 (0.88-1.38)	0.405	430	1.09 (0.85-1.40)	0.517	408
25(OH) vitamin D	0.63 (0.50-0.79)	<0.001	319	0.69 (0.53-0.90)	0.007	302
Wheeze in last 12 months						
Retinol	0.96 (0.71-1.29)	0.768	370	0.88 (0.65-1.19)	0.401	333
Ascorbic acid	0.88 (0.68-11.14)	0.341	332	0.92 (0.69-1.23)	0.578	305
α-tocopherol†	0.98 (0.76-1.26)	0.866	360	0.95 (0.73-1.24)	0.699	325
Total anti-oxidants	1.10 (0.85-1.42)	0.478	430	1.13 (0.84-1.51)	0.423	389
25(OH) vitamin D	0.71 (0.53-0.94)	0.017	319	0.81 (0.58-1.13)	0.217	289

†Adjusted for cholesterol. *Doctor-diagnosed asthma adjusted for child's gestation, and birth order, mother's height, education, smoking in pregnancy and history of asthma and father's history of asthma. Wheeze in the last 12 months analysis adjusted for child's gestation, pet exposure in first year of life and birth order, education, smoking in pregnancy and history of asthma and father's history of asthma

Relative risks were calculated as change in risk per SD change in vitamin status.

Table 98 Relative risk of doctor-diagnosed asthma and wheeze in the last 12 months according to maternal serum vitamin and anti-oxidant concentrations at 34 weeks' gestation

Standardised status	Unadjusted analyses RR (95% CI)	P value	n	Adjusted* analyses RR (95% CI)	P value	n
Transient wheeze						
Retinol	1.00 (0.90-1.11)	0.986	313	0.99 (0.89-1.10)	0.807	300
Ascorbic acid	0.96 (0.86-1.06)	0.380	279	0.99 (0.88-1.12)	0.888	267
α-tocopherol†	0.92 (0.83-1.02)	0.128	303	0.93 (0.84-1.04)	0.187	290
Total anti-oxidants	1.08 (0.98-1.19)	0.113	366	1.08 (0.98-1.19)	0.134	348
25(OH) vitamin D	0.90 (0.81-0.99)	0.029	279	0.90 (0.82-1.00)	0.052	265
Persistent wheeze						
Retinol	0.89 (0.67-1.19)	0.446	184	0.91 (0.68-1.23)	0.556	181
Ascorbic acid	0.88 (0.68-1.13)	0.318	166	0.92 (0.70-1.21)	0.577	161
α-tocopherol†	0.90 (0.72-1.12)	0.348	180	0.94 (0.74-1.18)	0.581	177
Total anti-oxidants	1.26 (0.98-1.62)	0.069	213	1.31 (1.02-1.68)	0.035	208
25(OH) vitamin D	0.65 (0.49-0.87)	0.003	142	0.73 (0.53-0.99)	0.045	139

†Adjusted for cholesterol. *Transient wheeze analysis adjusted for child's gestation, birth order and pet exposure and mother's height, education, smoking in pregnancy and history of asthma and rhinitis, and father's history of asthma. Persistent wheeze analysis adjusted for child's gestation, pet exposure in the first year of life and birth order, mother's age, education, smoking in pregnancy and history of asthma and rhinitis, and father's history of asthma
Relative risks were calculated as change in risk per SD change in vitamin status.

Table 99 Relative risk of transient and persistent wheeze according to maternal serum vitamin and anti-oxidant concentrations at 34 weeks' gestation

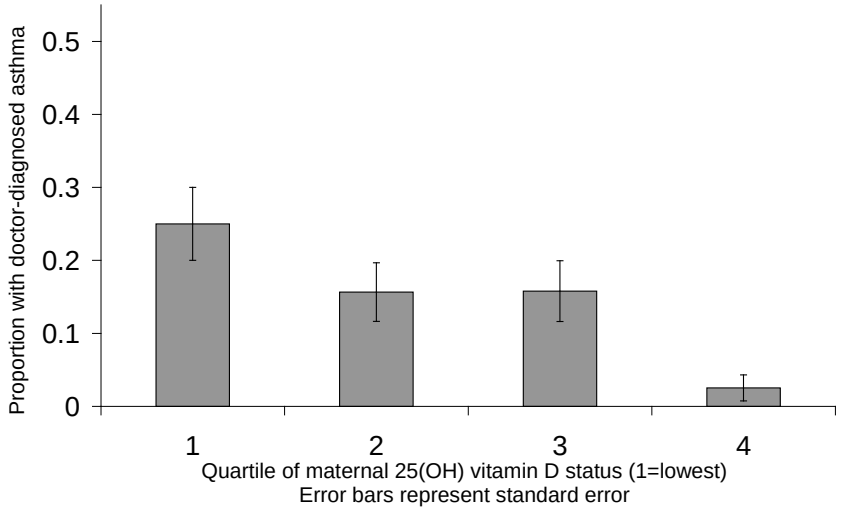
Standardised status	Unadjusted analyses			Adjusted* analyses		
	RR (95% CI)	P value	n	RR (95% CI)	P value	n
Atopic wheeze						
Retinol	1.19 (0.81-1.75)	0.370	218	1.31 (1.04-1.65)	0.021	193
Ascorbic acid	1.36 (0.76-2.46)	0.301	194	1.03 (0.51-2.07)	0.941	170
α-tocopherol†	1.17 (0.82-1.65)	0.384	215	1.10 (0.74-1.63)	0.636	190
Total anti-oxidants	1.09 (0.74-1.61)	0.650	261	1.25 (0.80-1.95)	0.322	230
25(OH) vitamin D	0.64 (0.42-0.97)	0.034	199	0.63 (0.40-1.99)	0.044	177
Non-atopic wheeze						
Retinol	0.68 (0.37-1.25)	0.212	213	0.59 (0.30-1.13)	0.112	204
Ascorbic acid	0.70 (0.50-0.98)	0.038	191	0.86 (059-1.27)	0.449	183
α-tocopherol†	0.89 (0.62-1.27)	0.511	210	0.90 (0.60-1.35)	0.618	201
Total anti-oxidants	1.42 (0.91-2.23)	0.124	254	1.39 (0.81-2.38)	0.228	241
25(OH) vitamin D	0.73 (0.49-1.10)	0.129	196	0.83 (0.53-1.31)	0.432	186

†Adjusted for cholesterol. *Atopic wheeze adjusted for child's sex, gestation, and birth order, mother's education, smoking in pregnancy and history of asthma and father's history of asthma. Non-atopic wheeze adjusted for child's gestation, pet exposure in the first year of life and birth order, mother's education, smoking in pregnancy and history of asthma and father's history of asthma
Relative risks were calculated as change in risk per SD change in vitamin status.

Table 100 Relative risk of atopic and non-atopic wheeze according to maternal serum vitamin and anti-oxidant concentrations at 34 weeks' gestation

The relative risk of doctor-diagnosed asthma was found to decrease by 31% per SD increase in serum 25(OH) vitamin D (p=0.007) (Table 98 & Figure 37). The relationship between maternal serum vitamin D status and recent doctor-diagnosed asthma approached but did not reach significance (RR 0.65, p=0.07). An inverse association between serum α-tocopherol and doctor-diagnosed asthma was found but this became less significant upon adjusting for confounders and did not reach significance at the 5% level (Table 98). No other serum measure of vitamin status was found to be significantly associated with the doctor-diagnosed asthma outcome.

Figure 37 Proportion of children with doctor-diagnosed asthma according to quartile of maternal 25(OH) vitamin D status at 34 weeks of pregnancy

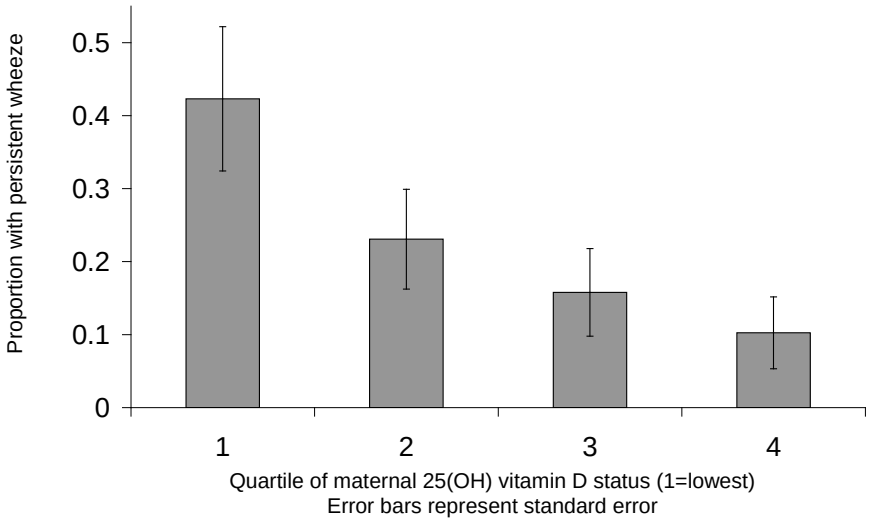


The relative risk of wheeze in the last 12 months was inversely associated with serum 25(OH) vitamin D in the unadjusted analysis but this association became non-significant following adjustment for confounders. There was no significant association between total serum anti-oxidant status and either doctor-diagnosed asthma or wheeze in the last 12 months (Table 98).

Transient wheeze was not significantly associated with anti-oxidant status or any serum vitamin marker except 25(OH) vitamin D (Table 99 & Figure 38). There was an inverse association between 25(OH) vitamin D and transient wheeze which narrowly failed to reach significance upon adjusting for confounders (RR 0.90, p=0.052) (Table 99).

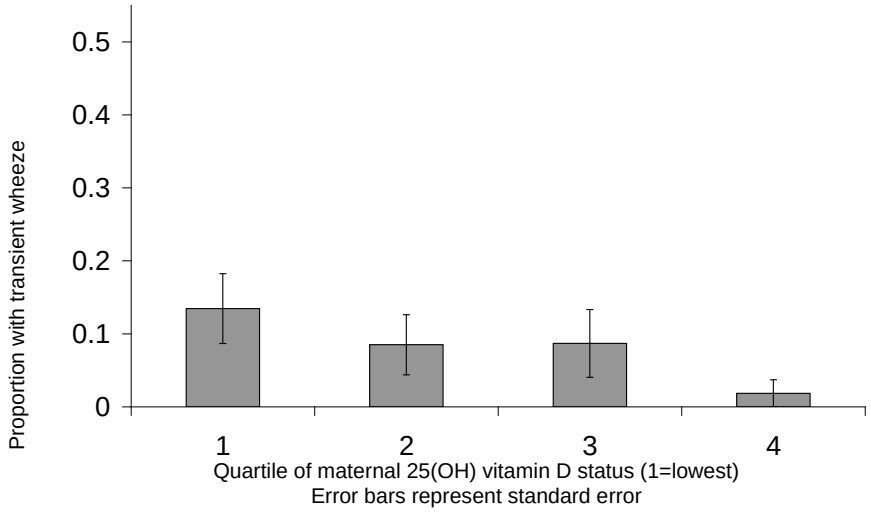
25(OH) vitamin D was also inversely associated with the persistent wheeze outcome (Figure 38 & Table 99). In the adjusted analysis the relative risk of persistent wheeze increased by 27% per SD decrease in serum 25(OH) vitamin D (p=0.045) (Table 99). An association was found between higher serum total anti-oxidant level and persistent wheeze, (RR 1.31, p=0.035) (Table 99).

Figure 38 Proportion of children with persistent wheeze according to quartile of maternal 25(OH) vitamin D status at 34 weeks of pregnancy



The atopic wheeze outcome was significantly positively associated with serum retinol concentration and inversely with serum 25(OH) vitamin D (RR 1.31, p=0.021 and RR 0.63, p=0.044 respectively) (Table 100, Figure 40). There were no significant associations between atopic wheeze and serum ascorbic acid, α-tocopherol or total anti-oxidants (Table 100).

Figure 39 Proportion of children with atopic wheeze according to quartile of maternal 25(OH) vitamin D status at 34 weeks of pregnancy



Non-atopic wheeze was not significantly associated with any serum measure of vitamin or anti-oxidant status following adjustment for confounders (Table 100).

8.6.2.3 Maternal nutrient status – plasma phospholipid phosphatidylcholine fatty acid composition

Exploratory analysis: seeking associations between the percentage composition of maternal plasma phospholipids and the childhood wheeze phenotypes.

Rationale: in section 6.5.2.3.2, atopy was found to be positively associated with maternal arachidonic acid status and inversely associated with α-linolenic acid status. These findings are consistent with Black and Sharpe's hypothesis that a low n-3:n-6 PUFA ratio might promote an allergic phenotype (Black & Sharpe 1997). Oily fish intake in childhood has been found to be inversely associated with current asthma (Hodge *et al.* 1996), n-3 PUFA supplementation has been shown to alter the profile of cytokines in cord blood (Prescott *et al.* 2007). Moreover, the profile of plasma fatty acids in childhood has been found to be associated with wheeze in infancy (Mihrshahi *et al.* 2004).

Results: higher maternal arachidonic acid status was associated with an increased risk of atopic wheeze at 6 years and a lower risk of transient wheeze. Lower proportions of α-linolenic and EPA in maternal plasma phospholipids were associated with increased doctor-diagnosed asthma risk. Lower maternal EPA status was also associated at 6 years of age with increased risks of wheeze in the last 12 months and non-atopic wheeze.

	Unadjusted analyses			Adjusted* analyses		
	RR (95% CI)	**P value**	**n**	**RR (95% CI)**	**P value**	**n**
Doctor-diagnosed asthma						
n-3 PUFAs	0.85 (0.69-1.04)	0.117	447	0.92 (0.73-1.16)	0.483	425
n-6 PUFAs	0.97 (0.76-1.24)	0.818	447	1.00 (0.80-1.25)	0.976	425
n-3:6 ratio	0.88 (0.71-1.08)	0.219	447	0.93 (0.74-1.18)	0.567	425
α-Linolenic acid	0.79 (0.64-0.97)	0.028	425	0.75 (0.60-0.94)	0.013	403
Linoleic acid	0.88 (0.71-1.09)	0.227	447	0.92 (0.75-1.12)	0.392	425
DHA	0.87 (0.70-1.08)	0.202	447	0.98 (0.77-1.26)	0.899	425
EPA	0.73 (0.56-0.96)	0.026	435	0.73 (0.54-0.99)	0.042	413
Arachidonic acid	1.05 (0.85-1.30)	0.672	447	1.05 (0.84-1.31)	0.690	425
Wheeze in the last 12 months						
n-3 PUFAs	0.88 (0.72-1.07)	0.197	447	0.95 (0.75-1.20)	0.669	402
n-6 PUFAs	1.24 (0.97-1.59)	0.086	447	1.20 (0.94-1.53)	0.140	402
n-3:6 ratio	0.85 (0.70-1.05)	0.126	447	0.91 (0.72-1.15)	0.454	402
α-Linolenic acid	0.93 (0.71-1.22)	0.616	425	0.90 (0.68-1.18)	0.434	382
Linoleic acid	1.09 (0.85-1.40)	0.509	447	1.09 (0.85-1.41)	0.495	402
DHA	0.89 (0.72-1.10)	0.290	447	1.00 (0.78-1.28)	0.983	402
EPA	0.70 (0.54-0.90)	0.005	435	0.70 (0.52-0.94)	0.017	319
Arachidonic acid	1.07 (0.86-1.34)	0.538	447	1.04 (0.80-1.36)	0.761	402

***Doctor-diagnosed asthma adjusted for child's gestation, and birth order, mother's height, education, smoking in pregnancy and history of asthma and father's history of asthma. Wheeze in the last 12 months analysis adjusted for child's gestation, pet exposure in first year of life and birth order, education, smoking in pregnancy and history of asthma and father's history of asthma**
Relative risks were calculated as change in risk per SD change in plasma phospholipids fatty acid composition.

Table 101 Relative risk of doctor-diagnosed asthma and wheeze in the last 12 months according to percentage fatty acid composition of maternal plasma phospholipid phosphatidylcholine at 34 weeks' gestation

	Unadjusted analyses			Adjusted* analyses		
	RR (95% CI)	**P value**	**n**	**RR (95% CI)**	**P value**	**n**
Transient wheeze						
n-3 PUFAs	0.94 (0.86-1.03)	0.178	381	0.96 (0.88-1.05)	0.414	363
n-6 PUFAs	0.98 (0.90-1.06)	0.587	381	0.97 (0.90-1.06)	0.519	363
n-3:6 ratio	0.95 (0.87-1.04)	0.304	381	0.98 (0.89-1.07)	0.587	363
α-Linolenic acid	1.08 (0.98-1.19)	0.104	360	1.06 (0.96-1.17)	0.223	342
Linoleic acid	1.03 (0.94-1.13)	0.555	381	1.04 (0.94-1.14)	0.445	363
DHA	0.91 (0.83-1.00)	0.049	381	0.94 (0.85-1.04)	0.217	363
EPA	0.98 (0.89-1.07)	0.618	369	0.98 (0.89-1.07)	0.621	351
Arachidonic acid	0.92 (0.84-1.01)	0.068	381	0.90 (0.82-0.99)	0.038	368
Persistent wheeze						
n-3 PUFAs	0.88 (0.71-1.10)	0.251	219	1.00 (0.77-1.28)	0.972	214
n-6 PUFAs	1.14 (0.88-1.46)	0.323	219	1.04 (0.79-1.38)	0.768	214
n-3:6 ratio	0.87 (0.70-1.09)	0.223	219	0.99 (0.76-1.28)	0.915	214
α-Linolenic acid	1.05 (0.82-1.34)	0.715	209	1.04 (0.83-1.31)	0.717	204
Linoleic acid	1.11 (0.86-1.43)	0.409	219	1.07 (0.83-1.38)	0.615	214
DHA	0.86 (0.69-1.08)	0.196	219	0.99 (0.76-1.29)	0.956	214
EPA	0.75 (0.59-0.96)	0.021	216	0.78 (0.59-1.02)	0.066	211
Arachidonic acid	0.92 (0.75-1.13)	0.449	219	0.87 (0.70-1.08)	0.204	214

***Transient wheeze analysis adjusted for child's gestation, birth order and pet exposure and mother's height, education, smoking in pregnancy and history of asthma and rhinitis, and father's history of asthma. Persistent wheeze analysis adjusted for child's gestation, pet exposure in the first year of life and birth order, mother's age, education, smoking in pregnancy and history of asthma and rhinitis, and father's history of asthma**
Relative risks were calculated as change in risk per SD change in plasma phospholipids fatty acid composition.

Table 102 Relative risk of transient and persistent wheeze according to percentage fatty acid composition of maternal plasma phospholipid phosphatidylcholine at 34 weeks' gestation

	Unadjusted analyses			Adjusted* analyses		
	RR (95% CI)	**P value**	**n**	**RR (95% CI)**	**P value**	**n**
Atopic wheeze						
n-3 PUFAs	0.97 (0.71-1.34)	0.861	267	1.01 (0.70-1.45)	0.959	234
n-6 PUFAs	1.28 (0.91-1.79)	0.154	267	1.19 (0.87-1.63)	0.281	234
n-3:6 ratio	0.92 (0.67-1.27)	0.618	267	0.96 (0.68-1.36)	0.825	234
α-Linolenic acid	0.83 (0.58-1.19)	0.314	251	0.66 (0.44-1.01)	0.055	218
Linoleic acid	1.02 (0.72-1.45)	0.919	267	0.88 (0.64-1.22)	0.450	234
DHA	1.01 (0.72-1.43)	0.944	267	1.09 (0.73-1.64)	0.674	234
EPA	0.80 (0.55-1.14)	0.217	260	0.81 (0.54-1.19)	0.282	227
Arachidonic acid	1.17 (0.85-1.61)	0.334	267	1.46 (1.00-2.12)	0.050	234
Non-atopic wheeze						
n-3 PUFAs	0.70 (0.48-1.02)	0.064	259	0.84 (0.54-1.30)	0.432	246
n-6 PUFAs	1.09 (0.69-1.73)	0.699	259	1.01 (0.63-1.64)	0.955	246
n-3:6 ratio	0.72 (0.49-1.06)	0.099	259	0.86 (0.55-1.35)	0.512	246
α-Linolenic acid	0.80 (0.48-1.33)	0.385	244	0.84 (0.53-1.34)	0.468	231
Linoleic acid	1.08 (0.66-1.78)	0.763	259	1.03 (0.66-1.59)	0.911	246
DHA	0.72 (0.48-1.07)	0.100	259	0.87(0.53-1.43)	0.581	246
EPA	0.50 (0.29-0.88)	0.017	252	0.53 (0.30-0.94)	0.030	239
Arachidonic acid	0.95 (0.67-1.35)	0.781	259	0.93 (0.59-1.45)	0.738	246

***Atopic wheeze adjusted for child's sex, gestation, and birth order, mother's education, smoking in pregnancy and history of asthma and father's history of asthma. Non-atopic wheeze adjusted for child's gestation, pet exposure in the first year of life and birth order, mother's education, smoking in pregnancy**
Relative risks were calculated as change in risk per SD change in plasma phospholipids fatty acid composition.

Table 103 Relative risk of atopic and non-atopic wheeze according to percentage fatty acid composition of maternal plasma phospholipid phosphatidylcholine at 34 weeks' gestation

Associations were found between both doctor-diagnosed asthma and wheeze in the last 12 months and a lower percentage of EPA in plasma phospholipid phosphatidylcholine (RR 0.73, p=0.042 and RR 0.70, p=0.017 respectively). Doctor-diagnosed asthma was also significantly associated with a lower percentage of α-linolenic acid as a proportion of plasma phospholipids (Table 101). Similar directions of effect were seen when the relationships between recent doctor-diagnosed asthma and plasma phospholipid phosphatidylcholine were considered. Maternal α-linolenic acid status had a significant inverse association with recent doctor-diagnosed asthma (RR 0.65, p=0.010) and an inverse association between maternal EPA status and this outcome approached significance (RR 0.63, p=0.052).

Transient wheeze was significantly inversely associated with plasma phopholipid phosphatidylcholine arachidonic acid percentage after adjustment for confounders (RR 0.90, p=0.038). Percentage DHA in plasma phospholipids was also inversely associated with transient wheeze but this association became non-significant upon adjusting for confounders (Table 102).

Persistent wheeze was not significantly associated with any fatty acid's percentage contribution to plasma phospholipd phosphatidylcholine following adjustment for confounders, although persistent wheeze was inversely associated with EPA in the unadjusted analysis and this association approached significance in the adjusted analysis (RR 0.78, p=0.066) (Table 102).

Atopic wheeze was significantly positively associated with plasma phospholipid phosphatidylcholine arachidonic acid. A 46% increase in risk of atopic wheeze was seen for each SD increase in percentage of arachidonic acid in plasma phospholipids (p=0.050). An inverse association was seen between atopic wheeze and plasma phospholipid α-linolenic acid but this association did not reach significance at the 5% level (RR 0.66, p=0.055) (Table 103).

8.6.2.4 Maternal dietary patterns

Exploratory analysis: to investigate whether the prudent dietary pattern is associated with any of the childhood wheeze phenotypes.

Rationale: in section 1.1.1.1, atopy was found to be positively associated with a 'prudent' dietary pattern and measures of expiratory flow (FEV_1, FEV_1/FVC and $FEF_{25-75\%}$) were found to be positively associated with this dietary pattern (sections 7.5.2.3 & 7.5.3.2.3). The significance of these associations was reduced upon adjusting for confounders. An inverse association has been found between a Mediterranean dietary pattern score during pregnancy and early childhood and the risk of atopy and wheeze (Chatzi *et al.* 2008). Analysis of data-driven maternal dietary patterns in pregnancy in the ALSPAC study did not reveal any associations between childhood wheeze phenotypes and dietary patterns determined by principal component analysis which remained significant after adjusting for confounders (Shaheen *et al.* 2009). This analysis aims to explore whether the prudent dietary pattern is associated with any of the wheeze phenotypes and to determine whether any such associations are due to a causal link or to confounding influences.

Results: no significant associations were found between prudent diet score and any of the wheeze outcomes after adjusting for confounders.

Atopic wheeze was significantly positively associated with prudent diet score before pregnancy and at 34 weeks' gestation before adjusting for confounders but not after this adjustment. Non-atopic wheeze was inversely associated with prudent diet score at 34 weeks of pregnancy but, again, this association was significant only before adjusting for confounding factors (Table 104).

Prudent diet score	Unadjusted analyses			Adjusted* analyses		
	RR (95% CI)	P value	n	RR (95% CI)	P value	n
Doctor-diagnosed asthma						
Pre-pregnancy	0.87 (0.70-1.08)	0.216	469	1.05 (0.84-1.33)	0.653	443
11 weeks' gestation	0.95 (0.75-1.19)	0.629	381	1.23 (0.95-1.60)	0.113	370
34 weeks' gestation	0.88 (0.70-1.10)	0.244	459	1.10 (0.85-1.42)	0.481	442
Wheeze in last 12 months						
Pre-pregnancy	0.94 (0.75-1.18)	0.589	469	1.01 (0.80-1.27)	0.950	418
11 weeks' gestation	0.89 (0.70-1.13)	0.328	381	1.05 (0.79-1.39)	0.745	344
34 weeks' gestation	0.96 (0.76-1.21)	0.733	459	1.04 (0.79-1.35)	0.790	417
Transient wheeze						
Pre-pregnancy	0.95 (0.88-1.03)	0.242	398	1.00 (0.91-1.11)	0.955	377
11 weeks' gestation	1.02 (0.93-1.12)	0.728	325	1.07 (0.96-1.19)	0.215	316
34 weeks' gestation	0.95 (0.88-1.04)	0.257	389	0.99 (0.89-1.10)	0.858	376
Persistent wheeze						
Pre-pregnancy	0.87 (0.67-1.13)	0.307	229	1.04 (0.82-1.31)	0.755	223
11 weeks' gestation	0.96 (0.71-1.30)	0.813	186	1.15 (0.85-1.56)	0.376	183
34 weeks' gestation	0.88 (0.67-1.16)	0.381	226	1.02 (0.74-1.40)	0.914	223
Atopic wheeze						
Pre-pregnancy	1.39 (1.03-1.89)	0.032	278	1.29 (0.88-1.91)	0.193	243
11 weeks' gestation	1.36 (0.94-1.96)	0.102	224	1.50 (0.94-2.40)	0.086	200
34 weeks' gestation	1.49 (1.08-2.06)	0.016	272	1.21 (0.81-1.80)	0.354	242
Non-atopic wheeze						
Pre-pregnancy	0.70 (0.46-1.07)	0.102	271	0.93 (0.58-1.50)	0.774	258
11 weeks' gestation	0.79 (0.52-1.20)	0.268	219	1.00 (0.62-1.60)	0.986	212
34 weeks' gestation	0.59 (0.42-0.82)	0.002	266	0.77 (0.51-1.16)	0.210	257

***Analyses adjusted according to confounding factors identified for individual wheeze outcomes**
Relative risks were calculated as change in risk per SD change in size or growth measurement.

Table 104 Relative risk of wheeze phenotypes according to prudent diet score before and during pregnancy

8.6.3 Pre and postnatal growth and childhood wheeze phenotypes

Exploratory analysis: to determine whether any of the childhood wheeze phenotypes are associated with particular patterns of pre- or postnatal growth and, specifically, to assess the effects of faltering growth in late pregnancy.

Rationale: many studies have found birthweight to be inversely associated with childhood wheeze (Braback & Hedberg 1998; Carrington & Langley-Evans 2006; Lewis *et al.* 1995; Seidman *et al.* 1991; Shaheen *et al.* 1999; Svanes *et al.* 1998) suggesting that slower prenatal growth may be associated with adverse influences on immune and/or respiratory development. In section 4.4.2.2 slower growth in late pregnancy was found to be associated with atopic wheeze at 3 years of age, this analysis aims to identify whether a similar association can be found between late pregnancy growth faltering and asthma and other childhood wheeze phenotypes. Wheeze at 3 years was also found in chapter four (section 4.4.2.6) to be associated with greater adiposity gain in the first year of life and others have shown that weight gain in the first year of life is associated with poorer lung function at one year (Turner *et al.* 2008). It appears that postnatal patterns of growth may also be significant in the development of wheeze phenotypes.

Results: larger fetal size at 11 weeks gestation was associated with an increased risk of atopy and atopic wheeze at 6 years. Faltering abdominal circumference growth between 11 and 19 weeks' gestation was significantly associated with persistent wheeze and associations with wheeze in the last 12 months, atopic wheeze and non-atopic wheeze approached significance. Abdominal circumference at birth was significantly inversely associated with doctor-diagnosed asthma by age 6 years and an inverse association approaching significance was also found with persistent wheeze. Adiposity gain between birth and 6 months was significantly positively associated with transient wheeze and persistent wheeze.

	Unadjusted analyses			Adjusted* analyses		
	RR (95% CI)	**P-value**	**n**	**RR (95% CI)**	**P-value**	**n**
Fetal size variable						
11 weeks' gestation						
Head circumference	1.37 (1.09-1.71)	0.006	147	1.31 (1.05-1.63)	0.015	136
Abdominal circumference	1.34 (1.07-1.68)	0.010	151	1.31 (1.05-1.63)	0.017	140
19 weeks' gestation						
Head circumference	1.31 (1.03-1.65)	0.026	242	1.26 (0.99-1.60)	0.061	225
Abdominal circumference	1.19 (0.98-1.45)	0.084	242	1.18 (0.96-1.44)	0.110	225
34 weeks' gestation						
Head circumference	1.09 (0.86-1.37)	0.478	239	1.07 (0.84-1.35)	0.596	215
Abdominal circumference	0.92 (0.74-1.15)	0.486	239	0.93 (0.74-1.16)	0.501	224
Birth size variables						
Crown-heel length	1.00 (0.83-1.20)	0.978	349	1.00 (0.83-1.21)	0.993	325
Weight	1.05 (0.88-1.27)	0.570	354	1.11 (0.92-1.33)	0.265	328
Head circumference	1.05 (0.88-1.26)	0.564	357	1.01 (0.83-1.22)	0.931	331
Abdominal circumference	1.02 (0.85-1.24)	0.805	357	1.08 (0.89-1.31)	0.430	331
Subscapular skinfold	1.07 (0.91-1.27)	0.426	357	1.11 (0.94-1.31)	0.241	331
Conditional fetal growth						
11-19 weeks						
Head circumference	0.85 (0.60-1.22)	0.384	147	0.78 (0.52-1.18)	0.243	136
Abdominal circumference	0.91 (0.68-1.21)	0.508	151	0.91 (0.66-1.26)	0.572	140
19-34 weeks						
Head circumference	0.94 (0.74-1.19)	0.595	230	0.94 (0.74-1.19)	0.614	215
Abdominal circumference	0.85 (0.69-1.06)	0.142	238	0.87 (0.71-1.06)	0.164	223
Conditional Infant growth						
0 – 6 months						
Length	0.99 (0.84 -1.17)	0.923	346	0.89 (0.75-1.07)	0.211	322
Weight	0.96 (0.82-1.13)	0.660	352	0.89 (0.75-1.07)	0.212	326
Subscapular skinfolds	0.90 (0.76-1.08)	0.257	355	0.94 (0.79-1.11)	0.461	329
6 – 12 months						
Length	1.02 (0.83-1.27)	0.832	340	1.07 (0.87-1.31)	0.521	318
Weight	0.95 (0.78-1.15)	0.612	344	0.94 (0.75-1.16)	0.549	320
Subscapular skinfolds	1.02 (0.86-1.21)	0.790	342	1.00 (0.84-1.18)	0.966	319

N = 374 *Adjusted for child's gender and maternal education, history of asthma, atopic status and parity
Relative risks were calculated as change in risk per SD change in size or growth measurement.

Table 105 Relative risk of skin sensitisation at age 6 years, according to prenatal and early postnatal growth

	Unadjusted analyses			Adjusted* analyses		
	RR (95% CI)	P-value	n	RR (95% CI)	P-value	n
Fetal size variable						
11 weeks' gestation						
Head circumference	1.00 (0.70-1.42)	0.994	187	0.90 (0.60-1.36)	0.624	177
Abdominal circumference	0.95 (0.69-1.31)	0.765	192	0.82 (0.59-1.13)	0.221	183
19 weeks' gestation						
Head circumference	1.17 (0.89-1.53)	0.260	306	1.00 (0.75-1.33)	1.000	289
Abdominal circumference	0.97 (0.76-1.24)	0.792	305	0.77 (0.60-0.98)	0.035	288
34 weeks' gestation						
Head circumference	1.09 (0.83-1.42)	0.553	289	1.06 (0.80-1.41)	0.666	274
Abdominal circumference	0.94 (0.72-1.23)	0.642	303	0.89 (0.68-1.17)	0.401	288
Birth size variables						
Crown-heel length	0.88 (0.71-1.10)	0.262	438	0.83 (0.68-1.02)	0.084	416
Weight	0.82 (0.66-1.01)	0.067	443	0.81 (0.65-1.00)	0.045	420
Head circumference	0.96 (0.77-1.20)	0.736	446	1.00 (0.80-1.24)	0.998	423
Abdominal circumference	0.78 (0.63-0.97)	0.023	446	0.79 (0.65-0.96)	0.020	423
Subscapular skinfold	0.84 (0.65-1.08)	0.167	446	0.90 (0.71-1.16)	0.418	423
Conditional fetal growth						
11-19 weeks						
Head circumference	1.54 (0.89-2.66)	0.122	187	1.23 (0.63-2.38)	0.546	177
Abdominal circumference	1.05 (0.73-1.50)	0.805	191	0.79 (0.54-1.14)	0.206	182
19-34 weeks						
Head circumference	0.99 (0.74-1.32)	0.935	289	1.08 (0.79-1.48)	0.618	274
Abdominal circumference	0.96 (0.73-1.24)	0.735	301	1.01 (0.79-1.30)	0.926	286
Conditional Infant growth						
0 – 6 months						
Length	1.12 (0.88-1.42)	0.345	430	0.95 (0.75-1.20)	0.676	408
Weight	1.29 (1.06-1.57)	0.009	439	1.10 (0.89-1.35)	0.383	416
Subscapular skinfolds	1.14 (0.91-1.42)	0.259	441	1.07 (0.84-1.36)	0.604	418
6 – 12 months						
Length	0.99 (0.74-1.31)	0.932	420	0.90 (0.66-1.22)	0.491	400
Weight	0.95 (0.70-1.29)	0.744	428	1.05 (0.78-1.42)	0.753	408
Subscapular skinfolds	0.97 (0.75-1.26)	0.830	425	1.06 (0.85-1.32)	0.614	404

N = 469 * Adjusted for child's gestation, and birth order, mother's height, education, smoking in pregnancy and history of asthma and father's history of asthma
Relative risks were calculated as change in risk per SD change in size or growth measurement.

Table 106 Relative risks for the association between growth and whether the child had received a diagnosis of asthma from a doctor by age 6 years, compared with children who had never received such a diagnosis

	Unadjusted analyses			Adjusted* analyses		
	RR (95% CI)	**P-value**	**n**	**RR (95% CI)**	**P-value**	**n**
Fetal size variable						
11 weeks' gestation						
Head circumference	1.09 (0.79-1.48)	0.607	187	1.27 (0.81-1.99)	0.303	164
Abdominal circumference	1.26 (0.90-1.76)	0.184	192	1.34 (0.88-2.04)	0.174	170
19 weeks' gestation						
Head circumference	1.15 (0.85-1.57)	0.363	306	1.08 (0.78-1.50)	0.630	271
Abdominal circumference	1.04 (0.78-1.38)	0.780	305	0.92 (0.68-1.23)	0.557	271
34 weeks' gestation						
Head circumference	1.22 (0.92-1.62)	0.175	289	1.15 (0.84-1.56)	0.375	257
Abdominal circumference	0.94 (0.70-1.25)	0.658	303	0.94 (0.70-1.27)	0.689	270
Birth size variables						
Crown-heel length	0.88 (0.69-1.13)	0.327	438	0.96 (0.76-1.22)	0.737	391
Weight	0.87 (0.68-1.12)	0.288	443	0.91 (0.70-1.18)	0.467	396
Head circumference	0.97 (0.77-1.23)	0.830	446	1.03 (0.82-1.30)	0.792	399
Abdominal circumference	0.81 (0.64-1.03)	0.080	446	0.85 (0.66-1.08)	0.181	399
Subscapular skinfold	0.99 (0.80-1.22)	0.935	446	1.09 (0.87-1.38)	0.449	399
Conditional fetal growth						
11-19 weeks						
Head circumference	0.97 (0.61-1.56)	0.913	187	0.84 (0.49-1.45)	0.532	164
Abdominal circumference	0.84 (0.59-1.20)	0.343	191	0.67 (0.45-1.02)	0.061	170
19-34 weeks						
Head circumference	1.14 (0.84-1.54)	0.392	289	1.16 (0.83-1.61)	0.387	257
Abdominal circumference	0.93 (0.71-1.22)	0.609	301	1.01 (0.76-1.34)	0.946	269
Conditional Infant growth						
0 – 6 months						
Length	1.04 (0.84-1.29)	0.722	430	0.97 (0.79-1.21)	0.815	385
Weight	1.05 (0.85-1.29)	0.667	439	1.05 (0.84-1.31)	0.679	393
Subscapular skinfolds	1.05 (0.85-1.30)	0.659	441	1.04 (0.81-1.34)	0.769	396
6 – 12 months						
Length	1.08 (0.79-1.45)	0.633	420	1.08 (0.79-1.47)	0.649	379
Weight	0.91 (0.68-1.22)	0.540	428	0.96 (0.69-1.33)	0.802	386
Subscapular skinfolds	0.89 (0.69-1.16)	0.387	425	0.91 (0.69-1.21)	0.517	383

N = 469 *Adjusted for child's gestation, pet exposure in first year of life and birth order, education, smoking in pregnancy and history of asthma and father's history of asthma
Relative risks were calculated as change in risk per SD change in size or growth measurement.

Table 107 Relative risks for the association between growth and whether the child had wheezed in the last 12 months, compared with children who had not wheezed during this period

	Unadjusted analyses			Adjusted* analyses		
	RR (95% CI)	**P-value**	**n**	**RR (95% CI)**	**P-value**	**n**
Fetal size variable						
11 weeks' gestation						
Head circumference	1.05 (0.90-1.22)	0.528	158	0.99 (0.84-1.15)	0.855	150
Abdominal circumference	1.06 (0.90-1.25)	0.477	161	1.01 (0.85-1.21)	0.885	154
19 weeks' gestation						
Head circumference	1.08 (0.95-1.24)	0.239	255	0.99 (0.86-1.14)	0.884	241
Abdominal circumference	1.05 (0.93-1.19)	0.440	254	0.98 (0.85-1.13)	0.810	240
34 weeks' gestation						
Head circumference	1.03 (0.91-1.17)	0.641	241	1.02 (0.90-1.15)	0.790	229
Abdominal circumference	1.10 (0.97-1.23)	0.126	252	1.10 (0.98-1.25)	0.108	240
Birth size variables						
Crown-heel length	1.01 (0.92-1.11)	0.843	373	0.98 (0.88-1.09)	0.735	355
Weight	1.03 (0.94-1.13)	0.494	378	1.01 (0.91-1.11)	0.861	359
Head circumference	1.04 (0.95-1.14)	0.363	380	1.04 (0.95-1.14)	0.353	361
Abdominal circumference	1.03 (0.93-1.13)	0.567	380	1.00 (0.90-1.10)	0.980	361
Subscapular skinfold	1.03 (0.94-1.12)	0.545	380	1.04 (0.95-1.15)	0.391	361
Conditional fetal growth						
11-19 weeks						
Head circumference	0.99 (0.82-1.20)	0.929	158	0.94 (0.78-1.13)	0.509	150
Abdominal circumference	1.09 (0.91-1.31)	0.358	160	1.04 (0.86-1.27)	0.675	153
19-34 weeks						
Head circumference	1.00 (0.88-1.14)	0.870	241	1.04 (0.91-1.18)	0.581	229
Abdominal circumference	1.07 (0.96-1.19)	0.195	251	1.11 (1.00-1.24)	0.051	239
Conditional Infant growth						
0 – 6 months						
Length	1.03 (0.94-1.12)	0.528	369	1.00 (0.91-1.10)	0.993	351
Weight	1.11 (1.02-1.20)	0.016	377	1.07 (0.99-1.17)	0.097	358
Subscapular skinfolds	1.13 (1.05-1.22)	0.001	377	1.12 (1.04-1.21)	0.003	358
6 – 12 months						
Length	0.94 (0.85-1.05)	0.280	360	0.92 (0.81-1.03)	0.182	343
Weight	0.93 (0.83-1.04)	0.210	366	0.95 (0.84-1.07)	0.391	350
Subscapular skinfolds	0.98 (0.89-1.08)	0.757	362	0.99 (0.90-1.08)	0.760	345

N = 398 *Adjusted for child's gestation, birth order and pet exposure and mother's height, education, smoking in pregnancy and history of asthma and rhinitis, and father's history of asthma
Relative risks were calculated as change in risk per SD change in size or growth measurement.

Table 108 Relative risks for the association between growth and whether the child had transient wheeze, compared with children who had never wheezed

	Unadjusted analyses			Adjusted* analyses		
	RR (95% CI)	**P-value**	**n**	**RR (95% CI)**	**P-value**	**n**
Fetal size variable						
11 weeks' gestation						
Head circumference	1.10 (0.79-1.51)	0.578	93	1.0.1 (0.70-1.46)	0.964	90
Abdominal circumference	1.24 (0.92-1.67)	0.153	99	1.16 (0.82-1.65)	0.402	96
19 weeks' gestation						
Head circumference	1.08 (0.81-1.43)	0.591	159	0.98 (0.74-1.30)	0.906	154
Abdominal circumference	1.02 (0.80-1.30)	0.882	158	0.93 (0.73-1.19)	0.583	153
34 weeks' gestation						
Head circumference	1.06 (0.80-1.40)	0.692	149	1.01 (0.77-1.31)	0.951	145
Abdominal circumference	0.93 (0.72-1.22)	0.619	158	0.95 (0.75-1.20)	0.657	154
Birth size variables						
Crown-heel length	0.85 (0.66-1.08)	0.190	218	0.95 (0.75-1.22)	0.708	212
Weight	0.83 (0.66-1.05)	0.116	221	0.86 (0.67-1.10)	0.217	215
Head circumference	0.93 (0.74-1.16)	0.513	222	0.98 (0.78-1.22)	0.832	216
Abdominal circumference	0.79 (0.63-0.99)	0.045	222	0.80 (0.63-1.01)	0.056	216
Subscapular skinfold	1.02 (0.82-1.28)	0.836	222	1.05 (0.83-1.32)	0.681	216
Conditional fetal growth						
11-19 weeks						
Head circumference	0.86 (0.49-1.50)	0.590	93	0.64 (0.37-1.13)	0.123	90
Abdominal circumference	0.88 (0.63-1.23)	0.465	98	0.68 (0.48-0.97)	0.033	95
19-34 weeks						
Head circumference	1.00 (0.76-1.32)	0.990	149	1.00 (0.75-1.34)	0.981	145
Abdominal circumference	0.94 (0.73-1.21)	0.651	156	1.01 (0.78-1.30)	0.964	152
Conditional Infant growth						
0 – 6 months						
Length	1.09 (0.88-1.34)	0.453	213	1.07 (0.87-1.33)	0.521	207
Weight	1.30 (1.02-1.66)	0.036	218	1.35 (1.06-1.72)	0.013	212
Subscapular skinfolds	1.32 (1.06-1.65)	0.014	219	1.30 (1.03-1.65)	0.025	213
6 – 12 months						
Length	0.95 (0.70-1.28)	0.736	208	0.97 (0.72-1.30)	0.822	204
Weight	0.83 (0.62-1.11)	0.200	213	0.97 (0.70-1.35)	0.869	209
Subscapular skinfolds	0.90 (0.70-1.16)	0.414	213	0.93 (0.71-1.22)	0.604	206

N = 229 *Adjusted for child's gestation, pet exposure in the first year of life and birth order, mother's age, education, smoking in pregnancy and history of asthma and rhinitis, and father's history of asthma
Relative risks were calculated as change in risk per SD change in size or growth measurement.

Table 109 Relative risks for the association between growth and persistent wheeze, compared with children who had never wheezed

	Unadjusted analyses			Adjusted* analyses		
	RR (95% CI)	**P-value**	**n**	**RR (95% CI)**	**P-value**	**n**
Fetal size variable						
11 weeks' gestation						
Head circumference	1.53 (0.97-2.39)	0.066	113	1.42 (0.81-2.47)	0.218	98
Abdominal circumference	1.56 (1.05-2.31)	0.027	118	1.67 (1.07-2.59)	0.023	103
19 weeks' gestation						
Head circumference	1.47 (0.94-2.30)	0.092	187	1.31 (0.75-2.29)	0.335	166
Abdominal circumference	1.17 (0.82-1.66)	0.383	187	1.04 (0.69-1.57)	0.838	166
34 weeks' gestation						
Head circumference	1.28 (0.83-1.99)	0.268	180	1.04 (0.68-1.60)	0.860	159
Abdominal circumference	0.86 (0.60-1.24)	0.427	186	0.76 (0.53-1.09)	0.132	165
Birth size variables						
Crown-heel length	1.08 (0.74-1.57)	0.692	263	1.15 (0.78-1.69)	0.493	232
Weight	0.98 (0.70-1.38)	0.926	270	1.00 (0.72-1.39)	0.978	237
Head circumference	1.07 (0.76-1.51)	0688	270	0.89 (0.62-1.27)	0.522	237
Abdominal circumference	0.83 (0.59-1.16)	0.279	270	0.96 (0.71-1.29)	0.769	237
Subscapular skinfold	1.07 (0.81-1.43)	0.624	270	1.18 (0.82-1.70)	0.362	237
Conditional fetal growth						
11-19 weeks						
Head circumference	1.14 (0.57-2.26)	0.716	113	1.07 (0.45-2.54)	0.884	98
Abdominal circumference	0.80 (0.54-1.17)	0.246	118	0.58 (0.32-1.03)	0.063	103
19-34 weeks						
Head circumference	1.04 (0.67-1.63)	0.884	180	0.89 (0.59-1.34)	0.571	159
Abdominal circumference	0.82 (0.55-1.21)	0.314	185	0.80 (0.57-1.12)	0.193	164
Conditional Infant growth						
0 – 6 months						
Length	0.92 (0.67-1.26)	0.612	261	0.66 (0.45-0.98)	0.039	230
Weight	1.01 (0.78-1.31)	0.924	270	0.93 (0.60-1.30)	0.666	237
Subscapular skinfolds	1.04 (0.78-1.39)	0.779	269	1.22 (0.86-1.72)	0.259	236
6 – 12 months						
Length	1.05 (0.65-1.71)	0.847	258	1.28 (0.82-2.00)	0.272	228
Weight	0.85 (0.58-1.25)	0.408	262	0.78 (0.50-1.22)	0.277	231
Subscapular skinfolds	1.03 (0.74-1.44)	0.860	259	0.88 (0.60-1.30)	0.525	228

N = 278 *Adjusted for child's sex, gestation, and birth order, mother's education, smoking in pregnancy and history of asthma and father's history of asthma
Relative risks were calculated as change in risk per SD change in size or growth measurement.

Table 110 Relative risks for the association between growth and whether the child had atopic wheeze, compared with children who had never wheezed and are not atopic

	Unadjusted analyses			Adjusted* analyses		
	RR (95% CI)	P-value	n	RR (95% CI)	P-value	n
Fetal size variable						
11 weeks' gestation						
Head circumference	0.83 (0.59-1.17)	0.287	107	0.78 (0.40-1.55)	0.482	102
Abdominal circumference	1.36 (0.82-2.25)	0.229	110	2.55 (1.04-6.25)	0.040	105
19 weeks' gestation						
Head circumference	0.93 (0.55-1.57)	0.782	175	0.82 (0.43-1.58)	0.560	169
Abdominal circumference	0.92 (0.55-1.54)	0.753	175	0.90 (0.51-1.58)	0.706	169
34 weeks' gestation						
Head circumference	1.04 (0.63-1.73)	0.869	169	1.17 (0.68-2.03)	0.571	163
Abdominal circumference	1.11 (0.61-2..01)	0.735	174	1.29 (0.79-2.12)	0.309	168
Birth size variables						
Crown-heel length	0.73 (0.48-1.10)	0.130	256	1.02 (0.70-1.48)	0.934	243
Weight	0.68 (0.43-1.08)	0.101	261	0.80 (0.47-1.37)	0.412	248
Head circumference	0.81 (0.56-1.16)	0.248	262	1.04 (0.69-1.57)	0.865	249
Abdominal circumference	0.73 (0.44-1.20)	0.214	262	0.78 (0.46-1.35)	0.379	249
Subscapular skinfold	0.74 (0.48-1.15)	0.185	262	0.82 (0.50-1.35)	0.439	249
Conditional fetal growth						
11-19 weeks						
Head circumference	0.88 (0.30-2.65)	0.826	107	0.39 (0.18-0.84)	0.016	102
Abdominal circumference	0.78 (0.46-1.34)	0.368	110	0.48 (0.21-1.12)	0.088	105
19-34 weeks						
Head circumference	1.07 (0.66-1.72)	0.787	169	1.33 (0.81-2.18)	0.260	163
Abdominal circumference	1.10 (0.73-1.84)	0.521	174	1.49 (0.94-2.37)	0.088	168
Conditional Infant growth						
0 – 6 months						
Length	1.56 (1.15-2.10)	0.004	255	1.78 (1.16-2.72)	0.008	242
Weight	1.44 (1.05-1.96)	0.023	260	1.52 (0.95-2.44)	0.082	247
Subscapular skinfolds	1.16 (0.80-1.69)	0.444	261	1.10 (0.72-1.68)	0.675	248
6 – 12 months						
Length	1.08 (0.68-1.73)	0.746	252	1.28 (0.75-2.19)	0.366	240
Weight	0.95 (0.56-1.62)	0.857	253	1.25 (0.75-2.06)	0.393	242
Subscapular skinfolds	0.84 (0.55-1.30)	0.439	251	0.83 (0.56-1.24)	0.357	239

N = 271 *Adjusted for child's gestation, pet exposure in the first year of life and birth order, mother's education, smoking in pregnancy
Relative risks were calculated as change in risk per SD change in size or growth measurement.

Table 111 Relative risks for the association between growth and whether the child non-atopic wheeze, compared with children who had never wheezed and are not atopic

The relative risk of skin sensitisation at six years of age was significantly positively associated with both head and abdominal circumference at 11 weeks' gestation (RR 1.31, p=0.015 and RR 1.31, p=0.017). Abdominal circumference at 34 weeks' and abdominal circumference growth between 19 and 34 weeks' gestation were inversely associated with skin sensitisation but, although these findings echoed the directions of those found at 3 years (Table 16), these associations with the six year outcomes did not reach significance (Table 105).

The relative risk of doctor-diagnosed asthma was significantly inversely associated with birthweight. For every SD increase in birthweight asthma risk decreased by 19% (p=0.045). Abdominal circumference at birth was also significantly inversely associated with asthma risk (RR 0.79, p=0.020) and an inverse association with crown-heel length also approached significance. This pattern was similar to the inverse relationships seen between the doctor-diagnosed asthma outcome at 3 years and birth anthropometry (Table 20). Although smaller size at birth was associated with asthma diagnosed by 6 years no significant associations were found with faltering growth between either 11 and 19 weeks' or 19 and 34 weeks' gestation. The only other significant association was an inverse association with abdominal circumference at 19 weeks of pregnancy (RR 0.77, p=0.035) (Table 106).

Wheeze in the last 12 months was not significantly associated with any measure of size or pre- or postnatal growth. The birth measure with the strongest association with the risk of wheeze was abdominal circumference (RR 0.85, p=0.181). The measure of prenatal growth most significantly associated with wheeze in the last 12 months was abdominal circumference growth between 11 and 19 weeks' gestation (RR 0.67, p=0.061). There were no significant associations between this outcome and measures of postnatal growth (Table 107).

The relative risk of transient wheeze was significantly positively associated with conditional adiposity gain between birth and six months. A 12% increase in risk was seen for each SD increase in subscapular skinfold gain (p=0.003). Weight gain between birth and 6 months was also positively associated with the transient wheeze outcome, although this association was not significant following adjustment for confounders. Transient wheeze risk was not associated with measures of prenatal growth or birth anthropometry (Table 108). This pattern of association with postnatal growth alone was

similar to that seen at 3 years for any wheeze and non-atopic wheeze (Table 21 & Table 17).

Inverse relationships were found between the relative risk of persistent wheeze and abdominal circumference at birth and abdominal circumference growth between 11 and 19 weeks' pregnancy. Of these, only the association between early pregnancy abdominal growth and the persistent wheeze outcome remained significant in the adjusted analysis (RR 0.68, p=0.03). Postnatal weight and adiposity gain between birth and 6 months were both positively associated with the risk of persistent wheeze (RR 1.35, p=0.013 and RR 1.30, p=0.025 respectively) (Table 109).

An association between larger 11 week abdominal circumference and increased relative risk of atopic wheeze was seen in the six year data. However, the associations between atopic wheeze and rapid early growth trajectory and late pregnancy abdominal circumference growth faltering that were apparent at 3 years (Table 17) were not seen between these patterns of growth and atopic wheeze at 6 years. Prenatal growth velocity data were available for far fewer participants at 6 than 3 years of age; it is possible that the 6 year study was underpowered to detect associations between wheeze outcomes and variables such as prenatal growth for which data were not available for every participant. The relative risk of atopic wheeze was significantly positively associated with abdominal circumference at 11 weeks' gestation (RR 1.67, p=0.023) and growth in length between birth and 6 months (RR 0.66, p=0.039). Inverse associations approaching significance were also found between atopic wheeze and abdominal circumference at 34 weeks' and abdominal circumference growth between 11 and 19 and 19 and 34 weeks' gestation (Table 110).

The relative risk for non-atopic wheeze was significantly associated with abdominal circumference at 11 weeks' gestation (RR 2.55, p=0.040), head circumference growth between 11 and 19 weeks (RR 0.39, p=0.016) and growth in length between birth and 6 months (RR 1.78, p=0.008) (Table 111). An inverse association with abdominal circumference growth between 11 and 19 weeks' gestation and a positive association between weight gain between birth and 6 months approached, but did not reach, significance. There were no significant associations between birth anthropometry and the relative risk of non-atopic wheeze (Table 111). The associations with faltering head

circumference growth and more rapid postnatal weight gain were similar to those found for the non-atopic wheeze outcome at 3 years (Table 18). Birth anthropometry was not significantly associated with non-atopic wheeze at 6 years of age (Table 111).

8.7 SUMMARY DISCUSSION

This chapter explored possible associations between the childhood wheeze phenotypes and both maternal nutrition and fetal growth. Analyses were undertaken to confirm or refute the findings of published studies and the findings were compared to those described in previous chapters. The exploratory analyses were guided by consideration of biologically plausible mechanisms.

Summary of main findings:

- Wheeze and asthma were reported by participants in this study at rates comparable to children of a similar age in other UK birth cohorts.

- The most prevalent wheeze phenotype was transient wheeze. The transient wheeze phenotype was more prevalent in this study and the late-onset wheeze phenotype less prevalent than in other cohorts where these phenotypes have been described.

- Higher maternal pre-pregnancy percentage body fat and BMI were significantly associated with non-atopic wheeze in the offspring at age six years. Higher maternal body fat and arm muscle area before and during pregnancy were also significantly associated with the non-atopic wheeze outcome. Maternal body fat during pregnancy was significantly associated with recent doctor-diagnosed asthma. Less significant associations were also seen between higher maternal body fat and atopic wheeze, transient wheeze, wheeze in the last 12 months and doctor-diagnosed asthma ever. Maternal arm muscle area was also significantly associated with transient wheeze.

- Lower maternal pre-pregnancy vitamin D intake was significantly associated with doctor-diagnosed asthma, wheeze in the last 12 months and non-atopic wheeze in the offspring at age 6 years. Significant inverse associations were also found between maternal intakes of vitamins A and E and non-atopic wheeze. Significant positive associations were found between maternal vitamin C intake and doctor-diagnosed asthma, persistent wheeze and atopic wheeze.

- Adjusting maternal vitamin intakes for total energy intake increased the strength of associations between wheeze outcomes and maternal intakes of vitamins A and C and decreased the strength of the associations between maternal intake of vitamin D and these outcomes. After energy adjustment, vitamin D intake was no longer significantly associated with doctor-diagnosed asthma or wheeze in the last 12 months but remained significantly inversely associated with non-atopic wheeze. Significant positive associations were found between energy-adjusted vitamin A intake during pregnancy and persistent and atopic wheeze, energy-adjusted vitamin C intake and doctor-diagnosed asthma, persistent wheeze and atopic wheeze, and between energy-adjusted vitamin E intake and persistent wheeze.

- Maternal serum 25(OH) vitamin D status at 34 weeks' gestation was significantly inversely associated with doctor-diagnosed asthma, atopic wheeze and persistent wheeze in the offspring at age 6 years. Inverse associations approaching significance were also found between maternal serum 25(OH) vitamin D and transient wheeze. A significant positive association was found between maternal serum retinol and atopic wheeze and maternal total anti-oxidant status and persistent wheeze.

- A significant positive association was found between the percentage of arachidonic acid in maternal plasma fatty acid phospholipids at 34 weeks' gestation and atopic wheeze in the offspring at age 6 years. Significant inverse associations were found between maternal α-linolenic acid and both doctor-diagnosed asthma and recent doctor-diagnosed asthma whilst EPA status was significantly inversely associated with the relative risk of doctor-diagnosed asthma. Maternal EPA status was also significantly inversely associated with

wheeze in the last 12 months and non-atopic wheeze. An unexpected inverse association was found between maternal arachidonic acid status and the relative risk of transient wheeze.

- No significant associations were found between prudent diet score and any of the wheeze outcomes after adjusting for confounders. Atopic wheeze was significantly positively associated with prudent diet score before and during pregnancy before adjusting for confounders. Before adjusting for confounders, non-atopic wheeze was inversely associated with prudent diet score at 34 weeks of pregnancy.

- Larger fetal size at 11 weeks gestation was associated with an increased risk of atopy and atopic wheeze at 6 years. Faltering abdominal circumference growth between 11 and 19 weeks' gestation was significantly associated with persistent wheeze and associations with wheeze in the last 12 months, atopic wheeze and non-atopic wheeze approached significance. The association between wheeze and late pregnancy faltering of abdominal circumference growth that was seen at 3 years was not found for any of the 6-year outcomes. Abdominal circumference at birth was significantly inversely associated with doctor-diagnosed asthma by age 6 years and an inverse association approaching significance was also found with persistent wheeze. Adiposity gain between birth and 6 months was significantly positively associated with transient wheeze and persistent wheeze.

Chapter Nine
Overall Discussion

9.1 PRINCIPAL FINDINGS

Specific objectives for this thesis were outlined in chapter one (section 1.8). A number of *a priori* hypotheses were developed based upon previous epidemiological findings and biologically plausible mechanisms. Exploratory analyses were performed to explore areas where, currently, there is less data available to inform *a priori* hypotheses. The principal findings relating to the study's objectives were as follows:

Pre and postnatal growth and childhood wheeze and atopy

- Rapid abdominal circumference growth early in pregnancy and growth faltering in late pregnancy were associated with an increased risk of atopy at age 3 years; abdominal circumference growth faltering in late pregnancy was also associated with atopic wheeze. Greater weight and adiposity gain in the first year of life were associated with an increased risk, at 3 years, of atopic and non-atopic wheeze.
- Larger fetal size at 11 weeks gestation was associated with an increased risk of atopy and atopic wheeze at 6 years. Faltering abdominal circumference growth between 11 and 19 weeks' gestation was significantly associated with persistent wheeze, and associations with wheeze in the last 12 months, atopic wheeze and non-atopic wheeze approached significance. The association between wheeze and late pregnancy faltering of abdominal circumference growth that was seen at 3 years was not found for any of the 6-year outcomes. Abdominal circumference at birth was significantly inversely associated with doctor-diagnosed asthma by age 6 years and an inverse association approaching

significance was also found with persistent wheeze. Adiposity gain between birth and 6 months was significantly positively associated with transient wheeze and persistent wheeze.

Maternal nutrition and atopy at age 6 years

- Higher maternal arachidonic acid status at 34 weeks' gestation was associated with higher FENO and an increased risk of skin sensitisation in the offspring at age 6 years. Lower α-linolenic acid status in late pregnancy was also associated with an increased risk of skin sensitisation, and lower linoleic acid with greater FENO.
- No significant relationships were found between atopy in the offspring and maternal body composition, maternal intake of vitamin E or D, or maternal serum level of vitamin E or D. Higher maternal vitamin E from foods was associated with a greater multiplicative increase in FENO.

Maternal nutrition and lung function at age 6 years

- Lower energy-adjusted vitamin A intake in late pregnancy was associated with lower FEV_1 and lower $FEF_{25-75\%}$, but also less BHR in the offspring at age 6 years.
- No significant relationships were found between lung function in the offspring and maternal body composition, non-energy adjusted maternal intake of vitamin A or C, or maternal serum level of vitamin A or C.
- A lower maternal prudent diet score in late pregnancy was associated with a lower FEV_1/FVC and a lower $FEF_{25-75\%}$.

Maternal nutrition and childhood wheeze phenotypes

- Greater maternal pre-pregnancy BMI and greater maternal body fat, before and during pregnancy, were strongly associated with an increased risk of non-atopic wheeze at age 6 years.
- Lower maternal pre-pregnancy vitamin D intake was associated with increased risks of doctor-diagnosed asthma, wheeze in the last 12 months and non-atopic wheeze at age 6 years. Lower pre-pregnancy maternal intakes of vitamins A and E were also associated with an increased risk of non-atopic wheeze as was lower intake of vitamin E in late pregnancy. Greater pre-pregnancy vitamin C intake

was associated with an increased risk of persistent wheeze at age 6, and higher intake of this vitamin in late pregnancy was associated with increased risks of atopic wheeze and doctor-diagnosed asthma.

- Lower maternal serum 25(OH) vitamin D status at 34 weeks' gestation was associated with doctor-diagnosed asthma, atopic wheeze and persistent wheeze at 6 years. Higher maternal serum retinol and maternal total anti-oxidant status were associated with atopic wheeze and persistent wheeze respectively.
- Higher maternal arachidonic acid status was associated with an increased risk of atopic wheeze at 6 years and a lower risk of transient wheeze. Lower proportions of α-linolenic and EPA in maternal plasma phospholipids were associated with increased doctor-diagnosed asthma risk. Lower maternal EPA status was also associated at 6 years of age with increased risks of wheeze in the last 12 months and non-atopic wheeze.

9.2 GROWTH

The *a priori* hypothesis concerning growth in early life stated that factors which impair fetal growth, and are often followed by postnatal adiposity gain, may also alter immune and respiratory development. There were two principal findings concerning growth in early life and childhood wheeze and atopy. Firstly, slower abdominal circumference growth in fetal life was associated with atopy and atopic wheeze at 3 years and with persistent wheeze at 6 years. Secondly, greater weight and adiposity gains in the first year of life were associated with both atopic and non-atopic wheeze at 3 years and with transient and persistent wheeze at 6 years. Together these findings provide support for the *a priori* hypothesis, however, due to the observational nature of this study, these associations do not prove causality.

9.2.1 Background to the *a priori* hypothesis

Following the original observation of an inverse association between birthweight and respiratory disease in adulthood (Barker *et al.* 1991; Svanes *et al.* 1998), further work has shown that individuals who were small at birth tend to have reduced lung function from early in infancy (Friedrich *et al.* 2006; Hoo *et al.* 2004; Lucas *et al.* 2004; Lum *et al.* 2001). This suggests infant lung function is not purely genetically determined but can be influenced by environmental factors during prenatal and early postnatal life (Dezateux *et al.* 1999; Martinez *et al.* 1991; Stick *et al.* 1996).

As outlined in section 1.4.1, although many investigators have found a relationship between lower birthweight and subsequent wheeze (Braback & Hedberg 1998; Carrington & Langley-Evans 2006; Lewis *et al.* 1995; Seidman *et al.* 1991; Shaheen *et al.* 1999; Svanes *et al.* 1998), others have found evidence for an association with higher birthweight (Remes *et al.* 2008; Yuan *et al.* 2002), and some no relationship at all (Kelly *et al.* 1995; Oliveti *et al.* 1996; Rona *et al.* 1993; Sears *et al.* 1996; Strachan *et al.* 1996a; Taveras *et al.* 2006). This lack of agreement may reflect methodological differences, including lack of adjustment for gestational age and other confounding factors. The lack of consensus is unsurprising, however, given that birth anthropometry provides only an indirect indicator of the quality of the intrauterine environment.

9.2.2 Comparison with other studies

The broad conclusions, of this thesis are supported by data from the Aberdeen cohort which suggest lower fetal growth is associated with later asthma and wheeze. Compared to those children whose fetal size measurements were above the median value at 10 and 20 weeks' gestation, the relative risk of asthma, diagnosed by five years of age, was significantly higher in those children who were below the median values for crown-rump length at 10 weeks' and bi-parietal diameter at 20 weeks' gestation (Turner *et al.* 2008). The Aberdeen study did not measure growth in the true sense as change in any given fetal size parameter, however, and did not account for precise gestational age. In contrast, within the SWS antenatal ultrasounds were performed at 11, 19 and 34 weeks' gestation in order to more precisely characterise the relationship between early growth and asthma-related outcomes, and measures of postnatal growth were also considered.

9.2.3 Prenatal growth and wheeze and atopic outcomes

A novel finding of this study was the association of a faster early pregnancy fetal growth trajectory and increased risk of atopy and atopic wheeze at age 3 years (sections 4.4.2.1 & 4.4.2.2). Larger fetal size at 11 weeks gestation was also associated with increased risks of atopy and atopic wheeze at age 6 years (section 8.6.3). Slower mid or late pregnancy fetal growth was associated with wheeze outcomes at both 3 and 6 years of age (sections 4.4.2 & 8.6.3). However, both the timing of the period of slower growth and the specific wheeze outcome with which it was associated differed between the 3 and 6-year analysis. Abdominal growth velocity in late pregnancy (between 19 and 34 weeks' gestation) was significantly inversely associated with atopy and atopic wheeze

risk at 3 years of age (section 4.4.2.1 & 4.4.2.2). Atopy and atopic wheeze measured at 6 years of age were not associated with late pregnancy abdominal growth faltering, although persistent wheeze risk at 6 years was significantly associated with slower fetal abdominal growth during early pregnancy (between 11 and 19 weeks' gestation, section 8.6.3). Slowed growth during mid and late pregnancy is likely to occur in those fetuses growing along a faster growth trajectory as their nutritional requirements are greater. Slower growth in mid and late pregnancy can be regarded as faltering from the growth trajectory established in early pregnancy. Unpublished observations from the MRC Epidemiology Resource Centre suggest that fetuses with fast growth between 7 and 11 weeks gestation may often show faltering of abdominal growth between 11 and 19 weeks. The differential associations between wheeze persisting until 6 years and slower early fetal growth and between atopy and atopic wheeze at 3 years and faltering of fetal growth in late pregnancy may reflect an earlier onset of growth faltering in the subset of subjects studied at age 6 years; if effects upon respiratory and immune function are restricted to critical periods in development, the timing of growth slowing may be important in determining the phenotypic outcome.

Slower head circumference growth between 11 and 19 weeks' gestation was significantly associated with an increased risk of non-atopic wheeze by 3 years of age (section 4.4.2.3. Slower head circumference growth in early pregnancy was also significantly associated with an increased risk of non-atopic wheeze at age 6 years (section 8.6.3). The magnitude of this effect was greater at 6 years than at 3 years of age (RR 0.39, $p=0.016$ vs. 0.90, $p=0.041$, respectively).

There was an approximately two to threefold greater number of children included in the 3-year than the 6-year analysis. Exclusion criteria and outcome definitions also differed slightly between the two analyses. All children born at less than 37 weeks' gestation were excluded from the 3-year analysis, whilst, to maximise power, at 6 years only those born before 35 weeks' gestation were excluded. Atopic status at 6 years was determined by skin testing to tree pollens in addition to the allergens tested at 3 years (cat, dog, house dust mite, milk, grass pollens, and egg). Atopic and non-atopic wheeze at 6 years were defined on the basis of presence or absence of skin sensitisation at 6 years and wheeze in the preceding year; in the 3-year analysis, these outcomes included those children who had been reported to wheeze at any time up to and including 3 years.

Differences between the associations found at 3 and 6 years were not explained by differences in gestation-based exclusion criteria of the 3 and 6-year analyses. The associations found between abdominal growth faltering and both atopy and atopic wheeze at 3 years remained significant when the data were re-analysed excluding only those children born before 35 weeks (Appendix 1, Table 128). The addition of tree pollen to the skin testing allergens is also unlikely to have contributed significantly to the differences between the 3 and 6 year results as only one child at 6 years was classified as atopic based upon skin sensitisation to this allergen alone.

Failure to detect at 6 years the significant association found between late pregnancy abdominal growth faltering and atopy that was found at 3 years may be due simply to the reduced power of the six year analysis. However, differences between the phenotypic characteristics of those children classified as atopic at 3 years compared to those so classified at 6 years of age might provide a plausible biological reason for the differences between the two analyses. Of those children classed as atopic at 6 years, 48% were not atopic at 3 years whilst of those classified as atopic at 3 years only 75% remained so at 6 years (Table 47, Chapter 6). It is possible that faltering fetal growth is more closely associated with early (prior to 3 years) than late (between 3 and 6 years) sensitisation to allergens.

A biological explanation for the seemingly stronger association between slow head circumference growth and non-atopic wheeze at 6 years compared to that at 3 years may also be speculated. Low growth in early pregnancy was associated with both non-atopic and persistent wheeze. A low early growth trajectory may identify those at risk of developing these phenotypes due to a common mechanism. Although for the majority of children with non-atopic wheeze at 3 years of age this was a transient problem, most of those with non-atopic wheeze at 6 years had persistent wheeze. Indeed, no child developed late-onset non-atopic wheeze (Table 42, Chapter 5). Non-atopic wheeze in infancy might reflect multiple factors, including susceptibility to and response to infection, whilst narrower airways attributable to impaired respiratory development associated with poor fetal growth may play a more significant role in the aetiology of persistent non-atopic wheeze at 6 years.

9.2.4 Birth anthropometry and wheeze and atopic outcomes

Doctor-diagnosed asthma both by 3 and by 6 years was significantly inversely associated with both birthweight and abdominal circumference (sections 4.4.2.6 & 8.6.3). Atopy at 3 years of age was significantly positively associated with crown-rump length at birth. These associations remained significant after adjusting for confounding variables including gestational age. Birthweight was positively associated with atopy at both 3 and 6 years, although this did not reach significance (RR 1.08, p=0.241, section 4.4.2.1, and RR 1.11, p=0.265, section 8.6.3).

Despite the lack of precision associated with substituting birth anthropometry for measures of prenatal growth and the general lack of consensus regarding the relationship between weight at birth and later asthma, these findings find some support from within the existing literature. Many studies of asthma and wheeze have found size at birth, as in this thesis, to be inversely related to these outcomes (Braback & Hedberg 1998; Carrington & Langley-Evans 2006; Lewis *et al.* 1995; Seidman *et al.* 1991; Shaheen *et al.* 1999; Svanes *et al.* 1998). Larger size at birth has also been consistently found in association with atopy (Bolte *et al.* 2004; Godfrey *et al.* 1994; Gregory *et al.* 1999; Katz *et al.* 2003).

Recent studies suggest the relationship between birthweight and wheeze outcomes might be non-linear (Remes *et al.* 2008). It is possible that birthweight may be a marker for separate developmental effects upon immune and respiratory development. For example, Lewis (1995) found low birthweight to be associated with wheeze before 5 years of age but not after. Lewis proposed that low birthweight may be an independent risk factor for low airway calibre and hence early wheeze. Conversely, Remes demonstrated high birthweight to be associated with asthma in atopic children and concluded that the positive relationship between atopy and birthweight accounted for much of this association (Remes *et al.* 2008). The finding in this thesis of a positive association between greater abdominal circumference growth velocity in early pregnancy and atopy and atopic wheeze is certainly consistent with the hypothesis that rapid fetal growth increases the risk of atopy. This increased risk may be due to a direct effect of growth promoting factors upon immune development or the increased vulnerability of a fetus on a high growth trajectory to growth faltering during nutrient restriction (Harding *et al.* 1992). Follow-up of this cohort to 16 years would enable

testing of Lewis's finding that wheeze which persists until this age is not associated with low birthweight.

9.2.5 Postnatal growth and wheeze and atopic outcomes

Weight and adiposity gain in the first year of life were significantly positively associated with the risk of wheezing before 3 years of age (sections 4.4.2.2 & 4.4.2.3). Despite the lower power of the six year analysis, a positive association between infant weight and adiposity gain and wheeze before 3 years was also detected in the 6-year analysis as an association between infant adiposity gain and transient wheeze (section 8.6.3). In addition, weight and adiposity gain in the first year of life were significantly positively associated with persistent wheeze at age 6 years (section 8.6.3)

Unless exposed to severe or prolonged restriction of *in utero* nutrient supply, infants suffering prenatal growth faltering, may attempt to 'compensate' by increased postnatal weight gain (Healy *et al.* 1956; Tanner 1981). It is possible that above average postnatal growth represents a marker for intrauterine growth restriction or, alternatively, rapid postnatal growth may itself impair lung development. Moreover it also remains possible that some of the associations between early weight gain and later atopy and wheeze may reflect an association between early weight gain and weight in later childhood (Ong *et al.* 2000).

An association was found in a Chilean study between rapid growth in the first year of life and asthma; both rapid gains in weight and length were positively associated with asthma symptoms in adulthood (Rona *et al.* 2005). Together these findings are consistent with cross-sectional studies of older children which show associations between higher BMI and airway hyperresponsiveness, wheeze and asthma (Oddy *et al.* 2004; von Mutius *et al.* 2001). However this study was conducted at a time when infant nutrition in Chile was poorer than that in most of the developed world and no adjustments were made for confounders such as smoking during pregnancy and family history. Moreover, this study did not measure adiposity directly.

In the Project Viva cohort, higher weight for length at 6 months, when adjusted for birthweight, was found to be associated with a greater risk of recurrent wheezing by age 3 years (Taveras *et al.* 2008). Similarly, in subgroup analysis greater change in weight for

length between birth and 6 months was also positively associated with recurrent wheeze. There was no association between weight for length at 6 months and a diagnosis of atopy based upon serum total and specific IgE levels. A weak association only was found between asthma and adiposity in the Project Viva data. It is possible that this reflects a lack of diagnostic precision at age 3 years, however, this thesis appears to confirm that adiposity has little effect upon atopy and is more closely associated with wheeze than a specific diagnosis of asthma.

There is evidence from several sources that poorer infant lung function is associated with greater early postnatal weight gain. Infants who gain greater weight between birth and 5-14 weeks have been shown to have diminished lung function compared to those who gained less weight (Lucas *et al.* 2004). Moreover, change in lung function has been demonstrated to be inversely related to weight gain between 1 month and 1 year of age (Turner *et al.* 2008). However, there is also evidence to suggest rapid early weight gain is associated with altered immune development which may, in turn, predispose to atopy. For example, total serum IgE has been found in Filipino adolescents to be positively associated with weight velocity in the first 6 months of life (McDade *et al.* 2004).

9.2.6 Mechanisms linking growth with childhood wheeze and atopy

Animal studies support the key hypothesis that lung development and later function is sensitive to factors associated with fetal growth restriction, notably fetal hypoxaemia, reduced nutrient supply and hypercortisolaemia. For example, prenatal growth restriction in sheep, a long-gestation species, is associated with reduced lung weight to bodyweight ratio (Maloney *et al.* 1982), reduced alveolarisation (Maritz *et al.* 2001) and reduced airway luminal area and airway wall cartilage (Wignarajah *et al.* 2002). Growth restriction during prenatal development may permanently affect lung architecture and have lasting adverse effects on the lung and chest wall sufficient to affect postnatal lung function. Fetal growth restricted lambs have be shown to have altered lung structure (Maritz *et al.* 2001) and decreased respiratory and increased chest wall compliance (Joyce *et al.* 2001) which persist into postnatal life.

Animal studies also demonstrate that poor fetal growth results in impaired thymic development (Kochanowski & Sherman 1982; Lang *et al.* 2000; Winick & Noble 1966) and an imbalance in the Th1/Th2 lymphocyte balance (Bass *et al.* 1991). Correlations

between seasonal patterns of food availability and measures of infant thymus size (Collinson *et al.* 2003), cord blood lymphocyte count (Collinson *et al.* 2008) and infectious deaths in young adulthood (Moore *et al.* 1997) provide some evidence that the human immune system may be sensitive to programming during pregnancy.

A number of cross-sectional epidemiological studies have shown excess adiposity to be linked with asthma; whilst this may be due to common risk factors, either condition may be mechanistically important in the development of the other. Potential mechanisms whereby excess adiposity may contribute to the development of asthma include genetic mechanisms (Hallstrand *et al.* 2005; Thomsen *et al.* 2007), sex-specific endocrine effects (Castro-Rodriguez *et al.* 2001), direct effects upon the mechanical functioning of the lung (Fredberg *et al.* 1997; Unterborn 2001; Zerah *et al.* 1993), changes in the immune response (Weisberg *et al.* 2003) or increased susceptibility to gastric reflux (Gunnbjornsdottir *et al.* 2004). In children additional factors such as infant feeding, might be associated with both asthma and obesity (Oddy *et al.* 2004; Oddy & Sherriff 2003).

9.3 MATERNAL NUTRITION

9.3.1 Maternal body composition and wheeze and atopic outcomes

Maternal pre-pregnancy total body fat and BMI were significantly positively associated with non-atopic wheeze in the offspring at age 6 years (section 8.6.1). Maternal total body fat and arm muscle area during pregnancy were also significantly positively associated with non-atopic wheeze, and transient wheeze was significantly positively associated with maternal arm muscle area (section 8.6.1). Positive associations approaching significance were found between pre-pregnancy body fat and transient wheeze, and between body fat during pregnancy and doctor-diagnosed asthma, wheeze in the last 12 months and atopic wheeze at 6 years of age (section 8.6.1). No significant associations were found between any measure of maternal body composition and atopy or lung function at 6 years of age, although FEV_1/FVC was found to be weakly inversely associated with maternal body fat at 34 weeks of pregnancy (section 8.6.1).

There were two main *a priori* hypotheses regarding maternal body composition. Firstly, given that both obesity and atopic diseases have increased in prevalence over the same time course (Heslehurst *et al.* 2007), it was hypothesised that maternal body fat before or

during pregnancy would be positively associated with atopy. Genetic or environmental links may underlie this relationship. For example, maternal obesity might predispose to childhood atopy or wheeze via an increased risk of obesity in childhood or, alternatively, the *in utero* environment may be altered in obese women in a manner which predisposes their children towards atopy. Secondly, given that birthweight is known to be predictive of lung function and that low maternal fat and muscle mass prior to conception are associated with impaired fetal growth (Sanin Aguirre *et al.* 2004), it was hypothesised that pre-pregnancy maternal fat and muscle mass would be positively associated with lung function. The results of this thesis did not confirm these hypotheses. There was no evidence that children of obese mothers were at greater risk of atopy (section 6.5.1), as measured by skin sensitisation or FENO, and neither was low maternal fat or muscle mass associated with impaired lung function (sections 7.5.1 & 7.5.3.1). However, a strong positive association was found between mothers' fat mass, both before and during pregnancy, and non-atopic wheeze in their children.

Many social and lifestyle characteristics including education, smoking and breast-feeding choice may be associated with both maternal obesity and respiratory disease in childhood. Controlling for a number of covariates reduces the possibility of confounding, although residual confounding may still occur as a consequence of unaccounted for social and lifestyle factors. Until recently, the relationship between maternal body composition and childhood wheeze or atopy had received little attention and no previous study has performed a detailed analysis of maternal body composition in relation to offspring lung function. Recently, data from in excess of 30,000 mother-child pairs enrolled with the Norwegian Mother and Child population-based cohort study (MoBa) were examined in a multivariate analysis controlling for relevant socioeconomic, lifestyle, health and obstetric variables (Haberg *et al.* 2009). The risk of wheeze at 18 months was found to increase linearly with maternal pre-pregnancy BMI.

The results from the MoBa cohort are consistent with the findings of this thesis. Few of the children experiencing wheeze at 18 months of age are likely to be atopic, the majority of wheeze at this age being associated with viral infection, possibly in conjunction with an underlying susceptibility to airway obstruction due to small calibre airways. The most consistent finding was the positive association between total maternal body fat and non-atopic wheeze; this relationship was found both before and during

pregnancy and, importantly, remained significant after adjusting for childhood BMI. The weak inverse association between maternal body fat in late pregnancy and FEV_1/FVC provides some support for an effect of maternal body composition upon airway caliber. This is further supported by the positive association between maternal fat and muscle masses and the transient wheeze phenotype.

The measure of maternal body composition most frequently associated with wheeze outcomes was total body fat, although positive associations were also seen between percentage body fat and wheeze. The relationship between lean mass and wheeze and atopic outcomes was more difficult to interpret as no proportional measure of lean mass was available. It is likely that the positive associations seen between maternal arm muscle area and non-atopic and transient wheeze reflect a general increase in body size, fatter women also had larger measures of arm muscle area (the Pearson's coefficients for correlation between fat mass and arm muscle area in early and late pregnancy were 0.66 and 0.59 respectively, $p<0.001$). The less significant associations between maternal body fat and doctor-diagnosed asthma, wheeze in the last 12 months and atopic wheeze at 6 years of age may represent evidence for a weak effect of maternal adiposity upon the development of these phenotypes or, alternatively, may have occurred due to residual confounding by lifestyle or socioeconomic factors.

Obesity predisposes women to metabolic, vascular and inflammatory dysregulation (Ramsay *et al.* 2002) and is associated with obstetric complications including gestational diabetes, pre-eclampsia, hypertension and caesarian delivery (Sebire *et al.* 2001). In turn, pregnancy complications, preterm delivery, low birthweight and mode of delivery are associated with childhood wheeze (Nafstad *et al.* 2000; Rusconi *et al.* 2007). It is possible that the association between obesity and wheeze outcomes may be mediated by pregnancy complications or birth outcomes. However, accounting for these factors in the Norwegian MoBa study did not completely explain the relationship between obesity and wheeze. It is known that elevated levels of inflammatory mediators are associated with obesity (Madan *et al.* 2009; Retnakaran *et al.* 2003). These mediators may influence fetal development directly or via alteration of placental function.

Data from this thesis suggest that maternal adiposity is more closely associated with non-atopic than atopic wheeze phenotypes and that lung function may be more affected

by maternal body composition than atopy. Leptin, a hormone secreted by adipocytes may be speculated to have a role in this relationship as leptin levels correlate with BMI (Considine 2005) and leptin receptors are known to be involved in regulation of lung growth (Tsuchiya *et al.* 1999). There is also evidence that umbilical cord leptin levels are raised in infants with intrauterine growth retardation (Shekhawat *et al.* 1998).

9.3.2 Maternal dietary intakes and wheeze and atopic outcomes

The link between fetal and maternal nutrition is indirect; fetal growth is dependent upon the uptake of nutrients from a complex maternal supply line. Nutrients supplied to the fetus reflect placental function and maternal physiological adaptations to pregnancy as well as maternal intake and absorption of nutrients and fetal demand (Bloomfield & Harding 1998). Factors such as maternal smoking, fetal exposure to adrenocortical hormones and physical constraints could potentially link fetal growth retardation to later poor respiratory health. It is often argued that dietary macronutrient or micronutrient deficiency is rarely responsible for clinically significant impaired growth (Mathews *et al.* 1999). However, even if birthweight remains in the normal range this may conceal a birthweight below genetic potential due to suboptimal maternal or fetal nutrition (Altman & Hytten 1989). It is known that nutritional deprivation redistributes maternal cardiac output away from the uterine vasculature resulting in a chronic fetal stress response (Morriss *et al.* 1980). It remains likely that maternal nutrition plays a significant role in determining fetal growth and development. Maternal energy and protein deficiency have been found to be associated with growth retardation (Godfrey *et al.* 1996; Kramer & Kakuma 2003). Moreover, transport mechanisms exist in the placenta for antioxidants (Schenker *et al.* 1998) and PUFAs (Dutta-Roy 2000) and it has also been suggested that food and inhalant allergens encountered by the mother may cross the placenta (Szepfalusi *et al.* 2000). This establishes a biological basis for a prenatal effect of dietary factors upon the development of respiratory system and the immune response to allergens.

9.3.2.1 Dietary patterns

A higher prudent diet score at 34 weeks of pregnancy was associated with both higher FEV_1/FVC and $FEF_{25-75\%}$ (section 7.5.3.2.3). There were no significant associations between either atopic status or wheeze phenotype and prudent diet score, either before or during pregnancy. Atopic wheeze, non-atopic wheeze and FEV_1 were significantly

positively, and skin sensitisation significantly inversely, related to prudent diet score, both before and during pregnancy (chapters 6-8). The associations between prudent diet score and wheeze phenotypes became non-significant upon adjusting for confounders but those between maternal prudent diet score in pregnancy and lung function in the offspring at 6 years remained significant following adjustment.

Measures of airflow obstruction appeared more closely correlated with prudent diet score in this thesis than atopy. This may be cautiously interpreted as evidence for a beneficial effect of adhering to current dietary guidelines upon lung development. As mothers' diets are likely to be correlated with those of their children (Devereux *et al.* 2006; Devereux *et al.* 2007; Robinson *et al.* 2007) this may be an effect of either maternal or childhood diet, or both. Alternatively this observation may have arisen as a consequence of residual confounding. Maternal smoking during pregnancy may not be adequately corrected for using a binary variable, for example, and this may have a greater bearing on the association between prudent diet and lung function than that with atopy.

It appears that adopting a generally healthier diet may have little effect upon asthma or allergy prevention and that much of the association between the principal component analysis-derived dietary patterns is due to confounding by sociodemographic factors. Certainly, principle component analysis of dietary patterns within the ALSPAC cohort found results largely consistent with this thesis; after controlling for confounders, these authors concluded that dietary patterns did not predict asthma and related childhood outcomes (Shaheen *et al.* 2009). The ALSPAC study did not find any relationship between dietary patterns and lung function after correcting for confounders. Before adjustment, atopy, asthma, early wheezing and FEV_1 were positively associated with a 'health conscious' dietary pattern. The similarity of the findings of this thesis and those of ALSPAC is, perhaps, unsurprising given the considerable overlap between the constituents of a health conscious diet (rich in salad, fruit, fruit juices, rice, pasta, cereals, fish, pulses, cheese and non-white bread) and a prudent diet (comprised of high intakes of fruit, vegetables, wholemeal bread, rice and pasta, but low intakes of white bread, added sugar, and tinned vegetables). These findings contrast, however, with others which suggest a predefined 'Mediterranean' diet, if eaten during pregnancy, might protect against wheeze and allergy (Chatzi *et al.* 2008).

The Mediterranean diet is characterised by high intakes of fruits, vegetables, bread, wholegrain cereals, legumes and nuts, low to moderate intakes of dairy products and eggs and low intakes of red meat. It is not immediately clear why the Mediterranean diet study's findings differ from those based upon principal component analysis-derived dietary patterns, as the Mediterranean diet is certainly similar to both the health conscious and prudent dietary patterns. The results of the relatively small Mediterranean birth cohort may have been driven by a strong relationship between a particular food, for example fish, or a particular nutrient such as n-3 polyunsaturated fatty acids, rather than by an effect of the diet as a whole.

9.3.2.2 Whole foods

Maternal citrus fruit intake was not significantly positively associated with either skin sensitisation or FENO in childhood, nor was the hypothesised inverse association between maternal oily fish intake and FENO or skin sensitisation found (section 6.5.2.5).

Previous studies have found maternal intake of oily fish during the second trimester of pregnancy to protect against asthma and allergic disease at 5 years (Willers *et al.* 2007) and that citrus fruit and sweet pepper intake in the last month of pregnancy might increase the risk of skin sensitisation at 2 years (Sausenthaler *et al.* 2007). Moreover Willer's study suggested a protective effect of maternal apple intake upon the development of asthma (Willers *et al.* 2007). Biological mechanisms based upon anti-oxidant and anti-inflammatory properties of these foods have been proposed to explain the beneficial effects associated with their consumption.

Apple and sweet pepper intake could not be measured precisely using the FFQ as large numbers of single fruits or vegetables where not included in the design of the FFQ as this is known to promote overestimation of consumption (Emmett 2009). Analysis of specific foods was therefore limited to the effects of maternal intake of oily fish and citrus fruits upon markers of atopy. No support was found for the *a priori* hypothesis that atopy would be associated with lower intakes of oily fish and higher intakes of citrus fruits. The lack of agreement between this and previous studies may reflect differences in protocol or the smaller size of the birth cohort studied in this thesis. It is difficult to suggest a biologically plausible mechanism for the positive association found

to approach significance between FENO and oily fish intake. No such association was found in the Aberdeen study, although FENO was measured in only 167 participants (Willers *et al.* 2007). In contrast, an adult study where FENO was considered in relation to fish oil intake found FENO to be decreased by low dose n-3 PUFA supplementation (Schubert *et al.* 2009). Whilst the presence of other PUFAs in oily fish, including n-6 PUFAs, must be acknowledged, it is possible that this result occurred as a consequence of multiple analyses or residual confounding by socially determined environmental or lifestyle factors which may affect both diet (Robinson *et al.* 2004) and prevalence of atopy (Heinrich *et al.* 1998b).

9.3.2.3 Vitamins

9.3.2.3.1 *Summary of findings and comparison with other studies*

Lower maternal pre-pregnancy vitamin D intake was significantly associated with increased risk of doctor-diagnosed asthma, wheeze in the last 12 months and non-atopic wheeze in the offspring at age 6 years (section 8.6.2.1). Lower pre-pregnancy maternal intakes of vitamins A and E were also significantly associated with non-atopic wheeze as was lower intake of vitamin E in late pregnancy. Greater pre-pregnancy vitamin C intake was significantly associated with persistent wheeze and greater intake of this vitamin in late pregnancy was significantly associated with atopic wheeze and doctor-diagnosed asthma at 6 years of age (section 8.6.2.1).

Longitudinal studies have suggested that lower maternal intakes of vitamin C (Martindale *et al.* 2005) and higher intakes of vitamins E (Devereux *et al.* 2006; Litonjua *et al.* 2006; Martindale *et al.* 2005) and D (Camargo, Jr. *et al.* 2007; Devereux *et al.* 2007) may decrease the risk of wheeze symptoms in early childhood. These results are not without controversy, indeed a local study found high maternal vitamin D status to be associated with both higher intakes of vitamin D and an increased risk of wheeze and eczema in childhood (Gale *et al.* 2008). As maternal intake of vitamin C was positively associated with specific wheeze outcomes and vitamin E and D intakes were inversely associated with wheezing in childhood (section 8.6.2.1), data from this study broadly confirm the conclusions of previous studies regarding wheeze phenotypes. However, the *a priori* hypotheses investigated in chapters six and seven which explored whether intake of specific vitamins might be associated predominantly with a predisposition towards atopy or poorer lung function met with variable support.

Intake or status	SWS Cohort (n=469) (Inskip *et al.* 2006)	Aberdeen Cohort (n=1751) (Devereux *et al.* 2006; Devereux *et al.* 2007; Martindale *et al.* 2005)	Project Viva Cohort (n=1194) (Camargo, Jr. *et al.* 2007; Litonjua *et al.* 2006)
Vitamin D	Pre-pregnancy intake inversely associated with doctor-diagnosed asthma, wheeze in the last year and non-atopic wheeze at 6 years	Pregnancy intake inversely associated with ever wheeze, wheeze in previous year and persistent wheeze and positively associated with BDR at 5 years	Pregnancy intake inversely associated with recurrent wheeze at 3 years
Vitamin E	Pre-pregnancy and pregnancy inversely associated with non-atopic wheeze	Pregnancy intake inversely associated with wheeze in absence of cold at 2 years and inversely associated with wheeze in previous year, asthma ever, persistent wheeze and FENO at 5 years	Pregnancy intake inversely associated with any wheezing and recurrent wheezing at 2 years of age
Vitamin A	Pre-pregnancy intake inversely associated with non-atopic wheeze and pregnancy intake positively associated with greater FEV_1 and greater $FEF_{25-75\%}$ at 6 years	No association found between pregnancy intake and wheeze at 2 or 5 years	Pregnancy intake found to be inversely associated with wheeze in first 2 years in univariate but not multivariate analysis.
Vitamin C	Pre-pregnancy intake positively associated with, persistent wheeze and pregnancy intake positively associated with atopic wheeze and doctor-diagnosed asthma	Pregnancy intake positively associated with wheeze at 2 years but not at 5 years	Pregnancy intake found to be inversely associated with wheeze in first 2 years in univariate but not multivariate analysis.
Vitamin D	34 week 25(OH) vitamin D status inversely associated with doctor-diagnosed asthma, atopic wheeze and persistent wheeze	Vitamin D status not examined	Vitamin D status not examined
Vitamin E	No association between 34 week tocopherol status and atopy or wheeze outcomes	12 week tocopherol positively associated with post broncho-dilator FEV_1 and inversely with atopic sensitisation Tocopherol at delivery positively associated with FENO	Vitamin E status not examined
Vitamin A	34 week serum retinol status positively associated with atopic wheeze	ß-carotene found not to be associated with wheeze	Vitamin A status not examined
Vitamin C	No association between 34 week ascorbate status and wheeze outcomes	Early pregnancy ascorbate positively associated wheeze in second year of life	Vitamin C status not examined

Table 112 Comparison of the principal findings of the SWS, Aberdeen and Project Viva birth cohort studies

9.3.2.3.2 *Maternal nutrient intake and lung function in the offspring*

No positive association between maternal vitamin A or C intake and any measure of childhood lung function reached significance (sections 7.5.2.1 & 7.5.3.2.1), despite strong evidence from experimental studies in animals to suggest increased intakes of these vitamins improve lung development (Massaro & Massaro 1996; Proskocil *et al.* 2005).

9.3.2.3.3 *Maternal vitamin intake and atopy in the offspring*

Despite previous findings suggesting CBMC response to antigens are increased following low pregnancy intake of vitamin E (Devereux *et al.* 2002), no relationship was found between lower total vitamin E intake and atopy (section 6.5.2.1). Finally, although supplementation studies suggest higher intakes of vitamin D may predispose to atopy (Hypponen *et al.* 2004) there was no evidence of an association between high total intakes of vitamin D and an increased risk of either skin sensitisation or elevated FENO (section 6.5.2.1).

A significant positive relationship was found between food-derived vitamin E intake at 34 weeks' of gestation and FENO measured in the offspring at age 6 years (section 6.5.2.1). This association was not supported by a consistent positive association between skin sensitisation and food-derived vitamin E intake or associations between FENO and food-derived vitamin E intake before pregnancy or at 11 weeks' gestation. The association between late pregnancy food-derived vitamin E and FENO may have arisen due to residual confounding similar to that proposed to account for the relationship between late pregnancy oily fish intake and FENO, or it may have occurred as a consequence of performing multiple analyses. It is also possible that the significant relationship with food-derived vitamin E intake reflects some effect upon FENO of those foods which constitute common sources of vitamin E.

9.3.2.3.4 *Energy adjustment*

After energy adjustment, maternal intake of vitamin C remained positively associated with wheeze outcomes and vitamin E and D intakes remained inversely associated with wheezing in childhood (Appendix 1, Tables 118-123). Energy adjustment of the total vitamin intake data revealed greater support for an association between higher intakes of vitamin A and more favourable lung development; FEV_1 and $FEF_{25-75\%}$ were both significantly positively associated with energy adjusted vitamin A intake at 34 weeks of

pregnancy. The weak positive association between unadjusted vitamin A intake and these measures did not reach significance, possibly due to misclassification of dietary intake by the FFQ. Energy-adjustment also unmasked an unexpected positive association between vitamin A intake at 34 weeks of pregnancy and atopic wheeze.

Energy adjustment marginally altered the levels of significance associated with the vitamin intake findings such that borderline associations between wheeze phenotypes and vitamin D intake became non-significant whilst borderline associations with vitamin C intake became significant; variable effects were noted for associations between wheeze and intakes of vitamins A and E. Energy adjustment may change the nature of the relationship between nutrient intake and respiratory outcomes for two reasons. Firstly, energy adjustment serves to adjust reported intakes for total intake and can correct for over or under reporting by individual participants, thus giving a more consistent ranking according to relative vitamin intake. Secondly, adjusting for energy intake provides an estimate of the nutrient richness of individuals' diets and perhaps, as energy intake is often related to body size, some estimate of size-adjusted vitamin intake. Energy adjustment may, therefore, be considered to lessen any confounding effect of total energy, or its determinants, such as body size or physical activity, from the relationship between nutrient exposure and disease risk (Willett *et al.* 1997).

The effects of energy adjustment may vary according to the nutrient under consideration. Correlation coefficients for FFQ and food diary derived estimates of intake have been shown to be similar for vitamins A, C, D and E, however, vitamin C has been shown to be subject to greater over-reporting than intake of the other vitamins (Robinson *et al.* 1996). Any nutrient for which the requirement is thought to be related to body size may be best investigated using adjusted values, whilst if a threshold effect is suspected unadjusted values may enable this to be detected more easily. For any given nutrient, the differences introduced by energy adjustment will be greater if the intake of that nutrient does not increase in proportion with total energy intake. This may explain why the significance of the associations between wheeze and vitamin D intake declined with energy adjustment as a significant proportion of vitamin D is derived from supplements and, therefore, it is not appropriate to correct this for total energy intake.

9.3.2.3.5 *Differences between early and late pregnancy*

It is difficult to draw clear conclusions regarding critical periods for the effect of maternal nutrition upon fetal development as the FFQ sampled maternal diet during two time periods only. The most noticeable pattern was that wheeze phenotypes inversely associated with vitamin D intake were all associated with pre-pregnancy intakes of this vitamin. This may reflect the importance of pre-pregnancy stores of this fat-soluble vitamin or may merely be a result of the greater completion rates of the pre-pregnancy FFQ and hence the greater power associated with analyses based upon pre-pregnancy data.

9.3.2.3.6 *Vitamin D*

In the Aberdeen cohort, total vitamin D and food-derived vitamin D intake at 32 weeks of pregnancy were inversely associated with ever wheezing, wheeze at 2 years, wheeze at 5 years and persistent wheeze but not asthma. In Project Viva, higher maternal vitamin D intake was inversely related to recurrent wheeze at 3 years, with a stronger effect in doctor-confirmed or recurrent wheeze. However, asthma *per se* was not measured due to the difficulties associated with making this diagnosis in young children. The finding of an inverse relationship between maternal vitamin D intake and asthma in this thesis may be attributable to greater diagnostic accuracy in children of 6 years and older compared to younger children. Recently, a Finnish birth cohort has confirmed this inverse relationship. In 1669 children with HLA-DQB1-conferred susceptibility to diabetes, energy-adjusted total maternal vitamin D intake and vitamin D from food were inversely related to asthma (Erkkola *et al.* 2009).

9.3.2.3.7 *Vitamin E*

In Project Viva, maternal vitamin E intake during pregnancy was inversely associated with wheeze and recurrent wheeze at 2 years (Litonjua *et al.* 2006). In the Aberdeen birth cohort, a consistent inverse relationship was found between vitamin E intake and wheeze and atopic outcomes, at both 2 and 5 years. Negative associations were reported between maternal vitamin E intake and 'wheeze in the absence of a cold' in the second year of life, eczema at age 2 years in children of atopic mothers, wheeze at 5 years, asthma ever and persistent wheeze. The findings relating to the first two years of life were interpreted as evidence for an effect of maternal vitamin E intake upon the development of atopic immunity, rather than any effect upon lung development or immune responses to infection. In this thesis, vitamin E intake was inversely associated

with non-atopic wheeze rather than an atopic phenotype (see sections 6.5.2.1 & 8.6.2.1). An argument can be made for an influence of maternal vitamin E intake upon lung development, but given that no significant associations between either atopy (section 6.5.2.1) or lung function (sections 7.5.2.1 & 7.5.3.2.1) were found, this is largely speculative. Indeed when energy-adjusted vitamin intakes were analysed, as they were in the Aberdeen study, an inverse association was found between vitamin E intake and the persistent wheeze phenotype. This suggests any association between vitamin E intake and wheeze may not be limited to wheeze in the absence of atopy as 53% of the children classified as suffering from persistent wheeze were atopic.

9.3.2.3.8 *Vitamins A and C*

An inverse association between maternal vitamin A intake in late pregnancy and non-atopic wheeze has not been found in previous studies. Whilst this may be a chance finding, due to multiple comparisons or residual confounding, it is consistent with a biologically plausible mechanism relating vitamin A intake to lung growth. This is supported by the positive associations found between energy-adjusted vitamin A intake in pregnancy and spirometric measures of forced expiratory flow (Table 116). Conversely, the positive associations found between maternal vitamin C intake and persistent wheeze, atopic wheeze and doctor-diagnosed asthma may be chance findings or due to reverse causation (section 8.6.2.1), selective reporting or confounding by other nutrients or lifestyle. Although it was suggested that the positive association seen between vitamin C and wheeze at 2 years in the Aberdeen cohort may have arisen due to a pro-oxidant effect, an association between vitamin C intake and wheeze was not confirmed at 5 years. The finding of a positive association at 6 years in this cohort may reflect differences in socio-economic and lifestyle factors compared to the Aberdeen cohort, or differences in correction for these effects, or could have arisen from pro-oxidant actions of vitamin C.

9.3.2.3.9 *Associations between maternal vitamin intakes and BDR and BHR in the offspring*

Fewer participants contributed BDR or BHR data than simple spirometry measures. Relationships between maternal nutrition and BDR were particularly difficult to interpret as, although this measure effectively discriminated those children who had received a diagnosis of asthma from those who had not, the relationship between doctor-diagnosed asthma and BDR was the inverse of that expected. Asthmatic children on average had lower BDRs than non-asthmatic children, due possibly, as suggested in

chapter seven (section 7.5), to a combination of good asthma control in the asthmatic participants and undiagnosed asthma in the non-asthmatics. BDR was also measured in 238 children from the Aberdeen study. BDR measured as percentage of initial FEV_1 (rather than as a percentage of predicted FEV_1 as in this thesis) was not found to be significantly associated with maternal vitamin E intake but a positive association was found with vitamin D intake. This result was surprising given the otherwise protective effect of vitamin D intake upon wheeze phenotypes. This contradictory result was interpreted as evidence for an association between higher vitamin D intake and greater potential for lung growth as greater BDR has been shown to predict higher FEV_1 values later in childhood (Tantisira *et al.* 2006). However, no information was presented regarding the relationship between BDR and wheeze or asthma, and it is possible that the BDR was an equally poor marker of the asthmatic phenotype in the Aberdeen study as it appeared to be in this thesis.

Bronchial hyperresponsiveness was positively associated with energy-adjusted maternal vitamin A intake at 34 weeks' gestation; that is, higher intakes of vitamin A were associated with lower inverse log. slope values which, in turn, represent greater BHR (section 7.5.3.2.1 & Table 117). This effect was supported by both a significant positive association between late pregnancy energy-adjusted vitamin A intake and atopic wheeze, and by positive associations which approached significance between energy-adjusted intakes of vitamin A and BHR, both before pregnancy and at 11 weeks' gestation. However, such an association has not been observed in either the Aberdeen or Project Viva cohorts. Anti-oxidant vitamins have been demonstrated to decrease BHR (Chang & Crapo 2002; Chang & Crapo 2003). Nonetheless, a study using an animal model has demonstrated worsening of asthma severity following high vitamin A intake (Schuster *et al.* 2008). Furthermore, in murine species, vitamin A supplementation has been shown to provoke an enhancement of Th1 differentiation (Albers *et al.* 2003; Cui *et al.* 2000).

9.3.2.3.10 *Supplementation*

A final consideration regarding the effects of maternal vitamin intake is that of supplementation. A variable proportion of women reported taking supplemental vitamin A, C, D and E before and during pregnancy. Pre-pregnancy vitamin supplement use was reported by 38% of women and 42% and 29% of women supplemented with at least one of vitamins A, C, D or E in early or late pregnancy respectively. Significant associations found with food-derived vitamin intakes or in subgroup analysis of

unsupplemented women but not in total intake or supplemented women may reflect some effect of foods containing the vitamin in question rather than the vitamin *per se*. Conversely, associations found with total intake and in supplemented women but not with food-derived vitamin intake or in unsupplemented women may reflect confounding by some lifestyle or other factor associated with supplementation or an effect only seen at high levels of vitamin intake, achievable only through supplementation.

The effect of supplementation was difficult to analyse as the distribution of supplemental intakes was highly skewed and the numbers of children in each arm of the binary outcome groups were often small, particularly in the subgroup of women reporting supplement use. When significant associations were found between total vitamin intake and respiratory or atopic outcomes a consistent direction of effect was seen when vitamin intakes from food sources alone were considered (Appendix 1, Table 126). These associations were not always significant and this may be a reflection of the fact that the outcome is determined by total vitamin intake regardless of source, thus food-derived vitamin intake is a poorer predictor of outcome than total intake. Similarly, when the associations between respiratory and atopic outcomes and total vitamin intakes were compared in those women who did or did not take supplements, directions of effect remained consistent for all relationships previously found to be significant save for that between pre-pregnancy vitamin D and doctor-diagnosed asthma. Although these associations did not always remain significant, most likely due to the reduced power inherent to smaller sample sizes, this analysis provides some evidence that the associations were not driven solely by high supplemental intakes or confounded by some other factor associated with supplementation.

The relationship between total vitamin C intake in late pregnancy and doctor-diagnosed asthma was significant for total but not food-derived intake and in supplemented but not unsupplemented women (Tables 124 & 125). Social confounding may explain this observation, those women most likely to follow a 'health conscious' pattern of vitamin supplementation may be more likely to consult a doctor regarding symptoms of wheeze and the children of these women may, therefore, be more often diagnosed with asthma.

In contrast, a positive association was found in chapter six between food-derived vitamin E and FENO (section 6.5.2.2), although total vitamin E intake and FENO were not significantly associated. This may have occurred as a consequence of multiple analyses or, alternatively, may reflect effects of other nutrients found in vitamin E containing foods.

9.3.3 Maternal nutritional status and wheeze and atopic outcomes

9.3.3.1 Serum vitamin levels

Lower maternal serum 25(OH) vitamin D status at 34 weeks' gestation was inversely associated with doctor-diagnosed asthma, atopic wheeze and persistent wheeze in the offspring at age 6 years (section 8.6.2.2). An inverse association with transient wheeze approached significance. FENO was inversely associated with serum 25(OH)-vitamin D until corrected for relevant confounders. There were no associations between vitamin status and skin sensitisation (section 6.5.2.3.1) or lung function (sections 7.5.2.2 & 7.5.3.2.2), although a weak positive association between BDR and total antioxidant status approached significance (section 6.5.2.3.1). Higher maternal serum retinol and maternal total anti-oxidant status were positively associated with atopic wheeze and persistent wheeze respectively (section 8.6.2.2).

9.3.3.1.1 *Vitamin D status*

The analyses in chapters six and eight were designed to test the hypothesis that high maternal vitamin D status might be associated with childhood asthma and that this might occur as a result of a developmental bias of the immune system towards atopy. The results from this study do not support this hypothesis or the findings of a previous study where high maternal 25(OH)-vitamin D status was found to be associated with asthma and eczema in childhood (Gale *et al.* 2008). The original study included participants born be3tween 1991 and 1992 to mothers who had slightly lower vitamin D intakes and serum 25(OH)-vitamin D levels than the women included in this thesis. The original study was limited by larger losses to follow up and did not correct for all relevant confounders. Gale *et al.*'s findings have since been brought into question by more recent studies of vitamin D intake (Camargo, Jr. *et al.* 2007; Devereux *et al.* 2007).

Several longitudinal studies have reported an inverse association between childhood wheeze and vitamin D intake but this study is the first to support this by demonstrating an inverse relationship between maternal 25(OH)-vitamin D status and wheeze in

childhood. Potentially, this is an important finding which may provide a basis from which to explore the mechanism underlying this effect. Maternal serum 25(OH)-vitamin D was associated with doctor-diagnosed asthma, atopic wheeze and persistent wheeze in the offspring at 6 years. Each of these outcome groups contains a high proportion of atopic individuals (Table 42, Chapter 5), these findings may be interpreted as evidence that adequate vitamin D status protects against wheeze via effects upon atopic immunity. Certainly, animal studies suggest that vitamin D affects many of the cell types involved in atopic immunity and this is reinforced by genetic studies of vitamin D receptor polymorphisms showing associations with both asthma and atopy (Poon *et al.* 2004; Raby *et al.* 2004). However, a direct effect upon skin sensitisation was not found in chapter six for either vitamin D intake or status. Whilst it is possible that skin sensitisation is not the best marker for the immune mechanism underlying atopic wheeze, alternative explanations must be considered. Inadequate maternal vitamin D status was associated with the more severe wheeze phenotypes rather than the more common transient wheeze. An alternative explanation for the association between maternal vitamin D status and these wheeze phenotypes might be a persistent change in lung structure which is not outgrown in early childhood.

It is unclear whether high levels of 25(OH)-vitamin D in maternal serum are protective against eosinophilic inflammation. Certainly a strong inverse association between serum 25(OH)-vitamin D and FENO was seen in the unadjusted analysis, however, this association became non-significant after adjusting for maternal education, eczema, and atopy, and child's gender, birth order and month of birth (section 6.5.2.3.1). The confounding factor which most influenced the significance of this relationship was month of birth. Maternal serum 25(OH)-vitamin D levels displayed a clear seasonality, peaking in the summer months, as would be expected due to greater sun exposure. It is possible that correcting for month of birth was an overcorrection which removed an important source of variation in vitamin D exposure. However, on univariate analysis, month of birth appeared to be significantly associated with FENO suggesting that month of birth confounds the relationship between serum 25(OH)-vitamin D and FENO. Children were invited to attend clinic according to their date of birth such that their dates of attendance could be expected to correlate with their dates of birth, there was little evidence for this, however. The Pearson's coefficient for correlation between month of birth and month of test was -0.08, $p=0.2$. This lack of strong correlation is

not unexpected given that the mean (SD) age of the children attending the clinic was 6.72 years (0.22 years), thus the time period between their sixth birthday and their clinic visit ranged from 3 to 15 months rather than clustering tightly around each child's month of birth. Seasonality of allergen exposure or respiratory infection may generate seasonal variation in FENO measurements which might cause month of test to be a confounder (Roberts *et al.* 2004; Spanier *et al.* 2008). Alternatively, month of birth *per se* might confound the relationship because the risk of developing allergic disorders is known to be seasonal (Wjst *et al.* 2005). Month of birth will remain a confounder if allergic disorders display seasonality due to early allergen exposure (Harley *et al.* 2009), however, it is possible that vitamin D exposure may lie on the causal pathway and contribute to the seasonal pattern of allergy risk.

The 25(OH)-vitamin D results were the most consistent; the vitamin D intake data supported an inverse association with asthma and significant associations were found with a number of phenotypically similar wheeze outcomes, the outcomes significantly associated with serum 25(OH)-vitamin D were atopic wheeze, doctor-diagnosed asthma (43% atopic) and persistent wheeze (53% atopic).

9.3.3.1.2 *Other measures of maternal serum vitamin status*

No other serum measure of maternal vitamin status was inversely associated with any wheeze outcome. Indeed, higher maternal serum retinol and maternal total anti-oxidant status were positively associated with atopic wheeze and persistent wheeze respectively (section 8.6.2.2). These findings are difficult to reconcile with the anti-oxidant hypothesis and may be the result of reverse causation or residual confounding. It is interesting to note, however, that the serum retinol findings with regard to atopic wheeze are consistent with the positive association seen previously between vitamin A intake and both atopic wheeze and BHR. No significant associations were found between either serum ascorbate and α-tocopherol and any wheeze phenotype or any measure of atopy or lung function. This is contrary to the findings of the Aberdeen cohort where vitamin status was measured in 1089 women at 12 weeks' gestation. Significant positive associations were found between plasma ascorbate and wheeze in the second year of life and between plasma α-tocopherol and post-bronchodilator FEV_1 at 5 years. Significant negative associations were found between plasma α-tocopherol and skin sensitisation at 5 years (Table 63).

It is unclear why the findings from the Aberdeen cohort regarding vitamin E were not replicated in this study. This may be a consequence of the smaller size of the cohort studied in this thesis. Alternatively, methodological or population differences might explain the discrepancies between the two studies. For example, intakes of vitamin E in the SWS were greater than those in Aberdeen, and it is possible that protective effects of α-tocopherol are less evident in a vitamin E replete population. The timing of blood sampling may also have contributed to the contrasting findings. The authors of the Aberdeen study argued for a dual effect of vitamin E upon lung function and suggested that vitamin E exposure early in pregnancy may be more likely to influence lung function than exposure later in pregnancy, which may have a greater effect upon the immune system. A positive association between maternal plasma α-tocopherol at delivery and FENO was forwarded to support this statement; however, this finding was only statistically significant in children of atopic mothers.

The general lack of significant associations between childhood wheeze phenotypes and serum measures of maternal vitamin status, other than vitamin D, initially appears disappointing given that several significant associations were found between childhood wheeze and intakes of vitamins A, C and E. Whilst it is possible that some of these associations may be confounded by lifestyle factors or by influences of other nutrients closely correlated with intakes of these vitamins, it should also be considered that a single measure of maternal serum vitamin status may not be the best marker of fetal exposure. Maternal serum vitamin measures are known to change during pregnancy, notably concentrations of ascorbate and retinol decrease whilst serum α-tocopherol increases (Cikot *et al.* 2001; Roes *et al.* 2006). The reasons for these changes are unclear but may include alterations in body composition, glomerular filtration, fetal and maternal demand and hormonal mechanisms. The relationship between maternal dietary intake and cord blood vitamin levels is known to be affected by factors such as smoking (Bolisetty *et al.* 2002) and gestation (Baydas *et al.* 2002). Findings from the Aberdeen study, for example, have demonstrated that maternal diet influences cord plasma levels of vitamin C but not vitamins A and E (Scaife *et al.* 2006). Finally, it must be remembered that nutrient levels measured in serum do not necessary reflect nutrient stores or indeed nutrient availability to target tissues.

9.3.3.2 Plasma phospholipid fatty acids

Atopy at 6 years was significantly associated with higher maternal arachidonic acid status. The children of mothers with higher percentage composition of arachidonic acid in plasma phosphotidylcholine had both greater rates of skin sensitisation and higher FENO values. A significant positive association was also found between the percentage of arachidonic acid in maternal plasma phosphotidylcholine and atopic wheeze in the offspring at age 6 years (section 8.6.2.2). Significant inverse associations were found between maternal α-linolenic and EPA status and the relative risk of doctor-diagnosed asthma. Maternal EPA status was also inversely associated with wheeze in the last 12 months and non-atopic wheeze at 6 years of age. An unexpected negative association was found between maternal arachidonic acid status and the relative risk of the transient wheeze phenotype.

Changes in dietary fat intake within the Western diet have preceded and paralleled changes in asthma prevalence (Black & Sharpe 1997). Due to substituting vegetable oils for saturated fats in the human diet and as a result of changes in animal feeds, intakes of n-6 PUFAs have increased whilst those of n-3 PUFAs have decreased. An increased ratio of n-6:n-3 PUFAs is seen in many atopic disorders and it has been proposed that changes in the relative proportions of n-3 and n-6 derived immune mediators occur, due to competition between n-3 and n-6 PUFAs for the same desaturation enzymes. This might bias immune development towards the Th2 phenotype (Calder 1998). Supplementation studies provide some support for a protective effect of n-3 PUFAs. For example, the Childhood Asthma Prevention Study found a 9.8% reduction in wheeze before 18 months in children receiving a n-3 PUFA supplement compared to control subjects (Mihrshahi *et al.* 2004) and a large population based intervention study also found n-3 PUFA supplementation in late pregnancy to be beneficial in relation to childhood asthma (Olsen *et al.* 2008). This simple form of the lipid hypothesis was used to formulate an *a priori* hypothesis for this thesis, however, recent work suggests this approach may be relatively unsophisticated. For example, data from Project Viva suggest increased cord blood levels of both AA and EPA have similar effects on cord blood lymphocyte proliferation and IFNγ production (Gold *et al.* 2006).

Several findings from this thesis are consistent with the simple lipid hypothesis. Certainly, the positive association between arachidonic acid and atopic wheeze and the

inverse associations between both α-linolenic acid and EPA and doctor-diagnosed asthma support the hypothesis that an elevated n-6:n-3 ratio might promote atopy. Moreover, higher arachidonic acid concentrations were significantly associated with greater rates of skin sensitisation and higher FENO values. The inverse association found between EPA and non-atopic wheeze and, likewise, that between arachidonic acid and transient wheeze might reflect an association between susceptibility to respiratory infection and compromised maternal EFA status. The lack of association between any measure of lung function and plasma fatty acid status argues against any significant effect of fatty acid status upon airway caliber or lung development.

The metabolism of maternal essential fatty acids and their longer chain, more unsaturated, derivatives during normal pregnancy is incompletely characterised. A recent longitudinal study of the EFA status of women during normal pregnancy has demonstrated that total plasma fatty acids, including LCPs, increase during pregnancy but that percentage composition of LCPs falls (Al *et al.* 1995). It is proposed that LCPs are mobilised from maternal stores to meet the needs of the fetus and that LCP status may not be adequately replaced between closely spaced pregnancies. Case-control studies, for example of asthma, allergic rhinitis and eczema, have found higher levels of the n-6 precursor, linoleic acid, lower levels of EPA and DHA, and an increased arachidonic acid:EPA ratio (Sala-Vila *et al.* 2008); however, reduced n-6 PUFAs and increased precursor EFAs have been found to coexist with low n-3 PUFA levels in some atopic disorders (Horrobin 2000). To account for the decreased levels of both n-3 and n-6 PUFAs in relation to their precursors, inefficiency of the δ-6-desaturase enzyme has been proposed to be associated with atopic disorders (Manku *et al.* 1982). Altered fatty acid metabolism and transfer is known to occur in pregnancies complicated by obesity (King 2006), it is possible that alterations in the profile of PUFAs supplied to the fetus may contribute to the association seen between maternal body composition and childhood wheeze. Further evidence for a subtle relationship between supply of PUFAs and immune system development can be found in the strong positive associations which exist between head circumference at birth and both later atopy (Fergusson *et al.* 1997; Godfrey *et al.* 1994) and cord blood n-6 PUFAs (Leaf *et al.* 1992).

One of the largest studies to date to consider the relationship between maternal fatty acid status and childhood wheeze and atopic outcomes was the population-based Avon

Longitudinal Study of Parents and Children (ALSPAC) (Newson *et al.* 2004). Samples were taken from 2945 women at 20 weeks' gestation. No associations were demonstrated between maternal blood erythrocyte membrane PUFA concentration and early wheezing (0 - 6 months and 30 - 42 months) or eczema (18-30 months). Cord blood erythrocyte linoleic:α-linolenic ratio was related to late onset wheeze and the arachadonic acid:EPA ratio to eczema. However, after adjusting for multiple comparisons, these associations were no longer significant. It was concluded that fetal exposures to LCPs are unlikely to be important determinants of early childhood wheezing and atopic disease. Recently, a smaller Dutch study of maternal and cord blood plasma phospholipid concentrations also concluded that plasma arachidonic acid concentrations before and around birth are not strongly associated with atopic disease in children at age 7 years (Dirix *et al.* 2009).

There are several reasons why previous studies' findings might differ from those presented here. Firstly, ALSPAC measured red cell phospholipid fatty acids rather than plasma phospholipids. The haem content of red cells can potentially cause oxidative damage to the sample and make fatty acid measurement unreliable. Moreover, red cell fatty acids reflect a mean concentration over the cells' 120 day lifespan, this may not be sensitive to subtle fluctuations of fatty acid level within critical periods during gestation. Secondly, wheeze status was determined early in childhood, at 42 months, in the ALSPAC study. Wheeze is common early in childhood and may not be linked to later asthma. Thirdly, ALSPAC blood samples were taken at any point between 20 weeks' gestation and delivery; samples in this thesis were taken over a much narrower time frame centred upon 34 weeks (mean 34.5 weeks, range 31.9 – 36.9 weeks). As fat accretion in the fetus increases exponentially from 30 weeks (Southgate & Hay 1975), earlier sampling may not provide an adequate estimate of maternal fatty acid status during the most relevant period of development. Moreover, given the marked changes in maternal fatty acid profile that occur throughout gestation, failure to correct adequately for the exact time of sampling in the ALSPAC study may have biased towards the null hypothesis. Finally, if multiple comparisons had been corrected for in this thesis it is likely that many of the significant results would have lost significance in a similar fashion to those in the ALSPAC study. The results were not adjusted, however, as the analyses were based upon *a priori* hypotheses.

9.3.4 Potential mechanisms of action

Undoubtedly changes in agricultural practice, food processing, storage and patterns of food choices have made a substantial impact upon the modern diet. Marked changes have occurred in both essential fatty acid and anti-oxidant intake, and lifestyle and behavioural changes have also served to reduce sun exposure and hence the photosynthesis of vitamin D within the skin.

9.3.4.1 Immune modulation

Early life interactions between allergens and Th-cells are critical in determining whether a Th2 biased response emerges. There are a number of mechanisms by which nutrients could influence the first interactions between allergens and the immune system. Dietary influences might skew the immune response towards a Th2 phenotype via effects on antigen presenting cells, via regulatory T cells or via direct effects upon the cytokine profile of Th-cells. For example, reduced Vitamin E can increase Th2 differentiation under the influence of PGE_2 production by macrophages; low vitamin E levels also have effects on Tr-cells (Li-Weber *et al.* 2002) and can down regulate IL-4 by inhibiting binding of the transcription factors nuclear factor-kB and activator protein (AP)-1 to the IL-4 promoter region. Moreover, it has been reported that naïve T-cells ($D45RA^{high}$) are more responsive to vitamin E than memory Th-cells ($D45RO^{high}$) (Malmberg *et al.* 2002).

Vitamin D can influence both antigen-presenting cells such as macrophages (Griffin *et al.* 2003) and the generation of regulatory T cells (Meehan *et al.* 1992). Many laboratory studies suggest vitamin D induces a shift in the balance between Th subsets towards Th2 dominance (Cantorna *et al.* 2004). In human cord blood cells, however, vitamin D has been found to inhibit not only IL-2 generated IFNγ production but also to suppress IL-4 and IL-4 induced expression of IL-13 (Pichler *et al.* 2002). These differences may be related to the timing of exposure. The response of naïve T cells to vitamin D exposure may differ from that of mature cells (Annesi-Maesano *et al.* 2001) or the effect may depend upon the vitamin D status of the individual. Vitamin D transcriptive activity in immune cells may represent a final common pathway for the contrasting effects of vitamin A and D intake upon the atopic phenotype. It has been proposed, for example, that vitamin A can antagonise the actions of vitamin D by competition at the retinoid X receptor (Rohde & DeLuca 2005).

An increase in the proportion of n-6 to n-3 PUFAs could lead, via metabolism of arachidonic acid by cyclooxygenase-2 to increased PGE_2 and thus bias of T-cell differentiation towards the Th2 phenotype. Related changes would be reduced IFNγ (Miles *et al.* 2003; Roper *et al.* 1995), increased class-switching to IgE and increased production of IL-4 and IL-5 (Kalinski *et al.* 1997). It has also been proposed that n-3 PUFAs can affect the activity of antigen-presenting cells (Sanderson *et al.* 1997) and that both n-3 and n-6 PUFAs can modulate T-cell function directly through effects on cell membrane fluidity, cell signaling and gene transcription (Calder 2002). Whilst some of the reported associations are consistent with the simple lipid hypothesis, the similarity of the associations with n-3 and n-6 PUFAs are at variance with this hypothesis. This finding is however, consistent with the observations from animal studies which show murine spleen lymphocyte responses are reduced by both n-3 and n-6 supplementation (Wallace *et al.* 2001). Possible explanations for these observations could be direct effects of n-3 and n-6 PUFA on Th-cells and/or non-PGE_2 metabolites. Alternatively, n-3 and n-6 PUFA may influence regulatory T-cells.

9.3.4.2 Lung development

Maternal nutrient intake during pregnancy could influence early life respiratory epithelial and mesenchymal development with sub-optimal nutrient status being associated with impaired airway development (Devereux 2006). In rat models maternal vitamin E supplementation accelerates growth in hypoplastic lungs, increases lung complexity, surface area and bud count (Islam *et al.* 1999). Expression of genes coding for extracellular matrix proteins is modulated by α-tocopherol. Reduced α-tocopherol status is associated with upregulation of metallomatrix proteinase-1 (Ricciarelli *et al.* 1999), which, in turn has been associated with reduced FEV_1 (Culpitt *et al.* 2005). It is also possible that fetal airway development might be sensitive to vitamin E status due to modulation by this vitamin of genes implicated in cell signaling and cell cycle regulation (Azzi *et al.* 2004). Vitamin D has also been identified as a local mediator of epiethelial-mesenchymal cell interactions in the developing rat lung (Nguyen *et al.* 1996) and has been linked to fetal lung development in animal models (Nguyen *et al.* 1996) and higher vitamin intakes in adolescent (Burns *et al.* 2007) and adult (Black & Scragg 2005) life are associated with better lung function. Vitamin D may have a therapeutic role in asthma by enhancing responsiveness to glucocorticoids for induction of IL-10 (Xystrakis *et al.* 2006).

9.4 IMPLICATIONS

The data contained in this thesis provide new evidence for the importance of the early life environment in the development of the respiratory and immune systems. The data support a life-course approach to preventing disease later in childhood and in adulthood. Aspects of maternal nutrition were strongly associated with asthma and wheeze phenotypes in the offspring at age 6 years, and selected maternal factors appeared to significantly influence both atopy and lung function. Relatively large differences in the relative risk of asthma were observed within the range of vitamin intakes seen in this cohort. This suggests dietary manipulation could have a significant effect upon the health of the population as a whole. For example, an increase in the geometric mean pre-pregnancy vitamin D intake from 3.81 to 7.11 mcg could be expected to be associated with a 25% reduction in the risk of doctor-diagnosed asthma.

Findings from this and similar studies could be used to inform future public health strategy. Given the association between maternal body fat and non-atopic wheeze in the offspring, recommending that women hoping to conceive optimise their body weight would appear sensible advice. Additionally, ensuring adequate intakes of vitamins D and E may also be beneficial. This study did not confirm previous concerns regarding high vitamin D intake and atopic outcomes. Following appropriate intervention studies, current recommendations regarding vitamin D intake in pregnancy may be revised to account for the protective effect of this vitamin upon respiratory health.

As a note of caution, however, for some nutrients, interpreting low circulating levels in maternal serum as evidence of a dietary deficiency may prove to be an oversimplification. For example, the reduction seen in maternal LCP levels during pregnancy represents a physiological partitioning of these PUFAs in favour of the fetus, rather than insufficient intake. Maternal nutrition may contribute to the efficacy of this physiological adaptation but this may be more complicated processes than simply supplying PUFA substrate. For example, a number of nutrients serve as co-factors to support the partitioning of fatty acids into very low density lipoproteins in the maternal liver. In this manner, multiple associations are likely to underlie the relationship between maternal nutrition and measurable endpoints.

9.5 FURTHER WORK

The analyses performed in this thesis will be repeated once data collection from six-year-old children in the Developmental Influences upon Childhood Respiratory Health Study is complete. The total number of children in the final dataset will be approximately double that reported upon here and the power of the analyses will consequentially be greater. With increased power there will be greater scope to refine the adjustment for likely confounders, even for variables such as maternal atopy where some participants have missing data. Correcting for maternal atopy without substantial loss of power would enable reverse causation to be considered, that is the possibility that atopic mothers may alter their diet and deliberately remove foods perceived as allergenic from their diets during pregnancy. This could be addressed by testing for interactions between food intake and maternal history of atopic disease. There is evidence form the Aberdeen cohort that the effects of maternal nutrient intake were greatest in breast fed infants. As further data are collected within the SWS, it will be possible to adjust the effects of maternal diet for infant feeding practices, including breast feeding. It will also be possible to assess whether the effects of maternal diet are confounded by diet during childhood. It was not thought essential to correct for childhood diet in this analysis, however, as data from the Aberdeen study suggest that, although maternal and childhood intakes are weakly correlated, childhood diet did not correlate with any atopic or wheeze phenotype (Devereux *et al.* 2006; Devereux *et al.* 2007). Moreover, when results from the Project Viva study were corrected for childhood diet, the effects of maternal diet remained significant (Camargo, Jr. *et al.* 2007).

Further epidemiological work directly developing ideas from this thesis includes:

- **Further characterisation of the effect of vitamin D intake upon asthma and wheeze outcomes.**

  Firstly, vitamin D intake was treated as a continuous variable in this analysis to maximise power. However, once the complete dataset has been collected, it should be possible to repeat the analysis using quartiles or quintiles of intake and to investigate any non-linearity of the effect of vitamin D intake. Secondly, there is some evidence form Project Viva that the effect of vitamin D intake is

greatest in mothers whose last menstrual period occurred in the winter, suggestive of a greater effect of dietary vitamin D intake when sun exposure is low. Larger numbers would permit investigation of whether the effect of vitamin D intake is modified according to season of birth in this cohort.

- **Investigation of the apparent dual effect of vitamin A upon non-atopic and atopic outcomes and testing of the hypothesis that this may reflect opposing effects upon early lung development and the development of an atopic immune response.**

  The SWS provides an important opportunity to assess the contribution of early environmental influences upon early lung function. The SWS infant lung function cohort has robust $FEV_{0.4}$ data which may be helpful in establishing the relationship between vitamin exposure and early lung function. Many of the infants in whom infant lung function was measured have been followed throughout childhood, up to and including 6 years of age. This subset may prove particularly useful in determining the significance of interactions between early lung function and allergic sensitisation in the evolution of asthma and wheeze phenotypes.

- **Exploration of the interactions between different nutrients and investigation of the best means of providing dietary supplementation.**

  Firstly, further work is needed to determine whether maternal EFA status is best improved by increasing the intake of fish in the diet or by providing a supplement of one or more fatty acids, or whether n-6 fatty acid intake should be limited. Intakes of many nutrients are highly correlated as they are found together in the same foods. Supplementation may, certainly, be an easy route to achieve a constant elevation of plasma phospholipids but fish intake may have additional advantages relating to the balance of fatty acids found in fish, synergistic effects of other nutrients, and effects due to the replacement of other foods in the diet by fish. Secondly, multivariate analyses to establish independent effects of the various nutrient intakes found to be associated with particular wheeze outcomes were not performed in this thesis as rarely were different nutrients found to be associated with identical wheeze outcomes.

However, with greater power further significant associations may be discovered and these might be suitable for multivariate analysis.

- **Exploration of the interaction between the effects of maternal and childhood obesity upon the development of wheeze phenotypes.**
  DXA scan data will soon be available to permit detailed characterisation of childhood body composition. As the children in this cohort grow older, the aim will be to assess them by questionnaire at age 8 years and again around puberty. This will provide opportunities to investigate further the links between adiposity and asthma and to explore the gender differences in disease phenotype that become apparent around puberty.

The findings of this and similar epidemiological studies can be used to generate hypotheses and guide research in two broad areas. Firstly, the mechanisms underlying the associations identified in epidemiological studies warrant further investigation. Appropriate animal models have been used to identify significant factors influencing the functional development of the immune and respiratory systems early in life. Intrauterine intervention studies using animal models may further understanding regarding both the stage of prenatal life during which environmental exposures influence development and whether any interaction occurs with postnatal exposures. The second area warranting further investigation is the translation of observational epidemiological findings to intervention studies. Intervention studies during pregnancy are ethically challenging and may need to be limited to women who are proven to be vitamin deficient. Such studies are nevertheless invaluable in guiding public health policy.

9.6 LIMITATIONS OF THIS STUDY AND ITS INTERPRETATION

This study prospectively recruited a cohort of women of child-bearing age who were broadly representative of the UK population across a range of socioeconomic, education, dietary and lifestyle factors. These women were extensively characterised both before and during pregnancy. The prospective design enabled data to be collected from both planned and unplanned pregnancies. However there were a number of limitations.

9.6.1 General recruitment issues

Women were invited to participate via their general practitioners and by an advertising campaign. Participation was dependent upon a degree of self-motivation and it is possible that those volunteering to take part were those finding it easy to access medical services and were consequentially less socially isolated than those who did not volunteer. It is also likely that participants may be better motivated and more adherent to medical advice than those who did not participate. Those women originally participating are likely to have been healthier than a random cross section of the population within an equivalent age range.

Only surviving children who had not been lost to follow up contributed data. Retention within the study is likely to be affected by the influence of health and social class upon participants' abilities to maintain contact with the study and to devote time to participation. Certainly, participating women were less likely to smoke during pregnancy than those who did not participate. However, this would only alter this study's results if the relationships between maternal factors and offspring health differed between those that did or did not smoke. It is possible that smoking may increase anti-oxidant exposure and thus increase the requirement for anti-oxidant vitamins. This effect however would make any positive effect of anti-oxidant vitamins upon respiratory health more difficult to detect in the study population and bias towards the null hypothesis.

Participants may differ from non-participants if they modify their behaviour due to the Hawthorne effect, that is, because they know they are being studied. This could either be a true modification of dietary or other lifestyle factors or a misrepresentation of true habits when surveyed. This effect will depend upon participants' preconceptions of a healthy diet and lifestyle, but would generally tend to blur associations and bias towards the null hypothesis. Although all cohort studies are vulnerable to reporting bias and behaviour modification, the SWS has the advantages that participants were not necessarily aware of the exact hypotheses being studied and, moreover, that long-term follow up within the study permits checks upon internal consistency of information.

A further problem common to all cohort studies is that any association detected between any given exposure and outcome might be mediated by a confounding factor

associated with both variables. For example, an association was seen between a high prudent diet score and the likelihood of skin sensitisation in the offspring which, actually, was likely to reflect well known associations between higher maternal education and both a healthy diet and increased risk of atopy. In order to minimise confounding, the biological plausibility of associations was considered and a number of likely confounding factors corrected for in multivariate analyses.

It is possible that residual confounding exists. This may be a result of failing to consider important confounding factors or failing to provide adequate adjustment. Other studies have found significant associations between maternal paracetamol exposure in pregnancy and childhood wheeze and atopic outcomes, for example, yet this was not corrected for in this study. The decision not to consider paracetamol exposure in this study was largely a pragmatic one based upon the unavailability of suitable data at the time of writing. However, this will only confound the studies results if there is reason to suspect a significant association with both exposure and outcome variables. Thus, whilst paracetamol exposure has been proven to be associated with wheeze and atopic outcomes, it will only confound the effects seen with the nutritional exposures explored in this study if paracetamol use in pregnancy, or indeed other possibly unknown factors, varies according to nutrient intake. Other factors that were not corrected for due to lack of reliable data include selenium intake, due to difficulty capturing accurate data via questionnaire, and prenatal infection and antibiotic use, due to current unavailability of this data. Ethnicity was not corrected for as 94% of the population was white and other ethnicities were difficult to classify and represented at low frequencies.

Many nutritional and behavioural choices are undoubtedly socially patterned, it is likely that many exposures tend to vary together in a manner explained by social class and this makes social class an important confounder. As the important facets of social class are unknown it is difficult to be confident that social class is adequately corrected for. Some studies have corrected for household income, others according to a form of deprivation index and others still for social class classified according to occupation. Within the SWS maternal education has been used as a proxy for social class as the traditional classification based upon occupation is believed to be outdated and to poorly reflect the status of women. Maternal education by contrast has been found to be a strong predictor of both maternal and children's diets. Maternal education also provides a

categorical variable with multiple classes to more accurately differentiate individual women along this axis. Other confounding variables may benefit from a more precise adjustment, for example smoking is currently represented as a binary variable rather then a quantitative measure of exposure, for example in pack years or cigarettes per day.

Finally, it is possible that some multivariate models might overcorrect. This has been discussed previously in the context of vitamin D status. Maternal vitamin D clearly shows seasonal variation and this variation might be causal contributor to variation in asthma incidence according to month of birth. This presents a dilemma regarding whether month of birth should be corrected for. In this study month of birth was corrected for in the analysis of the FENO outcome only as month of birth was not found on univariate analysis to be significantly associated with any other outcome in this study.

It is possible that some of the associations found within this study occurred due to chance as multiple analyses were undertaken. In many cases these analyses were based upon previously published or clearly stated *a priori* hypotheses. Where large numbers of outcomes and exposures were studied this was clearly stated to be an exploratory analysis. It can be argued that correction for multiple testing is required under these circumstances. However, some epidemiologists hold the view that interpreting the significance of a given association differently according to how many other associations were investigated defies common sense and increases the chances of type II errors (the chance of accepting a false null hypothesis) (Perneger 1998). A further difficulty associated with correcting for multiple testing lies in deciding which analyses to correct for; taken to an extreme if all analyses within the SWS, even on unrelated outcomes, were corrected for, the chance of a type II error would be hugely inflated.

9.6.2 Maternal factors

9.6.2.1 Maternal body composition

The research nurses were regularly assessed in anthropometric measurement and retrained where necessary. All measurements were made with appropriately maintained and calibrated instruments. Together these factors maximised the accuracy of anthropometric measurements before and during pregnancy. However, the accuracy of the 'weight gain in pregnancy' measurement was limited by variation between different

women in the interval between initial interview and pregnancy. A negative pregnancy weight gain was recorded for some women; this was unrepresentative of their true weight gain during pregnancy and reflected weight loss between the initial interview and prior to conception. Additionally, measures of body composition based on derived values, for example arm muscle area or percentage body fat, were dependent upon the underlying assumptions used to calculate these values, for example the cross-sectional area of bone or the representativeness of the population sample used to derive the body fat reference equation. Caution is advised concerning the application of skinfold thickness equations during pregnancy.

9.6.2.2 Maternal dietary intakes

Misclassification of dietary exposure is an important potential limitation of this and other epidemiological studies. Semi-quantitative food frequency questionnaires are regarded as reasonably accurate because frequency generally explains most of the variation in total food intake (Tjonneland *et al.* 1992). Random misclassification of exposure would tend to bias effect estimates towards the null value, so this is unlikely to invalidate any effects that are detected. The list of foods contained within the FFQ was carefully compiled to be comprehensive and to match those foods commonly eaten by the study cohort. Overall intakes were checked for consistency by comparing the total energy intake recorded on the FFQ with predicted intakes. FFQs capture data regarding infrequently eaten foods but if the portion sizes are based upon average intake may underestimate foods eaten regularly or in large quantities (Emmett 2009). FFQs are also less able to quantify total intake of nutrients whose values vary according to agricultural or food processing practices, for example selenium the intake of which is dependent upon soil selenium content. Furthermore, FFQs provide no information regarding non-dietary exposure to nutrients, for example vitamin D derived from photosynthesis in the skin.

The FFQ used by the SWS has been validated for intakes of energy, protein, carbohydrate, fat, calcium, iron, zinc, copper, retinol equivalents, thiamine, riboflavin, niacin equivalents, pyridoxine, vitamin B12, folate, vitamin C, vitamin D and vitamin E (Robinson *et al.* 1996). This validation showed that, although absolute levels of intake can be subject to over-reporting, the FFQ provides an assessment of diet that can be used to rank the nutrient intakes of individuals.

9.6.2.3 Maternal serum samples

The use of biomarkers of maternal vitamin status complements the vitamin intake data. Direct measurement of vitamin status avoids bias due to misreporting and provides information regarding non-dietary sources of nutrients such as vitamin D. However, serum levels are not informative regarding the flux of nutrients between body stores and relevant tissues, neither are maternal serum levels direct correlates of fetal exposure as this is determined by placental transfer and fetal uptake.

9.6.2.3.1 *Vitamin status*

Maternal serum samples were collected in late pregnancy and spun down within 24 hours, serum was then separated and stored at -80°C. 25(OH)-vitamin D is stable over this period. Any degradation of samples is likely to be random rather than systematic. 25(OH)-vitamin D was measured with a highly sensitive and specific radio-immunoassay; as with all radio-immunoassay techniques there could have been underestimation of the D2 form. This problem is likely to be relevant particularly in vegetarian participants as this is the form of vitamin D derived from plant sources. It was not possible, however, to separate women according to vegetarianism for this thesis.

The ascorbic acid estimations are likely to be the least accurate of the biomarkers as they were not taken under ideal conditions. Ideally, fasting samples should be used to assess ascorbic acid status but the participants were not instructed to fast before their blood samples were taken. Additionally, ascorbic acid in untreated serum is very unstable and within 24 hours can degrade completely at room temperature. The process is accelerated on exposure to light. Prior to storage, freshly prepared metaphosphoric acid was added to the samples to protect the lactone ring and inhibit oxidation.

9.6.2.3.2 *Fatty acid status*

The major limitation of gas chromatography is that complex lipids, such as triacylglycerols and phospholipids, cannot be studied intact and so potentially important information regarding the combinations of fatty acids in these molecules is lost. Accurate quantification of fatty acids depends on the quality of integration of the peaks generated by the gas chromatography mass spectrometer, small peaks are typically integrated with less accuracy than larger ones. Absolute concentrations of each fatty acid can be calculated by comparison with the peak size produced by a given quantity of

a known standard. Interbatch errors associated with calculations based upon the addition of a standard are removed by using relative fatty acid concentrations. The fatty acid measurements used in this thesis were performed by a group with an international reputation in this area.

9.6.3 Fetal ultrasound

Fetal USS measures were performed by three ultrasonographers only. Each ultrasonographer was highly trained and regular checks were performed to ensure consistency of measurement. Characterisation of fetal growth patterns in this thesis was superior to that of other studies where scans were performed less frequently. Fetal growth was directly measured as comparable measurements of fetal size were measured at multiple time points during gestation, this avoided the use of birth anthropometry as a proxy measure. The effects of regression to the mean were accounted for by using the method of Royston to measure growth conditional upon original size (Royston 1995). It is possible that those women with the most complete fetal ultrasound data may have been more adherent to prenatal care and may have differed in other characteristics from women with less complete data. This would only have affected the results of the study if the relationship between fetal growth and wheeze and atopic outcomes differed significantly between the two groups and there is no reason to suggest this may be the case.

9.6.4 Respiratory outcome measures

As summarised in chapter three, the six year outcome measures were rigorously validated. Regular checks of measurement techniques were conducted and operators received regular training in skin prick testing, spirometry, FENO measurement and methacholine challenge testing. Where possible, the number of operators recording each measurement was restricted in order to minimise differences due to operator technique.

Careful quality control of the spirometry data collected from the home visit ensured only valid flow-volume curves were accepted. Flow-volume curves were assessed blind to any questionnaire or clinical data related to the exposures or outcomes of interest. The data were vulnerable to a systematic bias towards low measures of forced expiratory flow because the majority of children in this study had not performed

spirometry before. The nurses coaching the children in spirometry were instructed to encourage the children as much as possible in order to minimise this bias. This appeared successful as the forced expiratory flows obtained in this study are comparable with published standards, many of which were recorded from children experienced in performing spirometry.

The more sophisticated measures of lung function were more difficult to obtain. The main technical concerns were the validity of the BDR measure, the low dose of methacholine administered and the use of two different FENO analysers. The significant inverse association between BDR and asthma was unexpected. The greater BDR in the non-asthmatic group may have arisen as a consequence of a greater training effect in those children that had never performed this manoeuvre before. Although it is also possible to explain this finding in terms of sub-clinical asthma in the non-asthmatic group and well controlled disease in the asthmatic group, uncertainty concerning the interpretation of this measure limited its utility. Although the methacholine dose used in this study was likely to be less than that recommended in the ATS/ERS guidelines, clear variation in BHR was demonstrated between participants and there was a trend for greater BHR in children with asthma at 6 years ($p=0.19$). It is difficult to compare prevalences of BHR between studies due to methodological differences. For example, a study from the Isle of Wight which used an almost identical protocol to the present study to assess BHR in 10-year-olds found a PC_{20} less than 4 mg/ml in 22% of their cohort (Arshad *et al.* 2005). However, 13% of the Isle of Wight cohort had a current diagnosis of asthma and asthmatic children were preferentially invited to attend for methacholine challenge. Only 6.5% of the children studied in this thesis had a PC_{20} less than 4 mg/ml but this is consistent with the lower rate of current asthma recorded (7%). A formal sensitivity analysis of FENO measurement would benefit from greater numbers of children using the respective analysers. The analyses in Appendix 1, Table 129 showed that the FENO analyser used had little influence upon the overall direction and magnitude of association when the significant associations found in chapter six were analysed separately according to the analyser used.

Finally, reliance on questionnaire data may lead to diagnostic misclassification of outcomes. The questionnaires however, were based upon the well established and validated ISAAC format, however, and replies to such questionnaires have been shown

to be reproducible (Luyt *et al.* 1994). Using research nurses to administer the questionnaire was superior to self-completion as this prevented bias towards responses from more literate participants.

9.7 CONCLUSIONS

Elements of maternal body composition, dietary intake and dietary status are associated with the childhood wheeze phenotypes. Moreover patterns of pre- and postnatal growth are also associated with these outcomes. Although the mechanisms underlying these associations cannot be proven by epidemiological study, there is evidence to suggest that early growth and development of the immune and respiratory systems are sensitive to early life influences. These observations suggest that optimising the early life environment would serve to reduce the burden of illness associated with asthma and childhood wheeze.

Chapter Ten

Appendices

Appendix 1 Supplementary data

Association between energy-adjusted vitamin intakes and atopy outcomes

Standardised energy-adjusted intake	Unadjusted analyses RR (95% CI)	P value	n	Adjusted* analyses RR (95% CI)	P value	n
Vitamin E						
Pre-pregnancy	1.05 (0.90-1.23)	0.506	374	0.96 (0.81-1.14)	0.649	346
11 weeks	1.04 (0.88-1.22)	0.655	302	1.01 (0.85-1.19)	0.945	282
34 weeks	1.11 (0.97-1.28)	0.140	365	1.09 (0.95-1.25)	0.228	340
Vitamin D						
Pre-pregnancy	0.94 (0.80-1.11)	0.469	374	0.92 (0.79-1.09)	0.349	346
11 weeks	1.08 (0.89-1.31)	0.412	302	1.04 (0.86-1.26)	0.685	282
34 weeks	1.17 (1.01-1.37)	0.042	365	1.16 (0.98-1.36)	0.078	340

***Adjusted for child's gender and maternal education, history of asthma, atopic status and parity**
Relative risks were calculated as change in risk per SD change in energy-adjusted vitamen intake.

Table 113 Relative risk of skin sensitisation according to energy-adjusted maternal vitamin D and E intakes before and during pregnancy

Standardised energy-adjusted intake	Unadjusted analyses Exp Beta (95% CI)	P value	n	Adjusted* analyses Exp Beta (95% CI)	P value	n
Vitamin E						
Pre-pregnancy	0.98 (0.91-1.07)	0.661	248	0.96 (0.89-1.03)	0.275	234
11 weeks	0.96 (0.87-1.06)	0.408	198	0.92 (0.83-1.02)	0.110	188
34 weeks	1.00 (0.92-1.08)	0.988	246	0.99 (0.91-1.06)	0.696	232
Vitamin D						
Pre-pregnancy	0.95 (0.88-1.03)	0.238	248	0.96 (0.89-1.04)	0.307	234
11 weeks	0.96 (0.88-1.06)	0.430	198	0.97 (0.88-1.06)	0.464	188
34 weeks	0.98 (0.90-1.06)	0.589	246	0.96 (0.89-1.04)	0.339	232

***Adjusted for child's gender and month of birth and maternal education, history of eczema, atopic status and parity**
Regression coefficients were calculated as change in FENO per SD change in energy-adjusted vitamin intake.

Table 114 Multiplicative increase in FENO according to energy-adjusted maternal vitamin D and E intakes before and during pregnancy

Association between energy-adjusted vitamin intakes and lung function outcomes

Standardised energy-adjusted intake	Unadjusted analyses Beta (95% CI)	P value	n	Adjusted* analyses Beta (95% CI)	P value	n
Vitamin A						
Pre-pregnancy	0.05 (−0.04-0.15)	0.263	310	0.09 (−0.02-0.19)	0.054	296
11 weeks	−0.01 (−0.12-0.10)	0.865	245	0.01 (−0.13-0.10)	0.798	239
34 weeks	0.09 (0.00-0.18)	0.060	305	0.11 (0.02-0.20)	0.021	295
Vitamin C						
Pre-pregnancy	0.05 (−0.05-0.14)	0.336	310	0.05 (−0.05-0.14)	0.335	295
11 weeks	0.02 (−0.09-0.12)	0.764	245	0.00 (−0.11-0.11)	0.989	239
34 weeks	0.03 (−0.07-0.13)	0.599	305	0.04 (−0.06-0.14)	0.438	295

***Adjusted for child's gender, gestation, age at last breast feed and birth order, mother's education, smoking in pregnancy and history of asthma and father's history of asthma**
Relative risks were calculated as change in risk per SD change in energy-adjusted vitamin intake.

Table 115 Regression analysis of FEV_1 according to energy-adjusted maternal vitamin A and C intakes before and during pregnancy

Standardised energy-adjusted intake	Unadjusted analyses Beta (95% CI)	P value	n	Adjusted* analyses Beta (95% CI)	P value	n
FEV_1/FVC z-score						
Vitamin A						
Pre-pregnancy	0.07 (−0.04-0.18)	0.203	310	0.04 (−0.07-0.15)	0.500	297
11 weeks	0.01 (−0.11-0.14)	0.832	245	0.02 (−0.11-0.14)	0.808	240
34 weeks	0.08 (−0.02-0.19)	0.114	305	0.09 (−0.02-0.20)	0.111	296
Vitamin C						
Pre-pregnancy	0.01 (−0.10-0.12)	0.903	310	−0.01 (−0.12-0.11)	0.884	297
11 weeks	0.01 (−0.10-0.13)	0.809	245	0.00 (−0.12-0.13)	0.984	240
34 weeks	0.08 (−0.03-0.20)	0.165	305	0.07 (−0.05-0.19)	0.250	296
$FEF_{25-75\%}$ z-score						
Vitamin A						
Pre-pregnancy	0.08 (−0.04-0.20)	0.186	310	0.07 (−0.05-0.20)	0.228	297
11 weeks	0.00 (−0.13-0.14)	0.966	245	0.03 (−0.11-0.16)	0.708	240
34 weeks	0.11 (0.00-0.23)	0.055	305	0.14 (0.02-0.25)	0.023	296
Vitamin C						
Pre-pregnancy	0.00 (−0.12-0.12)	0.948	310	−0.01 (−0.13-0.12)	0.892	297
11 weeks	0.03 (−0.10-0.17)	0.621	245	0.00 (−0.13-0.14)	0.945	240
34 weeks	0.09 (−0.04-0.22)	0.156	305	0.09 (−0.04-0.22)	0.189	296

***FEV_1/FVC z-score analysis adjusted for child's gestation, mother's education, smoking in pregnancy and history of asthma and rhinitis and father's history of asthma. $FEF_{25-75\%}$ z-score analysis adjusted for child's gender, gestation and birth order, mother's education, smoking in pregnancy and history of asthma and rhinitis and father's history of asthma**
Regression coefficients were calculated as change in lung function measure per SD change in energy-adjusted vitamin intake.

Table 116 Regression analysis of childhood spirometry according to energy-adjusted maternal vitamin A and C intakes before and during pregnancy

Standardised energy-adjusted intake	Unadjusted analyses Beta (95% CI)	P value	n	Adjusted* analyses Beta (95% CI)	P value	n
BDR z-score						
Vitamin A						
Pre-pregnancy	0.05 (−0.11-0.22)	0.522	143	0.07 (−0.10-0.25)	0.411	131
11 weeks	−0.12 (−0.28-0.05)	0.159	116	−0.11 (−0.27-0.06)	0.192	109
34 weeks	−0.04 (−0.21-0.13)	0.630	138	−0.05 (−0.22-0.13)	0.595	131
Vitamin C						
Pre-pregnancy	0.01 (−0.14-0.16)	0.874	143	−0.02 (−0.19-0.14)	0.762	131
11 weeks	0.00 (−0.17-0.17)	0.980	116	−0.10 (−0.28-0.09)	0.291	109
34 weeks	0.02 (−0.14-0.19)	0.784	138	−0.03 (−0.21-0.15)	0.743	131
Inverse log. slope						
Vitamin A						
Pre-pregnancy	−0.19 (−0.37-0.01)	0.041	107	−0.17 (−0.36-0.02)	0.082	102
11 weeks	−0.26 (−0.51-0.01)	0.044	82	−0.26 (−0.51-0.00)	0.052	81
34 weeks	−0.15 (−0.31-0.01)	0.074	107	−0.18 (−0.36-0.01)	0.039	102
Vitamin C						
Pre-pregnancy	−0.04 (−0.27-0.18)	0.697	107	−0.01 (−0.25-0.24)	0.951	102
11 weeks	−0.15 (−0.39-0.09)	0.215	82	−0.13 (−0.39-0.13)	0.328	81
34 weeks	−0.19 (-0.36-0.02)	0.027	107	−0.16 (−0.34-0.03)	0.091	102

***BDR z-score analysis adjusted for child's gestation and birth order, mother's education, smoking in pregnancy and history of asthma and rhinitis and father's history of asthma. BHR z-score analysis adjusted for child's gestation and birth order, mother's education, smoking in pregnancy and history of asthma and father's history of asthma**
Regression coefficients were calculated as change in lung function measure per SD change in energy-adjusted vitamin intake.

Table 117 Regression analysis of BDR and BHR according to energy-adjusted maternal vitamin A and C intakes before and during pregnancy

Association between energy-adjusted vitamin intakes and wheeze phenotypes

Standardised energy-adjusted intake	Unadjusted analyses RR (95% CI)	P value	n	Adjusted* analyses RR (95% CI)	P value	n
Vitamin A						
Pre-pregnancy	0.89 (0.71-1.11)	0.295	469	0.93 (0.75-1.16)	0.551	443
11 weeks	1.00 (0.82-1.21)	0.989	381	1.03 (0.86-1.23)	0.748	370
34 weeks	1.01 (0.81-1.26)	0.933	459	1.09 (0.88-1.35)	0.427	442
Vitamin C						
Pre-pregnancy	0.97 (0.78-1.21)	0.801	469	1.05 (0.86-1.29)	0.601	443
11 weeks	1.14 (0.88-1.46)	0.3202	381	1.32 (1.03-1.69)	0.030	370
34 weeks	1.15 (0.89-1.47)	0.285	459	1.29 (1.04-1.59)	0.019	442
Vitamin D						
Pre-pregnancy	0.73 (0.55-0.96)	0.027	469	0.76 (0.57-1.00)	0.052	443
11 weeks	0.93 (0.71-1.23)	0.626	381	0.97 (0.74-1.27)	0.812	370
34 weeks	0.82 (0.63-1.07)	0.150	459	0.89 (0.67-1.17)	0.393	442
Vitamin E						
Pre-pregnancy	1.03 (0.83-1.29)	0.766	469	1.03 (0.83-1.28)	0.787	443
11 weeks	0.91 (0.74-1.13)	0.389	381	0.92 (0.73-1.15)	0.467	370
34 weeks	1.11 (0.90-1.38)	0.313	459	1.16 (0.96-1.41)	0.129	442

***Adjusted for child's gestation, and birth order, mother's height, education, smoking in pregnancy and history of asthma and father's history of asthma**
Relative risks were calculated as change in risk per SD change in energy-adjusted vitamin intake

Table 118 Relative risk of doctor-diagnosed asthma according to energy-adjusted maternal vitamin intake before and during pregnancy

Standardised energy-adjusted intake	Unadjusted analyses RR (95% CI)	P value	n	Adjusted* analyses RR (95% CI)	P value	n
Vitamin A						
Pre-pregnancy	0.95 (0.76-1.19)	0.678	469	1.03 (0.83-1.28)	0.789	418
11 weeks	1.14 (0.91-1.42)	0.247	381	1.19 (0.99-1.45)	0.068	344
34 weeks	1.15 (0.90-1.47)	0.259	459	1.14 (0.88-1.46)	0.319	417
Vitamin C						
Pre-pregnancy	1.13 (0.92-1.39)	0.252	469	1.21 (0.99-1.50)	0.069	418
11 weeks	1.00 (0.76-1.30)	0.978	381	1.12 (0.84-1.50	0.429	344
34 weeks	1.05 (0.81-1.36)	0.708	459	1.08 (0.81-1.43)	0.611	417
Vitamin D						
Pre-pregnancy	0.77 (0.60-0.98)	0.030	469	0.81 (0.65-1.02)	0.078	418
11 weeks	0.89 (0.66-1.20)	0.436	381	0.90 (0.67-1.21)	0.470	344
34 weeks	0.86 (0.67-1.12)	0.261	459	0.90 (0.69-1.16)	0.408	417
Vitamin E						
Pre-pregnancy	1.12 (0.90-1.39)	0.301	469	1.16 (0.92-1.45)	0.213	418
11 weeks	0.80 (0.61-1.03)	0.088	381	0.79 (0.59-1.04)	0.090	344
34 weeks	0.96 (0.76-1.21)	0.729	459	0.94 (0.74-1.19)	0.599	417

***Adjusted for child's gestation, pet exposure in first year of life and birth order, education, smoking in pregnancy and history of asthma and father's history of asthma**
Relative risks were calculated as change in risk per SD change in energy-adjusted vitamin intake

Table 119 Relative risk of wheeze in the last 12 months according to energy-adjusted maternal vitamin intake before and during pregnancy

Standardised energy-adjusted intake	Unadjusted analyses RR (95% CI)	P value	n	Adjusted* analyses RR (95% CI)	P value	n
Vitamin A						
Pre-pregnancy	0.97 (0.89-1.06)	0.560	398	0.99 (0.90-1.09)	0.842	377
11 weeks	1.06 (0.96-1.16)	0.235	325	1.04 (0.95-1.14)	0.431	316
34 weeks	1.02 (0.93-1.12)	0.656	389	1.02 (0.93-1.11)	0.736	376
Vitamin C						
Pre-pregnancy	0.94 (0.86-1.03)	0.169	398	0.96 (0.88-1.05)	0.370	377
11 weeks	1.03 (0.93-1.14)	0.521	325	1.06 (0.96-1.18)	0.243	316
34 weeks	0.98 (0.90-1.07)	0.683	389	1.02 (0.93-1.12)	0.640	376
Vitamin D						
Pre-pregnancy	0.99 (0.91-1.08)	0.822	398	1.01 (0.92-1.11)	0.822	377
11 weeks	1.01 (0.92-1.12)	325	325	1.03 (0.92-1.14)	0.636	316
34 weeks	0.92 (0.84-1.01)	0.081	389	0.94 (0.86-1.04)	0.228	376
Vitamin E						
Pre-pregnancy	0.96 (0.87-1.05)	0.346	398	0.96 (0.87-1.05)	0.350	377
11 weeks	1.03 (0.95-1.13)	0.484	325	1.03 (0.95-1.13)	0.446	316
34 weeks	1.00 (0.92-1.09)	0.990	389	1.02 (0.93-1.12)	0.690	376

***Adjusted for child's gestation, birth order and pet exposure and mother's height, education, smoking in pregnancy and history of asthma and rhinitis, and father's history of asthma**
Relative risks were calculated as change in risk per SD change in energy-adjusted vitamin intake

Table 120 Relative risk of transient wheeze according to energy-adjusted maternal vitamin intake before and during pregnancy

Standardised intake energy adjusted	Unadjusted analyses RR (95% CI)	P value	n	Adjusted* analyses RR (95% CI)	P value	n
Vitamin A						
Pre-pregnancy	0.97 (0.77-1.22)	0.778	229	1.13 (0.91-1.41)	0.266	223
11 weeks	1.22 (0.97-1.52)	0.088	186	1.23 (1.01-1.49)	0.038	183
34 weeks	1.13 (0.89-1.43)	0.309	226	1.10 (0.87-1.39)	0.422	223
Vitamin C						
Pre-pregnancy	1.08 (0.88-1.32)	0.452	229	1.24 (1.03-1.48)	0.021	223
11 weeks	0.99 (0.78-1.26)	0.952	186	1.14 (0.89-1.45)	0.289	183
34 weeks	1.02 (0.80-1.30)	0.872	226	1.17 (0.92-1.49)	0.206	223
Vitamin D						
Pre-pregnancy	0.77 (0.60-1.00)	0.053	229	0.85 (0.67-1.08)	0.176	223
11 weeks	0.92 (0.70-1.22)	0.566	186	0.90 (0.69-1.19)	0.466	183
34 weeks	0.81 (0.62-1.06)	0.117	226	0.82 (0.63-1.07)	0.141	223
Vitamin E						
Pre-pregnancy	1.10 (0.91-1.33)	0.326	229	1.26 (1.02-1.54)	0.028	223
11 weeks	0.89 (0.67-1.17)	0.399	186	0.92 (0.70-1.21)	0.552	183
34 weeks	0.96 (0.78-1.19)	0.695	226	0.98 (0.78-1.24)	0.885	223

***Persistent wheeze analysis adjusted for child's gestation, pet exposure in the first year of life and birth order, mother's age, education, smoking in pregnancy and history of asthma and rhinitis, and father's history of asthma**
Relative risks were calculated as change in risk per SD change in energy-adjusted vitamin intake

Table 121 Relative risk of persistent wheeze according to energy-adjusted maternal vitamin intake before and during pregnancy

Standardised energy-adjusted intake	Unadjusted analyses RR (95% CI)	P value	n	Adjusted* analyses RR (95% CI)	P value	n
Vitamin A						
Pre-pregnancy	1.06 (0.79-1.41)	0.707	278	1.05 (0.77-1.43)	0.778	243
11 weeks	1.15 (0.90-1.48)	0.251	224	1.18 (0.89-1.57)	0.242	200
34 weeks	1.24 (0.86-1.81)	0.249	272	1.44 (1.01-2.05)	0.045	242
Vitamin C						
Pre-pregnancy	1.29 (0.98-1.71)	0.071	278	1.07 (0.72-1.59)	0.731	243
11 weeks	1.16 (0.82-1.63)	0.407	224	1.06 (0.56-2.01)	0.856	200
34 weeks	1.54 (1.18-2.01)	0.001	272	1.56 (1.08-2.27)	0.018	242
Vitamin D						
Pre-pregnancy	0.97 (0.70-1.35)	0.874	278	0.98 (0.61-1.40)	0.932	243
11 weeks	1.12 (0.71-1.79)	0.608	224	1.05 (0.69-1.58)	0.827	200
34 weeks	1.22 (0.87-1.73)	0.251	272	1.18 (0.83-1.69)	0.356	242
Vitamin E						
Pre-pregnancy	1.41 (1.15-1.72)	0.001	278	1.31 (0.99-1.73)	0.058	243
11 weeks	0.86 (0.60-1.24)	0.417	224	0.79 (0.55-1.15)	0.221	200
34 weeks	1.23 (0.99-1.53)	0.061	272	1.15 (0.89-1.48)	0.299	242

***Adjusted for child's sex, gestation, and birth order, mother's education, smoking in pregnancy and history of asthma and father's history of asthma**

Relative risks were calculated as change in risk per SD change in energy-adjusted vitamin intake

Table 122 Relative risk of atopic wheeze according to energy-adjusted maternal vitamin intake before and during pregnancy

Standardised energy-adjusted intake	Unadjusted analyses RR (95% CI)	P value	n	Adjusted* analyses RR (95% CI)	P value	n
Vitamin A						
Pre-pregnancy	0.82 (0.59-1.13)	0.220	271	0.86 (0.44-1.16)	0.336	258
11 weeks	1.02 (0.70-1.49)	0.907	219	1.23 (0.76-1.97)	0.395	212
34 weeks	0.83 (0.55-1.26)	0.376	266	0.89 (0.59-1.34)	0.583	257
Vitamin C						
Pre-pregnancy	0.90 (0.62-1.30)	0.560	271	0.95 (0.64-1.43)	0.822	258
11 weeks	0.88 (0.58-1.33)	0.542	219	1.07 (0.59-1.92)	0.824	212
34 weeks	0.78 (0.47-1.31)	0.353	266	0.95 (0.55-1.64)	0.867	257
Vitamin D						
Pre-pregnancy	0.45 (0.29-0.70)	0.257	271	0.49 (0.31-0.78)	0.002	258
11 weeks	0.67 (0.33-1.34)	0.257	219	0.61 (0.28-1.36)	0.228	212
34 weeks	0.59 (0.33-1.08)	0.086	266	0.66 (0.36-1.20)	0.175	257
Vitamin E						
Pre-pregnancy	0.64 (0.41-1.00)	0.052	271	0.73 (0.48-1.11)	0.138	258
11 weeks	0.88 (0.57-1.35)	0.552	219	0.98 (0.64-1.49)	0.913	212
34 weeks	0.76 (0.49-1.20)	0.242	266	0.71 (0.44-1.17)	0.177	257

***Adjusted for child's gestation, pet exposure in the first year of life and birth order, mother's education, smoking in pregnancy and history of asthma and father's history of asthma**
Relative risks were calculated as change in risk per SD change in energy-adjusted vitamin intake

Table 123 Relative risk of non-atopic wheeze according to energy-adjusted maternal vitamin intake before and during pregnancy

Regression analyses to investigate the effect of maternal vitamin supplementation upon lung function in the offspring at age 6 years

Standardised intake	Unadjusted analyses Beta (95% CI)	P value	n	Adjusted* analyses Beta (95% CI)	P value	n
34-week energy-adjusted vitamin intake A and FEV_1						
Total vitamin A	0.09 (0.00-0.18)	0.060	305	0.11 (0.02-0.20)	0.021	295
Food-derived	0.08 (−0.01-0.17)	0.085	305	0.10 (0.01-0.19)	0.032	295
34-week energy-adjusted vitamin A intake and $FEF_{25-75\%}$						
Total vitamin A	0.11(0.00-0.23)	0.055	305	0.14 (0.02-0.25)	0.023	296
Food-derived	0.11 (−0.01-0.22)	0.071	305	0.13 (0.01-0.25)	0.028	296
34-week energy-adjusted vitamin A intake and BHR						
Total vitamin A	−0.15 (−0.32-0.01)	0.074	107	−0.18 (−0.36-−0.01)	0.039	102
Food-derived	−0.13 (−0.30-0.04)	0.119	107	−0.17 (−0.35-0.00)	0.020	91

Analysis conducted only for vitamins for which the total intake was found to be significantly associated with a measure of lung function The food-derived vitamin intakes were not adjusted for supplement intake as food and supplement-derived intakes are likely to be correlated.
***Analyses adjusted for confounders specific to each outcome**
Regression coefficients were calculated as change in lung function measure per SD change in energy-adjusted vitamin intake

Table 124 Regression analysis of maternal vitamin intake and lung function in the offspring at age 6 years, comparing total and food-derived maternal vitamin intakes

Standardised intake	Unadjusted analyses Beta (95% CI)	P value	n	Adjusted* analyses Beta (95% CI)	P value	n
34-week energy-adjusted vitamin A intake and FEV_1						
Supplements	−0.06 (−0.36-0.24)	0.684	24	0.02 (−0.35-0.40)	0.900	24
No supplements	0.09 (−0.01-0.19)	0.067	281	0.12 (0.02-0.22)	0.015	271
34-week energy-adjusted vitamin A intake and $FEF_{25-75\%}$						
Supplements	−0.08 (−0.40-0.24)	0.598	24	0.08 (−0.36-0.52)	0.696	24
No supplements	−0.13 (−0.26-0.00)	0.048	281	0.16 (0.03-0.28)	0.014	272
34-week energy-adjusted vitamin A intake and BHR						
Supplements	0.19 (−0.69-1.07)	0.631	11	0.36 (−0.56-1.28)	0.335	11
No supplements	−0.17 (−0.34-0.00)	0.055	96	−0.22 (−0.40-−0.03)	0.020	91

Analysis conducted only for vitamins for which the total intake was found to be significantly associated with a measure of lung function. The food-derived vitamin intakes were not adjusted for supplement intake as food and supplement-derived intakes are likely to be correlated.
***Analyses adjusted for confounders specific to each outcome**
Regression coefficients were calculated as change in lung function measure per SD change in energy.

Table 125 Regression analysis of total maternal vitamin intake and lung function in the offspring at age 6 years, comparing women taking vitamin supplements with those who did not

Regression analyses to investigate the effect of supplementation upon wheeze phenotypes in the offspring at age 6 years

Standardised intake	Unadjusted analyses RR (95% CI)	P value	n	Adjusted* analyses RR (95% CI)	P value	n
Initial vitamin A intake and non-atopic wheeze						
Total	0.73 (0.51-1.06)	0.095	271	0.73 (0.53-1.00)	0.047	258
Food-derived	0.81 (0.57-1.16)	0.257	271	0.82 (0.59-1.13)	0.226	258
Initial vitamin C intake and persistent wheeze						
Total	1.06 (0.87-1.30)	0.554	229	1.22 (1.02-1.48)	0.035	223
Food-derived	1.09 (0.86-1.38)	0.465	229	1.21 (0.95-1.55)	0.118	223
34-week vitamin C intake and atopic wheeze						
Total	1.49 (1.12-1.98)	0.006	272	1.46 (1.01-2.11)	0.047	242
Food-derived	1.44 (1.07-1.95)	0.016	272	1.32 (0.89-1.90)	0.169	242
34-week vitamin C intake and doctor-diagnosed asthma						
Total	1.16 (0.91-1.48)	0.228	459	1.25 (1.01-1.54)	0.036	442
Food-derived	1.13 (0.88-1.45)	0.342	459	1.18 (0.96-1.46)	0.120	442
Initial vitamin D intake and doctor-diagnosed asthma						
Total	0.75 (0.58-0.96)	0.021	469	0.75 (0.58-0.96)	0.024	443
Food-derived	0.72 (0.57-0.90)	0.04	469	0.72 (0.57-0.91)	0.006	443
Initial vitamin D intake and wheeze in the last 12 months						
Total	0.77 (0.61-0.96)	0.022	469	0.79 (0.63-1.00)	0.046	418
Food-derived	0.73 (0.59-0.90)	0.003	469	0.73 (0.59-0.90)	0.003	418
Initial vitamin D intake and non-atopic wheeze						
Total	0.47 (0.32-0.68)	<0.001	271	0.46 (0.30-0.71)	<0.001	258
Food-derived	0.52 (0.37-0.72)	<0.001	271	0.51 (0.35-0.76)	0.001	258
Initial vitamin E intake and non-atopic wheeze						
Total	0.59 (0.36-0.97)	0.038	271	0.65 (0.45-0.95)	0.027	258
Food-derived	0.66 (0.43-1.02)	0.063	271	0.74 (0.55-1.00)	0.051	258
34-week vitamin E intake and non-atopic wheeze						
Total	0.71 (0.43-1.13)	0.150	266	0.59 (0.38-0.93)	0.023	257
Food-derived	0.76 (0.50-1.15)	0.188	266	0.62 (0.42-0.911	0.015	257

Analysis conducted only for vitamins for which the total intake was found to be significantly associated with a wheeze phenotype. The food-derived vitamin intakes were not adjusted for supplement intake as food and supplement-derived intakes are likely to be correlated.
***Analyses adjusted for confounders specific to each outcome**
Relative risks were calculated as change in risk per SD change in vitamin intake.

Table 126 Regression analysis of maternal vitamin intake and wheeze phenotypes in the offspring at age 6 years, comparing total and food-derived maternal vitamin intakes

Standardised 34-week total vitamin A intake	Unadjusted analyses Beta (95% CI)	P value	n	Adjusted* analyses Beta (95% CI)	P value	n
Initial vitamin A intake and non-atopic wheeze						
Supplements	*Too few women supplemented to support analysis*					
No supplements	0.78 (0.55-1.11)	0.174	209	0.84 (0.60-1.17)	0.303	198
Initial vitamin C intake and persistent wheeze						
Supplements	1.00 (0.70-1.44)	0.982	82	1.16 (0.85-1.57)	0.345	82
No supplements	1.10 (0.82-1.64)	0.387	147	1.60 (1.04-2.44)	0.031	141
34-week vitamin C intake and atopic wheeze						
Supplements	0.94 (0.53-1.68)	0.842	58	1.20 (0.36-3.98)	0.767	55
No supplements	1.79 (1.23-2.59)	0.02	214	1.48 (0.91-2.42)	0.117	187
34-week vitamin C intake and doctor-diagnosed asthma						
Supplements	1.80 (1.22-2.66)	0.003	99	1.83 (1.34-2.49)	<0.001	97
No supplements	1.07 (0.79-1.46)	0.646	360	1.14 (0.87-1.49)	0.334	345
Initial vitamin D intake and doctor-diagnosed asthma						
Supplements	0.88 (0.53-1.45)	0.616	130	1.02 (0.60-1.74)	0.931	126
No supplements	0.68 (0.50-0.95)	0.025	339	0.68 (0.48-0.96)	0.028	317
Initial vitamin D intake and wheeze in the last 12 months						
Supplements	0.59 (0.41-0.86)	0.06	130	0.61 (0.44-0.84)	0.002	120
No supplements	0.69 (0.50-0.96)	0.026	339	0.73 (0.50-1.06)	0.096	298
Initial vitamin D intake and non-atopic wheeze						
Supplements	*Too few women supplemented to support analysis*					
No supplements	0.48 (0.32-0.73)	<0.001	196	0.48 (0.28-0.83)	0.009	184
Initial vitamin E intake and non-atopic wheeze						
Supplements	*Too few women supplemented to support analysis*					
No supplements	0.65 (0.34-1.27)	0.206	193	0.77 (0.45-1.31)	0.328	182
34-week vitamin E intake and non-atopic wheeze						
Supplements	*Too few women supplemented to support analysis*					
No supplements	0.72 (0.41-1.24)	0.231	222	0.57 (0.34-0.94)	0.029	214

Analysis conducted only for vitamins for which the total intake was found to be significantly associated with a wheeze phenotype
***Analyses adjusted for confounders specific to each outcome**
Relative risks were calculated as change in risk per SD change in vitamin intake.

Table 127 Regression analysis of total maternal vitamin intake and wheeze phenotypes in the offspring at age 6 years, comparing women taking vitamin supplements with those who did not

Re-analysis of significant associations between conditional fetal growth and relative risk of atopy and atopic wheeze at 3 years of age

	Unadjusted analyses				Adjusted* analyses*			
	RR	(95% CI)	P-value	n	RR	(95% CI)	P-value	n
Atopy								
11-19 weeks								
Abdominal circumference	1.38	(1.08-1.76)	0.009	449	1.40	(1.07-1.82)	0.014	393
19-34 weeks								
Abdominal circumference	0.83	(0.71-0.96)	0.014	735	0.83	(0.70-0.97)	0.023	650
Atopic wheeze								
11-19 weeks								
Abdominal circumference	1.42	(1.05-1.92)	0.024	197	1.32	(0.94-1.85)	0.114	172
Abdominal circumference	0.82	(0.66-1.01)	0.066	321	0.80	(0.65-1.00)	0.046	288

***Atopy adjusted for gender, maternal eczema, maternal atopy, maternal education and birth order Atopic wheeze adjusted for gender, smoking during pregnancy, maternal asthma and maternal rhinitis, maternal atopy, paternal asthma, maternal education and birth order**
Relative risks were calculated as change in risk per SD change in size or growth measurement.

Table 128 Re-analysis of the relationship between conditional fetal abdominal circumference growth and atopy and atopic wheeze at 3 years of age, excluding only children born at less than 35 weeks' gestation

Sensitivity analysis comparing those children recording FENO values on the NIOX® analyser with those children recording values on the NIOX MINO® analyser

Percentage of total fatty acids	Unadjusted analyses Exp beta (95% CI)	P value	n	Adjusted* analyses Exp beta (95% CI)	P value	n
Results combined from both analysers						
Linoleic acid	0.93 (0.86-1.02)	0.124	237	0.87 (0.80-0.94)	0.001	224
Arachidonic acid	1.11 (1.02-1.21)	0.019	237	1.12 (1.03-1.21)	0.007	224
Results from NIOX® analyser only						
Linoleic acid	0.94 (0.83-1.06)	0.321	131	0.90 (0.81-1.01)	0.068	126
Arachidonic acid	1.14 (1.02-1.28)	0.024	131	1.18 (1.05-1.32)	0.005	126
Results from NIOX MINO® analyser only						
Linoleic acid	0.94 (0.83-1.06)	0.298	106	0.85 (0.75-0.96)	0.010	98
Arachidonic acid	1.03 (0.90-1.16)	0.691	106	1.02 (0.89-1.16)	0.795	98

***Adjusted for child's gender and month of birth and maternal education, history of eczema, atopic status and parity**
Regression coefficients were calculated as change in risk per SD change in plasma phospholipids fatty acid composition.

Table 129 Multiplicative increase in FENO according to fatty acid composition of maternal plasma phospholipid phosphatidylcholine at 34 weeks' gestation

Appendix 2 Developmental influences upon childhood respiratory health protocol

Developmental influences upon childhood respiratory health

6-year assessment Protocol

Developmental influences upon childhood respiratory health

SWS cohort study at age 6 years

Protocol

Research questions and hypotheses

The study has three main research questions.

1. **What are the links between asthma and obesity in childhood? Is the link:**

- Common antenatal environmental exposures?
- Common postnatal environmental exposures?
- Common genetics?

2. **Do maternal genotype and phenotype impact on the child's phenotype independent of infant genotype?**
3. **Does pre- and postnatal nutrition affect the development of asthma and other wheezing illnesses in the child?**

The research questions will be addressed by investigating the following hypotheses:

1. The association between asthma and obesity is the result of particular *prenatal* environmental influences (maternal high fat mass, low energy intake and smoking during pregnancy) that increase the risk of both disorders.
2. The association between asthma and obesity is the result of particular *postnatal* environmental influences (high infant weight gain and low childhood physical activity) that increase the risk of both disorders.
3. The association between asthma and obesity is the result of polymorphisms in particular candidate genes that increase the risk of both disorders.
4. Maternal genotype and phenotype determine obesity and asthma in the child independent of the child's genotype.
5. Impaired maternal nutrition during pregnancy (specifically low maternal fat and/or muscle mass and low intakes of vitamins A and/or C) is associated with impaired lung function (defined by spirometry) at 6 years of age
6. High maternal fat mass, high vitamin D status and low maternal vitamin E intake in pregnancy are associated with atopy (positive skin prick test) at 6 years of age

7. Maternal nutrition and faltering of fetal growth in late gestation relate to each of the childhood wheeze syndromes (transient viral-associated wheeze, atopic asthma and non-atopic asthma).

Background

The prevalence of asthma and obesity increased in parallel during the 1980s and 90s, and mounting evidence suggests a link between obesity and the development of asthma (Wannamethee *et al.* 2005; Weiss & Shore 2004). It has been proposed that environmental or genetic factors common to both disorders are responsible (Schaub & von Mutius 2005). The proposed study will investigate whether particular aspects of the prenatal environment (maternal high fat mass, low energy intake and smoking during pregnancy) and/or postnatal environment (high infant weight gain and low physical activity) are associated with asthma and obesity at 6 years of age. It will also investigate the genetic influences that determine asthma and obesity. It has been suggested that polymorphisms of the beta-2-adrenergic receptor (ADRB2), ADAM33, IL6, leptin, TNFA and PPARG genes may contribute to both asthma and obesity, but there is currently little evidence to support or refute a role for these candidate genetic influences.

The proposed study will use prospectively collected, longitudinal growth and respiratory data in 950 6-year olds enrolled in the Southampton Women's Survey (Inskip *et al.* 2005). The children's mothers were extensively characterised before and during pregnancy; body composition (detailed anthropometry), dietary intakes (food frequency questionnaires and food diaries), physical activity, atopic disorders and smoking were recorded. Longitudinal fetal growth measurements were collected by ultrasound at 11, 19 and 34 weeks. Children have been monitored for growth and features of respiratory morbidity and atopy at 6 months and 1, 2 and 3 years. Additionally, 131 of the cohort had lung function measured in early infancy, showing impaired lung development in infants that had had lower rates of fetal growth and higher weight gain in the first weeks after birth (Lucas *et al.* 2004). The assessment at 6 years will allow us to collect a detailed dataset that combines information on asthma, body composition and physical activity in childhood. To add to information that is being collected on lung function and respiratory symptoms, we will measure adiposity and regional body composition (anthropometry, densitometry and DXA scanning), collect objective physical activity

data using a combined accelerometer and heart rate monitor, and characterise the genetic variation of particular candidate genes linked with asthma and/or obesity. For 6 candidate genes, haplotype tagging sets of Single Nucleotide Polymorphisms (SNPs) will be selected based on information available from the Seattle SNPs variation discovery resource (http://pga.mbt.washington.edu/) (ADRB2, IL6, PPARG, TNFA and leptin) and from our own data (TNFA and ADAM33). These SNPs together with putative functional SNPs (e.g. PPARG Pro12Ala and IL-6 -174) will be typed in child and parental DNA by a combination of methods.

Collection of these data will allow us to relate prenatal (maternal high fat mass, low energy intake and smoking during pregnancy) and postnatal (high infant weight gain and low physical activity) exposures and genotypes to respiratory outcomes and adiposity at age 6 years, and to examine whether maternal genotype and phenotype impact on the child's phenotype independent of infant genotype. We hope that understanding the links between these disorders will enable us to develop strategies to reduce the chance of individuals developing asthma.

Study design and methodology

The Southampton Women's Survey

The Southampton Women's Survey (SWS) was started in 1998. It is a study of a population sample of non-pregnant women aged 20 to 34 years resident in the city of Southampton, UK. They are representative of the British population in terms of ethnicity and deprivation. From this group, 1477 of those who have become pregnant and delivered infants would be eligible for the assessment of the children at age 6 years proposed in this study. We conservatively estimate that 65% will participate, giving 950 children.

Existing data from the SWS cohort

Maternal nutritional data

Uniquely, maternal body composition and diet have been assessed before and during pregnancy. Body composition is assessed by 4-site skinfold thicknesses and other anthropometric measures, allowing estimation of fat and muscle mass. Diet is assessed using a 100-item administered food frequency questionnaire to record the average frequency of consumption over a 3-month period preceding the interview. Although such questionnaires can be subject to bias, validation using 4-day food diaries and

measurement of maternal micronutrient concentrations has indicated that our questionnaire gives an assessment of diet that can be used to rank the nutrient intakes of individuals. Dietary supplement use is assessed in detail over the same period, allowing us to derive maternal intakes of vitamins A, C, D and E during pregnancy. Vitamin intakes in a previous cohort of Southampton pregnancies showed marked variability. Maternal 25-OH vitamin D concentrations in early and late pregnancy are being measured and are combined with information on ultraviolet B exposure calculated from the hours of sunshine from a local Meteorological Office weather station with an adjustment for seasonal energy variation in ultraviolet B radiation (http://www.soda-is.com/index.html); in our previous study, the correlation coefficient between this measure of ultraviolet B exposure and maternal 25-OH vitamin D concentration in late pregnancy was 0.60, $P<0.001$ (unpublished data).

Fetal growth data

Longitudinal fetal growth measurements have been collected by ultrasound at 11, 19 and 34 weeks, together with detailed neonatal anthropometry. Using the method of Royston, we will generate Z-scores of crown-rump length, head and abdominal circumferences adjusted for duration of gestation in early, mid and late pregnancy and at birth, and calculate the velocities of growth unconditional and conditional upon the initial measurement of size. We will use longitudinal ultrasound measurements of fetal anthropometry at 11 and 19 weeks gestation to define the velocity of the initial trajectory of growth, and the change in abdominal measurements between 34 weeks and delivery to describe growth faltering in late pregnancy.

Other information available about this cohort from birth to 3 years of age

At 6, 12, 24 and 36 month visits, the principal carer has been questioned about the child's illnesses since the previous visit. These questions focused on respiratory, allergic and gastrointestinal symptoms and illness. Specifically, the questionnaire asked about episodes of wheezing or whistling in the chest. We also have prospectively collected data about other important exposures, including environmental cigarette smoke, pets and childcare. Skin prick testing has been undertaken at 1 and 3 years of age at a time when subjects had not taken any anti-histamine for at least 72 hours. Testing to cat, dog, grass pollens, house dust mite, milk and egg allergens (ALK, Horshølm, Denmark) was undertaken with a single headed lancet. Weal diameters were measured and a positive result defined as one that is at least 3mm in diameter in the presence of valid controls. The controls are valid if the negative (saline) control was zero and positive

control (histamine) is at least 3mm. Additionally, we have DNA stored for each subject. Lastly, premorbid infant lung function data, domestic dust samples and urinary cotinine measurements are available from a subset of 150 participants.

Respiratory and growth data to be collected at age 6 years

Recruitment

All families are already recruits of SWS. Families currently enrolled with the SWS, whose child is between 6 and 7 years during the study period will be identified from the SWS database.

Some families will have moved house and not informed the SWS of their change of contact details. We will therefore have a press release aimed at local media to inform them of the aims of the 6-year-old assessment, and to encourage families to contact the team if they need to update contact information.

Families who are enrolled with SWS will be contacted by post to inform them of the 6-year follow-up. A member of the research team will then contact the family by telephone or email to ask if they would like to participate in this part of the study. This follows the format of previous SWS contacts and appointment making. No undue pressure will be placed on families to participate.

Children will be assessed between the ages of six and seven years. They will have a home visit from a SWS nurse and will be invited for further investigations to the Wellcome Trust Clinical Research Facility (WTCRF), Southampton, with paediatric facilities for more detailed respiratory and body composition investigations.

Inclusion criteria

All children enrolled on the SWS between their 6th and 7th birthdays during the study period.

Exclusion criteria
All carers will be invited to complete the questionnaire.

Exclusions for specific investigations are as follows:

- Methacholine challenge: Baseline FEV_1< 70% predicted; unstable asthma; current respiratory infection.
- Skin testing: antihistamine use within 72 hours.

Consent

Consent will be taken by a nurse or doctor who has a detailed understanding of the study protocol. Prior to consent, the child and parent will have received age-appropriate information sheets at least a week before the appointment. The person taking consent will ensure that the parent and child understand the aims and procedures. The parent and child will have as much time as is necessary to ask questions. If both the child and parent are in agreement that the research should proceed we will ask their parents to sign a consent form.

Where will the studies take place?

Home	WTCRF
Height, Weight, skin folds	DXA
Simple spirometry	Methacholine test OR reversibility studies using salbutamol
Buccal brushing (genetics)	Exhaled nitric oxide
Actiheart	Blood pressure and heart rate
Allergy tests (at WTCRF if food allergic)	Allergy tests if food allergic

Questionnaire

- The questionnaire will be administered by a member of the research team to the child's carer at home.
- If carers live outside the Southampton area, the questionnaire will be administered over the telephone.

- The questionnaire is attached in appendices 2 & 3. It is primarily designed to assess the child's respiratory and atopic status. It also includes questions to assess current diet and activity.

Body composition

- Measurements of height, weight and skinfold thicknesses will be made by a trained nurse or doctor.
- DXA scan. This will be performed by technicians trained and experienced in its use. Approximately 500 of the children have had previous DXA measurements in the SWS.

Activity

- Physical activity over a 5-7 day period using an Actiheart combined accelerometer and heart rate monitor; we have successfully used these in over 50 SWS children at age 4 years.
- The Actiheart will be applied by small stickers to the child's torso during the home visit and instructions describing reapplication will be given. The child will be asked to wear the monitor for up to a week, during which time they should pursue their normal activities. They will be provided with a pre-paid package to return the Actiheart to the research team for analysis, or it can be returned when they attend the WTCRF for a visit.
- The Actiheart will be accompanied by a questionnaire about activity and exercise for the parent/carer to complete and return in the pre-paid package with the Actiheart monitor

Atopy

- Skin prick testing to house dust mite, cat, dog, mixed grass pollen, mixed tree pollen, egg and milk. Subjects with a test result greater than or equal to 3mm with appropriate controls will be defined as atopic.

Lung Function

- Lung function, including flow volume loops will be measured using Koko incentive software.
- Within the WTCRF, children will be invited to have a more detailed assessment of their lung function by either (a) methacholine challenge or (b) reversibility with salbutamol. All children with a history of wheeze and approx 100 children without

wheeze will be invited to have a methacholine challenge. Those who decline and all other patients will be invited to have reversibility studied using salbutamol.

- Children will be prescribed 6 puffs of salbutamol to be administered via meter-dose-inhaler (MDI) and spacer (100mcg per puff) to access reversibility of airway obstruction. Lung function measurement will be repeated 15 minutes after the salbutamol dose.
- Methacholine challenge will be used to document bronchial hyperresponsiveness as an objective marker for asthma. In this test, the patient inhales an aerosol of one or more concentrations of methacholine. Results of lung function tests (e.g. FEV_1) performed before and after the inhalations are used to quantify the response. A positive test is defined as a decrease from the post-diluent FEV_1 value of at least 20%.

Exhaled nitric oxide

- Exhaled NO, as a non-invasive marker of airway inflammation will be measured using the single expiratory breath method with a chemiluminescence analyser (NIOX® desktop system, Aerocrine, Solna, Sweden) set at a rate of 50 ml/s. Measurements are repeated until two consecutive results within 10% are obtained; this generally requires 2–4 attempts. All measurements will be undertaken before spirometric testing. Exhaled NO values will be discarded if the ambient level was above 100 ppb.

Clinical Samples

- DNA buccal swabs will be taken from children and parents who consent.
- Samples will be collected by trained nurses and doctors.
- Samples will be labelled with the child's unique SWS identification number for subsequent linking with information in the SWS database. The results will not be linked to individual names.
- The samples will be stored in the SWS freezers in the MRC Epidemiology Resource Centre, Southampton General Hospital. They will be stored until all analyses are completed. Prof Cyrus Cooper, Director of the Centre, and subsequent Directors will have custodial responsibility.
- Stored cord blood and parental DNA (LREC 340/97; 307/97; 018/99) as well as newly collected DNA specimens will be analysed for asthma and obesity genotyping. Stored linked-anonymised samples will be analysed for all eligible

children, whether or not they are recruited for this 6 year assessment. This will allow linking of the genotype data to respiratory and growth outcomes in early life as well as at 6 years.

Analysis

Primary outcome measures will be current wheeze at age 6-years, estimated fat mass and distribution, atopy and FEV_1, together with $FEF_{25-75\%}$ (more sensitive to small airway disease, although less reproducible). Controlling for potential confounders, binary outcomes (wheeze, atopy) will be analysed by logistic regression, and continuous outcomes (FEV_1, $FEF_{25-75\%}$) by linear regression after transformation to normalise them as necessary. As secondary outcomes, we will investigate clinical wheeze phenotypes defined as (1) transient viral-associated wheeze: presence of wheeze only with viral upper respiratory tract infections within the first 5 years of life; (2) atopic asthma: wheeze between viral upper respiratory tract infections or with exercise that responds to a bronchodilator in an atopic child; (3) non-atopic asthma: as for atopic asthma but in a child who is not atopic.

Hypothesis 1:

Primary *prenatal* exposure variables will be maternal pre-pregnancy fat mass, energy intake in pregnancy and smoking. To investigate the secondary exposures of low early trajectories of fetal growth and faltering of growth in late gestation, we will generate Z-scores of fetal size adjusted for duration of gestation in early, mid and late pregnancy and at birth, and calculate velocities of growth unconditional and conditional upon the earlier measurement of size (Royston 1995). In those with infant lung function data we will explore whether any relationship between maternal influences and childhood asthma was already apparent in early postnatal life.

Hypothesis 2

Primary *postnatal* exposure variables will be rapid weight gain in infancy (change in Z-score of weight for height, conditional and unconditional upon size at birth) and lower physical activity at age 6 years. Secondary postnatal exposures will be infant feeding mode, duration of breast-feeding and postnatal smoke exposure. Cord blood leptin will

be used as a measure of adiposity at birth, to examine whether any associations truly reflect postnatal influences.

Hypotheses 3 & 4

We will relate genotypes directly to outcomes, and analyse associations between the environmental exposures and outcomes, stratifying for category of genotype. We will also utilise the parental DNA to undertake family-based analyses of association that avoids potential confounding by population stratification using FBAT methodology.

Hypothesis 5

FEV_1 and $FEF_{25-75\%}$ will be transformed to normalise them as necessary. Multiple regression analysis will be used to investigate whether they are influenced by low maternal fat and/or muscle mass and low maternal intake of vitamins A and C. We will explore whether the children whose fetal growth faltered in late gestation (as measured by serial ultrasound scans) are those whose impaired maternal nutrition during pregnancy most affects their childhood lung function, and whether there is an interaction between vitamin C intake and smoking. Lastly, we plan to use the infant lung function data, available for a subgroup, to allow us to explore whether any relationship between maternal nutrition in pregnancy and childhood lung function is already apparent in the first few weeks of life.

Hypothesis 6

Logistic regression will be used to investigate the effects of high maternal fat mass and high vitamin D status, and of low maternal intake of vitamin E during pregnancy on atopic status at age 6 years. Atopy will be defined as at least one positive skin prick test.

Hypothesis 7

The clinical wheeze phenotypes will be defined as (1) transient viral-associated wheeze: presence of wheeze only with viral upper respiratory tract infections within the first 5 years of life; (2) atopic asthma: wheeze between viral upper respiratory tract infections or with exercise that responds to a bronchodilator in an atopic child; (3) non-atopic asthma: as for atopic asthma but in a child who is not atopic. Children with a history of wheeze will be assigned to one of these categories according to the timing of symptoms

and the presence of atopy. An exploratory analysis will be undertaken to examine how maternal nutrition (as defined by body composition, vitamin D status and vitamin A, E and C intakes) and fetal growth (as measured by serial ultrasound scans) differ between these three wheeze phenotypes and atopic and non-atopic children who have no history of wheeze. This will allow us to explore whether impaired maternal nutrition during pregnancy and impaired fetal growth are important in the development of each of these wheeze phenotypes. Lastly, the infant lung function data, available for a subgroup, will allow us to explore how impaired lung function develops in each of these childhood wheeze phenotypes.

Sample size and power calculations

Assuming a 65% follow-up of the 1477 children gives a sample size of about 950. Our experience of similar longitudinal cohorts indicates that we may well achieve a higher follow-up, giving greater statistical power than shown here. Assuming a 5% level of significance, for objectives 1 and 2, we have 97% power to detect a difference of 0.25 SDs in the continuous outcomes of FEV_1 and estimated fat mass, between the top and bottom halves of the distribution of each continuous exposure variable (maternal fat mass, energy intake, infant weight gain and physical activity). This falls to 90% power for a 1% level of significance. Analysis of the continuous outcomes without dichotomisation will provide greater power. For objectives 1, 3 and 4, in relation to the dichotomous exposure variables of smoking and genetic polymorphisms, the table below gives the power to detect a difference of 0.25 SDs in the same continuous outcomes for various different prevalences of the exposure variable. We anticipate that the prevalence of the genetic polymorphisms ranges from 50%-10%; the prevalence of smoking in pregnancy in this population is 17%:

Frequency of exposure	Statistical power
50%	**97%**
40%	**97%**
30%	**94%**
20%	**87%**
17%	**83%**
15%	**79%**
10%	**64%**

For hypothesis 5, we have 87% power to detect a difference of 0.2 standard deviations (SDs) in FEV_1 between the top half and the bottom half of the distribution of each exposure variable. Defining impaired fetal growth or impaired maternal nutrition as those in the lowest 20% of the distribution, we have 87% power to detect a difference of 0.25 SDs in FEV_1 between these groups and the remaining children.

For hypothesis 6, for any normally distributed exposure measurement we have 81% power to detect a difference of 0.25 SDs between the exposure of those with atopy (assuming a 16% prevalence of atopy at age 6 years) and those without. Dichotomising the exposure gives 80% power to detect a relative risk of atopy of 1.55 for those in the bottom half of the exposure distribution compared with the top half.

For hypothesis 7, we will perform an exploratory analysis. As an example of our power, if we measure lung function at six years in 70 children with infant lung function data and the prevalence of wheezing at six years is 20%, we will have 80% power to identify a 20% difference in infant $FEV_{0.4}$ between those who wheeze at six years of age and those who do not.

Key Milestones

Respiratory and body composition assessments at 6 years of age will occur during the initial 27 months. During year 1 the whole cohort will be genotyped. A final report, and drafts of publications will be submitted to LREC by October 2011.

The research team

Professor Warner and Drs Lucas, Roberts and Holloway are academic researchers within the Infection, Inflammation and Repair (IIR) Division of Southampton School of Medicine. Professors Godfrey and Cooper and Dr Inskip work within the MRC Epidemiology Resource Centre. The applicants have a track record of successful collaboration (Lucas *et al.* 2004). Dr Lucas is a respiratory paediatrician with a research interest in lung development. Professor Godfrey's research within SWS is characterising the interactions between prenatal, postnatal and genetic influences on health outcome. Professor Warner's research has focused on the early origins of asthma. Dr Holloway heads the Asthma Genetics Group and recently reported ADAM33 as an asthma-susceptibility gene. Dr Inskip, a statistician/epidemiologist, coordinates the SWS, studying the effects of pre-conceptional factors on fetal and postnatal growth. Dr

Roberts is a respiratory paediatrician, with an expertise in epidemiology. Professor Cooper is Director of the MRC Epidemiology Resource Centre and has expertise in developmental influences on body composition.

The team will include nurses from the WTCRF who are experienced in research with children. Training will be provided to the nurses in any aspects of the protocol, as necessary. Home visits will generally be conducted by SWS nurses who have been involved in earlier visits to SWS families and have developed and nurtured relationships between the participants and the Survey.

We have employed Dr Katy Pike as a Clinical Research Fellow to assist in the clinical investigation of the cohort and the analysis of the data, under the supervision of the PI. Dr Pike is a Paediatric SpR with an interest in paediatric respiratory medicine. Training in research governance, ethics, child protection, respiratory physiology etc will be provided.

Investigators

University Child Health, IIR/ DOHaD

Dr Jane Lucas, Senior Lecturer/ Honorary Respiratory Paediatrician

Dr Graham Roberts, Senior Lecturer/ Honorary Respiratory Paediatrician

MRC Epidemiology Resource Centre

Dr Hazel Inskip, Statistician/ Senior Lecturer

Professor Keith Godfrey, Professor in Epidemiology & Human Development

Dr Sian Robinson, Senior Research Fellow, Nutritionist

Professor Cyrus Cooper, Director, MRC Epidemiology Resource Centre.

Human Genetics Division, IIR

Dr John Holloway, Lecturer in Pharmacology

References

Inskip, H. M., Godfrey, K. M., Robinson, S. M., Law, C. M., Barker, D. J., Cooper, C. *et al.* 2006, Cohort profile: The Southampton Women's Survey, *International Journal of Epidemiology*. 35(1):42-48.

Lucas, J. S., Inskip, H. M., Godfrey, K. M., Foreman, C. T., Warner, J. O., Gregson, R. K. *et al.* 2004, Small size at birth and greater postnatal weight gain: relationships to diminished infant lung function, *American Journal of Respiratory & Critical Care Medicine*. 170(5):534-40.

Royston, P. 1995, Calculation of unconditional and conditional reference intervals for foetal size and growth from longitudinal measurements, *Statistics in Medicine*. 14(3);1417-1436.

Schaub, B. & von Mutius, E. 2005, Obesity and asthma, what are the links? *Current Opinion in Allergy & Clinical Immunology*. 5(2):185-93.

Wannamethee, S.G., Shaper, A.G., & Whincup, P.H. 2005, Body fat distribution, body composition, and respiratory function in elderly men. *American Journal of Clinical Nutrition*. 82(5):996-1003.

Weiss, S.T. & Shore, S. 2004, Obesity and asthma: directions for research. *American Journal of Respiratory & Critical Care Medicine*. 169(8):963-68.

Appendix 3 Southampton Women's Survey six-year home visit questionnaire – respiratory data

6 Year

QUESTIONNAIRE

HOME VISIT

Mother's forename only: _____

Child's forename only: _____

[Nurse to refer to six-year visit record card to ensure child's name is correct, and record any changes thereon. Also to request additional telephone numbers, email addresses etc, for tracing purposes if family move]

Child's date of birth dd mm yy

Sex M=Male F=Female

Date of interview dd mm yy

Interviewer

Discuss the visit with the mother and child and obtain completed consent and assent forms

To be completed by the nurse if the mother was not the person interviewed:

1.1 Why was the mother not available?

1. Has left the family home
2. Still lives in family home, but was unavailable for interview
3. Has died
4. Is ill or in hospital
8. Other, specify _____
9. Don't know

1.2 Who was interviewed?

1. Study child's father
2. Mother's partner (if not father)
3. Study child's grandparent
4. Other family member
5. Mother "figure" (eg father's partner/step-mother)
6. Family friend
8. Other, specify _____

2. NEONATAL HISTORY

Now I'm going to ask you some questions about what happened to your child around the time of birth.

2.1 Was your child admitted to a Special Care Baby Unit?

1. No go to section 3
2. Yes

☐

2.2 Was he/she admitted for breathing problems? ☐

1. No
2. Yes
9. Don't know

2.3 How long was your child in the Special Care Baby Unit?

mths	wks	days
☐☐	☐☐	☐☐

2.4 Did he/she need any help with his/her breathing (ventilator/life-support machine CPAP)?

1. No go to section 3
2. Yes

☐

2.5 Did he/she require invasive ventilation (tube into lungs) or non-invasive (e.g.CPAP)?

1. Non- invasive (e.g. CPAP)
2. Invasive (e.g. tube into lungs)
3. Both

☐

2.6 For how long was he/she ventilated

mths	wks	days
☐☐	☐☐	☐☐

(Note if ventilated both non-invasively and invasively, give combined time here)

3. FAMILY HISTORY

3.1 Have you or any other members of the child's family ever been diagnosed by a doctor with any of the disorders on the list? (complete each box with a 0 for no or a 1 for yes)

	Mother	Father	Sibling	Half - sibling
3.2 Asthma				
3.3 Wheezing				
3.4 Eczema				
3.5 Hayfever				
3.6 Food allergy				
3.7 Drug allergy				
3.8 Bee or wasp sting allergy				
3.9 Cystic Fibrosis				

Prompts
Asthma: wheeze or whistling in the chest with exercise or other triggers that is rapidly relieved with a reliever inhaler. Only if doctor-diagnosed.
Wheeze: whistling in the chest when breathing out.
Eczema: A skin condition resulting in dry, itchy, red skin. If it is infected the skin may become wet. (Doctor-diagnosed only).
Hayfever: runny, itchy eyes or/and nose in the spring or summer, not caused by a cold.

Note: Only record 'Yes' if the person has definitely had the problem. If the person has, for example, never been stung by a bee or a wasp then the answer is 'No'.

MOTHER'S HEIGHT AND WEIGHT

3.1 Mother's height ☐☐☐•☐ cm

3.2 Mother's ☐☐☐•☐ kg

4. ASTHMA

I would now like to ask a few questions about illnesses your child has had

4.1 Has your child ever had asthma?

0. No go to section 5
1. Yes ☐

4.2 Was the asthma diagnosed by a doctor?

0. No go to section 5
1. Yes ☐

4.3 How old was he/she when he/she was first diagnosed?

yrs ☐ mths ☐☐ wks ☐☐

4.4 Has he/she ever been admitted to hospital for asthma?

0. No
1. Yes ☐

4.5 Has he/she received inhalers or other medication for asthma prescribed by a doctor in the past 12 months?

0. No go to section 5
1. Yes ☐

5. OTHER RESPIRATORY ILLNESSES AND SYMPTOMS

5.1 Has he/she ever been diagnosed as having bronchiolitis by a doctor?

0. No go to 5.4
1. Yes ☐

5.2 How old was he/she when he/she was first diagnosed?

yrs ☐ mths ☐☐ wks ☐☐

5.3 Has he/she ever been admitted to hospital for this?

0. No
1. Yes ☐

5.4 Has he/she ever been diagnosed as having pneumonia or a chest infection by a doctor?

0. No go to 5.8
1. Yes ☐

5.5 Has he/she ever been admitted to hospital for this?

0. No
1. Yes ☐

5.6 Has he/she been diagnosed as having pneumonia or a chest infection by a doctor in the past 12 months?

0. No go to 5.8
1. Yes ☐

5.7 Has he/she been admitted to hospital for pneumonia or a chest infection in the past 12 months?

0. No
1. Yes ☐

5.8 Has he/she ever had a persistent cough every day for more than 3 weeks?

0. No go to 5.12
1. Yes ☐

5.9 Has he/she ever been admitted to hospital for this?

0. No
1. Yes ☐

5.10 Has he/she had a persistent cough every day for more than 3 weeks in the past 12 months?

0. No go to 5.12
1. Yes ☐

5.11 Has he/she been admitted to hospital for a persistent cough in the past 12 months?

0. No
1. Yes ☐

5.12 Does your child have any other respiratory problems (eg cystic fibrosis)?

0. No go to 5.8 ☐
1. Yes, if yes specify_____

5.13 Has your child regularly snored at night (3 nights a week or more) for at least 6 months over the past year?

0. No
1. Yes ☐

5.14 Has your child had his/her adenoids or tonsils removed?

0. No
1. Adenoids only
2. Tonsils only
3. Adenoids and tonsils ☐

6. FURTHER QUESTIONS ABOUT ASTHMA AND WHEEZE
(based on core ISAAC questions and proposed standardised BPRS questionnaire)

6.1 Has your child ever had wheezing or whistling in the chest at any time in the past?

0. No go to 6.13 ☐
1. Yes

6.2 Were these wheezy or whistling episodes associated with colds?

0. No go to 6.4 ☐
1. Yes

6.3 Has he/she ever wheezed or whistled in the chest between colds?

0. No ☐
1. Yes

6.4 Has your child had wheezing or whistling in the chest in the last 12 months?

0. No go to 6.12 ☐
1. Yes

6.5 How many attacks of wheezing has your child had in the last 12 months?

0. None
1. 1 - 3
2. 4 - 12
3. > 12

☐

6.6 In the last 12 months, how often, on average, has your child's sleep been disturbed due to wheezing?

0. Never woken with wheeze
1. Woken with wheeze less than one night per week
2. Woken with wheeze on one or more nights per week

☐

6.7 In the last 12 months, has your child's chest sounded wheezy during or after exercise?

0. No
1. Yes

☐

6.8 In the last 12 months has wheezing ever been severe enough to limit your child's speech to only one or two words at a time between breaths?

0. No
1. Yes

☐

6.9 Does your child wheeze? (please put 0 for No or 1 for Yes in each box)

In winter	
In spring	
In summer	
In autumn	

6.10 What else makes him/her wheeze? (please put 0 for No or 1 for Yes in each box)

Change of weather	
Emotion (e.g. excited / upset)	
Smoky rooms	
Exercise	
Pollen Season	
During vacuum cleaning or bed making	
Perfume	
Certain foods (specify):	
Moulds	
Hairy / furry animals (specify):	
Other (specify):	

6.11 In the last 12 months how many of the following has your child had? (please complete with 0s if none have occurred)

Hospital admissions with asthma/wheeze	
Visits to Casualty Dept with asthma/wheeze	
Visits to GP or 'out of hours' doctor with asthma/ wheeze	
Days off school due to asthma/wheeze	
Nights woken with asthma / wheeze (with or without colds) – approximate number	

Go to 6.13

6.12 At what age did your child last wheeze?

years ☐

6.13 In the last 12 months, has your child had a cough at night, apart from a cough associated with a cold or chest infection?

0. No
1. Yes ☐

6.14 Has your child ever been prescribed an asthma reliever inhaler?

0. No
1. Yes ☐

6.15 Did it help his/her breathing (wheezing or coughing) to improve?

0. No
1. Yes ☐

(QUESTIONS 7-9 ARE NOT INCLUDED HERE AS THEY WERE USED TO COLLECT TO NON-RESPIRATORY DATA)

10. MEDICATION

Now I would like to ask about medicines and other treatments your child has taken

Oral steroids

10.1 Has he/she ever taken Oral steroids for any condition? (e.g. Prednisolone)

0. No go to 10.5
1. Yes ☐

10.2 How many courses has he/she ever taken? ☐☐

10.3 How many courses has he/she taken in the last 12 months? ☐☐

10.4 How long ago did the last course finish? ☐ yrs ☐☐ mths ☐☐ wks

(Complete all 4 boxes above with 8s if the course is still on-going)

Antihistamines

10.5 Has he/she taken antihistamines in the last 12 months?
(e.g. Ketotifen, Loratidine, Piriton, Zirtek etc.) ☐

0. No go to 10.7
1. Yes

10.6 How often does he/she use these ?

1. All the time
2. During hayfever season only
3 Only occasionally ☐

Current/recent asthma or medication

10.7 In the past three months has he/she used any inhalers or antihistamines, or taken any medicines for asthma, or any chest symptoms

0. No go to 10.9
1. Yes ☐

10.8 Please ask the mother/carer for all those medicines that the child has taken and ask to see them if possible. Then fill in the table below, using the FFQ codes for how often they have been taken

Name of medicine	Medicine Code	Number of puffs/spoons /tablets/etc taken for each dose	How often does he/she take this dose? FFQ code 1-8	Number of times per day, if more than once a day
	☐☐☐☐	☐☐	☐	☐☐
	☐☐☐☐	☐☐	☐	☐☐
	☐☐☐☐	☐☐	☐	☐☐
	☐☐☐☐	☐☐	☐	☐☐

Has your child taken any other medications in the past three months? Please include both prescribed medicines and those bought over the counter. (Note: do not include vitamins or food supplements, but do include cough remedies, paracetamol etc).

0. No go section 11
1. Yes ☐

10.10 What medicines has he/she taken? (please specify)

Medicine 1_____

Medicine 2_____

Medicine 3_____

Medicine 4_____

14. RESPIRATORY SYMPTOMS ON DAY OF SPIROMETRY

14.1 Has your child had a cold in the last 3 weeks? ☐

0. No go to 14.4
1. Yes

14.2 Does he/she still have symptoms of the cold? ☐

0. No
1. Yes go to 14.4

14.3 How many days is it since he/she last had symptoms of the cold? ☐☐

14.4 Has your child coughed in the last 7 days? ☐

0. No go to 14.6
1. Yes

14.5 *What type of cough was it?

1. A cough that produced sputum
2. A cough that sounded "wet" but didn't produce sputum
0. A cough that sounded dry

*(may need to explain that we mean coughing something up from the chest)

14.6 Has your child wheezed in the last 7 days? ☐

0. No
1. Yes

14.7 Has your child used a bronchodilator (e.g. ventolin, bricanyl, salbutamol, terbutaline) in the last 12 hours? (Nurse: please note that many mothers will have said that their children do not use such medication in their answers to section 10. Be aware

of this but nonetheless please confirm prior to spirometry that there has been no bronchodilator use).

0. No go to section 15
1. Yes

14.8 How long ago was it used? ☐☐ hrs ☐☐ mins

(If less than four hours ago, do not do spirometry and go to section 16)

15. SPIROMETRY

Please record the room temperature . ☐ °C

Ask the mother/carer which ethnic group the child belongs to:

1. White
2. Black Caribbean
3. Black African
4. Black Other
5. Indian
6. Pakistani
7. Bangladeshi
8. Chinese
9. Other Asian group
10. Other (specify) _____

☐☐

Perform the spirometry on the laptop using the Koko incentive software.

16. CHILD EXAMINATION - ANTHROPOMETRY

16.1 Measurement Date dd ☐☐ mm ☐☐ yy ☐☐

16.2 Time (24 hr clock) ☐☐☐☐

16.3 Measurer ☐☐

16.4 Helpers (Parent = 90) ☐☐ ☐☐

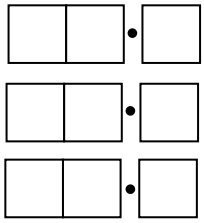
16.5 Which hand does the child write with?

2. Right
3. Left
4. Ambidextrous
5. Don't know

☐

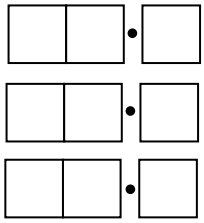
Mark up and measure the non-dominant arm and side of body. If ambidextrous or not known measure the left side

16.5 Occipito-frontal circumference



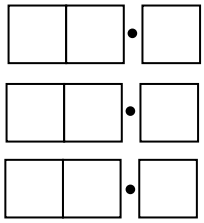
Wriggling (0 No, 1 Yes) ☐

16.6 Left mid-upper arm



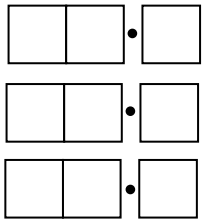
Wriggling (0 No, 1 Yes) ☐

16.7 Waist circumference



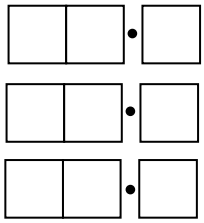
Wriggling (0 No, 1 Yes) ☐

16.8 Chest circumference



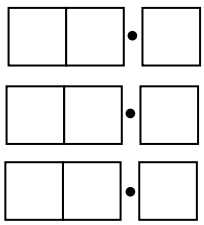
Wriggling (0 No, 1 Yes) ☐

16.9 Hip circumference



Wriggling (0 No, 1 Yes) ☐

16.10 Height (barefoot)



Wriggling (0 No, 1 Yes) ☐

16.11 Sitting height

cm

cm

cm

Wriggling (0 No, 1 Yes)

16.12 Stadiometer used

16.13 Child's weight (preferably in underwear only) kg

16.14 Approx weight of any clothes (except underwear) kg

16.15 Scales used

SKINFOLD THICKNESSES

16.16 Triceps skinfold

mm mm

mm mm

mm mm

Wriggling (0 No, 1 Yes)

16.16 Subscapular skinfold

mm mm

mm mm

mm mm

Wriggling (0 No, 1 Yes)

16.18 Skinfold calipers used

17. SKIN PRICK TESTING (performed on the child's arm)
(If the child has a food allergy or moderate/severe asthma, do not perform the skin prick testing at home)

17.1 Has your child had any antihistamine syrup in the last 7 days?

0. No
1. Yes

17.2

Skin Prick Test (av diameter)	mm
Cat	
Dog	
Egg	
Negative control	
Grass pollen mix	
House dust mite	
Milk	
Tree pollen mix	
Positive control	

(If there is no reaction please enter 0)

17.3 Skin prick tester

Appendix 4 Southampton Women's Survey six-year clinic visit questionnaire – respiratory data

6 Year

QUESTIONNAIRE

CLINIC VISIT

Mother's forename only: _____

Child's forename only: _____

[Nurse to refer to six-year visit record card to ensure child's name is correct, and record any changes thereon. Also to request additional telephone numbers, email addresses etc, for tracing purposes if family move]

Child's date of birth dd ☐☐ mm ☐☐ yy ☐☐

Sex M=Male F=Female ☐

Date of interview dd ☐☐ mm ☐☐ yy ☐☐

Interviewer ☐☐

Discuss the visit with the mother and child and obtain completed consent and assent forms

To be completed by the nurse if the mother was not the person interviewed:

Why was the mother not available? ☐

5. Has left the family home
6. Still lives in family home, but was unavailable for interview
7. Has died
8. Is ill or in hospital
8. Other, specify _____
10. Don't know

Who was interviewed? ☐

7. Study child's father
8. Mother's partner (if not father)
9. Study child's grandparent
10. Other family member
11. Mother "figure" (e.g. father's partner/step-mother)
12. Family friend
8. Other, specify _____

1 RESPIRATORY SYMPTOMS ON DAY OF HOSPITAL VISIT

1.1 Has your child had a cold in the last 3 weeks? ☐

0. No go to 1.3
1. Yes

1.2 Does he/she still have symptoms of the cold? ☐

0. No
1. Yes go to 1.4

1.3 How many days is it since he/she last had symptoms of the cold? ☐☐

1.4 Has your child coughed in the last 7 days? ☐

0. No go to 1.5
1. Yes

1.5 What type of cough was it?

2. A cough that produced sputum
3. A cough that sounded "wet" but didn't produce sputum
4. A cough that sounded dry

1.6 Has your child wheezed in the last 7 days? ☐

0. No
1. Yes

1.7 Has your child used a bronchodilator (e.g. ventolin, bricanyl, salbutamol, terbutaline) in the last 12 hours? ☐

0. No
1. Yes

1.8 How long ago was it used? ☐☐ hrs ☐☐ mins

Clinical Investigations
Respiratory rate (bpm): _____
SaO2 (%): _____
Blood pressure (mmHg): _____
Mean resting HR (bpm): _____

SKIN PRICK TESTING To be performed in clinic if not done at the home visit

1.9 Has your child had any antihistamine syrup in the last 7 days?

0. No
1. Yes ☐

Skin Prick Test (av diameter)	mm
Cat	
Dog	
Egg	
Negative Control	
Grass pollen mix	
House dust mite	
Milk	
Tree pollen mix	
Positive Control	

Skin prick tester ☐☐

METHACHOLINE CHALLENGE

Nurse ☐☐

Doctor Supervising ☐☐

If methacholine not done give reason:

1. Child or parent declined
2. FEV_1 <75%
3. Poor spirometry technique
4. Methacholine not available
5. Paediatrician not available
6. Other (specify) ☐

SALBUTAMOL REVERSIBILITY

Nurse ☐☐

If reversibility not done give reason:

1. Methacholine challenge performed
2. Child or parent declined
3. Poor spirometry technique
4. Drugs or equipment not available
5. Other (specify) ☐

EXHALED NITRIC OXIDE

If exhaled nitric oxide not done give reason:

1. Child or parent declined
2. Staff or machine not available
3. Other (specify)

☐☐☐ ppb ☐☐☐ ppb ☐☐☐ ppb

Appendix 5 Food frequency questionnaire

FOOD FREQUENCY QUESTIONNAIRE

2: DIETARY QUESTIONS

2.1 Now I am going to ask you about the foods you have eaten over the past 3 months. To do this I have a list of foods and I would like you to tell me how often you have eaten each food. As before the list may include foods you <u>never</u> ate or you may find foods which you eat a lot are missing. These can be added on at the end. *(Define the 3 month period)*

	FOOD DESCRIPTION	FREQUENCY EATEN							
FOOD CODE		Never	Once every 2-3 Months	Once a Month	Once a Fortnight	1-2 Times per Week	3-6 Times per Week	Once a day	More than once a day
1	White Bread	1	2	3	4	5	6	7	
	When you ate bread/toast/sandwiches, how many slices/rolls did you eat at a typical meal? ☐☐·☐ *Rolls (count as 2 slices)* *French bread (2" counts as 1 slice)*								
2	Brown and wholemeal bread/rolls	1	2	3	4	5	6	7	
	How many slices/rolls did you eat at a typical meal? ☐☐·☐ *Rolls (count as 2 slices)*								

3	Crackers and cheese biscuits	1	2	3	4	5	6	7	
4	Wholemeal and rye crackers	1	2	3	4	5	6	7	
5	'Bran' breakfast cereals	1	2	3	4	5	6	7	
6	Other breakfast cereals	1	2	3	4	5	6	7	
7	Added bran to foods	1	2	3	4	5	6	7	
8	Cakes and gateaux	1	2	3	4	5	6	7	
9	Buns	1	2	3	4	5	6	7	
10	Pastries	1	2	3	4	5	6	7	

11	Biscuits-chocolate, digestive and ginger	1	2	3	4	5	6	7	
12	Other biscuits	1	2	3	4	5	6	7	
13	Fruit puddings	1	2	3	4	5	6	7	
14	Milk based puddings and sauces	1	2	3	4	5	6	7	
15	Other puddings	1	2	3	4	5	6	7	
16	Yogurt and fruit fools	1	2	3	4	5	6	7	
17	Potatoes – boiled and jacket	1	2	3	4	5	6	7	
	When you ate these how many potatoes did you eat at a typical meal? ☐☐.☐ *Large baking (count as 3)/new (count as 0.5)*								
18	Roast potatoes and chips	1	2	3	4	5	6	7	

	When you ate these how many potatoes did you eat at a typical meal? ☐☐.☐								
19	Yorkshire puddings and savoury pancakes	1	2	3	4	5	6	7	
20	Brown and white rice	1	2	3	4	5	6	7	
21	Pasta and dumplings	1	2	3	4	5	6	7	
22	Tinned vegetables	1	2	3	4	5	6	7	
23	Peas and green beans	1	2	3	4	5	6	7	
24	Carrots	1	2	3	4	5	6	7	
25	Parsnips, swede and turnip	1	2	3	4	5	6	7	
26	Sweetcorn and mixed veg	1	2	3	4	5	6	7	

27	Beans and pulses	1	2	3	4	5	6	7	
28	Tomatoes	1	2	3	4	5	6	7	
29	Spinach	1	2	3	4	5	6	7	
30	Broccoli, Brussels sprouts and spring greens	1	2	3	4	5	6	7	
31	Cabbage and cauliflower	1	2	3	4	5	6	7	
32	Peppers and watercress	1	2	3	4	5	6	7	
33	Onion	1	2	3	4	5	6	7	
34	Green salad	1	2	3	4	5	6	7	
35	Side salads in dressing	1	2	3	4	5	6	7	

36	Courgettes, marrow and leeks	1	2	3	4	5	6	7	
37	Mushrooms	1	2	3	4	5	6	7	
38	Vegetable dishes	1	2	3	4	5	6	7	
39	Vegetarian foods	1	2	3	4	5	6	7	
40	Tinned fruit not including grapefruit, prunes, figs or blackcurrants	1	2	3	4	5	6	7	
41	Cooked fruit not including blackcurrants	1	2	3	4	5	6	7	
42	Dried fruit	1	2	3	4	5	6	7	
43	Fresh apples and pears	1	2	3	4	5	6	7	
44	Fresh oranges and orange juice	1	2	3	4	5	6	7	

45	Grapefruit and grapefruit juice	1	2	3	4	5	6	7	
46	Blackcurrants, ribena and hi-juice blackcurrant drinks	1	2	3	4	5	6	7	
47	Other fruit juices (not squashes)	1	2	3	4	5	6	7	
48	Diet Coke and Pepsi not including caffeine free	1	2	3	4	5	6	7	
49	Coke and Pepsi	1	2	3	4	5	6	7	
50	Soft drinks not including diet drinks (low calorie or low sugar)	1	2	3	4	5	6	7	
51	Bananas	1	2	3	4	5	6	7	
52	Fresh peaches, plums, cherries and grapes	1	2	3	4	5	6	7	
53	Strawberries and raspberries	1	2	3	4	5	6	7	

54	Fresh pineapple, melon, kiwi and other tropical fruits	1	2	3	4	5	6	7	
55	Nuts	1	2	3	4	5	6	7	
56	Bacon and gammon	1	2	3	4	5	6	7	
57	Pork	1	2	3	4	5	6	7	
58	Chicken and turkey	1	2	3	4	5	6	7	
59	Lamb	1	2	3	4	5	6	7	
60	Beef	1	2	3	4	5	6	7	
61	Minced meat dishes	1	2	3	4	5	6	7	
62	Meat Pies	1	2	3	4	5	6	7	

63	Liver and kidney	1	2	3	4	5	6	7	
64	Paté and liver sausage	1	2	3	4	5	6	7	
65	Faggots and black pudding	1	2	3	4	5	6	7	
66	Sausages	1	2	3	4	5	6	7	
67	Ham and luncheon meat	1	2	3	4	5	6	7	
68	White fish	1	2	3	4	5	6	7	
69	Fish fingers and fish dishes	1	2	3	4	5	6	7	
70	Oily fish	1	2	3	4	5	6	7	
71	Shellfish	1	2	3	4	5	6	7	

72	Boiled and poached eggs	1	2	3	4	5	6	7	
73	Omelette and fried eggs	1	2	3	4	5	6	7	
74	Cottage Cheese	1	2	3	4	5	6	7	
75	Cheese	1	2	3	4	5	6	7	
76	Pizza, quiches and cheese flans	1	2	3	4	5	6	7	
77	Soup	1	2	3	4	5	6	7	
78	Mayonnaise and salad cream	1	2	3	4	5	6	7	
79	Pickles, chutney, tomato ketchup and brown sauce	1	2	3	4	5	6	7	
80	Chocolate	1	2	3	4	5	6	7	

81	Other sweets	1	2	3	4	5	6	7	
82	Ice cream and chocolate desserts	1	2	3	4	5	6	7	
83	Cream	1	2	3	4	5	6	7	
84	Crisps and savoury snacks	1	2	3	4	5	6	7	
85	Sweet spreads	1	2	3	4	5	6	7	
86A	Gravy granules and powders	1	2	3	4	5	6	7	
86B	Stock cubes and Marmite	1	2	3	4	5	6	7	
87	Drinking chocolate and milk shakes not including McDonald style milkshakes	1	2	3	4	5	6	7	
88	Decaffeinated coffee and tea	1	2	3	4	5	6	7	

89	Tea	1	2	3	4	5	6	7	
90	Coffee	1	2	3	4	5	6	7	
93	Spreading fat (1) _____ F	1	2	3	4	5	6	7	
94	Spreading fat (2) _____ F	1	2	3	4	5	6	7	
95	Spreading fat (3) _____ F	1	2	3	4	5	6	7	
96	Frying fat or oil (1) _____ F	1	2	3	4	5	6	7	
97	Frying fat or oil (2) _____ F	1	2	3	4	5	6	7	
98	Frying fat or oil (3) _____ F	1	2	3	4	5	6	7	
99	Other vegetable oil (1) e.g. salad dressings, _____ F marinades	1	2	3	4	5	6	7	

100	Other vegetable oil (2) e.g. salad dressings, _____ marinades	F	1	2	3	4	5	6	7	

2.2 Are there food or drinks which you have eaten or drunk **once a week or more** which are not on the list? Include breakfast bars such as Nutrigrain and Kellogg's

0. No/1. Yes

If Yes

Name of food/drink	1-2 times per week	3-6 times per week	Once a day	More than once a day

Now I would like to ask in more detail about some specific foods

2.3: Which types of milk have you used regularly in drinks and added to breakfast cereals over the last 3 months?

0. None
1. Whole pasteurised
2. Semi-skimmed pasteurised
3. Skimmed pasteurised
4. Whole UHT
5. Semi-skimmed UHT
6. Skimmed UHT
7. Other

Milk 1 ☐ Other (specify)_____

Milk 2 ☐ Other (specify)_____

Milk 3 ☐ Other (specify)_____

2.4 On average over the last 3 months how much of each milk have you consumed per day?

Milk 1 ☐ · ☐☐ pints

Milk 2 ☐ · ☐☐ pints

Milk 3 ☐ · ☐☐ pints

2.5 Have you added sugar to breakfast cereals, tea & coffee, puddings etc.?

0. No *go to 2.7* ☐
1. Yes

2.6 Approximately how many teaspoons of sugar have you added each day? ☐☐

2.7 When you eat meat, how much of the fat have you usually cut off (including chicken skin)?

1. all 100%
2. most 60%
3. some 30%
4. none 0%
9. not applicable

☐

2.8 Just thinking about the **past week** how many servings did you eat of:

Vegetables and vegetable-containing dishes (excluding potatoes)?	
fruit and pure fruit juices?	
meat and fish and their dishes?	

3: FOOD SUPPLEMENTS & DIETARY CHANGES

3.1 During the past three months have you taken any pills, tonics or tablets to supplement your diet? (e.g. vitamins, minerals, iron tablets, folic acid, fish oils etc.)

0. No
1. Yes

☐

If yes, please state which:
(for number per day, record number of tablets/capsules/teaspoons per day, as appropriate)

Supplement	Number per day	How many days in the last 90?	Did you start taking this: 1: Less than 1 month ago 2: 1-2 months ago 3: More than 2 months ago
☐☐☐☐			
☐☐☐☐			
☐☐☐☐			
☐☐☐☐			
☐☐☐☐			

Appendix 6 Maternal anthropometry protocol

MRC Epidemiology Research Centre

ANTHROPOMETRIC MEASUREMENT PROTOCOL

Author: S Duggleby

Height

Place the base-plate on the floor, selecting as firm and level a surface as possible, and preferably near a perpendicular, such as a door architrave, which helps the eye to ensure that the tape is vertical. Ask the subject to remove her shoes and stand on the base-plate with her back to the tape. She should be told to stand as tall and straight as possible with feet together and arms held loosely at the side and shoulders relaxed (to avoid lordosis). She should stand far enough forward on the base-plate such that the tape is not distorted when pulled to vertical. Check that the tape is inserted correctly into the base plate. Raise the tape vertically and place the head plate on the top of the subject's head, using the spirit level to check that the plate is horizontal.

The head should be placed in the Frankfurt Plane, such that an imaginary line joining the upper margin of the external auditory meatus and the lower border of the orbit of the eye is horizontal.

If a short person is measuring a very tall person, considerable error can be introduced when reading off the height scale: their eyes will read the scale at an angle (parallax effect). Measurers should be aware of this and aim to read the scale from as level a position as possible. If there is a lot of height disparity the measurer should try to get level with the scale, by standing on, for example, telephone directories. Read the height to the nearest 0.1cm and beware of digit preference. Make one measurement of height.

Weight

Take time to place the weighing scales on a level surface. Ensure the scales are at zero when they are switched on. Weigh without shoes. Aim for subject to be wearing shirt and trousers/skirt only, and to remove heavy items of clothing and heavy jewellery if possible. Weight to nearest 0.1 kg. Make one measurement of weight.

Mid upper arm circumference

The subject stands with her back to the measurer, arms hanging by her sides. The tip of the acromion (the point of the shoulder) is palpated and marked. Then with the subject's arm flexed at 90°, the olecranon (tip of the elbow) is palpated. Put the tape measure on the mark on the shoulder and drop it down to the tip of the elbow, by the side of the arm. Read the exact distance as if you had drawn an imaginary horizontal line from the bottom most point of the elbow to your tape measure. Mark a point on the arm halfway between the acromion and olecranon. This marks the vertical level at which the circumference will be measured. It is important that this measurement is made with the arm flexed, otherwise the tape takes an oblique course across the upper arm, and the mid-point is too high up. The subject is then asked to relax, with the arm hanging by her side. This is important as a very different reading may be obtained if the arm is not fully relaxed. The tape is placed around the upper arm, with the upper border of the tape on the mark. **Ensure tape is horizontal all round.** Make sure the tape is not pulled too tight: it should rest on the skin, but not indent it. Read the tape to the nearest 0.1 cm and beware of digit preference. Make one measurement of mid upper arm circumference.

Skinfolds

We measure four standard skinfolds sites: triceps, biceps, subscapular and upper suprailiac. These sites were selected from 93 sites originally assessed (Edwards 1950). They are easily located in relation to bony landmarks, the skinfold can be raised from underlying tissue and measurements are reproducible.

Apparatus

We use the Harpenden 'John Bull' calipers with external springs. They should be kept and transported within their plastic case. The blades of the calipers are 90 mm and open to 50 mm. The large dial reads up to 20 mm, and a smaller scale on the dial registers whether you have already gone once or twice around the scale. They exert a constant pressure of 10 g/mm. Divisions on the dial are every 0.2 mm, but it is usually possible to read to the nearest 0.1 mm. There is a screw adjuster on the side of the dial. Loosening this screw allows you to move the dial face relative to the needle, so that you can adjust the instrument to 0 with the blades closed. However, you should only let the appointed person for your study adjust the calipers.

Calipers can be tested and calibrated using machined metal blocks to check the readings once a month. They can be sent back to the manufacturers to be calibrated, but this is costly and should not be needed frequently if they are treated carefully.

Technique

There is no international consensus as to which side of the body should be used for skinfold measurements. Some use the non-dominant side; others always use the right or the left (Martorell *et al.* 1988). There is no statistically significant difference between measurements made on different sides of the body, even when there are considerable differences in muscularity, as in a tennis player (Wormersley & Durnin 1973; Gwinup *et al.* 1971). However, as we are interested in muscularity and will be measuring mid upper arm circumference, **we make all measurements on the non-dominant side**

For the technique of picking up a skinfold I quote from Noel Cameron: " the skinfold is often described as a 'pinch', but the action to obtain it is to sweep the index or middle finger and thumb together over the surface of the skin to collect the subcutaneous tissue pushed away from the underlying muscle fascia by this action. To 'pinch' the object suggests a very small and painful pincer movement of the fingers, and this is not the movement made. Firstly, the measurement of skinfolds should not cause undue pain to the subject…Secondly, a pincer or pinching action does not collect the quantity of subcutaneous tissue normally measured" (Cameron 1978). It is easier to use both hands initial, to massage up a 'tube' of skin with thumb and fingers of both hands. One hand then remains holding the skinfold throughout the measurement of the skinfold, and the measurer picks up and uses the calipers with the other hand.

The positioning of the blades of the calipers on the skinfold will vary with the size of the fold of skin, but in general should be at least one blade-breadth in from the apex of the skinfold. Be careful not to twist the calipers whilst striving to read the dial.

There are different techniques for timing the readings. Some say you should take the reading after 2, 4 or 5 seconds after closing the blades. Others think that you should wait until the needle on the dial has stopped moving (Cameron 1978; Fidanza 1991; Harrison *et al.* 1988; Garrow 1993). Experienced measurers say they have no difficulty counting 2 seconds in a reproducible way, but there is no doubt that at 2 seconds you are often trying

to read a rapidly moving target, and this is likely to produce differences between measurers. **We therefore use the 5 seconds rule** (Garrow 1993).

By doing this we measure compressed fat thicknesses. This may be important as people vary in the compressibility of their fat. Female fat is more compressible than male fat. However, it is important that we follow the same convention.

The calipers should be released fully before counting to 5. This is particularly important if a measurer has small hands because it is possible that some pressure will be maintained on the lever of the calipers not allowing them to exert full pressure. The dial should be read at 5 seconds even if it is still moving.

Do not drag the calipers off the fold at the end of the measurement, as this is uncomfortable and may damage the calipers. Consciously open the jaws to remove them.

Generally at least three measurements are taken at each site, releasing the skinfold and picking it up again each time. Some people keep measuring until three readings very close together are obtained. Some people use the average of three readings; others use the minimum or the maximum!

We make three readings and use the average. In some studies, the computer analyses the three values and decides if they match closely enough. If they don't, you may have to make two further readings. In general we aim to obtain 3 readings within 10% of one another within a maximum total of 5 readings.

The technique and bony landmarks used are the same in men and women.

Triceps skinfold (Cameron 1978; Fidanza 1991; Harrison *et al.* 1988)
The subject stands with their back to the measurer, arms hanging by their sides. The tip of the acromium (the point of the shoulder) is palpated and marked. With the subject's arm flexed at 90°, the olecranon (tip of the elbow) is palpated. Put the tape measure on the mark on the acromium and drop it down to the elbow, by the side of the arm. Read the exact distance as if you had drawn an imaginary horizontal line from the bottom most point of the elbow to your tape measure. Mark a point on the arm halfway between the

acrominon and elbow. This marks the vertical level at which the circumference will be measured. It is important that this measurement is made with the arm flexed, otherwise the tape takes an oblique course across the upper arm, and the midpoint is too high up. The subject is then asked to relax, with the arm hanging by their side. This is important as a very different reading may be obtained if the arm is not fully relaxed.

The tape is placed around the upper arm with the upper border of the tape at the level of the mark, as if to measure mid-upper arm circumference. With the tape in position a horizontal line is drawn on the skin posteriorly and anteriorly at the level of the first mark. The posterior line is used for the triceps fold and the anterior line for the biceps fold. To determine the side-to-side position at which the skinfold is measured, you must 'eyeball' the mid-point and the most dorsal (i.e. the part which sticks out furthest posteriorly) part of the arm at the level of your horizontal mark. Make a vertical mark to form a cross.

The skinfold is picked up in a vertical 'tube' with two hands, at least 1 cm above and below the cross. The skinfold calipers are applied at the level of the cross, with the cross on the apex of the fold.

It has been shown that the precise site is important, and that very different readings can be obtained, especially by displacement laterally and especially in obese subjects.

Biceps skinfold (Cameron 1978; Fidanza 1991)
The subject faces the measurer with their arms hanging down and the (non-dominant side) palm facing forward. An anterior horizontal line already marks the level at which the skinfold will be measured. As with the triceps skinfold, you need to 'eyeball' the point along this line where the arm bilges forward the most – the mid point of the belly of the biceps muscle. Mark a vertical line here to form a cross. There is sometimes a prominent blood vessel here, but you can ignore it, it will not be damaged by the calipers. The skinfold is picked up vertically and he calipers are applied at the level of the cross, with the cross on the apex of the fold.

Subscapular skinfold (Cameron 1978; Fidanza 1991; Harrison *et al.* 1988; Tanner *et al.* 1989)

The subject stands with the shoulders and arms relaxed. The lowermost tip of the scapula is identified. This is easy in slim subjects but may be difficult in the obese. It may help to follow the medial border of the scapula downwards until the inferior angle is felt. Alternatively, you can make the scapula stand out by asking the subject to put their arm behind their back, in a half-nelson. Once it is located, however, the subject must relax their arm again before you mark the skin with a cross, immediately below the lowermost tip of the scapula. The skinfold is picked up obliquely, in the natural cleavage of the skin and the calipers are applied at the level of the cross, with the cross on the apex of the fold.

Upper suprailiac skinfold (Fidanza 1991; Harrison *et al.* 1988)

Stand behind the subject. They should stand straight and relaxed with their arms folded in front of them. Locate the iliac crest, the large curving pelvic bone, just below the waist. In obese subjects you need to palpate firmly, and in all subjects, it helps if you feel both sides together. Draw a horizontal line just above the crest at the side. Next find the mid axillary line: ask the subject to lift up their arm. The apex of the axilla is the lowest point in the axillary 'hollow', just behind the thick fold made by the pectoral muscle. Drop an imaginary vertical line down from the apex of the axilla; this is the mid axillary line. Draw a line where this imaginary vertical line meets the horizontal line. Pick up a fold in the natural creases of skin and apply the calipers at the level of the cross, with the cross on the apex of the fold. It may help to ask the subject to tilt towards you to ease the tension on the skin while picking up the skinfold.

References

Cameron, N. 1978, The methods of auxological anthropometry. In: Falkner, F. & Tanner , J.M., eds. *Human growth*. London, Bailliere Tindall pp.35-90.

Edwards, D.A.W. 1950, Observations of the distribution of subcutaneous fat. *Clinical Science*. 9:259-270.

Fidanza, F. 1991, Anthropometric methodology. In: Fidanza F., ed. *Nutritional status assessment*. London, Chapman & Hall pp.1-62.

Garrow, J.S. 1993, Composition of the body. In: Garrow, J.S. & James, W.P.T., eds. *Human nutrition and dietetics*. New York, Churchill Livingstone pp.12-23.

Gwinup, G., Chelvam, R. & Steinberg, T. 1971, Thickness of subcutaneous fat and activity of underlying muscles. *Annals of Internal Medicine*. 74:408-411.

Harrison, G.G., Buskirk, E.R., Carter, J.E.L., Johnstone, F.E., Lohman, T.G., Pollock, M.L., *et al.* 1988, Skinfold thicknesses and measurement technique. In: Lohman, T.G., Roche, A.F., Martorell, R.., eds *Anthropometric standardization reference manual.* Champaign, Illinois: Human Kinetics Books pp.55-70.

Martorell, R.., Mendoza, F., Mueller, W.H., Pawson, I.G. 1988, Which side to measure: right or left? In: Lohman, T.G., Roche, A.F., Martorell, R., eds *Anthropometric standardization reference manual.* Champaign, Illinois: Human Kinetics Books pp.87-91.

Tanner, J.M., Hiernaux, J. & Jarman, S. 1989 Growth and physique studies. In: Weiner, J.S., Lourie, J.A., eds. *Human biology: a guide to field methods.* Oxford: Blackwell Scientific Publications pp.1-16.

Wormersley, J. & Durnin, J.V.G.A. 1973, An experimental study on variability of measurements of skinfold thickness on young adults. *Human Biology.* 45:281-292.

Appendix 7 Fetal ultrasound scan protocol

MRC Epidemiology Resource Centre

PROTOCOL FOR ANTHROPOMETRIC MEASUREMENTS

Author: P Mahon

All women participating in the Southampton Women's Survey who become pregnant will be offered ultrasound scans at 11, 19 and 34 weeks of gestation. The following measurements will be taken:

1) Crown-rump Length (CRL)
2) Bi-parietal Diameter (BPD)
3) Head Circumference (HC)
4) Femur Length (FL)
5) Abdominal Circumference (AC)
6) Thoracic Circumference (TC)

The following Ultrasound machines, calibrated to 1540 m/s will be used:

a) ACUSON 128 XP using the following multi hertz transducers:

C7 curvi-linear with 7 and 5 MHz frequencies,

C3 curvi -linear with 3.5 and 2.5 Mhz frequencies.

b) ACUSON ASPEN & SEQUIOA using the following transducers;

C7 curvi-linear with 7, 5 and 4 MHz frequencies,

C3 curvi-linear with 4, 3.5 and 2.5 MHz frequencies.

CRITERIA FOR BIOMETRY:

1) Crown Rump Length (11 wks)

The embryo can be visualised from 6 weeks postmenstrual age transabdominally. Correctly performed measurements of the CRL are the most accurate at estimating gestational age, as the fetus grows rapidly at this stage. However, the CRL depends on the operator's ability to obtain a true, unflexed, longitudinal section of the fetus with the end points clearly defined. Owing to fetal movement there can be no standardised technique. A longitudinal section of the uterus and gestation sac should be identified. Once the fetus has been identified, the transducer should be rotated until the long axis of the fetus is obtained. A measurement is then taken from the frozen image, from the

top of the head (crown) to the end of the trunk (rump), using the callipers. The Ultrasound machine then calculates the distance between the two callipers. The yolk sac should not be included in the measurement, as this will artefactually increase the gestational age. From 9 weeks the fetal spine can be identified and therefore any degree of flexion can be assessed. Any degree of flexion of the fetal spine will underestimate the CRL. With increasing gestational age the fetus is likely to be in a flexed position, and therefore it is likely to be inaccurate after 12 weeks postmenstrual age. (Chudleigh & Pearce 1992, Dewbury *et al.* 1993)

2) Bi-parietal Diameter (11, 19 & 34 wks)

The BPD measurement is a linear one, with well-defined landmarks for reproducibility. To measure the BPD a longitudinal section of the fetus with the spine or aorta is obtained. The transducer is then moved cranially so that the head and neck are visualised, and then rotated through 90 degrees keeping the same angle of asynclitism. The transducer is then moved up and down until the correct transverse section is obtained. The correct section is at the level of the fetal head where the cavum septum pellucidum (csp) breaks the midline echo, approximately one third of the way from the anterior border of the skull (Campbell *et al.* 1977). The BPD is the maximum diameter of the transverse section of the fetal skull, at the level of the csp, with the callipers placed on the outer aspect of the proximal skull surface and the inner aspect of the distal skull surface (outer-inner), at 90 degrees to the midline.

3) Head Circumference (11, 19 & 34 wks)

The same section as the BPD is used. The first calliper is placed on the outer aspect of the skull at the occiput and the second calliper on the outer aspect of the skull at the sinciput. Then using the ellipse key on the ultrasound machine the Head Circumference is measured and the machine calculates the circumference.

4) Femur Length (19 & 34 wks)

The femur length is also an accurate linear measurement. It is easily located. A cross section of the fetal abdomen is obtained and then the transducer is moved caudally, so that the iliac bones are seen. The transducer is than rotated until the full length of the femur has come into view and both ends are clearly seen. The measurement of the

femur is then taken from the centre of the 'U' shape at each end of the bone, which represents the length of the diaphysis (Chudleigh & Pearce 1992).

5) Abdominal circumference (11, 19 & 34 wks)

The section used for measuring the above should have the following features;
The outline is circular. There should be a short length of umbilical vein. This should be imaged so that it is centrally placed between the lateral abdominal walls and is a third of the way along an imaginary line drawn from the anterior abdominal wall to the fetal spine. The stomach is usually visualised as a transonic area in the left side of the abdomen (Chudleigh & Pearce 1992). A longitudinal length of the fetus is obtained. The transducer should then be moved until the fetal aorta is visualised from the fetal chest and through the abdomen. The transducer should then be rotated through 90 degrees at the level of the fetal stomach to obtain a cross-section. The transducer is then moved until the correct section is obtained as described above. The ellipse method will then be used to measure the circumference.

6) Thoracic Circumference (19 & 34 wks)

A transverse view of the fetal thorax is obtained and then the transducer is moved caudally or cranially until a section containing the 4-chamber heart view is obtained. The section is then slightly rotated to ensure that the section was 90 degrees to the spine. The image is then frozen and using the 'trace' mechanism, callipers are then placed on the edge of the skin surface and a circumference is drawn around the fetal thorax (Roberts & Mitchell, 1990).

References

Campbell, S. & Thoms, A. 1977, Ultrasound measurement of the fetal head to abdomen circumference ratio in the assessment of growth retardation, *British. Journal of Obstetrics & Gynaecol*ogy. 84(3):165-174.

Chudleigh, P. & Pearce, J. 1992, *Obstetric Ultrasound* 2nd edition Churchill Livingstone, Edinburgh.

Roberts, A.B. & Mitchell, J.M. 1990, Direct ultrasonographic measurement of fetal lung length in normal pregnancies and pregnancies complicated by prolonged rupture of membranes. *American Journal of Obstetrics & Gynecology.* 163:1560–6.

Dewbury, K., Meire, H., Cosgrove, D. & Goldberg, B., eds. 1993, *Clinical Ultrasound : a comprehensive text : Ultrasound in obstetrics and gynaecology.* Churchill Livingstone. Edinburgh.

Appendix 8 Height measurement protocol

Wellcome Trust Clinical Research Facility, Southampton

MEASURING CHILDREN'S HEIGHT PROTOCOL

Authors: Sr. R King & SN J Trewin

- Only RGN/RSCN or RN Child assessed as competent may measure children
- Check and calibrate stadiometer annually using manufacturer's guidelines

1. Explain procedure to child and parent(s). Explain to the child you want them to stand as tall and straight as possible.

2. Child to wear light clothing.

3. Take child's shoes off so that the measurer can see that heels are in the correct position.

4. Undo or adjust hairstyles and remove hair accessories that interfere with measurement.

5. Ask the child to stand on the stadiometer; facing forwards, tall and straight, arms hanging loosely at their sides.

6. Child's feet to be positioned together and flat, their heels to touch back plate. Child's knees to be straight, buttocks and shoulders to touch stadiometer, but the child should not lean against it.

7. Position the head in 'Frankfort plane', child to look straight ahead, parallel to the floor.

8. To ensure best position is achieved: explain to the child, what you are about to do.

9. Cup the child's head in your hands, placing the heels of your palms either side of the face. Firmly but gently, apply upward pressure lifting the child's head to

their maximum height. Perform the procedure smoothly and take care not to tilt the head at an angle.

10. Check for any bending of the knees, slumping of shoulders or raising of heels.

11. Lower headpiece of the stadiometer lightly onto the crown of the child's head.

12. Ask child to take a deep breath in, let it out (shoulders will relax) and then read the measurement whilst still holding chin.

13. Measurer's eyes should be level with counter/pointer and measurement read to the nearest millimetre. Record the measurement.

14. The child should be able to step off the stadiometer without ducking their head.

Bibliography

Department of Health 1996, *Health Survey for England.*

United Nations 1986, How to Weigh and Measure Children: *Assessing the Nutritional Status of Young Children in Household Surveys.*

Appendix 9 Skin prick testing protocol

Southampton General Hospital

SKIN PRICK TESTING PROTOCOL

Authors: Sr. D Keeton & Sr. R King

Equipment

- Appropriate allergens/substances being tested
  (check manufacturer's expiry date and once opened the date must be written on the bottle and the contents must be used within 6 months)
- Tissues
- Skin prick testing lancets
- Pen
- Timer
- Skin test reaction gauge
- Documentation sheet
- Sharps box
- Appropriate emergency equipment and rescue medication must be available

Allergens

- Negative control (50% glycerin) 806ED Hollister-Stier, Spokane, WA
- Grass mix #7 Kentucky Bluegrass, Orchard, Redtop, Timothy, Sweet Vernalgrass, Meadow fescue, Perennial Ryegrass 0850 Hollister-Stier 10 000 BAU/ml
- Cat hair 4815TR Hollister-Stier 10 000 BAU/ml
- Dog 408ED Hollister-Stier
- Dermatophagoides pteronyssinus 6692UP Hollister-Stier 30 000 AU/ml
- Cows milk (whole) 3390ED Hollister-Stier
- 145 Whole egg Alyostal
- ALK 197 Three tree Alunus Betula Corylus ALK Abelló Hørsholm, Denmark
- ALK 001 Histamine dihydrochloride (positive control)

Procedure

1. The SWS 6 year Respiratory Follow-up information leaflet should be given to the family before the test and time allowed to ask questions to ensure accurate information is given and parents are able to give informed verbal consent.

2. Gather equipment required to prevent unnecessary delays.

3. If taking antihistamines, check when last taken. Do not proceed if there is a chance that recent antihistamine medication will interfere with the test.

4. The nurse must wash her hands prior to commencing the procedure, and also once the procedure has been completed. The nurse must also risk assess the need for wearing protective clothing and/or gloves during the procedure.

5. The nurse administering the procedure to select an appropriate site on the forearm for the skin test, according to the child's preference and skin condition. The test should only be performed on clear, eczema-free skin where topical steroids and emollients have not been applied.

6. The site chosen should not be cleaned with antiseptics or alcohol.

7. Mark the skin with the initial letter of each allergen being tested. Each site should be a minimum of 2 cm apart.

8. Place one drop of each allergen solution in line with its marked place on the skin.

9. Push the lancet through the drop of allergen and apply the lancet at 90° to the skin without drawing blood. The lancet should then be immediately discarded into the sharps bin.

10. Repeat the procedure for each allergen and the controls using a new lancet for each allergen.

11. Carefully remove the surplus fluid from all sites simultaneously by placing a paper tissue over the drops. Take care not to cross contaminate the sites with other allergen solutions.

12. The results should be read 15 minutes after the positive was completed. The measurements are taken using skin test reaction gauge, measuring the wheal not including the flare, in two planes 90° to each other. Record the mean of these two measurements.

13. A wheal measuring at least 3mm in size and equal to or greater than the positive control is considered to be a positive result. A wheal diameter recorded following the negative control solution indicates that the child may suffer from dermographism (the skin is reacting to pressure rather than the solution) or is sensitive to the stabilisers in the allergen solutions and so invalidates the test. A negative reaction to the positive control allergen indicates that the child may have taken some antihistamine or has had some topical application that is preventing the skin from reacting and so invalidates the test

14. Calamine lotion can be applied after the test if the child complains of irritation.

Bibliography

Dreborg, S. & Frew, *A.* 1992, *Position Paper: Allergen Standardisation and Skin Tests* EAACI Subcommittee on Skin Tests.

Dreborg, S. 2001, Skin Testing in Allergen Standardisation and Research *Immunology & Allergy Clinics of North America.* 21(2):329-354.

Mygind, N. 1996, Skin Testing In: Mygind, N., Dahl, R. & Thestrup-Pederson, K., eds. *Essential Allergy* Blackwell, Oxford. pp.111-114.

Saarinen, J.V., Harvima, R.J., Horsmanheimo & M., Harvima, J.T., 2001, Modulation of the immediate allergic wheal reaction in the skin by drugs inhibiting the effects of leukotriene C4 and prostaglandin D2. *European Journal of Clinical Pharmacology.* 57: 1 – 4.

Appendix 10 Spirometry protocol

Wellcome Trust Clinical Research Facility, Southampton

SPIROMETRY PROTOCOL

Authors: Dr G Roberts, Sr. R King & SN J Trewin

Equipment Required

- Koko trolley with computer, isolation transformer & printer
- Clean mouth filter – Koko
- Koko spirometer
- Peak flow meter
- 3L Koko calibration tube and calibration mouthpiece

Information Required

- Volunteer's date of birth
- Volunteer study number
- Height
- Medication details

Rescue Medication

- Spacer - volunteer's own if available
- Bronchodilator-volunteer's own if available
- Sabutamol for nebuliser
- Acorn for sidestream nebuliser
- Oxygen supply nearby

Before testing

- N.B. Check oxygen supply is working and resuscitation equipment is readily available.
- Ensure that the study doctor is aware that testing is about to commence.
- Plug spirometer into the socket at the back of the hard drive.
- Switch on computer.
- Click on workstation only and enter user name and password.

- Click on Koko PFT system icon.

Calibration

1. Click on green calibration icon.
2. Enter room temperature and barometric pressure (multiply by 0.75 to convert to mmHg) and relative humidity N.B. these values are essential for an accurate calibration.
3. Enter name of person performing calibration. Click **OK**.
4. Click on the green circle.
5. Connect the calibration syringe to the pneumotach inlet port and pull the syringe handle all the way out. Click **OK**.
6. Follow instructions as per computer - ensure you perform one slow, one medium and one fast manoeuvre. Ensure the pneumotach is held still prior to the calibration to allow it to zero itself.

Entering patient details

1. Click on patient information icon.
2. Click on new patient.
3. Enter new patient details
4. Click **OK**. Details will then be displayed on the Patient Information screen.
5. Click Close.
6. Click on Perform FVC icon - this test directly measures inspiratory and expiratory flow volumes.
7. To select the technician and physician names, click on Set up then Test Information.
8. To select the incentive screen to be used click on Set up then Incentive.

Testing

1. Place a new mouthpiece on the Koko spirometer ensuring that the arrows on the equipment are pointing away from the mouthpiece.
2. To perform a test, ask the volunteer to sit and to place the noseclip on the lower part of the nose. Press the space bar.
3. The volunteer should hold the spirometer still (to allow the pneumotach to zero itself) until the red bar at the bottom of the screen turns black.

4. Follow the instructions on the bottom of the screen.
5. To save the attempt click on **Yes**. To reject it click **No**.
6. To view and print the results go to File then Display/Interpret/Print results.
7. If performing post treatment lung function tests, close this screen and then select Mode. Click on **Post Rx**. Perform the lung function tests as before. The flow volume plot will appear in red.
8. To enter the name of the bronchodilator used select Set up and go to Test information.

Bibliography

Arets, H. G., Brackel, H. J., & van der Ent, C. K. 2001, Forced expiratory manoeuvres in children: do they meet ATS and ERS criteria for spirometry?, *European Respiratory Journal*. 18(4):655-660.

Aurora, P., Stocks, J., Oliver, C., Saunders, C., Castle, R., Chaziparasidis, G., *et al.* 2004, Quality control for spirometry in preschool children with and without lung disease, *American Journal of Respiratory & Critical Care Medicine*. 169(10):1152-1159.

Beydon, N., Davis, S. D., Lombardi, E., Allen, J. L., Arets, H. G., Aurora, P *et al.* 2007, An official American Thoracic Society/European Respiratory Society statement: pulmonary function testing in preschool children, *American Journal of Respiratory & Critical Care Medicine*. 175(12): 1304-1345.

Miller, M. R., Hankinson, J., Brusasco, V., Burgos, F., Casaburi, R., Coates, A., *et al.* 2005, Standardisation of spirometry, *European Respiratory Journal*. 26(2): 319-338.

Appendix 11 Measurement of exhaled nitric oxide protocol - NIOX® analyser

Wellcome Trust Clinical Research Facility, Southampton

NIOX® PROTOCOL

Authors: Sr. R King & SN N Lancaster

Equipment

- NIOX® Nitric Oxide Analyser
- NIOX® Breathing Handle
- NIOX® Filter – single use

Initial calibration

(To be performed when switching between nasal and oral NIOX®)

1. Check tubing connected to cylinder and that tube is not bent or squashed.
2. Open valve 1 (check that dial registers pressure).
3. Open valve 2 until it registers pressure Bar 3.
4. Open valve 3.
5. The system will start to flush.
6. Close valve 3 and then valve 1.
7. Open valve 1 and then valve 3.
8. Repeat the above steps 10 to 20 times.
9. Finish with valve 1 and valve 3 closed.
10. Connect the metal end of the tubing from the cylinder to the back of the NIOX®

Entering patient details

1. Enter all details as prompted (some fields are optional).
2. Whilst details are being entered, NIOX® will start a self-test. On the screen it will say Self test in progress.
3. Click on Mode on the top toolbar and click on Clinical.

4. Check last calibration date (should be calibrated every 14 days. For calibration instructions, follow the steps below).
5. Check ambient NIOX® display – calculated during last self-test.

MACHINE SHOULD ALWAYS BE LEFT IN STANDBY MODE

If the machine has been shut off, full initial calibration is required after 30 minutes (as above).

4 hours stabilisation in clinical mode is needed before calibration (shown below) and clinical use.

Calibration

(The NIOX® should be calibrated every 14 days)

1. Select **Options**, then **Calibrate**.
2. Check gas certificate (attached to cylinder) and enter Nitric Oxide concentration analysis figure (200 ppb for oral NIOX®).
3. Select **Continue**.
4. The system will start to flush. The Calibration Progress will indicate this on the screen.
5. Leave patient filter on the breathing handle.
6. When prompted on the screen, connect patient end of the breathing handle to the calibration port behind door flap (white tube above metal disc). Click **Continue**.
7. Open valve one and valve three (as shown on the diagram) and adjust the gas regulator (valve two), until the marker is within the green area of the pressure indicator on screen. (Note: the marker is not steady, and will always fluctuate).
8. When completed, close off the main valve first, followed by small regulator valve. Click **Continue**.
9. Remove the breathing handle from the calibration port and close the hatch door.
10. The calibration data should now be displayed. The screen shows Calibration successful, click **OK**.

N.B.

15-30 °C ambient temperature

800-1060 barometric pressure

30-75% humidity

Uninterrupted power supply needed

Always leave the machine with filter in situ to prevent contamination and clogging of the system.

Never change the filter when other activities are happening.

Always use a new filter for each patient and throw away any old ones.

Always hold the edges of a filter when changing it.

Testing

1. Explain to the patient how you use the machine and what they will need to do (you can use previews on the screen by selecting the help menu).
2. Sit the patient comfortably so they can see the screen (same environment and position for each test for reproducibility).
3. Explain to the patient that this is not a forced expiratory manoeuvre like lung function, but a slow steady blow out.
4. Change the filter before the patient starts.
5. Select which incentive screen to be used – meter view or balloon view.
6. Instruct the patient to exhale first and then put the mouthpiece in their mouth and inhale for 2-3 seconds. This should trigger the display on the incentive screen.
7. Instruct the patient to exhale continuously until they are prompted to stop (either by the balloon landing or by progress bar at the bottom of the meter).
8. When exhaling they should try and keep the pressure of exhaling steady, this will be helped by the incentives on the screen, i.e. if breathing correctly the balloon flies between the two lines or the pointer on the meter points to the green area.
9. The patient should put down the mouthpiece between each measurement to prevent tampering with the filter.
10. Allow at least 30 seconds recovery time in between each test.
11. 3 valid measurements are required (i.e. agree within 10% of mean value of all tests).

12. A maximum of 6 attempts to be done to gain the 3 valid measurements.

Bibliography

Aerocrine AB. 2000, *NIOX® Daily Use Manual* – version 1.

American Thoracic Society & European Respiratory Society 2005, ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005, *American Journal of Respiratory & Critical Care Medicine*. 171(8):912-930.

Alving, K., Janson, C., Nordvall, L. 2006, Performance of a new hand-held device for exhaled nitric oxide measurement in adults and children. *Respiratory Research*. 7:6.

Baraldi, E., de Jongste, J. C., European Respiratory Society, & American Thoracic Society 2002, Measurement of exhaled nitric oxide in children, 2001, *European Respiratory Journal*. 20(1):223-237.

Frank, T.L., Adisesh, A., Pickering, A.C., Morrison, J. F., Wright, T., Francis, H. *et al.* 1998, Relationship between exhaled nitric oxide and childhood asthma. *American Journal of Respiratory Care Medicine*. 158:1032-6.

Appendix 12 Measurement of exhaled nitric oxide protocol - NIOX MINO® analyser

Wellcome Trust Clinical Research Foundation, Southampton

NIOX MINO® GUIDELINE

Authors: SN N Lancaster, Sr. R King & Dr K Pike

NB FENO measurement should be carried out before spirometry to ensure validity of result

Equipment

- NIOX MINO® Mains lead adaptor
- Patient mouthpiece with integral filter

Use of equipment

1. At the start of the session, attach AC/DC adaptor to the NIOX MINO® unit and connect to mains power supply. The unit takes approximately 5 minutes to warm up and indicates when ready by the presence of a smiling cloud on the display.
2. Attach a new filter.
3. Touch the display to select standard (10 second) mode or special (6 second) mode. (The 6 second mode is helpful for children under 130 cm tall or those unable to exhale for 10 seconds.) A large cloud on the display indicates standard mode and a smaller cloud is displayed when operating in special mode.
4. When top light is blue in colour the unit is ready for measurement.

Measuring FENO

1. Encourage participant to breathe in and out in a steady fashion. Explain that a continuous noise will sound when the participant exhales correctly during the test and that inconsistent exhalation will be signalled by an intermittent sound (fast or slow pips depending upon whether the participant is blowing too hard or too softly).

2. Ask the participant to inhale deeply through the filter until the cloud icon is inflated.

   (Comparing the technique to sucking through a straw may help with understanding.)
3. Immediately ask the participant to exhale through the filter slowly and continuously. Give the participant encouragement to slow down or speed up as indicated by the audible tone and the position of the cloud icon on the unit display.
4. On successful completion of the exhalation, the sound will cease with a high frequency 'ping'.
5. The result will be displayed on the unit display in 90 seconds.
6. In the event that the measurement is unsuccessful, the cloud icon rests in a dark zone on the display and the measuring line is incomplete. A low frequency sound indicates the need for a retest.

Bibliography

Aerocrine 2004, *NIOX MINO® manual, Bring the possibilities of optimal asthma management to your practice.*

American Thoracic Society & European Respiratory Society 2005, ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005, *American Journal of Respiratory & Critical Care Medicine.* 171(8):912-930.

Alving, K., Janson, C. & Nordvall, L. 2006, Performance of a new hand-held device for exhaled nitric oxide measurement in adults and children. *Respiratory Research.* 7:6.

Baraldi, E., de Jongste, J. C., European Respiratory Society, & American Thoracic Society 2002, Measurement of exhaled nitric oxide in children, 2001, *European Respiratory Journal.* 20(1):223-237.

Frank, T.L., Adisesh, A., Pickering, A.C., Morrison, J. F., Wright, T., Francis, H. *et al.* 1998, Relationship between exhaled nitric oxide and childhood asthma. *American Journal of Respiratory& Critical Care Medicine.* 158:1032-6.

Appendix 13 Methacholine challenge protocol

Wellcome Trust Clinical Research Facility, Southampton

METHACHOLINE CHALLENGE PROTOCOL

Authors: Dr G Roberts, Dr K Pike, Sr. R King & SN J Trewin

Equipment

- Koko Spirometer with dosimeter
- Normal (0.9%) saline
- Methacholine in required concentrations (see Table 1 below),
- Prescription chart
- Nebulised salbutamol
- Compressed air source at 2 bar
- Inhaled salbutamol (MDI with spacer)

Personnel

- Paediatric research nurse
- Paediatrician

Prerequisites

- Informed consent from parent(s), assent from subject
- Baseline FEV_1 of at least 70% predicted
- (If < 70%, reversibility testing will be performed instead – see below)
- Free from upper or lower respiratory tract infection in previous 2 weeks
- No use of short acting inhaled bronchodilator in previous 6 hours

Baseline Pulmonary Function

1. Baseline FEV_1 measured using KoKo spirometer with subject sitting.
2. Aim to obtain three FEV_1 values within 5% of the highest value; the highest FEV_1 is recorded.
3. If the first three FEV_1 values are not within 5%, spirometry can be performed, if the child is willing, continue until three values within 5% are obtained. If the

subject is unable to produce three FEV_1 within 5%, reversibility testing will be undertaken.

Methacholine Test

Saline Reference:

1. Baseline observations (heart rate, respiratory rate and saturation) Ask subject to take five breaths of 0.9% saline given via dosimeter holding the breath for 3 seconds after inspiration.
2. Repeat the spirometry a minute after completing saline dose to record a reference (post-saline) FEV_1 value.
3. If the first three FEV_1 values are not within 5%, spirometry can be performed, if the child is willing, until three values within 5% are obtained, or 5 minutes have passed and it is time to administer the next dose. If none of the five FEV_1 values are within 5% of each other, the highest FEV_1 value will be recorded.
4. Then:

   • If post-saline drop (from the reference) is ≤10% FEV_1, first methacholine dose can be administered.

   • If post-saline drop > 10% FEV_1, methacholine not given and go recovery.

Methacholine test:

1. Five breaths of methacholine (starting with Dose 1) administered via dosimeter holding the breath for 3 seconds after inspiration.
2. Spirometry repeated to record a post-methachacoline FEV_1 value. If the first two FEV_1 values are not within 5%, spirometry can be performed, if the child is willing, until two values within 5% are obtained, or 5 minutes have passed and it is time to administer the next dose. If none of the five FEV_1 values are within 5% of each other, the highest FEV_1 value will be recorded.
3. Then:

   • If post-methacholine FEV_1 drops by < 15% from the reference (saline) FEV_1 give the next dose of methacholine (see Table 1).

   • If post-methacholine FEV_1 drops by 15-19.9% from the control (saline) FEV_1 give HALF concentration of the next dose of methacholine (take half the usual

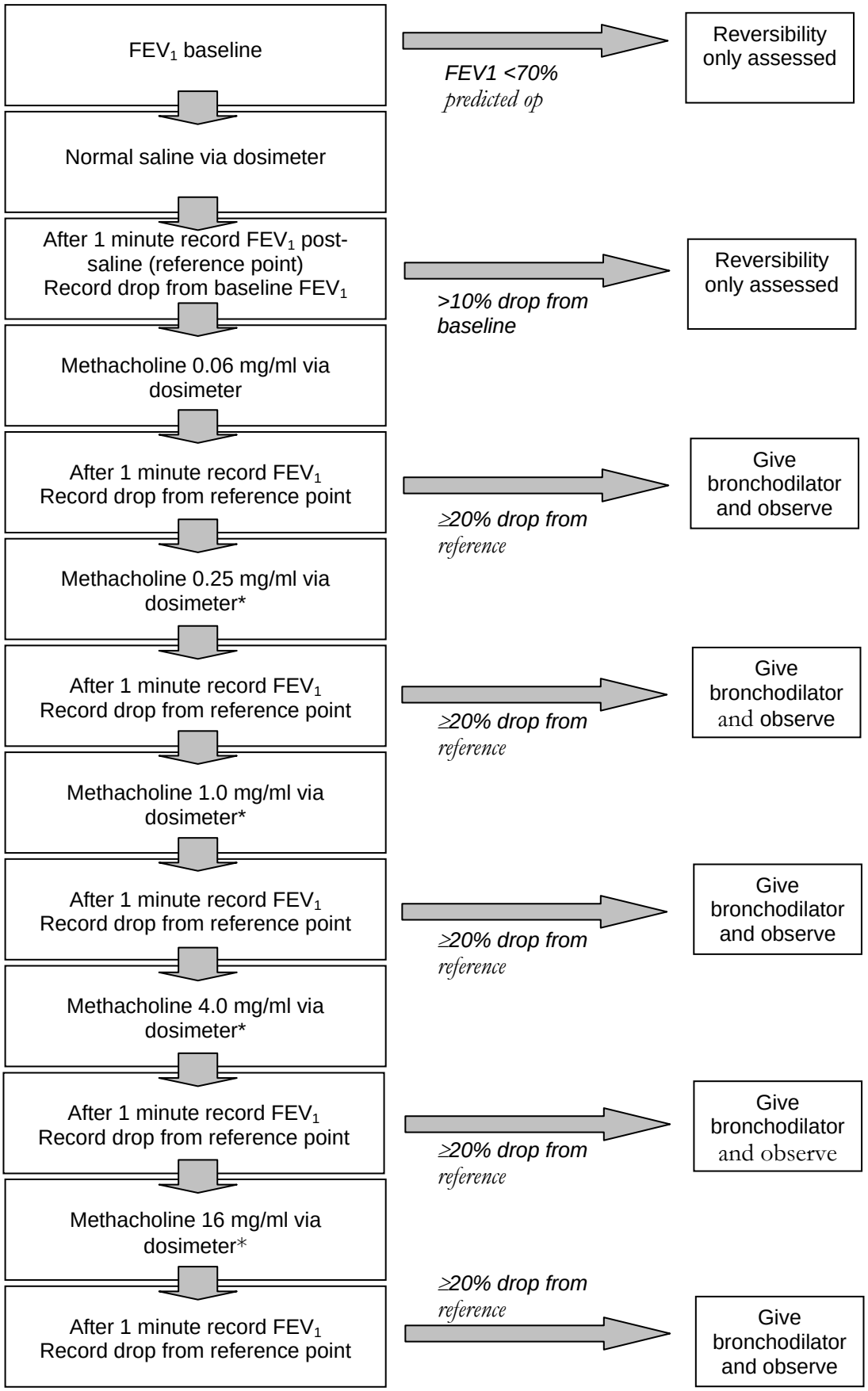
volume of methacholine solution and mix with a similar volume of 0.9% saline) (see Table 1).

• If there is a 20% or greater reduction from the reference (saline) FEV_1 proceed to recovery

4. If all the methacholine doses have been given (up to and including 16 mg/ml) and post-methacholine FEV_1 drop is still < 20% from the reference (saline) FEV_1, proceed to recovery
5. All subjects are given a bronchodilator (e.g. 600 mcg salbutamol via large volume spacer) and observed until their FEV_1 has returned to at least their baseline level.

The aim is to complete spirometry measurements after each methacholine dose within 5 minutes so whole challenge completed within 30 minutes.

Flow diagram for Methacholine Challenge



* FEV_1 drops by 15-19.9% from reference, give HALF the concentration of the next dose of methacholine (take half the usual volume of methacholine solution and mix with a similar volume of 0.9% saline)

Bibliography

Abramson, M.J., Saunders, N.A. & Hensley, M.J. 1990, Analysis of bronchial reactivity in epidemiological studies. *Thorax*. 45:24-29.

American Thoracic Society. 2000, Guidelines for Methacholine and Exercise Challenge Testing-1999. *American Journal of Respiratory and Critical Care Medicine*. 161:309-329.

Crapo, R. O., Casaburi, R., Coates, A. L., Enright, P. L., Hankinson, J. L., Irvin, C. G., *et al.* 2000, Guidelines for methacholine and exercise challenge testing, 1999, *American Journal of Respiratory & Critical Care Medicine*. 161(1) 309-329.

Joseph-Bowen, J., de Klerk, N.H., Firth, M.J., Kendall, G.E., Holt, P.G., Sly, P.D. 2004, Lung Function, Bronchial Responsiveness, and Asthma in a Community Cohort of 6-Year-Old Children. *American Journal of Respiratory and Critical Care Medicine*. 169:850–854.

Seppälä, O-P. 1991, The dose-response slope: a useful method for expressing the results of methacholine provocation tests in healthy subjects? *Respiratory Medicine*. 85:265-71.

Yan, K., Salome, C. & Woolcock, A.J. 1983, Rapid method for measurement of bronchial responsiveness. *Thorax*. 38:760-5.

Appendix 14 Salbutamol reversibility protocol

Wellcome Trust Clinical Research Facility, Southampton

REVERSIBILITY PROTOCOL

Authors: Dr G Roberts, Dr K Pike, Sr. R King & SN J Trewin

(for subjects with baseline FEV_1 < 70% predicted or where there is no consent for methacholine challenge)

1. Perform baseline spirometry with the KoKo software. Coach the child to obtain acceptable flow volume spirometry loops. Aim for three FEV_1 values within 150 ml of the best value. Spirometry will be performed until three values within 5% are obtained, or the child shows no further improvement or refuses further testing.
2. Once the best possible pre-brochodilator values have been obtained proceed to administer 600 mcg salbutamol from a MDI via a volumatic spacer.
3. Shake the MDI vigorously for several seconds and fit into spacer device.
4. Ask subject to breathe out to the end of normal expiration (end tidal volume).
5. Ask subject to place spacer mouthpiece into their mouth in an horizontal position ensuring good seal with lips.
6. At the start of breathing in, with a slow deep breath, the MDI canister should be actuated (one puff) and the patient should perform five slow, deep breaths.
7. Repeat steps 1-6 a total of six times (start timer).
8. Begin spirometry testing 15 minutes following salbutamol administration with subject standing.
9. Once more, encourage the child to obtain acceptable flow volume spirometry loops. Aim for three FEV_1 values within 150 ml of the best value. Spirometry will be performed until three values within 5% are obtained, or the child shows no further improvement or refuses further testing.

Bibliography

Aurora, P., Stocks, J., Oliver, C., Saunders, C., Castle, R., Chaziparasidis, G., *et al.* 2004, Quality control for spirometry in preschool children with and without lung disease, *American Journal of Respiratory & Critical Care Medicine*. 169(10):1152-1159.

Beydon, N., Davis, S. D., Lombardi, E., Allen, J. L., Arets, H. G., Aurora, P *et al.* 2007, An official American Thoracic Society/European Respiratory Society statement: pulmonary function testing in preschool children, *American Journal of Respiratory & Critical Care Medicine*. 175(12): 1304-1345.

Miller, M. R., Hankinson, J., Brusasco, V., Burgos, F., Casaburi, R., Coates, A., *et al.* 2005, Standardisation of spirometry, *European Respiratory Journal.* 26(2): 319-338.

Chapter Eleven

References

Abramson, M. J., Saunders, N. A., & Hensley, M. J. 1990, Analysis of bronchial reactivity in epidemiological studies, *Thorax*. 45(12):924-9.

Aerocrine. Guide to Interpretation of eNO Values [cited 12/08/2009] available from: http://www.aerocrine.com/en/ Healthcare-professionals/Benefits-of-FEsubNOsub/Interpretation-of-FEsubNOsub-values.

Aerocrine. Specifications for NIOX Flex [cited 12/08/2009] available from: http://www.nioxflex.com/specifications.html.

Agalliu, I., Eisen, E. A., Hauser, R., Redlich, C. A., Stowe, M. H., Cullen, M. R. *et al.* 2003, Truncating the dose range for methacholine challenge tests: three occupational studies, *Journal of Occupational & Environmental Medicine.* 45(8):841-7.

Al, M. D., van Houwelingen, A. C., Kester, A. D., Hasaart, T. H., de Jong, A. E., & Hornstra, G. 1995, Maternal essential fatty acid patterns during normal pregnancy and their relationship to the neonatal essential fatty acid status, *British Journal of Nutrition.* 74(1):55-68.

Albers, R., Bol, M., Bleumink, R., Willems, A. A., & Pieters, R. H. 2003, Effects of supplementation with vitamins A, C, and E, selenium, and zinc on immune function in a murine sensitization model, *Nutrition.* 19(11-12):940-946.

Altman, D. G. & Hytten, F. E. 1989, Intrauterine growth retardation: let's be clear about it, *British Journal of Obstetrics & Gynaecology*. 96(10):1127-32.

Alving, K., Janson, C., & Nordvall, L. 2006, Performance of a new hand-held device for exhaled nitric oxide measurement in adults and children, *Respiratory Research* 7:67.

American Thoracic Society/European Respiratory Society 2005, ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005, *American Journal of Respiratory & Critical Care Medicine*. 171(8):912-930.

Anderson, H. R., Ruggles, R., Strachan, D. P., Austin, J. B., Burr, M., Jeffs, D. *et al.* 2004, Trends in prevalence of symptoms of asthma, hay fever, and eczema in 12-14 year olds in the British Isles, 1995-2002: questionnaire survey, *BMJ.* 328(7447):1052-3.

Annesi-Maesano, I., Moreau, D., & Strachan, D. 2001, In utero and perinatal complications preceding asthma, *Allergy.* 56(6):491-7.

Antipatis, C., Grant, G., & Ashworth, C. J. 2000, Moderate maternal vitamin A deficiency affects perinatal organ growth and development in rats, *British Journal of Nutrition.* 84(1):125-32.

Arets, H. G., Brackel, H. J., & van der Ent, C. K. 2001, Forced expiratory manoeuvres in children: do they meet ATS and ERS criteria for spirometry? *European Respiratory Journal.* 18(4):655-660.

Arshad, S. H., Kurukulaaratchy, R. J., Fenn, M., & Matthews, S. 2005, Early life risk factors for current wheeze, asthma, and bronchial hyperresponsiveness at 10 years of age, *Chest.* 127(2):502-508.

Arshad, S. H., Stevens, M., & Hide, D. W. 1993, The effect of genetic and environmental factors on the prevalence of allergic disorders at the age of two years, *Clinical & Experimental Allergy.* 23(6):504-11.

Arshad, S. H., Tariq, S. M., Matthews, S., & Hakim, E. 2001, Sensitization to common allergens and its association with allergic disorders at age 4 years: a whole population birth cohort study, *Pediatrics.* 108(2):E33, 2001.

Asher, M. I., Keil, U., Anderson, H. R., Beasley, R., Crane, J., Martinez, F. *et al.* 1995, International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods, *European Respiratory Journal.* 8(3):483-491.

Aurora, P., Stocks, J., Oliver, C., Saunders, C., Castle, R., Chaziparasidis, G. *et al.* 2004, Quality control for spirometry in preschool children with and without lung disease, *American Journal of Respiratory & Critical Care Medicine.* 169(10):1152-1159.

Avital, A., Springer, C., Bar-Yishay, E., & Godfrey, S. 1995, Adenosine, methacholine, and exercise challenges in children with asthma or paediatric chronic obstructive pulmonary disease, *Thorax.* 50(5):511-516.

Azzi, A., Gysin, R., Kempna, P., Munteanu, A., Negis, Y., Villacorta, L. *et al.* 2004, Vitamin E mediates cell signaling and regulation of gene expression, *Annals of the New York Academy of Sciences.* 1031:86-95.

Bancalari, E., Claure, N., & Sosenko, I. R. 2003, Bronchopulmonary dysplasia: changes in pathogenesis, epidemiology and definition, *Seminars in Neonatology.* 8(1):63-71.

Baraldi, E., Carra, S., Dario, C., Azzolin, N., Ongaro, R., Marcer, G. *et al.* 1999, Effect of natural grass pollen exposure on exhaled nitric oxide in asthmatic children, *American Journal of Respiratory & Critical Care Medicin*l. 159(1):262-266.

Baraldi, E., de Jongste, J. C., European Respiratory Society, & American Thoracic Society 2002, Measurement of exhaled nitric oxide in children, 2001, *European Respiratory Journal.* 20(1): 223-237.

Barbee, R. A., Lebowitz, M. D., Thompson, H. C., & Burrows, B. 1976, Immediate skin-test reactivity in a general population sample, *Annals of Internal Medicine*. 84(2):129-133.

Barker, D. J., Bull, A. R., Osmond, C., & Simmonds, S. J. 1990, Fetal and placental size and risk of hypertension in adult life, *BMJ*. 301(6746):259-62.

Barker, D. J., Godfrey, K. M., Fall, C., Osmond, C., Winter, P. D., & Shaheen, S. O. 1991, Relation of birth weight and childhood respiratory infection to adult lung function and death from chronic obstructive airways disease, *BMJ*. 303(6804):671-5.

Barker, D. J. & Osmond, C. 1986, Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales, *Lancet*. 1(8489):1077-81.

Barros, A. J. & Hirakata, V. N. 2003, Alternatives for logistic regression in cross-sectional studies: an empirical comparison of models that directly estimate the prevalence ratio, *BMC Medical Research Methodology*. 3:21.

Bass, H., Adkins, B., & Strober, S. 1991, Thymic irradiation inhibits the rapid recovery of TH1 but not TH2-like functions of CD4+ T cells after total lymphoid irradiation, *Cellular Immunology*. 137(2): 316-328.

Baydas, G., Karatas, F., Gursu, M. F., Bozkurt, H. A., Ilhan, N., Yasar, A. *et al.* 2002, Antioxidant vitamin levels in term and preterm infants and their relation to maternal vitamin status, *Archives of Medical Research*. 33(3):276-80.

Bellia, V., Rizzo, A., Amoroso, S., Mirabella, A., & Bonsignore, G. 1983, Analysis of dose-response curves in the detection of bronchial hyperreactivity, *Respiration*. 44(1):10-8.

Benn, C. S., Jeppesen, D. L., Hasselbalch, H., Olesen, A. B., Nielsen, J., Bjorksten, B. *et al.* 2001, Thymus size and head circumference at birth and the development of allergic diseases, *Clinical & Experimental Allergy* .31(12):1862-6.

Benson, M. K. 1975, Bronchial hyperreactivity, *British Journal of Diseases of the Chest*. 69:227-39.

Benson, M. K. 1978, Bronchial responsiveness to inhaled histamine and isoprenaline in patients with airway obstruction, *Thorax*. 33(2): 211-213.

Bernstein, I. L. & Storms, W. W. 1995, Practice parameters for allergy diagnostic testing. Joint Task Force on Practice Parameters for the Diagnosis and Treatment of Asthma. The American Academy of Allergy, Asthma and Immunology and the American College of Allergy, Asthma and Immunology, *Annals of Allergy, Asthma, & Immunology*. 75(6 Pt 2): 543-625.

Beydon, N., Davis, S. D., Lombardi, E., Allen, J. L., Arets, H. G., Aurora, P. *et al.* 2007, An official American Thoracic Society/European Respiratory Society statement: pulmonary function testing in preschool children, *American Journal of Respiratory & Critical Care Medicine*. 175(12):1304-1345.

Bibi, H., Montgomery, M., Pasterkamp, H., & Chernick, V. 1991, Relationship between response to inhaled salbutamol and methacholine bronchial provocation in children with suspected asthma, *Pediatric Pulmonology*. 10(4):244-8.

Black, P. N. & Scragg, R. 2005, Relationship between serum 25-hydroxyvitamin D and pulmonary function in the third national health and nutrition examination survey, *Chest*. 128(6):3792-8.

Black, P. N. & Sharpe, S. 1997, Dietary fat and asthma: is there a connection? *European Respiratory Journal*. 10(1):6-12.

Bloomfield, F. H. & Harding, J. E. 1998, Experimental aspects of nutrition and fetal growth, *Fetal and Maternal Medicine Review*. 10:91-107.

Bofill, M., Janossy, G., Janossa, M., Burford, G. D., Seymour, G. J., Wernet, P. *et al.* 1985, Human B cell development. II. Subpopulations in the human fetus, *Journal of Immunology*. 134(3):1531-8.

Bolisetty, S., Naidoo, D., Lui, K., Koh, T. H., Watson, D., Montgomery, R. *et al.* 2002, Postnatal changes in maternal and neonatal plasma antioxidant vitamins and the influence of smoking, *Archives of Disease in Childhood Fetal & Neonatal Edition*. 86(1):F36-40.

Bolte, G., Schmidt, M., Maziak, W., Keil, U., Nasca, P., von Mutius, E. *et al.* 2004, The relation of markers of fetal growth with asthma, allergies and serum immunoglobulin E levels in children at age 5-7 years, *Clinical & Experimental Allergy*. 34(3):381-8.

Braback, L. & Hedberg, A. 1998, Perinatal risk factors for atopic disease in conscripts, *Clinical & Experimental Allergy*. 28(8):936-42.

Bradley, B. L., Azzawi, M., Jacobson, M., Assoufi, B., Collins, J. V., Irani, A. M. *et al.* 1991, Eosinophils, T-lymphocytes, mast cells, neutrophils, and macrophages in bronchial biopsy specimens from atopic subjects with asthma: comparison with biopsy specimens from atopic subjects without asthma and normal control subjects and relationship to bronchial hyperresponsiveness, *Journal of Allergy & Clinical Immunology*. 88(4):661-74.

Brain, J. D. & Valberg, P. A. 1979, Deposition of aerosol in the respiratory tract, *American Review of Respiratory Disease*. 120(6):1325-73.

Brooks, A. A., Johnson, M. R., Steer, P. J., Pawson, M. E., & Abdalla, H. I. 1995, Birth weight: nature or nurture? *Early Human Development*. 42(1):29-35.

Brown, H. M., Su, S., & Thantrey, N. 1981, Prick testing for allergens standardized by using a precision needle, *Clinical Allergy*. 11(1): 95-98.

Brussee, J. E., Smit, H. A., van Strien, R. T., Corver, K., Kerkhof, M., Wijga, A. H. *et al.* 2005, Allergen exposure in infancy and the development of sensitization, wheeze, and asthma at 4 years, *Journal of Allergy & Clinical Immunology*. 115(5): 946-952.

Burney, P. & Chinn, S. 1987, Developing a new questionnaire for measuring the prevalence and distribution of asthma, *Chest*. 91(Suppl.):79S-83S.

Burney, P. G., Laitinen, L. A., Perdrizet, S., Huckauf, H., Tattersfield, A. E., Chinn, S. *et al.* 1989, Validity and repeatability of the IUATLD (1984) Bronchial Symptoms Questionnaire: an international comparison, *European Respiratory Journal.* 2(10):940-5.

Burns, J. S., Dockery, D. W., Neas, L. M., Schwartz, J., Coull, B. A., Raizenne, M. *et al.* 2007, Low dietary nutrient intakes and respiratory health in adolescents, *Chest.* 132(1):238-45.

Burrows, B., Knudson, R. J., & Lebowitz, M. D. 1977, The relationship of childhood respiratory illness to adult obstructive airway disease, *American Review of Respiratory Disease.* 115(5): 751-760.

Burrows, B., Lebowitz, M. D., & Barbee, R. A. 1976, Respiratory disorders and allergy skin-test reactions, *Clinical Notes on Respiratory Diseases.* 15(3): 12-13.

Bussamra, M. H., Cukier, A., Stelmach, R., & Rodrigues, J. C. 2005, Evaluation of the magnitude of the bronchodilator response in children and adolescents with asthma, *Chest.* 127(2):530-5.

Calder, P. C. 1998, Fat chance of immunomodulation, *Immunology Today.* 6:244-247.

Calder, P. C. 2002, Fatty acids and gene expression related to inflammation, *Nestle Nutrition Workshop Series Clinical & Performance Program.* 7:19-36.

Calder, P. C. 2003, Polyunsaturated fatty acids and cytokine profiles: a clue to the changing prevalence of atopy? *Clinical & Experimental Allergy.* 33(4):412-415.

Calder, P. C. 2006, n-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases, *American Journal of Clinical Nutrition.* 83(Suppl.):1505S-1519S.

Camargo, C. A., Jr., Rifas-Shiman, S. L., Litonjua, A. A., Rich-Edwards, J. W., Weiss, S. T., Gold, D. R. *et al.* 2007, Maternal intake of vitamin D during pregnancy and risk of recurrent wheeze in children at 3 y of age, *American Journal of Clinical Nutrition.* 85(3):788-95.

Campbell, S. & Thoms, A. 1977, Ultrasound measurement of the fetal head to abdomen circumference ratio in the assessment of growth retardation, *British Journal of Obstetrics & Gynaecology.* 84(3):165-174.

Cantorna, M. T., Zhu, Y., Froicu, M., & Wittke, A. 2004, Vitamin D status, 1,25-dihydroxyvitamin D3, and the immune system, *American Journal of Clinical Nutrition.* 80(Suppl.):1717S-20S.

Carrington, L. J. & Langley-Evans, S. C. 2006, Wheezing and eczema in relation to infant anthropometry: evidence of developmental programming of disease in childhood, *Maternal & Child Nutrition.* 2(1):51-61.

Cartier, A., Malo, J. L., Begin, P., Sestier, M., & Martin, R. R. 1983, Time course of the bronchoconstriction induced by inhaled histamine and methacholine, *Journal of Applied Physiology: Respiratory, Environmental & Exercise Physiology.* 54(3):821-826.

Cartier, A., Thomson, N. C., Frith, P. A., Roberts, R., & Hargreave, F. E. 1982, Allergen-induced increase in bronchial responsiveness to histamine: relationship to the

late asthmatic response and change in airway caliber, *Journal of Allergy & Clinical Immunology*. 70(3):170-7.

Casan, P., Roca, J., & Sanchis, J. 1983, Spirometric response to a bronchodilator. Reference values for healthy children and adolescents, *Bulletin Europeen de Physiopathologie Respiratoire*. 6: 567-569.

Casas, R. & Bjorksten, B. 2001, Detection of Fel d 1-immunoglobulin G immune complexes in cord blood and sera from allergic and non-allergic mothers, *Pediatric Allergy & Immunology*. 12(2):59-64.

Castro-Rodriguez, J. A., Holberg, C. J., Morgan, W. J., Wright, A. L., & Martinez, F. D. 2001, Increased incidence of asthmalike symptoms in girls who become overweight or obese during the school years, *American Journal of Respiratory & Critical Care Medicine*. 163(6):1344-1349.

Chai, H., Farr, R. S., Froehlich, L. A., Mathison, D. A., McLean, J. A., Rosenthal, R. R. *et al.* 1975, Standardization of bronchial inhalation challenge procedures, *Journal of Allergy & Clinical Immunology*. 56(4):323-327.

Chailley-Heu, B., Chelly, N., Lelievre-Pegorier, M., Barlier-Mur, A. M., Merlet-Benichou, C., & Bourbon, J. R. 1999, Mild vitamin A deficiency delays fetal lung maturation in the rat, *American Journal of Respiratory Cell & Molecular Biology*. 21(1):89-96.

Chang, L. Y. & Crapo, J. D. 2002, Inhibition of airway inflammation and hyperreactivity by an antioxidant mimetic, *Free Radical Biology & Medicine*. 33(3):379-86.

Chang, L. Y. & Crapo, J. D. 2003, Inhibition of airway inflammation and hyperreactivity by a catalytic antioxidant, *Chest*. 123(Suppl.):446S.

Chaplin, D. D. 2003, Overview of the immune response, *Journal of Allergy & Clinical Immunology*. 111(Suppl.):S442-S459.

Chatzi, L., Torrent, M., Romieu, I., Garcia-Esteban, R., Ferrer, C., Vioque, J. *et al.* 2008, Mediterranean diet in pregnancy is protective for wheeze and atopy in childhood, *Thorax*. 63(6):507-13.

Chavasse, R., Johnson, P., Francis, J., Balfour-Lynn, I., Rosenthal, M., & Bush, A. 2003, To clip or not to clip? Noseclips for spirometry, *European Respiratory Journal*. 21(5):876-878.

Chinn, S. 1998, Methodology of bronchial responsiveness, *Thorax*. 53(11): 984-988.

Chinn, S., Arossa, W. A., Jarvis, D. L., Luczynska, C. M., & Burney, P. G. 1997, Variation in nebulizer aerosol output and weight output from the Mefar dosimeter: implications for multicentre studies, *European Respiratory Journal*. 10(2):452-6.

Chinn, S., Britton, J. R., Burney, P. G., Tattersfield, A. E., & Papacosta, A. O. 1987, Estimation and repeatability of the response to inhaled histamine in a community survey, *Thorax*. 42(1):45-52.

Chinn, S., Burney, P. G., Britton, J. R., Tattersfield, A. E., & Higgins, B. G. 1993, Comparison of PD20 with two alternative measures of response to histamine challenge in epidemiological studies, *European Respiratory Journal.* 6(5):670-9.

Chinn, S. & Schouten, J. P. 2005, Reproducibility of non-specific bronchial challenge in adults: implications for design, analysis and interpretation of clinical and epidemiological studies, *Thorax.* 60(5):395-400.

Chytil, F. 1992, The lungs and vitamin A, *American Journal of Physiology.* 262(5 Pt 1):L517-27.

Cikot, R. J., Steegers-Theunissen, R. P., Thomas, C. M., de Boo, T. M., Merkus, H. M., & Steegers, E. A. 2001, Longitudinal vitamin and homocysteine levels in normal pregnancy, *British Journal of Nutrition.* 85(1):49-58.

Clifford, R. D., Radford, M., Howell, J. B., & Holgate, S. T. 1989, Prevalence of respiratory symptoms among 7 and 11 year old schoolchildren and association with asthma, *Archives of Disease in Childhood.* 64(8):1118-25.

Cochrane, G. M., Prieto, F., & Clark, T. J. 1977, Intrasubject variability of maximal expiratory flow volume curve, *Thorax.* 32(2):171-176.

Cockcroft, D. W. & Berscheid, B. A. 1983, Slope of the dose-response curve: usefulness in assessing bronchial responses to inhaled histamine, *Thorax.* 38(1):55-61.

Cockcroft, D. W., Berscheid, B. A., & Murdock, K. Y. 1983a, Measurement of responsiveness to inhaled histamine using FEV_1: comparison of PC20 and threshold, *Thorax.* 38(7):523-6.

Cockcroft, D. W., Berscheid, B. A., & Murdock, K. Y. 1983b, Unimodal distribution of bronchial responsiveness to inhaled histamine in a random human population, *Chest.* 83(5):751-4.

Cockcroft, D. W., Davis, B. E., Todd, D. C., & Smycniuk, A. J. 2005, Methacholine challenge: comparison of two methods, *Chest.* 127(3):839-44.

Cockcroft, D. W., Killian, D. N., Mellon, J. J., & Hargreave, F. E. 1977, Bronchial reactivity to inhaled histamine: a method and clinical survey, *Clinical Allergy.* 7(3):235-43.

Collinson, A. C., Moore, S. E., Cole, T. J., & Prentice, A. M. 2003, Birth season and environmental influences on patterns of thymic growth in rural Gambian infants, *Acta Paediatrica.* 92(9):1014-1020.

Collinson, A. C., Ngom, P. T., Moore, S. E., Morgan, G., & Prentice, A. M. 2008, Birth season and environmental influences on blood leucocyte and lymphocyte subpopulations in rural Gambian infants, *BMC Immunology.* 9:18.

Considine, R. V. 2005, Human leptin: an adipocyte hormone with weight-regulatory and endocrine functions, *Seminars in Vascular Medicine.* 5(1):15-24.

Cook, D. G., Carey, I. M., Whincup, P. H., Papacosta, O., Chirico, S., Bruckdorfer, K. R. *et al.* 1997, Effect of fresh fruit consumption on lung function and wheeze in children, *Thorax.* 52(7):628-33.

Crapo, R. O., Casaburi, R., Coates, A. L., Enright, P. L., Hankinson, J. L., Irvin, C. G. *et al.* 2000, Guidelines for methacholine and exercise challenge testing, 1999, *American Journal of Respiratory & Critical Care Medicine.* 161(1):309-329.

Crenesse, D., Berlioz, M., Bourrier, T., & Albertini, M. 2001, Spirometry in children aged 3 to 5 years: reliability of forced expiratory maneuvers, *Pediatric Pulmonology.* 32(1):56-61.

Crozier, S. R., Robinson, S. M., Borland, S. E., Inskip, H. M., & SWS Study Group 2006, Dietary patterns in the Southampton Women's Survey, *European Journal of Clinical Nutrition.* 60(12):1391-9.

Cui, D., Moldoveanu, Z., & Stephensen, C. B. 2000, High-level dietary vitamin A enhances T-helper type 2 cytokine production and secretory immunoglobulin A response to influenza A virus infection in BALB/c mice, *Journal of Nutrition.* 130(5):1132-9.

Culpitt, S. V., Rogers, D. F., Traves, S. L., Barnes, P. J., & Donnelly, L. E. 2005, Sputum matrix metalloproteases: comparison between chronic obstructive pulmonary disease and asthma, *Respiratory Medicine.* 99(6):703-10.

Dawodu, A., Agarwal, M., Hossain, M., Kochiyil, J., & Zayed, R. 2003, Hypovitaminosis D and vitamin D deficiency in exclusively breast-feeding infants and their mothers in summer: a justification for vitamin D supplementation of breast-feeding infants, *Journal of Pediatrics.* 142(2):169-73.

de Meer, G., Marks, G. B., de Jongste, J. C., & Brunekreef, B. 2005, Airway responsiveness to hypertonic saline: dose-response slope or PD15? *European Respiratory Journal.* 25(1):153-8.

De, V., Sr., Houwelingen, A. C., Hornstra, G., Dhont, M., & Christophe, A. B. 2001, The composition of saturated fatty acids in plasma phospholipids changes in a way to counteract changes in the mean melting point during pregnancy, *Lipids.* 36(1):15-20.

DEFRA 1940, *British National Food Survey.*

Department for Environment, F. a. R. A. H. Consumption of selected household foods (GB) 1942 to 2000 [cited 12/08/2009] available from: https://statistics.defra.gov.uk/esg/publications/nfs/datasets/allfood.xls. Last amended 2009.

Devereux, G. 2006, The increase in the prevalence of asthma and allergy: food for thought, *Nature Reviews.Immunology.* 6(11):869-74.

Devereux, G., Barker, R. N., & Seaton, A. 2002, Antenatal determinants of neonatal immune responses to allergens, *Clinical & Experimental Allergy.* 32(1):43-50.

Devereux, G., Litonjua, A. A., Turner, S. W., Craig, L. C., McNeill, G., Martindale, S. *et al.* 2007, Maternal vitamin D intake during pregnancy and early childhood wheezing, *American Journal of Clinical Nutrition.* 85(3):853-9.

Devereux, G., Turner, S. W., Craig, L. C., McNeill, G., Martindale, S., Harbour, P. J. *et al.* 2006, Low maternal vitamin E intake during pregnancy is associated with asthma in

5-year-old children, *American Journal of Respiratory & Critical Care Medicine.* 174(5):499-507.

Deykin, A., Massaro, A. F., Coulston, E., Drazen, J. M., & Israel, E. 2000, Exhaled nitric oxide following repeated spirometry or repeated plethysmography in healthy individuals, *American Journal of Respiratory & Critical Care Medicine.* 161(4 Pt 1):1237-1240.

Dezateux, C., Stocks, J., Dundas, I., & Fletcher, M. E. 1999, Impaired airway function and wheezing in infancy: the influence of maternal smoking and a genetic predisposition to asthma, *American Journal of Respiratory & Critical Care Medicine.* 159(2):403-410.

Dezateux, C., Stocks, J., Wade, A. M., Dundas, I., & Fletcher, M. E. 2001, Airway function at one year: association with premorbid airway function, wheezing, and maternal smoking, *Thorax.* 56(9):680-6.

Dirix, C. E., Hogervorst, J. G., Rump, P., Hendriks, J. J., Bruins, M., & Hornstra, G. 2009, Prenatal arachidonic acid exposure and selected immune-related variables in childhood, *British Journal of Nutrition.* 102(3):387-97.

Dold, S., Wjst, M., von, M. E., Reitmeir, P., & Stiepel, E. 1992, Genetic risk for asthma, allergic rhinitis, and atopic dermatitis, *Archives of Disease in Childhood.* 67(8):1018-22.

Downie, D., Antipatis, C., Delday, M. I., Maltin, C. A., & Sneddon, A. A. 2005, Moderate maternal vitamin A deficiency alters myogenic regulatory protein expression and perinatal organ growth in the rat, *American Journal of Physiology - Regulatory Integrative & Comparative Physiology.* 288(1):R73-9.

Dreborg, S. 1987, *The skin prick test. Methodological studies and clinical applications,* doctoral dissertation, Linkoping University.

Dreborg, S. 1989, Skin prick test, *Allergy.* 40(Suppl.): 55-65.

Dundas, I., Chan, E. Y., Bridge, P. D., & McKenzie, S. A. 2005, Diagnostic accuracy of bronchodilator responsiveness in wheezy children, *Thorax.* 60(1):13-6.

Durnin, J. V. & Womersley, J. 1974, Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years, *British Journal of Nutrition.* 32(1):77-97.

Dutta-Roy, A. K. 2000, Transport mechanisms for long-chain polyunsaturated fatty acids in the human placenta, *American Journal of Clinical Nutrition.* 71(Suppl.):315S-22S.

EAACI 1989, Skin tests used in type I allergy testing Position paper. Sub-Committee on Skin Tests of the European Academy of Allergology and Clinical Immunology, *Allergy.* 44 (Suppl.):1-59.

Eder, W., Ege, M. J., & von Mutius, E. 2006, The asthma epidemic, *New England Journal of Medicine.* 355(21):2226-35.

Edwards, L. J., Coulter, C. L., Symonds, M. E., & McMillen, I. C. 2001, Prenatal undernutrition, glucocorticoids and the programming of adult hypertension, *Clinical & Experimental Pharmacology & Physiology.* 28(11):938-41.

Emanuel, I., Filakti, H., Alberman, E., & Evans, S. J. 1992, Intergenerational studies of human birthweight from the 1958 birth cohort. 1. Evidence for a multigenerational effect, *British Journal of Obstetrics & Gynaecology*. 99(1):67-74.

Emmett, P. 2009, Assessing diet in longitudinal birth cohort studies, *Paediatric and Perinatal Epidemiology*. 23(Suppl..):154-173.

Erkkola, M., Kaila, M., Nwaru, B. I., Kronberg-Kippila, C., Ahonen, S., Nevalainen, J. *et al.* 2009, Maternal vitamin D intake during pregnancy is inversely associated with asthma and allergic rhinitis in 5-year-old children, *Clinical & Experimental Allergy*. 39(6):875-82.

Ernst, P., Demissie, K., Joseph, L., Locher, U., & Becklake, M. R. 1995, Socioeconomic status and indicators of asthma in children, *American Journal of Respiratory & Critical Care Medicine*. 152(2):570-5.

Fantuzzi, G. 2005, Adipose tissue, adipokines, and inflammation, *Journal of Allergy & Clinical Immunology*. 115(5): 911-919.

Fergusson, D. M., Crane, J., Beasley, R., & Horwood, L. J. 1997, Perinatal factors and atopic disease in childhood, *Clinical & Experimental Allergy*. 27(12):1394-401.

Fields, P. E., Kim, S. T., & Flavell, R. A. 2002, Cutting edge: changes in histone acetylation at the IL-4 and IFN-gamma loci accompany Th1/Th2 differentiation, *Journal of Immunology*. 169(2):647-50.

Flaherman, V. & Rutherford, G. W. 2006, A meta-analysis of the effect of high weight on asthma, *Archives of Disease in Childhood*. 91(4):334-9.

Fogarty, A., Lewis, S., Weiss, S., & Britton, J. 2000, Dietary vitamin E, IgE concentrations, and atopy, *Lancet*. 356(9241):1573-4.

Food Standards Agency. Eat well, be well [cited 12/08/2009] available from: www.eatwell.gov.uk/agesandstages/pregnancy/.

Forastiere, F., Agabiti, N., Corbo, G. M., Pistelli, R., Dell'Orco, V., Ciappi, G. *et al.* 1994, Passive smoking as a determinant of bronchial responsiveness in children, *American Journal of Respiratory & Critical Care Medicine*. 149(2 Pt 1):365-70.

Forastiere, F., Corbo, G. M., Dell'Orco, V., Pistelli, R., Agabiti, N., & Kriebel, D. 1996, A longitudinal evaluation of bronchial responsiveness to methacholine in children: role of baseline lung function, gender, and change in atopic status, *American Journal of Respiratory & Critical Care Medicine*. 153(3):1098-104.

Frank, T. L., Adisesh, A., Pickering, A. C., Morrison, J. F., Wright, T., Francis, H. *et al.* 1998, Relationship between exhaled nitric oxide and childhood asthma, *American Journal of Respiratory & Critical Care Medicine*. 158(4):1032-1036.

Franklin, P. J., Taplin, R., & Stick, S. M. 1999, A community study of exhaled nitric oxide in healthy children, *American Journal of Respiratory & Critical Care Medicine*. 159(1,): 69-73.

Franklin, P. J., Turner, S. W., Le Souef, P. N., & Stick, S. M. 2003, Exhaled nitric oxide and asthma: complex interactions between atopy, airway responsiveness, and symptoms in a community population of children, *Thorax*. 58(12):1048-1052.

Fredberg, J. J., Inouye, D., Miller, B., Nathan, M., Jafari, S., Raboudi, S. H. *et al.* 1997, Airway smooth muscle, tidal stretches, and dynamically determined contractile states, *American Journal of Respiratory & Critical Care Medicine*. 156(6):1752-1759.

Friedrich, L., Stein, R. T., Pitrez, P. M., Corso, A. L., & Jones, M. H. 2006, Reduced lung function in healthy preterm infants in the first months of life, *American Journal of Respiratory & Critical Care Medicine*. 173(4):442-447.

Galambos, C. & Demello, D. E. 2008, Regulation of alveologenesis: clinical implications of impaired growth, *Pathology*. 40(2):124-40.

Galant, S. P. & Maibach, H. I. 1973, Reproducibility of allergy epicutaneous test techniques, *Journal of Allergy & Clinical Immunology*. 51(4):245-250.

Gale, C. R., Robinson, S. M., Harvey, N. C., Javaid, M. K., Jiang, B., Martyn, C. N. *et al.* 2008, Maternal vitamin D status during pregnancy and child outcomes, *European Journal of Clinical Nutrition*. 62:68-77.

Gilliland, F. D., Berhane, K. T., Li, Y. F., Gauderman, W. J., McConnell, R., & Peters, J. 2003, Children's lung function and antioxidant vitamin, fruit, juice, and vegetable intake, *American Journal of Epidemiology*. 158(6):576-84.

Gillman, M. W., Rich-Edwards, J. W., Rifas-Shiman, S. L., Lieberman, E. S., Kleinman, K. P., & Lipshultz, S. E. 2004, Maternal age and other predictors of newborn blood pressure, *Journal of Pediatrics*. 144(2):240-5.

Gluckman, P. D. & Hanson, M. A. 2004, Living with the past: evolution, development, and patterns of disease, *Science*. 305(5691):1733-6.

Godden, D. J., Ross, S., Abdalla, M., McMurray, D., Douglas, A., Oldman, D. *et al.* 1994, Outcome of wheeze in childhood. Symptoms and pulmonary function 25 years later, *American Journal of Respiratory & Critical Care Medicine*. 149(1):106-112.

Godfrey, K., Robinson, S., Barker, D. J., Osmond, C., & Cox, V. 1996, Maternal nutrition in early and late pregnancy in relation to placental and fetal growth, *BMJ*. 312(7028):410-4.

Godfrey, K. M., Barker, D. J., & Osmond, C. 1994, Disproportionate fetal growth and raised IgE concentration in adult life, *Clinical & Experimental Allergy*. 24(7):641-8.

Godfrey, S., Springer, C., Bar-Yishay, E., & Avital, A. 1999, Cut-off points defining normal and asthmatic bronchial reactivity to exercise and inhalation challenges in children and young adults, *European Respiratory Journal*. 14(3):659-68.

Gold, D. R., Willwerth, B. M., Tantisira, K. G., Finn, P. W., Schaub, B., Perkins, D. L. *et al.* 2006, Associations of cord blood fatty acids with lymphocyte proliferation, IL-13, and IFN-gamma, *Journal of Allergy & Clinical Immunology*. 117(4):931-8.

Gregory, Doull, Pearce, Cheng, Leadbitter, Holgate *et al.* 1999, The relationship between anthropometric measurements at birth: asthma and atopy in childhood, *Clinical & Experimental Allergy*. 29(3):330-333.

Griffin, M. D., Xing, N., & Kumar, R. 2003, Vitamin D and its analogs as regulators of immune activation and antigen presentation, *Annual Review of Nutrition*. 23:117-45.

Gunnbjornsdottir, M. I., Omenaas, E., Gislason, T., Norrman, E., Olin, A. C., Jogi, R. *et al.* 2004, Obesity and nocturnal gastro-oesophageal reflux are related to onset of asthma and respiratory symptoms, *European Respiratory Journal*. 24(1):116-121.

Guo, X., Warden, B. A., Paeratakul, S., & Bray, G. A. 2004, Healthy Eating Index and obesity, *European Journal of Clinical Nutrition*. 58(12):1580-6.

Haberg, S. E., Stigum, H., London, S. J., Nystad, W., & Nafstad, P. 2009, Maternal obesity in pregnancy and respiratory health in early childhood, *Paediatric and Perinatal Epidemiology*. 23(4):352-62.

Habib, M. P., Pare, P. D., & Engel, L. A. 1979, Variability of airway responses to inhaled histamine in normal subjects, *Journal of Applied Physiology: Respiratory, Environmental & Exercise Physiology*. 47(1):51-8.

Hallstrand, T. S., Fischer, M. E., Wurfel, M. M., Afari, N., Buchwald, D., & Goldberg, J. 2005, Genetic pleiotropy between asthma and obesity in a community-based sample of twins, *Journal of Allergy & Clinical Immunology*. 116(6):1235-1241.

Harding, J., Liu, L., Evans, P., Oliver, M., & Gluckman, P. 1992, Intrauterine feeding of the growth retarded fetus: can we help? *Early Human Development*. 29(1-3):193-7

Harik-Khan, R. I., Muller, D. C., & Wise, R. A. 2004, Serum vitamin levels and the risk of asthma in children, *American Journal of Epidemiology*. 159(4):351-7.

Harley, K. G., Macher, J. M., Lipsett, M., Duramad, P., Holland, N. T., Prager, S. S. *et al.* 2009, Fungi and pollen exposure in the first months of life and risk of early childhood wheezing, *Thorax*. 64(4):353-8.

Harvey, N. C., Javaid, M. K., Poole, J. R., Taylor, P., Robinson, S. M., Inskip, H. M. *et al.* 2008, Paternal skeletal size predicts intrauterine bone mineral accrual, *Journal of Clinical Endocrinology & Metabolism*. 93(5):676-1681.

Harvey, N. C., Poole, J. R., Javaid, M. K., Dennison, E. M., Robinson, S., Inskip, H. M. *et al.* 2007, Parental determinants of neonatal body composition, *Journal of Clinical Endocrinology & Metabolism*. 92(2):523-526.

Healy, M. J., Tanner, J. M., Mackenzie, JD., & Whitehouse, R. H. 1956, Aberdeen growth study I: the prediction of adult body measurements from measurements taken each year from birth to 5 years, *Archives of Disease in Childhood*. 31:372-381.

Heinrich, J., Nowak, D., Wassmer, G., Jorres, R., Wjst, M., Berger, J. *et al.* 1998a, Age-dependent differences in the prevalence of allergic rhinitis and atopic sensitization between an eastern and a western German city, *Allergy*. 53(1):89-93.

Heinrich, J., Popescu, M. A., Wjst, M., Goldstein, I. F., & Wichmann, H. E. 1998b, Atopy in children and parental social class, *American Journal of Public Health.* 88(9):1319-24.

Hemmingsson, T., Linnarsson, D., & Gambert, R. 2004, Novel hand-held device for exhaled nitric oxide-analysis in research and clinical applications, *Journal of Clinical Monitoring & Computing.* 18,(5-6):379-387.

Henderson, J., Granell, R., Heron, J., Sherriff, A., Simpson, A., Woodcock, A. *et al.* 2008, Associations of wheezing phenotypes in the first 6 years of life with atopy, lung function and airway responsiveness in mid-childhood, *Thorax.* 63(11):974-80.

Heslehurst, N., Ells, L. J., Simpson, H., Batterham, A., Wilkinson, J., & Summerbell, C. D. 2007, Trends in maternal obesity incidence rates, demographic predictors, and health inequalities in 36,821 women over a 15-year period, *British Journal of Obstetrics & Gynaecology.* 114(2):187-94.

Hislop, A. A. 2001, Airway and blood vessel interaction during lung development, *Journal of Anatomy.* 4:325-334.

Hodge, L., Salome, C. M., Peat, J. K., Haby, M. M., Xuan, W., & Woolcock, A. J. 1996, Consumption of oily fish and childhood asthma risk, *Medical Journal of Australia.* 164(3):137-40.

Holick, M. F. 2003, Vitamin D: A millenium perspective, *Journal of Cellular Biochemistry.* 88(2):296-307.

Holick, M. F. 2004, Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease, *American Journal of Clinical Nutrition.* 80(Suppl.):1678S-88S.

Hollingsworth, J. W., Maruoka, S., Boon, K., Garantziotis, S., Li, Z., Tomfohr, J. *et al.* 2008, In utero supplementation with methyl donors enhances allergic airway disease in mice, *Journal of Clinical Investigation.* 118(10):3462-9.

Hollis, B. W. & Pittard, W. B., III 1984, Evaluation of the total fetomaternal vitamin D relationships at term: evidence for racial differences, *Journal of Clinical Endocrinology & Metabolism.* 59(4):652-7.

Holt, P. G., Macaubas, C., Prescott, S. L., & Sly, P. D. 2000, Primary sensitization to inhalant allergens, *American Journal of Respiratory & Critical Care Medicine.* 162(3 Pt 2):S91-4.

Hoo, A. F., Dezateux, C., Henschen, M., Costeloe, K., & Stocks, J. 2002, Development of airway function in infancy after preterm delivery, *Journal of Pediatrics.* 141(5):652-658.

Hoo, A. F., Stocks, J., Lum, S., Wade, A. M., Castle, R. A., Costeloe, K. L. *et al.* 2004, Development of lung function in early life: influence of birth weight in infants of nonsmokers, *American Journal of Respiratory & Critical Care Medicine.* 170(5):527-533.

Horrobin, D. F. 2000, Essential fatty acid metabolism and its modification in atopic eczema, *American Journal of Clinical Nutrition.* 71(Suppl.):367S-372S.

Hypponen, E., Sovio, U., Wjst, M., Patel, S., Pekkanen, J., Hartikainen, A. L. *et al.* 2004, Infant vitamin D supplementation and allergic conditions in adulthood: northern Finland birth cohort 1966, *Annals of the New York Academy of Sciences.* 1037:84-95.

Inskip, H. M., Godfrey, K. M., Robinson, S. M., Law, C. M., Barker, D. J., Cooper, C. *et al.* 2006, Cohort profile: The Southampton Women's Survey, *International Journal of Epidemiology.* 35(1):42-48.

Islam, S., Narra, V., Cote, G. M., Manganaro, T. F., Donahoe, P. K., & Schnitzer, J. J. 1999, Prenatal vitamin E treatment improves lung growth in fetal rats with congenital diaphragmatic hernia, *Journal of Pediatric Surgery.* 34(1):172-6.

Jarvis, D. & Burney, P. 1998, ABC of allergies. The epidemiology of allergic disease, *BMJ.* 316(7131):607-610.

Jenkins, M. A., Clarke, J. R., Carlin, J. B., Robertson, C. F., Hopper, J. L., Dalton, M. F. *et al.* 1996, Validation of questionnaire and bronchial hyperresponsiveness against respiratory physician assessment in the diagnosis of asthma, *International Journal of Epidemiology.* 25(3):609-16.

Jirapongsananuruk, O., Melamed, I., & Leung, D. Y. 2000, Additive immunosuppressive effects of 1,25-dihydroxyvitamin D3 and corticosteroids on TH1, but not TH2, responses, *Journal of Allergy & Clinical Immunology.* 106(5):981-5.

Jobsis, Q., Schellekens, S. L., Kroesbergen, A., Hop, W. C., & de Jongste, J. C. 1999, Sampling of exhaled nitric oxide in children: end-expiratory plateau, balloon and tidal breathing methods compared, *European Respiratory Journal.* 13(6):1406-1410.

Johnston, S. L., Clough, J. B., Pattemore, P. K., Smith, S., & Holgate, S. T. 1992, Longitudinal changes in skin-prick test reactivity over 2 years in a population of schoolchildren with respiratory symptoms, *Clinical & Experimental Allergy.* 22(10):948-957.

Jokic, R., Davis, E. E., & Cockcroft, D. W. 1998, Methacholine PC20 extrapolation, *Chest.* 114(6):1796-7.

Jones, A. C., Miles, E. A., Warner, J. O., Colwell, B. M., Bryant, T. N., & Warner, J. A. 1996, Fetal peripheral blood mononuclear cell proliferative responses to mitogenic and allergenic stimuli during gestation, *Pediatric Allergy & Immunology.* 7(3):109-16.

Jones, C. A., Vance, G. H., Power, L. L., Pender, S. L., Macdonald, T. T., & Warner, J. O. 2001, Costimulatory molecules in the developing human gastrointestinal tract: a pathway for fetal allergen priming, *Journal of Allergy & Clinical Immunology.* 108(2):235-41.

Jones, M. H., Davis, S. D., Grant, D., Christoph, K., Kisling, J., & Tepper, R. S. 1999, Forced expiratory maneuvers in very young children. Assessment of flow limitation, *American Journal of Respiratory & Critical Care Medicine.* 159(3):791-795.

Joyce, B. J., Louey, S., Davey, M. G., Cock, M. L., Hooper, S. B., & Harding, R. 2001, Compromised respiratory function in postnatal lambs after placental insufficiency and intrauterine growth restriction, *Pediatric Research.* 50(5):641-649.

Juniper, E. F., Frith, P. A., Dunnett, C., Cockcroft, D. W., & Hargreave, F. E. 1978, Reproducibility and comparison of responses to inhaled histamine and methacholine, *Thorax.* 33(6):705-10.

Kalinski, P., Hilkens, C. M., Snijders, A., Snijdewint, F. G., & Kapsenberg, M. L. 1997, IL-12-deficient dendritic cells, generated in the presence of prostaglandin E2, promote type 2 cytokine production in maturing human naïve T helper cells, *Journal of Immunology.* 159(1):28-35.

Katz, K. A., Pocock, S. J., & Strachan, D. P. 2003, Neonatal head circumference, neonatal weight, and risk of hayfever, asthma and eczema in a large cohort of adolescents from Sheffield, England, *Clinical & Experimental Allergy.* 33(6):737-45.

Kaur, B., Anderson, H. R., Austin, J., Burr, M., Harkins, L. S., Strachan, D. P. *et al.* 1998, Prevalence of asthma symptoms, diagnosis, and treatment in 12-14 year old children across Great Britain (international study of asthma and allergies in childhood, ISAAC UK), *BMJ.* 316(7125):18-124.

Kawano, Y., Morikawa, M., Watanabe, M., Ohshiba, A., Noma, T., & Odajima, H. 2005, Fetal growth promotion in allergic children, *Pediatric Allergy & Immunology.* 16(4):354-6.

Kelly, Y. J., Brabin, B. J., Milligan, P., Heaf, D. P., Reid, J., & Pearson, M. G. 1995, Maternal asthma, premature birth, and the risk of respiratory morbidity in schoolchildren in Merseyside, *Thorax.* 50(5):525-30.

Kharitonov, S. A., Gonio, F., Kelly, C., Meah, S., & Barnes, P. J. 2003, Reproducibility of exhaled nitric oxide measurements in healthy and asthmatic adults and children, *European Respiratory Journal.* 21(3):433-8.

Kharitonov, S. A., Yates, D., & Barnes, P. J. 1995, Increased nitric oxide in exhaled air of normal human subjects with upper respiratory tract infections, *European Respiratory Journal.* 8(2):295-297.

Kim, H. P. & Leonard, W. J. 2004, CREB/ATF-dependent T cell receptor-induced FoxP3 gene expression: a role for DNA methylation, *Journal of Experimental Medicine.* 7:1543-1551.

Kim, H. Y., Picciano, M. F., Wallig, M. A., & Milner, J. A. 1991, The role of selenium nutrition in the development of neonatal rat lung, *Pediatric Research.* 29(5):440-5.

King, J. C. 2006, Maternal obesity, metabolism, and pregnancy outcomes, *Annual Review of Nutrition.* 26:271-91.

Kjellman, N. I. 1977, Atopic disease in seven-year-old children. Incidence in relation to family history, *Acta Paediatrica Scandinavica.* 66(4):465-71.

Kochanowski, B. A. & Sherman, A. R. 1982, Cellular growth in iron-deficient rat pups, *Growth.* 46(2):126-134.

Kondo, N., Kobayashi, Y., Shinoda, S., Kasahara, K., Kameyama, T., Iwasa, S. *et al.* 1992, Cord blood lymphocyte responses to food antigens for the prediction of allergic disorders, *Archives of Disease in Childhood.* 67(8):1003-7.

Kramer, M. S. & Kakuma, R. 2003, Energy and protein intake in pregnancy, *Cochrane Database of Systematic Reviews*, vol. Issue 4. Art. No. CD000032. DOI: 10.1002/14651858.CD000032.

Krilis, S., Baldo, B. A., & Basten, A. 1985, Analysis of allergen-specific IgE responses in 341 allergic patients. Associations between allergens and between allergen groups and clinical diagnoses, *Australian & New Zealand Journal of Medicine*. 15(4):421-426.

Kulig, M., Bergmann, R., Klettke, U., Wahn, V., Tacke, U., & Wahn, U. 1999, Natural course of sensitization to food and inhalant allergens during the first 6 years of life, *Journal of Allergy & Clinical Immunology*. 103(6):1173-9.

Kurukulaaratchy, R. J., Fenn, M., Twiselton, R., Matthews, S., & Arshad, S. H. 2002, The prevalence of asthma and wheezing illnesses amongst 10-year-old schoolchildren, *Respiratory Medicine*. 96(3)163-169.

Laerum, B. N., Svanes, C., Wentzel-Larsen, T., Gulsvik, A., Iversen, M., Gislason, T. *et al.* 2005, The association between birth size and atopy in young North-European adults, *Clinical & Experimental Allergy*. 35(8):1022-7.

Lambert, R. K. & Pare, P. D. 1997, Lung parenchymal shear modulus, airway wall remodeling, and bronchial hyperresponsiveness, *Journal of Applied Physiology*. 83(1):140-7.

Lang, U., Baker, R. S., Khoury, J., & Clark, K. E. 2000, Effects of chronic reduction in uterine blood flow on fetal and placental growth in the sheep, *American Journal of Physiology - Regulatory Integrative & Comparative Physiology*. 279(1):R53-R59.

Laprise, C. & Boulet, L. P. 1997, Asymptomatic airway hyperresponsiveness: a three-year follow-up, *American Journal of Respiratory & Critical Care Medicine*. 156(2 Pt 1):403-9.

Lawlor, D. A., Ebrahim, S., & Davey, S. G. 2004, Association between self-reported childhood socioeconomic position and adult lung function: findings from the British Women's Heart and Health Study, *Thorax*. 59(3):199-203.

Le Souef, P. N., Sears, M. R., & Sherrill, D. 1995, The effect of size and age of subject on airway responsiveness in children, *American Journal of Respiratory & Critical Care Medicine*. 152(2):576-579.

Leadbitter, P., Pearce, N., Cheng, S., Sears, M. R., Holdaway, M. D., Flannery, E. M. *et al.* 1999, Relationship between fetal growth and the development of asthma and atopy in childhood, *Thorax*. 54(10):905-10.

Leaf, A. A., Leighfield, M. J., Costeloe, K. L., & Crawford, M. A. 1992, Long chain polyunsaturated fatty acids and fetal growth, *Early Human Development*. 30(3):183-91.

Lee, D. U., Agarwal, S., & Rao, A. 2002, Th2 lineage commitment and efficient IL-4 production involves extended demethylation of the IL-4 gene, *Immunity*. 16(5):649-60.

Lee, J. M., Smith, J. R., Philipp, B. L., Chen, T. C., Mathieu, J., & Holick, M. F. 2007, Vitamin D deficiency in a healthy group of mothers and newborn infants, *Clinical Pediatrics*. 46(1):42-4.

Lewis, S., Richards, D., Bynner, J., Butler, N., & Britton, J. 1995, Prospective study of risk factors for early and persistent wheezing in childhood, *European Respiratory Journal.* 8(3):349-56.

Li-Weber, M., Giaisi, M., Treiber, M. K., & Krammer, P. H. 2002, Vitamin E inhibits IL-4 gene expression in peripheral blood T cells, *European Journal of Immunology.* 32(9):2401-8.

Litonjua, A. A., Carey, V. J., Burge, H. A., Weiss, S. T., & Gold, D. R. 1998, Parental history and the risk for childhood asthma. Does mother confer more risk than father? *American Journal of Respiratory & Critical Care Medicine.* 158(1):176-81.

Litonjua, A. A., Rifas-Shiman, S. L., Ly, N. P., Tantisira, K. G., Rich-Edwards, J. W., Camargo, C. A., Jr. *et al.* 2006, Maternal antioxidant intake in pregnancy and wheezing illnesses in children at 2 y of age, *American Journal of Clinical Nutrition.* 84(4):903-11.

Litonjua, A. A. & Weiss, S. T. 2007, Is vitamin D deficiency to blame for the asthma epidemic? *Journal of Allergy & Clinical Immunology.* 120(5):1031-5.

Lommatzsch, M., Schloetcke, K., Klotz, J., Schuhbaeck, K., Zingler, D., Zingler, C. *et al.* 2005, Brain-derived neurotrophic factor in platelets and airflow limitation in asthma, *American Journal of Respiratory & Critical Care Medicine.* 171(2):115-20.

Loren, M. L., Leung, P. K., Cooley, R. L., Chai, H., Bell, T. D., & Buck, V. M. 1978, Irreversibility of obstructive changes in severe asthma in childhood, *Chest.* 74(2):126-129.

Lucas, J. S., Inskip, H. M., Godfrey, K. M., Foreman, C. T., Warner, J. O., Gregson, R. K. *et al.* 2004, Small size at birth and greater postnatal weight gain: relationships to diminished infant lung function, *American Journal of Respiratory & Critical Care Medicine.* 170(5):534-40.

Lum, S., Hoo, A. F., Dezateux, C., Goetz, I., Wade, A., DeRooy, L. *et al.* 2001, The association between birthweight, sex, and airway function in infants of nonsmoking mothers, *American Journal of Respiratory & Critical Care Medicine.* 164(11):2078-2084.

Luyt, D. K., Burton, P., Brooke, A. M., & Simpson, H. 1994, Wheeze in preschool children and its relation with doctor diagnosed asthma, *Archives of Disease in Childhood.* 71(1):24-30.

Madan, J. C., Davis, J. M., Craig, W. Y., Collins, M., Allan, W., Quinn, R. *et al.* 2009, Maternal obesity and markers of inflammation in pregnancy, *Cytokine.* 47(1):61-4.

Maden, M. & Hind, M. 2004, Retinoic acid in alveolar development, maintenance and regeneration, *Philosophical Transactions of the Royal Society of London - Series B: Biological Sciences.* 359(1445):799-808.

Malling, H. J. 1984, Skin prick testing and the use of histamine references, *Allergy.* 39(8):596-601.

Malmberg, K. J., Lenkei, R., Petersson, M., Ohlum, T., Ichihara, F., Glimelius, B. *et al.* 2002, A short-term dietary supplementation of high doses of vitamin E increases T

helper 1 cytokine production in patients with advanced colorectal cancer, *Clinical Cancer Research.* 8(6):1772-8.

Malmberg, L. P., Petays, T., Haahtela, T., Laatikainen, T., Jousilahti, P., Vartiainen, E. *et al.* 2006, Exhaled nitric oxide in healthy nonatopic school-age children: determinants and height-adjusted reference values, *Pediatric Pulmonology.* 41(7):635-42.

Maloney, J. E., Bowes, G., Brodecky, V., Dennett, X., Wilkinson, M., & Walker, A. 1982, Function of the future respiratory system in the growth retarded fetal sheep, *Journal of Developmental Physiology.* 4(5):279-297.

Manku, M. S., Horrobin, D. F., Morse, N., Kyte, V., Jenkins, K., Wright, S. *et al.* 1982, Reduced levels of prostaglandin precursors in the blood of atopic patients: defective delta-6-desaturase function as a biochemical basis for atopy, *Prostaglandins Leukotrienes & Medicine.* 9(6):615-28.

Maritz, G. S., Cock, M. L., Louey, S., Joyce, B. J., Albuquerque, C. A., & Harding, R. 2001, Effects of fetal growth restriction on lung development before and after birth: a morphometric analysis, *Pediatric Pulmonology.* 32(3):201-210.

Marriott, L. D., Robinson, S. M., Poole, J., Borland, S. E., Godfrey, K. M., Law, C. M. *et al.* 2008, What do babies eat? Evaluation of a food frequency questionnaire to assess the diets of infants aged 6 months, *Public Health Nutrition.* 11(7):751-756.

Marseglia, G. L., Cirillo, I., Vizzaccaro, A., Klersy, C., Tosca, M. A., La, R. M. *et al.* 2007, Role of forced expiratory flow at 25-75% as an early marker of small airways impairment in subjects with allergic rhinitis, *Allergy & Asthma Proceedings.* 28(1):74-78.

Martin, R., Harvey, N. C., Crozier, S. R., Poole, J. R., Javaid, M. K., Dennison, E. M. *et al.* 2007, Placental calcium transporter (PMCA3) gene expression predicts intrauterine bone mineral accrual, *Bone.* 40(5):1203-1208.

Martindale, S., McNeill, G., Devereux, G., Campbell, D., Russell, G., & Seaton, A. 2005, Antioxidant intake in pregnancy in relation to wheeze and eczema in the first two years of life, *American Journal of Respiratory & Critical Care Medicine.* 171(2):121-8.

Martinez, F. D. 2002, What have we learned from the Tucson Children's Respiratory Study? *Paediatric Respiratory Reviews.* 3(3):193-197.

Martinez, F. D. 2007, Genes, environments, development and asthma: a reappraisal, *European Respiratory Journal.* 29(1):179-84.

Martinez, F. D. & Helms, P. J. 1998, Types of asthma and wheezing., *European Respiratory Journal.* 27(Suppl.):3s-8s.

Martinez, F. D., Morgan, W. J., Wright, A. L., Holberg, C., & Taussig, L. M. 1991, Initial airway function is a risk factor for recurrent wheezing respiratory illnesses during the first three years of life. Group Health Medical Associates, *American Review of Respiratory Disease.* 143(2):312-316.

Martinez, F. D., Wright, A. L., Taussig, L. M., Holberg, C. J., Halonen, M., & Morgan, W. J. 1995, Asthma and wheezing in the first six years of life. The Group Health Medical Associates, *New England Journal of Medicine.* 332(3):133-8.

Massaro, G. D. & Massaro, D. 1996, Postnatal treatment with retinoic acid increases the number of pulmonary alveoli in rats, *American Journal of Physiology*. 270(2 Pt 1):L305-10.

Massaro, G. D., McCoy, L., & Massaro, D. 1988, Postnatal undernutrition slows development of bronchiolar epithelium in rats, *American Journal of Physiology*. 255(4 Pt 2):R521-6.

Matheu, V., Back, O., Mondoc, E., & Issazadeh-Navikas, S. 2003, Dual effects of vitamin D-induced alteration of TH1/TH2 cytokine expression: enhancing IgE production and decreasing airway eosinophilia in murine allergic airway disease, *Journal of Allergy & Clinical Immunology*. 112(3):585-92.

Mathews, F., Yudkin, P., & Neil, A. 1999, Influence of maternal nutrition on outcome of pregnancy: prospective cohort study, *BMJ*. 319(7206):339-43.

Matricardi, P. M., Franzinelli, F., Franco, A., Caprio, G., Murru, F., Cioffi, D. *et al.* 1998, Sibship size, birth order, and atopy in 11,371 Italian young men, *Journal of Allergy & Clinical Immunology*. 101(4 Pt 1):439-44.

McDade, T. W., Kuzawa, C. W., Adair, L. S., & Beck, M. A. 2004, Prenatal and early postnatal environments are significant predictors of total immunoglobulin E concentration in Filipino adolescents, *Clinical & Experimental Allergy*. 34(1):44-50.

McKeever, T. M., Lewis, S. A., Smit, H., Burney, P., Britton, J., & Cassano, P. A. 2004, Serum nutrient markers and skin prick testing using data from the Third National Health and Nutrition Examination Survey, *Journal of Allergy & Clinical Immunology*. 114(6):1398-402.

Meehan, M. A., Kerman, R. H., & Lemire, J. M. 1992, 1,25-Dihydroxyvitamin D3 enhances the generation of nonspecific suppressor cells while inhibiting the induction of cytotoxic cells in a human MLR, *Cellular Immunology*. 140(2):400-9.

Meinert, R., Frischer, T., Karmaus, W., & Kuehr, J. 1994, Influence of skin prick test criteria on estimation of prevalence and incidence of allergic sensitization in children, *Allergy*. 49(7):526-532.

Metzler, M. D. & Snyder, J. M. 1993, Retinoic acid differentially regulates expression of surfactant-associated proteins in human fetal lung, *Endocrinology*. 133(5):1990-8.

Migliaccio, G., Migliaccio, A. R., Petti, S., Mavilio, F., Russo, G., Lazzaro, D. *et al.* 1986, Human embryonic hemopoiesis. Kinetics of progenitors and precursors underlying the yolk sac-liver transition, *Journal of Clinical Investigation*. 78(1):51-60.

Mihrshahi, S., Peat, J. K., Webb, K., Oddy, W., Marks, G. B., Mellis, C. M. *et al.* 2004, Effect of omega-3 fatty acid concentrations in plasma on symptoms of asthma at 18 months of age, *Pediatric Allergy & Immunology*. 15(6):517-22.

Miles, E. A., Aston, L., & Calder, P. C. 2003, In vitro effects of eicosanoids derived from different 20-carbon fatty acids on T helper type 1 and T helper type 2 cytokine production in human whole-blood cultures, *Clinical & Experimental Allergy*. 33(5):624-32.

Miller, M. R., Hankinson, J., Brusasco, V., Burgos, F., Casaburi, R., Coates, A. *et al.* 2005, Standardisation of spirometry, *European Respiratory Journal*. 26(2):319-338.

Moore, S. E., Cole, T. J., Poskitt, E. M. E., Sonko, B. J., Whitehead, R. G. M. I. A., & Prentice, A. M. 1997, Season of birth predicts mortality in rural Gambia, *Nature*. 388(6641):434.

Morriss, F. H., Rosenfeld, C. R., Crandell, S. S., & Adcock, E. W., III 1980, Effects of fasting on uterine blood flow and substrate uptake in sheep, *Journal of Nutrition*. 110(12):2433-43.

Murray, A. B., Ferguson, A. C., & Morrison, B. 1981, Airway responsiveness to histamine as a test for overall severity of asthma in children, *Journal of Allergy & Clinical Immunology*. 68(2):119-24.

Naeye, R. L. 1987, Do placental weights have clinical significance? *Human Pathology*. 18(4):387-91.

Nafstad, P., Magnus, P., & Jaakkola, J. J. 2000, Risk of childhood asthma and allergic rhinitis in relation to pregnancy complications, *Journal of Allergy & Clinical Immunology*. 106(5):867-73.

National Asthma Campaign 2001, Out in the open: a true picture of asthma in the United Kingdom today, *Asthma Journal*. 6(Suppl.):3-14.

Nesby-O'Dell, S., Scanlon, K. S., Cogswell, M. E., Gillespie, C., Hollis, B. W., Looker, A. C. *et al.* 2002, Hypovitaminosis D prevalence and determinants among African American and white women of reproductive age: third National Health and Nutrition Examination Survey, 1988-1994, *American Journal of Clinical Nutrition*. 76(1):187-92.

Ness, A. R., Khaw, K. T., Bingham, S., & Day, N. E. 1996, Vitamin C status and respiratory function, *European Journal of Clinical Nutrition*. 50(9):573-9.

Newson, R. B., Shaheen, S. O., Henderson, A. J., Emmett, P. M., Sherriff, A., & Calder, P. C. 2004, Umbilical cord and maternal blood red cell fatty acids and early childhood wheezing and eczema, *Journal of Allergy & Clinical Immunology*. 114(3):531-7.

Nguyen, T. M., Guillozo, H., Marin, L., Tordet, C., Koite, S., & Garabedian, M. 1996, Evidence for a vitamin D paracrine system regulating maturation of developing rat lung epithelium, *American Journal of Physiology*. 271(3 Pt 1):L392-9.

Niggemann, B., Illi, S., Madloch, C., Volkel, K., Lau, S., Bergmann, R. *et al.* 2001, Histamine challenges discriminate between symptomatic and asymptomatic children. MAS-Study Group. Multicentre Allergy Study, *European Respiratory Journal*. 17(2):246-53.

Nystad, W., Samuelsen, S. O., Nafstad, P., Edvardsen, E., Stensrud, T., & Jaakkola, J. J. 2002, Feasibility of measuring lung function in preschool children, *Thorax*. 57(12):1021-1027.

O'Connor, G., Sparrow, D., Taylor, D., Segal, M., & Weiss, S. 1987, Analysis of dose-response curves to methacholine. An approach suitable for population studies, *American Review of Respiratory Disease*. 136(6):1412-7.

Oddy, W. H. & Sherriff, J. L. 2003, Breastfeeding, body mass index, asthma and atopy in children, *Asia-Pacific Journal of Public Health*. 15(Suppl.):S15-7.

Oddy, W. H., Sherriff, J. L., de Klerk, N. H., & Kendall, G. E. 2004, Breastfeeding, body mass index, and asthma and atopy in children, *Advances in Experimental Medicine & Biology.* 554:387-90.

Office of Health Economics 2003, *Compendium of Health Statistics,* 15th edn.

Okamoto, M., Mitsunobu, F., Ashida, K., Mifune, T., Hosaki, Y., Tsugeno, H. *et al.* 2000a, Effects of dietary supplementation with n-3 fatty acids compared with n-6 fatty acids on bronchial asthma, *Internal Medicine.* 39(2):107-11.

Okamoto, M., Mitsunobu, F., Ashida, K., Mifune, T., Hosaki, Y., Tsugeno, H. *et al.* 2000b, Effects of perilla seed oil supplementation on leukotriene generation by leucocytes in patients with asthma associated with lipometabolism, *International Archives of Allergy & Immunology.* 122(2):137-42.

Oliveti, J. F., Kercsmar, C. M., & Redline, S. 1996, Pre- and perinatal risk factors for asthma in inner city African-American children, *American Journal of Epidemiology.* 143(6):570-7.

Olsen, S. F., Osterdal, M. L., Salvig, J. D., Mortensen, L. M., Rytter, D., Secher, N. J. *et al.* 2008, Fish oil intake compared with olive oil intake in late pregnancy and asthma in the offspring: 16 years of registry-based follow-up from a randomized controlled trial, *American Journal of Clinical Nutrition.* 88(1):167-75.

Ong, K. K., Ahmed, M. L., Emmett, P. M., Preece, M. A., & Dunger, D. B. 2000, Association between postnatal catch-up growth and obesity in childhood: prospective cohort study, *BMJ.* 320(7240):967-71.

Ortega, R. M., Andres, P., Martinez, R. M., & Lopez-Sobaler, A. M. 1997, Vitamin A status during the third trimester of pregnancy in Spanish women: influence on concentrations of vitamin A in breast milk, *American Journal of Clinical Nutrition.* 66(3):564-8.

Ownby, D. R., Peterson, E. L., & Johnson, C. C. 2000, Factors related to methacholine airway responsiveness in children, *American Journal of Respiratory & Critical Care Medicine.* 161(5):1578-83.

Paredi, P., Kharitonov, S. A., Loukides, S., Pantelidis, P., du Bois, R. M., & Barnes, P. J. 1999, Exhaled nitric oxide is increased in active fibrosing alveolitis, *Chest.* 115(5):1352-1356.

Peat, J. K., Britton, W. J., Salome, C. M., & Woolcock, A. J. 1987, Bronchial hyperresponsiveness in two populations of Australian schoolchildren. III. Effect of exposure to environmental allergens, *Clinical Allergy.* 17(4):291-300.

Peat, J. K., Salome, C. M., Toelle, B. G., Bauman, A., & Woolcock, A. J. 1992a, Reliability of a respiratory history questionnaire and effect of mode of administration on classification of asthma in children, *Chest.* 102(1):153-7.

Peat, J. K., Salome, C. M., & Woolcock, A. J. 1992b, Factors associated with bronchial hyperresponsiveness in Australian adults and children, *European Respiratory Journal.* 5(8):921-9.

Pedroletti, C., Zetterquist, W., Nordvall, L., & Alving, K. 2002, Evaluation of exhaled nitric oxide in schoolchildren at different exhalation flow rates, *Pediatric Research.* 52(3):393-8.

Pellegrino, R., Sterk, P. J., Sont, J. K., & Brusasco, V. 1998, Assessing the effect of deep inhalation on airway calibre: a novel approach to lung function in bronchial asthma and COPD, *European Respiratory Journal.* 12(5):1219-1227.

Pellegrino, R., Viegi, G., Brusasco, V., Crapo, R. O., Burgos, F., Casaburi, R. *et al.* 2005, Interpretative strategies for lung function tests, *European Respiratory Journal.* 26(5):948-968.

Perneger, V. 1998, What's wrong with Bonferroni adjustments. *BMJ* 316:1236-1238.

Pesonen, M., Kallio, M. J., Siimes, M. A., & Ranki, A. 2007, Retinol concentrations after birth are inversely associated with atopic manifestations in children and young adults, *Clinical & Experimental Allergy.* 37(1):54-61.

Pichler, J., Gerstmayr, M., Szepfalusi, Z., Urbanek, R., Peterlik, M., & Willheim, M. 2002, 1 alpha,25(OH)2D3 inhibits not only Th1 but also Th2 differentiation in human cord blood T cells, *Pediatric Research.* 52(1):12-8.

Pierce, R. A. & Michael, S. J. 2000, Retinoid-enhanced alveolization: identifying relevant downstream targets, *American Journal of Respiratory Cell & Molecular Biology.* 23(2):137-41.

Poon, A. H., Laprise, C., Lemire, M., Montpetit, A., Sinnett, D., Schurr, E. *et al.* 2004, Association of vitamin D receptor genetic variants with susceptibility to asthma and atopy, *American Journal of Respiratory & Critical Care Medicine.* 170(9):967-73.

Prescott, S. L., Barden, A. E., Mori, T. A., & Dunstan, J. A. 2007, Maternal fish oil supplementation in pregnancy modifies neonatal leukotriene production by cord-blood-derived neutrophils, *Clinical Science.* 113(10):409-16.

Prescott, S. L., Macaubas, C., Smallacombe, T., Holt, B. J., Sly, P. D., & Holt, P. G. 1999, Development of allergen-specific T-cell memory in atopic and normal children, *Lancet.* 353(9148):196-200.

Proskocil, B. J., Sekhon, H. S., Clark, J. A., Lupo, S. L., Jia, Y., Hull, W. M. *et al.* 2005, Vitamin C prevents the effects of prenatal nicotine on pulmonary function in newborn monkeys, *American Journal of Respiratory & Critical Care Medicine.* 171(9):1032-9.

Purohit, A., Melac, M., Pauli, G., & Frossard, N. 2002, Comparative activity of cetirizine and mizolastine on histamine-induced skin wheal and flare responses at 24 hours, *British Journal of Clinical Pharmacology.* 53(3):250-254.

Raby, B. A., Lazarus, R., Silverman, E. K., Lake, S., Lange, C., Wjst, M. *et al.* 2004, Association of vitamin D receptor gene polymorphisms with childhood and adult asthma, *American Journal of Respiratory & Critical Care Medicine.* 170(10):1057-65.

Ramsay, J. E., Ferrell, W. R., Crawford, L., Wallace, A. M., Greer, I. A., & Sattar, N. 2002, Maternal obesity is associated with dysregulation of metabolic, vascular, and inflammatory pathways, *Journal of Clinical Endocrinology & Metabolism.* 87(9):4231-7.

Rayman, M. P. 1997, Dietary selenium: time to act, *BMJ.* 314(7078):387-8.

Reik, W., Dean, W., & Walter, J. 2001, Epigenetic reprogramming in mammalian development, *Science.* 293(5532):1089-93.

Remes, S. T., Patel, S. P., Hartikainen, A. L., Jarvelin, M. R., & Pekkanen, J. 2008, High birth weight, asthma and atopy at the age of 16 yr, *Pediatric Allergy & Immunology.* 6:541-543.

Retnakaran, R., Hanley, A. J., Raif, N., Connelly, P. W., Sermer, M., & Zinman, B. 2003, C-reactive protein and gestational diabetes: the central role of maternal obesity. *Journal of Clinical Endocrinology & Metabolism.* 88(8):3507-12.

Ricciarelli, R., Maroni, P., Ozer, N., Zingg, J. M., & Azzi, A. 1999, Age-dependent increase of collagenase expression can be reduced by alpha-tocopherol via protein kinase C inhibition, *Free Radical Biology & Medicine.* 27(7-8):729-37.

Rich-Edwards, J. W., Stampfer, M. J., Manson, J. E., Rosner, B., Hankinson, S. E., Colditz, G. A. *et al.* 1997, Birth weight and risk of cardiovascular disease in a cohort of women followed up since 1976, *BMJ* .315(7105):396-400.

Roberts, G., Hurley, C., Bush, A., & Lack, G. 2004, Longitudinal study of grass pollen exposure, symptoms, and exhaled nitric oxide in childhood seasonal allergic asthma, *Thorax.* 59(9):752-756.

Robinson, S., Godfrey, K., Osmond, C., Cox, V., & Barker, D. 1996, Evaluation of a food frequency questionnaire used to assess nutrient intakes in pregnant women, *European Journal of Clinical Nutrition.* 50(5):302-308.

Robinson, S., Marriott, L., Poole, J., Crozier, S., Borland, S., Lawrence, W. *et al.* 2007, Dietary patterns in infancy: the importance of maternal and family influences on feeding practice, *British Journal of Nutrition.* 98(5):1029-37.

Robinson, S. M., Crozier, S. R., Borland, S. E., Hammond, J., Barker, D. J., & Inskip, H. M. 2004, Impact of educational attainment on the quality of young women's diets, *European Journal of Clinical Nutrition.* 58(8):1174-80.

Roes, E. M., Hendriks, J. C., Raijmakers, M. T., Steegers-Theunissen, R. P., Groenen, P., Peters, W. H. *et al.* 2006, A longitudinal study of antioxidant status during uncomplicated and hypertensive pregnancies, *Acta Obstetricia et Gynecologica Scandinavica.* 85(2):148-55.

Rohde, C. M. & DeLuca, H. F. 2005, All-trans retinoic acid antagonizes the action of calciferol and its active metabolite, 1,25-dihydroxycholecalciferol, in rats, *Journal of Nutrition.* 135(7):1647-52.

Romieu, I., Mannino, D. M., Redd, S. C., & McGeehin, M. A. 2004a, Dietary intake, physical activity, body mass index, and childhood asthma in the Third National Health And Nutrition Survey (NHANES III), *Pediatric Pulmonology.* 38(1):31-42.

Romieu, I., Sienra-Monge, J. J., Ramirez-Aguilar, M., Moreno-Macias, H., Reyes-Ruiz, N. I., Estela, d. R.-N. *et al.* 2004b, Genetic polymorphism of GSTM1 and antioxidant

supplementation influence lung function in relation to ozone exposure in asthmatic children in Mexico City, *Thorax*. 59(1):8-10.

Rona, R. J., Gulliford, M. C., & Chinn, S. 1993, Effects of prematurity and intrauterine growth on respiratory health and lung function in childhood, *BMJ*. 306(6881):817-820.

Rona, R. J., Smeeton, N. C., Bustos, P., Amigo, H., & Diaz, P. V. 2005, The early origins hypothesis with an emphasis on growth rate in the first year of life and asthma: a prospective study in Chile, *Thorax*. 60(7):549-54.

Roper, R. L., Brown, D. M., & Phipps, R. P. 1995, Prostaglandin E2 promotes B lymphocyte Ig isotype switching to IgE, *Journal of Immunology*.154(1):162-70.

Rothman, K. J., Moore, L. L., Singer, M. R., Nguyen, U. S., Mannino, S., & Milunsky, A. 1995, Teratogenicity of high vitamin A intake, *New England Journal of Medicine*. 333(21):1369-73.

Royston, P. 1995, Calculation of unconditional and conditional reference intervals for foetal size and growth from longitudinal measurements, *Statistics in Medicine*. 14(13):1417-1436.

Rudolph, A. M. 1984, The fetal circulation and its response to stress, *Journal of Developmental Physiology*. 6(1):11-19.

Ruiz, R. G., Kemeny, D. M., & Price, J. F. 1992, Higher risk of infantile atopic dermatitis from maternal atopy than from paternal atopy, *Clinical & Experimental Allergy*. 22(8):762-6.

Rum, P. & Hornstra, G. 2002, The n-3 and n-6 polyunsaturated fatty acid composition of plasma phospholipids in pregnant women and their infants. relationship with maternal linoleic acid intake, *Clinical Chemistry & Laboratory Medicine*. 40(1):32-9.

Rusconi, F., Galassi, C., Corbo, G. M., Forastiere, F., Biggeri, A., Ciccone, G. *et al.* 1999, Risk factors for early, persistent, and late-onset wheezing in young children. SIDRIA Collaborative Group, *American Journal of Respiratory & Critical Care Medicine*. 160(5 Pt 1):1617-22.

Rusconi, F., Galassi, C., Forastiere, F., Bellasio, M., De Sario, M., Ciccone, G. *et al.* 2007, Maternal complications and procedures in pregnancy and at birth and wheezing phenotypes in children, *American Journal of Respiratory & Critical Care Medicine*. 175(1):16-21.

Sala-Vila, A., Miles, E. A., & Calder, P. C. 2008, Fatty acid composition abnormalities in atopic disease: evidence explored and role in the disease process examined, *Clinical & Experimental Allergy*. 38(9):1432-50.

Salam, M. T., Li, Y. F., Langholz, B., & Gilliland, F. D. 2005, Maternal fish consumption during pregnancy and risk of early childhood asthma, *Journal of Asthma*. 42(6):513-8.

Salome, C. M., Peat, J. K., Britton, W. J., & Woolcock, A. J. 1987, Bronchial hyperresponsiveness in two populations of Australian schoolchildren. I. Relation to respiratory symptoms and diagnosed asthma, *Clinical Allergy*. 17(4):271-81.

Salvatore, S., Keymolen, K., Chandra, R. K., & Vandenplas, Y. 2005, Dietary Intervention during Pregnancy and Allergic Diseases in the Offspring, *Nestle Nutrition Workshop Series.Paediatric Programme.* 55:221-31.

Sanderson, P., MacPherson, G. G., Jenkins, C. H., & Calder, P. C. 1997, Dietary fish oil diminishes the antigen presentation activity of rat dendritic cells, *Journal of Leukocyte Biology.* 62(6):771-7.

Sanin Aguirre, L. H., Reza-Lopez, S., & Levario-Carrillo, M. 2004, Relation between maternal body composition and birth weight, *Biology of the Neonate.* 86(1):55-62.

Sausenthaler, S., Koletzko, S., Schaaf, B., Lehmann, I., Borte, M., Herbarth, O. *et al.* 2007, Maternal diet during pregnancy in relation to eczema and allergic sensitization in the offspring at 2 years of age, *American Journal of Clinical Nutrition.* 85(2):530-537.

Scaife, A. R., McNeill, G., Campbell, D. M., Martindale, S., Devereux, G., & Seaton, A. 2006, Maternal intake of antioxidant vitamins in pregnancy in relation to maternal and fetal plasma levels at delivery, *British Journal of Nutrition.* 95(4):771-8.

Schaub, B. & von Mutius, E. 2005, Obesity and asthma, what are the links? 5, *Current Opinion in Allergy & Clinical Immunology.* 5(2):185-193.

Schenker, S., Yang, Y., Perez, A., Acuff, R. V., Papas, A. M., Henderson, G. *et al.* 1998, Antioxidant transport by the human placenta, *Clinical Nutrition.* 17(4):159-67.

Schubert, R., Kitz, R., Beermann, C., Rose, M. A., Lieb, A., Sommerer, P. C. *et al.* 2009, Effect of n-3 polyunsaturated fatty acids in asthma after low-dose allergen challenge, *International Archives of Allergy & Immunology.* 148(4):321-9.

Schuster, G. U., Kenyon, N. J., & Stephensen, C. B. 2008, Vitamin A deficiency decreases and high dietary vitamin A increases disease severity in the mouse model of asthma, *Journal of Immunology.* 180(3):1834-42.

Scott, G. C. & Kung, M. 1985, How many spirograms for a histamine challenge? *American Review of Respiratory Disease.* 132(2):268-71.

Sears, M. R., Burrows, B., Flannery, E. M., Herbison, G. P., Hewitt, C. J., & Holdaway, M. D. 1991, Relation between airway responsiveness and serum IgE in children with asthma and in apparently normal children, *New England Journal of Medicine.* 325(15):1067-71.

Sears, M. R., Greene, J. M., Willan, A. R., Wiecek, E. M., Taylor, D. R., Flannery, E. M. *et al.* 2003, A longitudinal, population-based, cohort study of childhood asthma followed to adulthood, *New England Journal of Medicine.* 349(15):1414-22.

Sears, M. R., Holdaway, M. D., Flannery, E. M., Herbison, G. P., & Silva, P. A. 1996, Parental and neonatal risk factors for atopy, airway hyper-responsiveness, and asthma, *Archives of Disease in Childhood.* 75(5):392-8.

Seaton, A., Godden, D. J., & Brown, K. 1994, Increase in asthma: a more toxic environment or a more susceptible population? *Thorax.* 49(2):171-4.

Sebire, N. J., Jolly, M., Harris, J. P., Wadsworth, J., Joffe, M., Beard, R. W. *et al.* 2001, Maternal obesity and pregnancy outcome: a study of 287,213 pregnancies in London, *International Journal of Obesity & Related Metabolic Disorders: Journal of the International Association for the Study of Obesity*. 25(8):1175-82.

Seidman, D. S., Laor, A., Gale, R., Stevenson, D. K., & Danon, Y. L. 1991, Is low birth weight a risk factor for asthma during adolescence? *Archives of Disease in Childhood*. 66(5):584-7.

Shaheen, S. O., Newson, R. B., Henderson, A. J., Emmett, P. M., Sherriff, A., Cooke, M. *et al.* 2004, Umbilical cord trace elements and minerals and risk of early childhood wheezing and eczema, *European Respiratory Journal*. 24(2):292-297.

Shaheen, S. O., Northstone, K., Newson, R. B., Emmett, P. M., Sherriff, A., & Henderson, A. J. 2009, Dietary patterns in pregnancy and respiratory and atopic outcomes in childhood, *Thorax*. 64(5):411-7.

Shaheen, S. O., Sterne, J. A., Montgomery, S. M., & Azima, H. 1999, Birth weight, body mass index and asthma in young adults, *Thorax*. 54(5):396-402.

Shekhawat, P. S., Garland, J. S., Shivpuri, C., Mick, G. J., Sasidharan, P., Pelz, C. J. *et al.* 1998, Neonatal cord blood leptin: its relationship to birth weight, body mass index, maternal diabetes, and steroids, *Pediatric Research*. 43(3):338-43.

Shenai, J. P., Chytil, F., Jhaveri, A., & Stahlman, M. T. 1981, Plasma vitamin A and retinol-binding protein in premature and term neonates, *Journal of Pediatrics*. 99(2):302-5.

Shenai, J. P., Kennedy, K. A., Chytil, F., & Stahlman, M. T. 1987, Clinical trial of vitamin A supplementation in infants susceptible to bronchopulmonary dysplasia, *Journal of Pediatrics*. 111(2):269-77.

Silkoff, P. E., McClean, P. A., Slutsky, A. S., Furlott, H. G., Hoffstein, E., Wakita, S. *et al.* 1997, Marked flow-dependence of exhaled nitric oxide using a new technique to exclude nasal nitric oxide, *American Journal of Respiratory & Critical Care Medicine*. 155(1):260-267.

Silverman, M. & Kuehni, C. E. 2007, Early lung development in COPD, *Lancet*. 370:717-718.

Sippel, J. M., Holden, W. E., Tilles, S. A., O'Hollaren, M., Cook, J., Thukkani, N. *et al.* 2000, Exhaled nitric oxide levels correlate with measures of disease control in asthma, *Journal of Allergy & Clinical Immunology*. 106(4):645-650.

Skull, S., Ngeow, J., Biggs, B., Street, A., & Ebeling, P. 2007, Vitamin D deficiency is common and unrecognised among recently arrived adult immigrants from the Horn of Africa, *Internal Medicine Journal*. 33:47-51.

Smith, J. M. 1976, The prevalence of asthma and wheezing in children, *British Journal of Diseases of the Chest*. 70(2):73-7.

Solway, J. & Fredberg, J. J. 1997, Perhaps airway smooth muscle dysfunction contributes to asthmatic bronchial hyperresponsiveness after all, *American Journal of Respiratory Cell & Molecular Biology*. 17(2):144-6.

Southgate, D. A. T. & Hay, E. N. 1975, Chemical and biochemical development of the fetus in *The Biology of Fetal Growth. Symposia of the Sociaety for the Study of Human Biology*, vol. 15 D.F.Roberts and A.M.Thomson, ed., pp. 195-209.

Spanier, A. J., Hornung, R. W., Kahn, R. S., Lierl, M. B., & Lanphear, B. P. 2008, Seasonal variation and environmental predictors of exhaled nitric oxide in children with asthma, *Pediatric Pulmonology.* 43(6):576-83.

Stanojevic, S., Wade, A., Stocks, J., Hankinson, J., Coates, A. L., Pan, H. *et al.* 2008, Reference ranges for spirometry across all ages: a new approach, *American Journal of Respiratory & Critical Care Medicine.* 177(3):253-60.

Stein, R. T., Holberg, C. J., Morgan, W. J., Wright, A. L., Lombardi, E., Taussig, L. *et al.* 1997, Peak flow variability, methacholine responsiveness and atopy as markers for detecting different wheezing phenotypes in childhood, *Thorax.* 52(11):946-52.

Stewart, A. W., Mitchell, E. A., Pearce, N., Strachan, D. P., Weilandon, S. K., & ISAAC Steering Committee.International Study for Asthma and Allergy in Childhood 2001, The relationship of per capita gross national product to the prevalence of symptoms of asthma and other atopic diseases in children (ISAAC), *International Journal of Epidemiology.* 30(1):173-179.

Stick, S. M., Burton, P. R., Gurrin, L., Sly, P. D., & Lesouef, P. N. 1996, Effects of maternal smoking during pregnancy and a family history of asthma on respiratory function in newborn infants, *Lancet.* 348(9034):1060-1064.

Stocks, J. & Dezateux, C. 2003, The effect of parental smoking on lung function and development during infancy, *Respirology.* 8(3):266-85.

Strachan, D. P. 1989a, Hay fever, hygiene, and household size, *BMJ.* 299(6710):1259-60.

Strachan, D. P. 1989b, Repeatability of ventilatory function measurements in a population survey of 7 year old children, *Thorax.* 44(6):474-9.

Strachan, D. P., Butland, B. K., & Anderson, H. R. 1996a, Incidence and prognosis of asthma and wheezing illness from early childhood to age 33 in a national British cohort, *BMJ.* 312(7040):1195-9.

Strachan, D. P., Taylor, E. M., & Carpenter, R. G. 1996b, Family structure, neonatal infection, and hay fever in adolescence, *Archives of Disease in Childhood.* 74(5):422-6.

Svanes, C., Omenaas, E., Heuch, J. M., Irgens, L. M., & Gulsvik, A. 1998, Birth characteristics and asthma symptoms in young adults: results from a population-based cohort study in Norway, *European Respiratory Journal.* 12(6):1366-70.

Szepfalusi, Z., Loibichler, C., Pichler, J., Reisenberger, K., Ebner, C., & Urbanek, R. 2000, Direct evidence for transplacental allergen transfer, *Pediatric Research.* 48(3):404-407.

Takahashi, Y. I., Smith, J. E., Winick, M., & Goodman, D. S. 1975, Vitamin A deficiency and fetal growth and development in the rat, *Journal of Nutrition.* 105(10):1299-310.

Tanner, J. M. 1981, Catch-up growth in man, *British Medical Bulletin*. 37(3):233-238.

Tantisira, K. G., Fuhlbrigge, A. L., Tonascia, J., Van Natta, M., Zeiger, R. S., Strunk, R. C. *et al.* 2006, Bronchodilation and bronchoconstriction: predictors of future lung function in childhood asthma, *Journal of Allergy & Clinical Immunology*. 117(6):1264-71.

Tattersfield, A. E., Knox, A. J., Britton, J. R., & Hall, I. P. 2002, Asthma., *Lancet*. 360(9342):1313-22.

Taudorf, E., Malling, H. J., Laursen, L. C., Lanner, A., & Weeke, B. 1985, Reproducibility of histamine skin prick test. Inter- and intravariation using histamine dihydrochloride 1, 5, and 10 mg/ml, *Allergy*. 40(5):344-349.

Taveras, E. M., Camargo, C. A., Jr., Rifas-Shiman, S. L., Oken, E., Gold, D. R., Weiss, S. T. *et al.* 2006, Association of birth weight with asthma-related outcomes at age 2 years, *Pediatric Pulmonology*. 41(7):643-8.

Taveras, E. M., Rifas-Shiman, S. L., Camargo, C. A., Jr., Gold, D. R., Litonjua, A. A., Oken, E. *et al.* 2008, Higher adiposity in infancy associated with recurrent wheeze in a prospective cohort of children, *Journal of Allergy & Clinical Immunology*. 121(5):1161-1166.e3.

Thomsen, S. F., Ulrik, C. S., Kyvik, K. O., Sorensen, T. I., Posthuma, D., Skadhauge, L. R. *et al.* 2007, Association between obesity and asthma in a twin cohort, *Allergy*. 62(10):1199-1204.

Tjonneland, A., Haraldsdottir, J., Overvad, K., Stripp, C., Ewertz, M., & Jensen, O. M. 1992, Influence of individually estimated portion size data on the validity of a semiquantitative food frequency questionnaire, *International Journal of Epidemiology*. 21(4):770-7.

Tsuchiya, T., Shimizu, H., Horie, T., & Mori, M. 1999, Expression of leptin receptor in lung: leptin as a growth factor, *European Journal of Pharmacology*. 365(2-3):273-9.

Turkeltaub, P. C. & Gergen, P. J. 1989, The risk of adverse reactions from percutaneous prick-puncture allergen skin testing, venipuncture, and body measurements: data from the second National Health and Nutrition Examination Survey 1976-80 (NHANES II), *Journal of Allergy & Clinical Immunology*. 84(6 Pt 1):886-890.

Turner, S., Campbell, D., Smith, N., Craig, L., McNeill, G., Forbes, S. *et al.*. 2008a Transient asthma symptoms are associated with reduced fetal measuremnts at ten and twenty weeks gestation E-communication: E3268 *European Respiratory Society Annual Congress* 2008.

Turner, S., Zhang, G., Young, S., Cox, M., Goldblatt, J., Landau, L. *et al.* 2008b, Associations between postnatal weight gain, change in postnatal pulmonary function, formula feeding and early asthma, *Thorax*. 63(3):234-239.

Turner, S. W., Craig, L. C., Harbour, P. J., Forbes, S. H., McNeill, G., Seaton, A. *et al.* 2007, Spirometry in 5-year-olds--validation of current guidelines and the relation with asthma, *Pediatric Pulmonology*. 42(12):1144-51.

Turner, S. W., Palmer, L. J., Rye, P. J., Gibson, N. A., Judge, P. K., Cox, M. *et al.* 2004, The relationship between infant airway function, childhood airway responsiveness, and asthma, *American Journal of Respiratory & Critical Care Medicine.* 169(8):921-7.

Tyson, J. E., Wright, L. L., Oh, W., Kennedy, K. A., Mele, L., Ehrenkranz, R. A. *et al.* 1999, Vitamin A supplementation for extremely-low-birth-weight infants. National Institute of Child Health and Human Development Neonatal Research Network, *New England Journal of Medicine.* 340(25):1962-8.

Unterborn, J. 2001, Pulmonary function testing in obesity, pregnancy, and extremes of body habitus, *Clinics in Chest Medicine.* 22(4):759-767.

Van Asperen, P. P., Kemp, A. S., & Mukhi, A. 1990, Atopy in infancy predicts the severity of bronchial hyperresponsiveness in later childhood, *Journal of Allergy & Clinical Immunology.* 85(4):790-5.

Verlato, G., Cerveri, I., Villani, A., Pasquetto, M., Ferrari, M., Fanfulla, F. *et al.* 1996, Evaluation of methacholine dose-response curves by linear and exponential mathematical models: goodness-of-fit and validity of extrapolation, *European Respiratory Journal.* 9(3):506-11.

Vieira, V. J., Ronan, A. M., Windt, M. R., & Tagliaferro, A. R. 2005, Elevated atopy in healthy obese women, *American Journal of Clinical Nutrition.* 82(3):504-9.

Vignola, A. M., Rennar, S. I., Hargreave, F. E., Fah, J. V., Bonsignore, M. R., Djukanovic, R. *et al.* 2002, Standardised methodology of sputum induction and processing. Future directions, *European Respiratory Journal.* 37(Suppl.):51s-55s.

Vilozni, D., Barak, A., Efrati, O., Augarten, A., Springer, C., Yahav, Y. *et al.* 2005, The role of computer games in measuring spirometry in healthy and "asthmatic" preschool children, *Chest..* 128(3):1146-1155.

Vollmert, C., Illig, T., Altmuller, J., Klugbauer, S., Loesgen, S., Dumitrescu, L. *et al.* 2004, Single nucleotide polymorphism screening and association analysis-exclusion of integrin beta 7 and vitamin D receptor (chromosome 12q) as candidate genes for asthma, *Clinical & Experimental Allergy.* 34(12):1841-50.

von Mutius, E., Schwartz, J., Neas, L. M., Dockery, D., & Weiss, S. T. 2001, Relation of body mass index to asthma and atopy in children: the National Health and Nutrition Examination Study III, *Thorax.* 56(11):835-8.

Waalkens, H. J., Merkus, P. J., Essen-Zandvliet, E. E., Brand, P. L., Gerritsen, J., Duiverman, E. J. *et al.* 1993, Assessment of bronchodilator response in children with asthma. Dutch CNSLD Study Group, *European Respiratory Journal.* 6(5):645-51.

Wallace, F. A., Miles, E. A., Evans, C., Stock, T. E., Yaqoob, P., & Calder, P. C. 2001, Dietary fatty acids influence the production of Th1- but not Th2-type cytokines, *Journal of Leukocyte Biology.* 69(3):449-57.

Wannamethee, S. G., Shaper, A. G., & Whincup, P. H. 2005, Body fat distribution, body composition, and respiratory function in elderly men, *American Journal of Clinical Nutrition.* 82(5):996-1003.

Warner, J. A., Miles, E. A., Jones, A. C., Quint, D. J., Colwell, B. M., & Warner, J. O. 1994, Is deficiency of interferon gamma production by allergen triggered cord blood cells a predictor of atopic eczema? *Clinical & Experimental Allergy*. 24(5):423-30.

Wegmann, T. G., Lin, H., Guilbert, L., & Mosmann, T. R. 1993, Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a TH2 phenomenon? *Immunology Today*. 14(7):353-6.

Weinmayr, G., Weiland, S. K., Bjorksten, B., Brunekreef, B., Buchele, G., Cookson, W. O. *et al.* 2007, Atopic sensitization and the international variation of asthma symptom prevalence in children, *American Journal of Respiratory & Critical Care Medicine*. 176(6):565-574.

Weisberg, S. P., McCann, D., Desai, M., Rosenbaum, M., Leibel, R. L., & Ferrante, A. W., Jr. 2003, Obesity is associated with macrophage accumulation in adipose tissue, *Journal of Clinical Investigation*. 112(12):1796-1808.

Weiss, S. T. 2005, Obesity: insight into the origins of asthma, *Nature Immunology*. 6(6):537-9.

Weiss, S. T. & Shore, S. 2004, Obesity and asthma: directions for research, *American Journal of Respiratory & Critical Care Medicine*. 169(8):963-968.

Whitaker, R. C., Wright, J. A., Pepe, M. S., Seidel, K. D., & Dietz, W. H. 1997, Predicting obesity in young adulthood from childhood and parental obesity, *New England Journal of Medicine*. 337(13):869-73.

Whitsett, J. A. & Wert, S. E. 2007, Molecular determinants of lung development in *Kendig's Disorders of the Respiratory Tract in Children*, V. Chernick & T. F. Boat, eds., WB Saunders, Philadelphia, PA, pp. 1-19.

Wignarajah, D., Cock, M. L., Pinkerton, K. E., & Harding, R. 2002, Influence of intrauterine growth restriction on airway development in fetal and postnatal sheep, *Pediatric Research*. 51(6):681-688.

Willers, S. M., Devereux, G., Craig, L. C., McNeill, G., Wijga, A. H., Abou El-Magd, W. *et al.* 2007, Maternal food consumption during pregnancy and asthma, respiratory and atopic symptoms in 5-year-old children, *Thorax*. 62(9):773-9.

Willett, W. C., Howe, G. R., & Kushi, L. H. 1997, Adjustment for total energy intake in epidemiologic studies, *American Journal of Clinical Nutrition*. 65(Suppl.):1220S-1228S.

Wilson, N. M., James, A., Uasuf, C., Payne, D. N., Hablas, H., Agrofioti, C. *et al.* 2001, Asthma severity and inflammation markers in children, *Pediatric Allergy & Immunology*. 12(3):125-132.

Winick, M. & Noble, A. 1966, Cellular response in rats during malnutrition at various ages, *Journal of Nutrition*. 89(3):300-306.

Wjst, M. 2005, Variants in the vitamin D receptor gene and asthma, *BMC Genetics*. 6(1):2.

Wjst, M., Dharmage, S., Andre, E., Norback, D., Raherison, C., Villani, S. *et al.* 2005, Latitude, birth date, and allergy, *PLoS Medicine / Public Library of Science.* 2(10):e294.

Wjst, M. & Dold, S. 1999, Genes, factor X, and allergens: what causes allergic diseases?, *Allergy.* 54(7):757-9.

Wjst, M., Dold, S., Reitmeir, P., Stiepel, E., & von, M. E. 1992, Month of birth and allergic disease at the age of 10, *Clinical & Experimental Allergy.* 22(11):1026-31.

Woods, R. K., Thien, F. C., & Abramson, M. J. 2002, Dietary marine fatty acids (fish oil) for asthma in adults and children, *Cochrane Database of Systematic Reviews.*(3):CD001283.

Woolcock, A. J., Salome, C. M., & Yan, K. 1984, The shape of the dose-response curve to histamine in asthmatic and normal subjects, *American Review of Respiratory Disease.* 130(1):71-5.

Wortsman, J., Matsuoka, L. Y., Chen, T. C., Lu, Z., & Holick, M. F. 2000, Decreased bioavailability of vitamin D in obesity, *American Journal of Clinical Nutrition.* 72(3):690-3.

Xystrakis, E., Kusumakar, S., Boswell, S., Peek, E., Urry, Z., Richards, D. F. *et al.* 2006, Reversing the defective induction of IL-10-secreting regulatory T cells in glucocorticoid-resistant asthma patients, *Journal of Clinical Investigation.* 116(1):146-55.

Yamada, K., Hung, P., Yoshimura, K., Taniguchi, S., Lim, B. O., & Sugano, M. 1996, Effect of unsaturated fatty acids and antioxidants on immunoglobulin production by mesenteric lymph node lymphocytes of Sprague-Dawley rats, *Journal of Biochemistry.* 120(1):138-44.

Yuan, W., Basso, O., Sorensen, H. T., & Olsen, J. 2002, Fetal growth and hospitalization with asthma during early childhood: a follow-up study in Denmark, *International Journal of Epidemiology.* 31(6):1240-1245.

Yunginger, J. W., Reed, C. E., O'Connell, E. J., Melton, L. J., III, O'Fallon, W. M., & Silverstein, M. D. 1992, A community-based study of the epidemiology of asthma. Incidence rates, 1964-1983, *American Review of Respiratory Disease.* 146(4):888-94.

Zerah, F., Harf, A., Perlemuter, L., Lorino, H., Lorino, A. M., & Atlan, G. 1993, Effects of obesity on respiratory resistance, *Chest.* 103(5):1470-1476.