Prenatal vitamin D for the prevention of preschool wheezing

Effect on early lung development

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Declaration of originality

All the work in this thesis is my own except where referenced.

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Abstract

Background: Asthma is the commonest chronic disease of childhood. The earliest manifestation is often preschool wheezing, which affects up to 50% of young children, but is difficult to evaluate practically. Observational studies suggest that low prenatal vitamin D status may be a risk factor for childhood wheezing.

Aims: To evaluate the measurement of preschool lung function using impulse oscillometry, and to test the hypotheses that prenatal vitamin D supplementation may promote lung development or prevent preschool wheezing.

Methods: We recruited 3 to 5 year olds from outpatient clinics to evaluate the quality of impulse oscillometry readings and the relationship between lung function and wheezing. We then evaluated impulse oscillometry in a group of 3-year-old children whose mothers had participated in a prenatal vitamin D supplementation trial. Next we evaluated the effect of prenatal vitamin D supplementation on lung function parameters and on the prevalence of wheeze and atopy in the first 3 years of life in this population (ISRCTN 68645785).

Findings: In 3 to 5 year old children, we successfully acquired high quality impulse oscillometry readings in 37/66 (56%). We found increased bronchodilator responses in those who had previously wheezed, with adjusted mean difference in respiratory resistance at 25Hz (95% confidence intervals) of -8.65 Kpa/Ls^{-1} (-16.63, -0.67), p=0.04. In 3-year-old children whose mothers participated in a prenatal vitamin D trial, we acquired high quality readings in 51/105 (49%), but found no relationship between wheezing history and bronchodilator response. In the randomised controlled trial of prenatal vitamin D supplementation, we evaluated 158/180 (88%) offspring at age 3 years and found no difference in wheeze, atopy, lung function or healthcare utilisation between vitamin D supplemented groups and controls.

Conclusions: It is possible to acquire high quality lung function data using impulse oscillometry for half of preschool children, however we found no consistent relationship

between lung function and wheezing history. There may be age related differences in the pathophysiology of wheezing and its relationship to lung function, which should be explored in future studies. Prenatal vitamin D supplementation in late pregnancy was not associated with differences in lung function or wheezing. Future research should explore the effect of higher doses and earlier administration of vitamin D in pregnancy.

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Publications

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M Griffiths, S Goldring, C Griffiths, L Cross, A Devine, A Martineau, S Robinson, S Shaheen, J Warner, R Boyle. Effects of prenatal vitamin D supplementation on healthcare utilisation in early life. A randomised control trial. Presented at the European Paediatric Allergy and Asthma Meeting, Barcelona, 13-15th October 2011

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1 Literature review

1.1 Abstract

Asthma is the commonest chronic disease of childhood. Its prevalence has increased rapidly over the last 50 years. The earliest manifestation is often preschool wheezing which affects up to 50% of children before the age of three years. Asthma is a heterogeneous disorder with considerable variation in causation between individuals. Two key pathways lead to asthma – an abnormal airway and an abnormal immune response to environmental antigens. Early atopic sensitization and respiratory tract infections make persistent asthma more likely.

There is a large body of evidence suggesting antenatal and early life influences are important in the development of asthma. Changing environmental factors acting on genetically susceptible individuals are likely to explain the increase in asthma prevalence in the last century. However, the precise environmental factors remain unidentified. Micronutrient status may be particularly important.

Early life vitamin D status is one proposed environmental factor. Opposing hypotheses argue that either high or low vitamin D status, at critical times in development have contributed to the asthma epidemic. Epidemiological data support both hypotheses. The nature of vitamin D's influence on immune and lung development, through its active metabolite calcitriol, illustrates how these hypotheses may not be mutually exclusive, and how such biological effects may be possible.

Modifying the vitamin D status of women of childbearing age is an attractive public health intervention for the primary prevention of asthma. The purpose of this thesis is to explore the potential role of vitamin D in early lung development.

1.2 Asthma and preschool wheezing

1.2.1 Introduction

The prevalence of asthma has increased dramatically over the second half of the 20th century (1, 2). It is estimated that 300 million people worldwide are affected by asthma and this figure is expected to rise to 400 million by 2025 (3, 4). In longitudinal birth cohort studies, up to half of all children experience at least one episode of wheezing before 6 years of age (5, 6). Of these nearly 40% experience persistent wheezing as adolescents (7). Asthma is estimated to affect 20% of children aged 6-7 in the United Kingdom, and 24% of 13-14 year olds (8), and is the commonest chronic disease of childhood (9). Many of these children have life long asthma (10). In the United Kingdom, the direct NHS costs attributable to asthma were over 700 million pounds in 2000 (11). Preschool wheezing itself was estimated to cost 0.15% of the total UK NHS budget in 2003 (12).

1.2.2 Defining asthma and preschool wheeze

Asthma is defined as a chronic inflammatory disorder of the airways associated with airway hyper-responsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness and coughing (9). Since airflow limitation and airway inflammation cannot be routinely assessed in preschool children, this definition is of limited use in young children, and applying the label asthma in children under 6 years is not recommended (13). In Europe, for clinical purposes, children under six years with wheezing may be categorised using a symptoms based approach (13). Children with 'episodic (viral) wheeze' have symptoms with viral colds only, and no symptoms between episodes; Children with 'multiple trigger wheeze' have symptoms in response to a number of triggers including tobacco smoke, allergen exposure, mist, crying, laughter or exercise. Pharmacological therapy is recommended based on this classification, although the evidence base in this age group is acknowledged to be weak (13). Furthermore, this classification is not robust over time, with more than half of children classified into either group switching to the other over the course

of a year (14). In the United States, an approach based on whether symptoms are intermittent or persistent is recommended (15).

1.2.3 Preschool wheezing phenotypes

Insight into the natural history of preschool wheezing comes from prospective cohort studies that have tracked the respiratory health of individuals from birth or early childhood. Such studies have been conducted worldwide and with varying degrees of biological and epidemiological data collected. The earliest prospective birth cohort study was the Tuscon Children's Respiratory Study (5).

In this study, 1246 newborn babies, not selected for asthma risk, were recruited between 1980 and 1984. At age six years, 51% had never wheezed, 20% had wheezing that began in the first three years and resolved by six years (early transient wheezing), 14% had wheezing that was present at three and six years (persistent wheezing) and 15% had wheezing that started after age three years (late onset wheezing) (5). Thus three different wheezing phenotypes were identified based on the presence of wheezing at three and six years. Risk factors associated with persistent wheeze included maternal asthma, male sex, atopic dermatitis, rhinitis apart from colds and elevated serum IgE levels in the first year of life (5).

Other birth cohorts have confirmed the presence of these wheezing phenotypes (6, 16, 17). By using latent class analysis, where children are categorised into patterns of wheezing over a fixed number of observation points, combined with detailed epidemiological data of individuals under study, further detail is emerging (6, 18, 19). The Avon Longitudinal Study of Parents and Children study (ALSPAC) identified two additional phenotypes in 6265 children followed from birth to 7 years (6). Prolonged early wheeze (around 9% of children), characterised by wheezing from 6 to 54 months and low prevalence from 69 months

onwards, and intermediate onset wheeze (around 2.5% of children) with onset between 18 and 42 months. In this study, phenotypes most strongly associated with atopy and airway responsiveness were characterised by onset after 18 months (6). The associations between preschool wheezing and asthma, atopy and lung function found in the ALSPAC study are summarised in Table 1.1.

Table 1.1 Associations between phenotypes and clinical outcomes in the ALSPAC study.

Phenotype	Asthma	Atopy	FEV1	FEF25-75	AHR
Transient early	√	X	↓	↓	↑
Prolonged early	√√	X	↓	↓↓	↑
Intermediate onset	√√√√	√√	↓↓	↓↓	↑↑
Late onset	√√√	√√	↓	↓	↑↑
Persistent	√√√√	√	↓↓	↓↓	↑↑

Asthma and atopy (skin prick test reactivity) assessed at age 7 years. Lung function and airway hyper-responsiveness assessed at age 8 years. The strength of association of each wheezing phenotype with each outcome is represented by the number of symbols (√,↓ or ↑) with a cross representing absence of an association with that outcome. AHR, airway hyper-responsiveness; FEF25-75, mid expiratory flow; FEV1 forced expiratory volume in 1 second. Reproduced from Henderson et al (6).

1.2.4 Role of atopic sensitization

Asthma in children often occurs with other allergic diseases such as eczema, allergic rhinitis and food allergy. The pattern of progression from eczema and food allergy in infancy to asthma and allergic rhinitis in older children is called 'the allergic march'. Atopic sensitisation is one of the most important risk factors for the development of asthma (20). Early sensitisation to allergens is associated with loss of lung function at school age (21, 22) and is a risk factor for persistence of asthma (21-23). Although both allergy and respiratory infections during early life are independently associated with developing asthma, the highest odds ratio is seen in children who have both (20, 24, 25). Early life sensitization to multiple aeroallergens carries the greatest risk for developing asthma (26).

However, wheezing occurs in both atopic and non-atopic children (27), and many atopic individuals do not develop asthma. In studies of school aged children in the United Kingdom, the percentage of atopic sensitization in asthmatic children ranged from 40-54%, and in non-asthmatics from 11 to 26% (28-30). In the European Community Respiratory Health Survey, the attributable fraction of asthma symptoms caused by atopy was 30%, but varied widely between centres from 4 to 61% (31). This highlights the heterogeneity of asthma, with approximately half of all asthma cases not attributable to atopic sensitisation, a key pathway in our understanding of asthma.

1.2.5 Role of early viral infection

In offspring of mothers with asthma, rhinovirus induced wheezing in the first three years of life was the greatest risk factor for developing asthma at age 6 years in one study (32). Rhinovirus infection, but not other viruses that induce wheezing, was also related to the number of sensitisations, total IgE level and nasal eosinophil count in the same cohort (33), with the evidence suggesting sensitization precedes rhinovirus infection, not vice versa (34).

1.2.6 Long term outcome of preschool wheezing

Longitudinal cohort studies demonstrate that early life wheezing that continues into school years generally persists into adulthood . For example, since 1964, the Melbourne Asthma Study (35) has followed up 401 randomly selected children from age 7 years who were classified as either controls (never wheezed), wheezy bronchitis (<5 episodes of wheezing associated with respiratory tract infection) or asthmatic (wheezing not associated with respiratory tract infection). In 1967, they were joined by 83 children with severe asthma defined as follows: onset of asthma before three years of age; persistent symptoms at age 10; barrel chest deformity or ratio of forced expiratory volume in one second to forced vital capacity < 50%. At 42 years of age, persistent asthma (defined as wheezing more than once per week) was present in 5% of the control group, 24% of the remaining cohort originally classified as asthmatic and 47% of severe asthmatics (35). Lung function showed a similar pattern with FEV1 values at age 42 years of 104%, 102%, 95% and 85% for adults originally classified as controls, wheezy bronchitis, asthma and severe asthma respectively. Other cohorts have found similar long-term rates of asthma and impaired lung function (10). More than one in four children who wheeze in early childhood have wheezing that persists into adulthood (10, 36).

1.2.7 Limitations of wheeze as the hallmark of asthma

Wheezing is defined as a continuous high-pitched sound with musical quality emitting from the chest during expiration (13). It represents narrowing of the intra-thoracic airways and expiratory flow limitation and has many causes other than ‘asthma’. From a parental perspective, wheezing is just one form of noisy breathing in their children (37). It is known that both parents (38, 39) and health professionals (40) differ in their interpretation of wheeze. Furthermore, researchers have questioned the role of wheeze as the hallmark of asthma in young children (41, 42). Parents reported wheeze in relation to only half the asthma exacerbations in young children during a RCT of asthma therapy, with cough the

individual symptom most strongly associated with an imminent asthma exacerbation (41). This suggests caregivers perceive a specific pattern of lung symptoms in relation to asthma, rather than an individual symptom such as wheeze (41). Furthermore, in the Copenhagen Prospective Study of Asthma in Childhood, total number of clinic visits for troublesome lung symptoms during the first three years of life was more predictive for asthma at age seven years, than whether wheeze was present on auscultation at these visits or not (42).

1.3 Pathophysiology of asthma

1.3.1 Introduction

Asthma is a disease of the airway characterized by inflammation, increased mucous production and airway remodelling leading to bronchial hyperactivity and airway obstruction (43). Airway inflammation is central to disease pathophysiology (44). As the disease progresses, the airway becomes susceptible to a wide range of environmental insults (allergens, viruses, air pollutants, drugs, chemical) leading to mucous cell metaplasia, smooth muscle proliferation, angiogenesis, fibrosis and nerve proliferation (44).

1.3.2 Allergic inflammation

Atopy is defined as the predisposition of individuals to produce IgE in response to environmental allergens, such as house dust mites. The healthy adaptive immune response is characterised by an appropriate balance between T helper 1 (Th-1) cells and T helper 2 (Th-2) cells. The former produce the cytokines interferon-gamma (IFN-γ) and interleukin 2 (IL-2), and the latter cytokines IL-4, IL-5 and IL-13 (45). T regulatory cells (Tregs) are crucial for maintaining balance, by cell-to-cell contact and the generation of IL-10 and transforming growth factor beta (TGF-β) (45). Over expression of Th-2 activity or a failure of control by Tregs will result in a higher probability of the development of allergic inflammation and sensitisation (45).

The nature of initial antigen exposure, and the subsequent co-stimulatory signalling during antigen-presentation by, for example, dendritic cells (DCs) to a naïve T cell determines the T cell response (45). In asthma, this is skewed towards Th-2 cells, resulting in increased production of cytokines that promote Th-2 cell survival (IL-4), IgE production (IL-4 and IL-13), mast cell differentiation and maturation (IL-3, IL-9 and IL-13), eosinophil maturation and

survival (IL-3, IL-5 and Granulocyte macrophage colony stimulating factor (GM-CSF)) and basophil recruitment (IL-3 and GM-CSF) (44).

Tregs are responsible for dampening immune responses and induction of allergen tolerance (44). Tregs are identified by co-expression of the transcription factor forkhead box protein 3 (FoxP3), CD4 and CD25. They exist as either naturally occurring or inducible forms (44). Naturally occurring Tregs influence allergic responses by secreting IL-10 and TGF-β which mediate suppression of DCs, direct inhibition of Th-1, Th-2 and Th-17 cells, suppression of allergen-specific IgE and induction of IgG4, inhibition of mast cells, basophils and eosinophils and prevention of effector T cell migration into the target tissue (44).

1.3.3 Breakdown of barrier function

The asthmatic epithelium is intrinsically defective with incomplete formation of tight junctions that facilitate penetration of inhaled allergens into the airway (46). Additional environmental stimuli such as respiratory viruses and air pollutants such as environmental tobacco smoke (ETS) can also disrupt tight junctions and impair barrier function (47).

1.3.4 Role of viral infection

The mechanism may relate to impaired Toll Like Receptor mediated responses by asthmatic epithelial cells that predispose to viral lung infection and allergic sensitization (44). These defects enable rhinovirus and other viruses to replicate, leading to cytotoxic cell death (rather than apoptosis), release of inflammatory products and enhanced viral shedding, events which act as a strong stimulus for recruitment of immature DCs and priming for allergen sensitization (44).

1.3.5 Airway remodelling

Accompanying these immunological changes are structural changes called airway remodelling. These consist of increased smooth muscle mass in the surrounding airway wall, deposition of extracellular matrix in the epithelial basement membrane, a loss of integrity of the airway epithelium, and goblet cell metaplasia leading to increased mucous production (43). Studies have confirmed that increased reticular basement membrane thickness and eosinophillic inflammation, the characteristic pathological features of adult asthma, are present in preschool children with confirmed wheeze between one and three years of age (48).

1.3.6 Role of the epithelium

The airway epithelium controls many aspects of allergic sensitization and is important in allergic inflammation, remodelling and bronchial hyper-reactivity (44). Airway epithelial cells (AECs) are equipped with pattern recognition receptors to enable defence against microorganisms, gases and allergens (43). Activation leads to the release of inflammatory mediators that initiate innate and adaptive immunological responses (43). For example, expression of Toll Like Receptor 4 (TLR-4) on the epithelium is crucial for promotion and activation of DCs and development of allergy to house dust mites (HDM), a response that is up regulated by viral infection of the airway epithelium with RSV or exposure to cigarette smoke (43). When AECs are chronically triggered by allergens, pollutants or Th-2 cytokines, they produce an array of cytokines and growth factors that may induce pathological changes (43). For example, TGF-β production activates underlying fibroblasts to differentiate into myofibroblasts that synthesize more collagen and extracellular matrix components (43). There is also evidence for epithelial mesenchymal transition, a process whereby AECs acquire characteristics of fibroblasts. The interaction between AECs and fibroblasts is known as the epithelial-mesenchymal trophic unit (43).

1.4 Lung function assessment in preschool children

1.4.1 Introduction

Objective measures of lung function in preschool children are important for evaluating the evolution of disease processes and response to interventions during this crucial developmental stage (49). However, practical evaluation of lung function in young children represents a major challenge (49). Preschool children are generally not able to perform the physiological manoeuvres required for techniques used in older children and adults. They are too old to sedate for lung function testing as is done in infants. They are easily distracted. The ideal pulmonary function test in preschool children should be applicable at any age (to allow for longitudinal studies), simple to perform, safe, reproducible, sensitive and specific enough to detect changes with growth, distinguish clearly between health and disease, and be acceptable to both the subject and parents (49). Impulse oscillometry (IOS) is one such technique that has been used in the clinical trial setting (50). IOS simply requires a child to breathe tidally through a mouthpiece for up to 30 seconds whilst measurements are taken. This section will outline the principles of impulse oscillometry and summarise previous work in preschool children.

1.4.2 Impulse oscillometry

The principle originated as the forced oscillation technique (FOT) described by Dubois et al in 1956 (51). As opposed to a traditional flow-volume loop, where the subject performs a maximal forced expiration to give physiological measurements such as the forced expiratory volume in one second (FEV1), the oscillation technique superimposes a small external pressure sine wave of a known frequency to the respiratory system, and the resulting pressure-flow relationship is measured (52). The ratio of the amplitude of the pressure wave, to the amplitude of the resulting flow wave, gives the respiratory impedance (Zrs), which represents the overall impediment to flow within the lung (49). Impedance is the

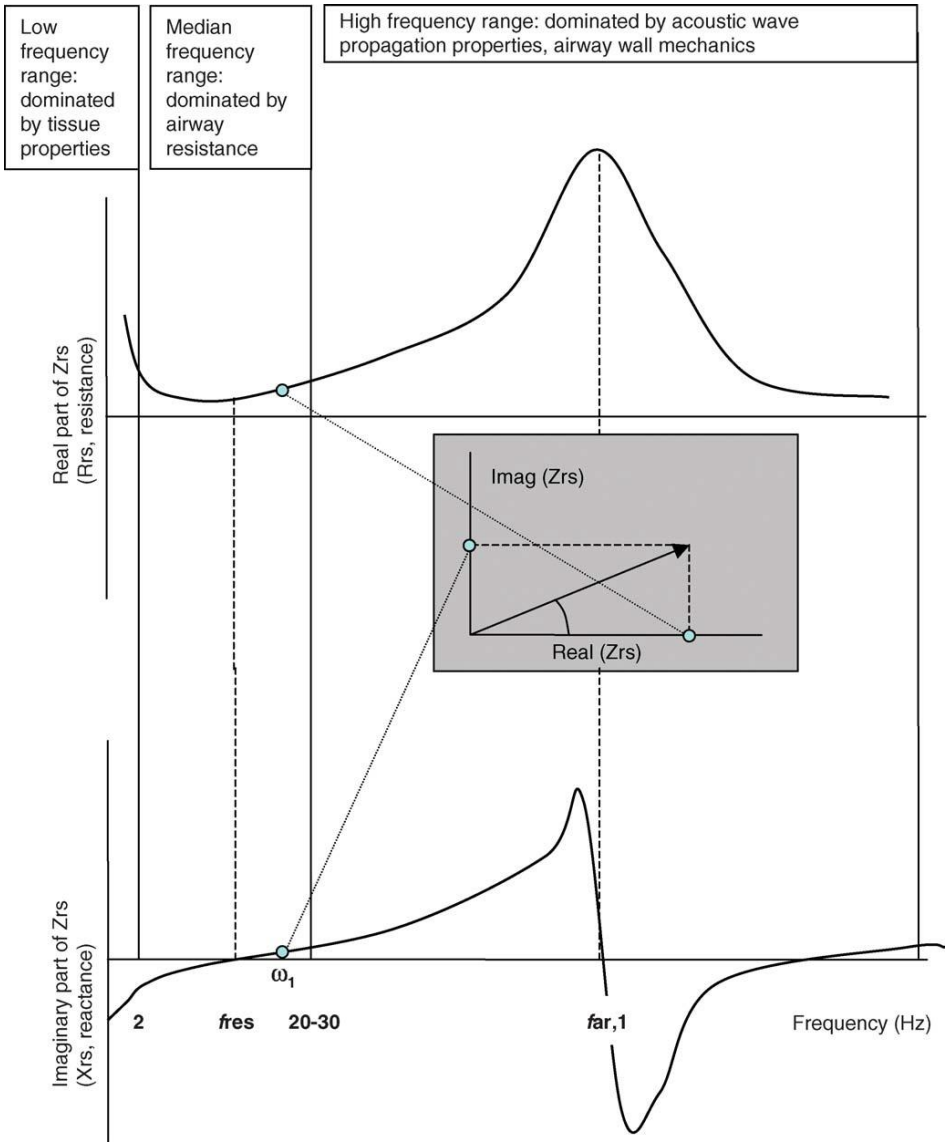
complex sum of lung resistance (Rrs) and reactance (Xrs), where Rrs represents frictional losses in the proximal and distal airways and lung parenchyma, of which airway resistance (Raw) is the most significant (49), and Xrs represents elastic and inertial properties within the lung.

These physical properties can be defined further as follows. Rrs equals pressure/flow ($KPa.L^{-1}.s$), lung elastance equals pressure/volume ($KPa.L^{-1}$) (the reciprocal of compliance) and lung inertance represents the relationship between pressure and volume acceleration. By applying different frequencies, the respiratory system can thus be evaluated for its oscillation properties.

Frey elegantly described these principles (52). Consider the lung as a single unit containing one resistive element, one elastic element and one inertial element. If an external pressure sine wave is applied, the resulting flow will be in phase providing inertia and elastance are zero. In this state the impedance is simply a function of the ratio of pressure and flow, or resistance. However, any increase in elastic forces causes a negative phase lag between pressure and flow, and any increase in inertial forces causes a positive phase lag between pressure and flow.

Mathematically, impedance can thus be represented as a vector with a given length (equivalent to the resistance) and phase angle (representing the phase lag between pressure and flow) which in turn can be represented as a complex mathematical number containing a real part (equivalent to the resistance) and an imaginary part (equivalent to the reactance). Thus, for a given frequency, Impedance (Zrs), [$kPa.L^{-1}.s$] = resistance (Rrs) [$kPa.L^{-1}.s$] + reactance (Xrs) [$kPa.L^{-1}$]. Figure 1-1, from Frey (52), summarises this representation of the human respiratory impedance spectrum.

Figure 1-1 Schematic representation of the human respiratory input impedance spectrum.



Reproduced from Frey (52).

1.4.3 Equipment

Initial techniques used monofrequencies that were time-consuming (51). With the development of Fast Fourier transformations, multiple frequencies could be analysed simultaneously (53). The impulse oscillation technique (IOS) generates multiple frequencies from a loudspeaker that are superimposed to generate a rectangular pulse applied to the airways every 200 milliseconds via a mouthpiece. Analysis of the superimposed pressure oscillations during normal spontaneous breathing allows calculation of Rrs and Xrs at multiple frequencies simultaneously. This has the advantages of reducing measurement time and generating continuous rather than discrete data.

1.4.4 Parameters

Respiratory resistance at different oscillating frequencies, for example at 5Hz, 10Hz and 15Hz, is represented by R5, R10 and R15. Likewise, for reactance the notation is X5, X10 and X15. Clinical applications generally use oscillating frequencies in the range 5 to 30 Hz. In this range, the impedance of the healthy respiratory system is dominated by airway resistance, and Rrs is considered a reasonable surrogate of airway resistance, Raw (49). Frequencies below 15Hz reflect the peripheral airways and frequencies above 20Hz, the central airways (54). There is a frequency dependence of resistance such that between four and 35 Hz, Rrs generally decreases with increasing frequency (49).

In the range from 5 to 30 Hz, the imaginary part of impedance, reactance Xrs, transitions from negative values where elastic properties dominate to positive values where inertial properties dominate (52). The resonant frequency, Fres, is defined as the point at which the magnitudes of elastic and inertial forces are equal and theoretically, Zrs equals Rrs (55).

1.4.5 Comparison of IOS with other lung function techniques

Several studies have compared IOS with other lung function techniques in young children, and found good correlation. In an observational study of 121 children aged 2 to 7 years, mean values of resistance measured by whole body plethysmography, the interrupter technique, and Rrs and Zrs measured by IOS were highly correlated (56). In children with acute asthma in the emergency department there was good correlation between percentage change in R8 after administration of bronchodilators and reversibility measured by spirometry, although the number of children under 7 years of age able to perform spirometry was limited (57). IOS using a facemask technique was more sensitive than the interrupter technique or whole body plethysmography in measuring metacholine induced lung function changes in children aged 2 to 7 years (58). Specific airway resistance (sRaw) measured by whole body plethysmography, respiratory resistance measured by the interrupter technique (Rint) and IOS were all able to discriminate bronchodilator responses in children aged 2 to 5 years with and without asthma, although sRaw provided the best discriminative power, with a sensitivity of 66% and specificity of 81% at the cut off level of a 25% decrease in sRaw after bronchodilator administration (59). In this study asthma was defined empirically based on typical asthma symptoms such as recurrent wheeze, cough and breathlessness; symptom relief with inhaled B2 agonist; relief with inhaled corticosteroids (ICS) and relapse during interruption of ICS therapy (59).

1.4.6 Determinants of lung function measured by IOS in healthy children

Several reference ranges for oscillatory mechanics in young children have been published and reviewed (49, 52, 60). Respiratory impedance is determined by height, with resistance decreasing and reactance increasing, as children get taller (61). The majority of studies do not show an effect of gender (52). It should be noted that most reference ranges have been performed on Caucasian children which may not be appropriate for African or Asian

subjects, where ethnic differences in lung function have been described (62).

1.4.7 Success rate of IOS in preschool children

Few studies have reported the success rate of impulse oscillometry in preschool children. Klug et al performed a community based study of 93 children aged 4 years or less, with a success rate of 57%, 65% and 82% for children aged 2,3 or 4 years respectively (56). In this study, IOS was performed along with the interrupter technique and whole body plethysmography at the same assessment. A facemask was used to increase compliance. Reasons for IOS failure were unacceptability of the facemask (90% of cases) and poor quality of measurements (10% of cases). In a study of 131 children aged 2 to 7 years, including 54 children aged 4 years or under, 13 children (9.9% of those eligible to perform IOS) refused to cooperate. Success rates were not reported by age (63). In a large community based study, 11 out of 233 (4.3%) children aged 3 to 10 years were unable to participate with IOS (64). This included 45 children aged 4 years or under, but again success rates were not given by age. Guilbert et al performed IOS yearly from 4 to 8 years as part of the annual assessment of a birth cohort of children at high risk for asthma (65). Success rates for IOS significantly increased with age. At 4 years only 21% of children could provide acceptable IOS tests. This increased to 58%, 74%, 79% and 86% at 5,6,7 and 8 years respectively. In the acute setting, the acceptability of IOS for assessing lung function in wheezy children presenting to the emergency room ranged from 20% in three year olds to more than 80% in 5 year olds (57).

1.4.8 Repeatability of IOS in preschool children

The coefficient of variation (CV) (within-occasion, within-test repeatability) is calculated as the standard deviation (SD) as a percentage of the mean (49), and the coefficient of repeatability (CR) (within-occasion, between-test) is defined as twice the SD of the mean difference between two series of measurements performed a few minutes apart, without any

intervention. The reported CV for IOS in young children ranges from 6.0% to 11% for resistance measurements (61) and up to 16% for reactance (66). Reported CR ranges from 6.1 to 10.2% (49) for resistance. The majority of children included in these studies were aged over 4 years.

1.4.9 Ability of IOS to discriminate between preschool children with and without wheezing

Studies that have looked for differences in lung function between preschool children with and without a history of wheezing or asthma using the Jaeger IOS system (Jaeger, Wurzburg, Germany) are show in Table 1.2.

Table 1.2 Studies investigating the ability of IOS to discriminate between preschool children with and without wheeze or asthma.

Ref	Design	Population	Age range	Parameters	Children with wheeze compared to controls	
					Baseline IOS	BDR
Hellinck 18 (67)	Case-control	247 healthy children vs 34 children with asthma recruited from Kindergarten	Age 3 to 6 years (Mean 4.5)	Rrs and Xrs at 5,10, 15, 20, 25 and 35 Hz, and Fres.	No difference	No difference
Nielsen 2001 (59)	Case-control	37 healthy controls from the community vs 55 children attending a hospital asthma clinic	Age 2 to 5 years (Mean 4.6)	R5 and X5	Significantly higher R5 and reduced X5	Significantly greater for R5 and X5
Marotta 2003 (68)	RCT of an environmental intervention for prevention of asthma	All children selected for higher risk of asthma at 9m to 2 years. At age 4, 42 children without, and 27 with asthma*	Age 4yrs (Median 4.1)	R5, R10, X5, X10, Fres	No difference	Significantly greater for R5 and R10
Song	Case-control	55 healthy children vs 77	Age 3 to 6	R5, R10, R20,	Significantly higher	Significantly greater

2008 (69)	study	children attending a hospital *asthma clinic	years (Mean 5.0)	R35, X5, Fres	R10	for R5, R10, R20 and R35
Komarow 2012 (70)	Case control study	29 non-asthmatic vs 88 children with asthma attending a hospital allergy clinic.	Age 3 to 18 years (Mean 7.7)	R5, R10, R20, X5, AX	No differences	Significant greater for R5, R10, X5 and AX
Malmberg 2008 (71)	Case control study	79 healthy controls vs 130 children with suspected asthma referred to a hospital allergy clinic.	Age 3 to 7 years (Mean 5.6)	R5, X5 and Fres	-	Significantly greater R5, X5 and Fres following a free running test
Shin 2012 (72)	Case control study	29 healthy controls vs 30 children with asthma	Age 2 to 6 years	R5, X5 and AX	-	Significantly greater for R5, X5 and AX

IOS = impulse oscillometry readings, BDR = IOS reading changes after administration of bronchodilator

*Asthma diagnosed if at least 4 out of 5 positive responses to the Modified ATS-B questions for asthma in childhood for the preceding 12 months, (wheeze with colds, wheeze apart from colds, dyspnoea associated with wheeze, wheeze after exercise, persistent cough).

Three studies in preschool children have found no differences in baseline IOS parameters between children with and without asthma (65, 67, 68, 70). However, two studies did find significant differences, with higher baseline R10 in asthmatics vs. controls in a Korean study (69), and higher R5 and lower X5 in asthmatics vs. controls in a Danish study (59).

The Bronchodilator response (BDR) measured by IOS is generally greater in children with asthma compared to controls (59, 68-70, 72). One, well conducted study that examined BDR in preschool children did not find a significant difference (67). The key difference with this study is that it was entirely community based, being set in a Kindergarten as opposed to recruiting children with asthma from hospital based clinics which may skew the severity of underlying illness. IOS is also able to discriminate a difference in response to exercise challenge, with greater changes in R5, X5 and Fres in asthmatics vs. controls after a free running test (71).

Differences in IOS parameters have been found in preschool children with a history of wheezing undergoing pharmacotherapy (50, 73). For example, in a randomised controlled trial of fluticasone vs. placebo in children with a positive asthma predictive index, children in the fluticasone group had significantly more symptom free days and significantly higher X5 than controls at the end of the study (50, 73).

1.5 Early life origins of asthma

1.5.1 Introduction

Asthma is a complex polygenic disease with a high degree of heritability. However, the rapid rise in asthma prevalence in the latter part of the 20th century in many industrialised countries cannot be explained by a population level genetic change (1). Asthma hypotheses propose that changing environmental exposures acting on genetically susceptible hosts - gene-environment interactions – have led to the asthma epidemic (1). The Developmental Origins of Health and Disease hypothesis states that intrauterine events can have lifelong health consequences (74). For asthma, four lines of evidence support this.

First, cohort studies from birth and early childhood show that physiological changes in early life track through to adulthood (7, 10, 36, 75). Reduced lung flow at one month of age predates the onset of early childhood wheezing and correlates with the persistence of asthma in adults (28). Airway hyper-responsiveness at age 11 years shows a significant association with measurements in late infancy (76). Thus impaired respiratory development is established at or shortly after birth, and tracks into adulthood.

Second, migration studies show that environmental influences that determine asthma risk act in early life. The prevalence of asthma in South Indian women in Leicestershire was significantly higher in those individuals who were born, or moved to the United Kingdom before the age of five years. After this period, individuals retain the asthma prevalence of their county of origin (77). This suggests that early life environmental factors are key to the initiation of asthma.

Third, associations between early life anthropometry data and the development of asthma suggest there are fetal origins to respiratory disease. Turner recently reviewed the literature

and performed a semi-structured review of 22 studies relating birth weight and asthma (78). There is consistent evidence of increased asthma among those of low birth weight (less than 2.5 kg), although the role of prematurity is not clear. There is also evidence of increased asthma among very heavy infants (>4.5 kg) (78). However, an infant's postnatal growth trajectory may be a better indicator of antenatal growth, with rapid infant growth suggesting growth suppression in utero (78). As the airways are established by mid pregnancy they may not be able to catch up to the same extent as somatic growth, resulting in a dissociation that is termed dysanapsis (78). Evidence for this comes from a Chilean cohort study that found that increased gain in weight and length during infancy is associated with increased asthma symptoms in young adults (79), a finding that has been replicated by a cohort from Southampton, who reported that increased weight and adiposity during infancy is associated with increased risk for wheeze at the age of three years (80). Similarly, reduced early fetal growth, based on ultrasound assessment of head circumference and abdominal girth, has been related to childhood asthma outcomes in two cohorts (80) (81, 82). This data suggests that factors which influence an individuals early life growth are also important in determining asthma risk.

Finally, studies of cord blood mononuclear cell proliferative responses in infants at high risk of atopy and asthma suggest that the immunological bias towards atopy is established in-utero (83) (84) and is predictive of asthma in later childhood (85).

Exactly how genetic and environmental factors interact to influence the development and expression of asthma is complex. Genes interact with other genes, and with environmental factors to determine asthma susceptibility (4). In addition, developmental aspects such as maturation of the immune response and the timing of infectious exposures during the first years of life are important risk modifying factors (4). Furthermore, asthma does not have a unifying pathological explanation (86). It is a heterogeneous disease with sub-phenotypes, each with specific determinants (45). This makes the challenge of identifying early life risk

factors, and potential interventions an even greater challenge than if there was single common pathway for the inception and expression of asthma.

1.5.2 Genetic factors

It has long been recognized that asthma runs in families and has a clear hereditary component (87). Twin studies show heritability rates of between 35% and 95% for asthma, with similar ranges for Total IgE, bronchial hyper-responsiveness and blood eosinophil counts (88).

Wheezing is more common amongst boys than girls in early years. Data from the National Child Development study followed a British national cohort of 17,414 children born during one week in March in 1958, with data available at ages 7, 11, 16 and 23 years. The male to female ratio for asthma or wheezy bronchitis rose from 1.23 in the 0 to 7 year period, to 1.48 at 12 to 16 years, and then reversed to 0.59 at 17 to 23 years (11). This is explained in part by differences in airway physiology. Male infants have smaller airways in relation to lung size than females, and females have a lower specific airway resistance and consistently higher threshold response to methacholine and decreased prevalence of airway hyper-responsiveness (AHR) than boys, relationships that reverse after adolescence (89).

1.5.3 Asthma genes

In 2007, Vercelli identified 33 genes associated with asthma and/or asthma related phenotypes that had been replicated in at least five independent candidate genome wide association studies (GWAS) or positional cloning studies (87). These genes were grouped into four main areas according to function as follows; Innate immunity and immune regulation; Th-2 cell differentiation and effector functions; Epithelial biology and mucosal immunity; Lung function, airway remodelling and disease severity. More recently, two meta-analysis of asthma GWAS have been published (90, 91). The following genes or regions had

p values at or near genome-wide levels of significance in both studies: the 17q21 locus, the IL1RL1/IL18R1 locus, TSLP, and IL33. These therefore represent four highly replicated, robust asthma susceptibility genes.

1.5.4 Limitations of genetic studies

Genotyping is not yet able to classify an individual's risk of asthma (90). In the GABRIEL GWAS study, the sensitivity and specificity of genotyping for predicting individual asthma risk was 35% and 75% respectively (90). This was based on a classification analysis, with subjects classified as having asthma if the predicted probability of disease was ≥0.5 using seven single nucleotide polymorphisms (SNPs) associated with childhood asthma. Thus these common genetic variants only account for a small proportion of asthma risk. Asthma's heterogeneous nature is likely to mean there are many genetically mediated pathways in asthma's development and manifestation (87). Furthermore, such studies do not take into account the complexities of gene-environment interactions. It is known that gene-environment interactions can critically modify the impact of a given gene on complex phenotypes. For example, the influence of the CD14-159CT polymorphism on IgE levels and asthma seems to depend on the relevant environmental exposures: cat and dog versus stable animals in a European population (92), and low versus high endotoxin in subjects of African descent (93). The same gene polymorphism can be associated with either disease or protection depending on the environment a subject is exposed to (45).

An additional factor explaining the limitation of genetic studies in accounting for asthma risk is that heritability estimates based on twin studies may be inflated. Twins, especially monozygotic twins, not only share all of their genes, but also a common uterine and early life environment. Thus heritability studies of twins may be telling us more about shared gene-environment interactions, than simply asthma 'genes' (88). An important question is to determine the nature of the biological switches that turn environmental exposures into

distinct immune response patterns (94).

1.5.5 Environmental determinants

Epidemiologic studies have highlighted many associations between environmental exposures and subsequent risk for asthma and allergy. Environmental modifiers include prenatal and postnatal environmental tobacco smoke exposure (95-98), growing up on a farm (99), exposure to cats, dogs and furry pets (100-103), family size and birth order (104, 105), day care attendance in early childhood (106), respiratory viral infections (32, 107), microbial exposures (108), pre and postnatal paracetamol exposure (109, 110), antibiotics (111, 112), mode of delivery (113, 114), breastfeeding (115, 116), diet and nutrition (117), air pollution (118), obesity (119), stress in family or other primary caregivers (120) and allergen exposure (121, 122). The following section will review prenatal determinants of asthma.

1.5.5.1 Maternal asthma

A meta-analysis of 33 childhood asthma studies has shown that maternal asthma increases the risk of disease in offspring to a greater extent than paternal asthma (123). This suggests either a specific role for maternal genetic components such as mitochondrial genes or those on the X chromosome, or that non-genetic in-utero and/or postnatal environmental factors mediated through the mother play a significant role in asthma susceptibility (123).

1.5.5.2 Maternal stress

Maternal stress in the pre and postnatal period is associated with increased wheezing in offspring (120). Mothers with higher stress in both periods appear to confer greatest risk (124). Higher prenatal maternal stress is associated with increased cord blood IgE (125) and altered innate and adaptive immune responses in cord blood mononuclear cells (126), an

association that may be modified by maternal atopic status (125). It is known that increased maternal stress in the third trimester is associated with altered methylation status of the human glucocorticoid receptor gene (NR3C1) in newborns, and increased salivary cortisol responses at 3 months (127), establishing the principle that maternal stress may alter biological responses in the newborn (128).

1.5.5.3 Maternal smoking

There is evidence to suggest that intrauterine exposure to environmental tobacco smoke is more strongly associated with asthma and poor lung function than passive exposure to second hand smoke after birth (129-131). In a systematic review of prospective cohort studies (98), overall, there was a 21% to 85% increase in asthma attributable to pre or postnatal passive smoke exposure, with the strongest effect from prenatal maternal smoking on asthma in children aged ≤2 years. A second review performed a pooled analysis of eight European birth cohorts (132). Cohort-specific effects of maternal smoking during pregnancy, but not during the first year, on wheeze and asthma at age four to six years were estimated. Among the 21,600 children included in the analysis, 735 children (3.4%) were exposed to maternal smoking exclusively during pregnancy. This was significantly associated with wheeze and asthma at age four to six years compared to no exposure. The likelihood increased in a dose-dependent linear manner with daily maternal cigarette consumption during the first trimester of pregnancy (132). Studies of asymptomatic infants have reported decrements in lung function shortly after birth in infants exposed to maternal smoking during pregnancy (96, 133, 134). In utero exposure to maternal smoking is independently associated with decreased lung function in children of school age, especially for small airway flows (130). It is possible that such deficits persist into later childhood (135, 136) and adult life (137).

One proposed hypothesis for the effect of maternal smoking on asthma risk in childhood was

the effect of polymorphisms in maternal antioxidant genes (Glutathione S-transferase (GST) M1) (138). However this was not supported in a recent study (139). Nicotine is the key chemical component of cigarette smoke, and recent studies have shown that nicotine can permanently affect the developing lung such that its final structure and function are adversely affected (140). Of note, the increased risk of asthma conferred by 17q21 genetic variants is restricted to early-onset asthma and the risk is further increased by early-life exposure to environmental tobacco smoke (141).

Grandmaternal smoking is also associated with increased asthma risk in childhood (142), suggesting a trans generational effect may operate. In a recently published rat model of *in utero* nicotine exposure, potential mechanisms were described (143). F1 offspring of nicotine-treated pregnant rats exhibited asthma-like changes to lung function, and associated epigenetic changes to DNA and histones. These alterations were blocked by co-administration of the peroxisome proliferator-activated receptor-γ agonist, rosiglitazone, implicating downregulation of this receptor in the nicotine effects. F2 offspring of F1 mated animals exhibited similar changes in lung function to that of their parents, even though they had never been exposed to nicotine (143).

1.5.5.4 Maternal paracetamol use

The use of paracetamol during pregnancy is associated with an increased risk of childhood asthma (109, 110, 144, 145). Features of studies are an association with paracetamol use during all trimesters of pregnancy and an association with persistent asthma, severe asthma, and with atopy.

Several mechanisms have been proposed to explain why early life paracetamol exposure may increase susceptibility to asthma and other allergic disorders. Paracetamol may decrease the amount of reduced glutathione present, resulting in impaired respiratory

antioxidant defences and airway inflammation (146-148), or may modulate the effect of glutathione levels on Th-1 and Th-2 cytokine response patterns (147, 149). Depleting glutathione in APCs in animal models causes a shift from Th-1 to Th-2 cytokine production that could pre-dispose to atopic diseases such as asthma. Other possible mechanisms are that by reducing fever, paracetamol use may reduce the cytokine storm that occurs as part of the febrile response, and that paracetamol may influence the production of prostaglandin E2 (150).

1.5.5.5 Fetal growth

Tedner reviewed 29 studies reporting on foetal growth, birth characteristics and allergy/ respiratory outcomes in offspring, excluding studies reporting only on gestational age or children born prematurely (151). Most studies show a correlation between low birth weight and increased risk of asthma or decreased lung function (151). For example, in one longitudinal study, reduced foetal size from the first trimester was associated with increased risk for asthma and obstructed lung function at age 10 years (81). In this study, for each millimetre increase in first trimester size, asthma risk reduced by 6% and FEV1 was higher by an average of 6 ml (81).

1.5.5.6 Obstetric factors

A meta-analysis (152) of 19 articles found that preterm babies (GA< 37 weeks) have an increased risk of asthma compared with term babies (152). Maternal complications during pregnancy and at delivery may also increase the risk of wheezing in childhood. In a large study of 15,609 children, maternal hypertension or preeclampsia, use of antibiotics for urinary tract infections, maternal antibiotic administration at delivery and offspring of a mother with diabetes were all risk factors for preschool wheezing (153). Neither amniocentesis/chorionic villus sampling, weight gain in pregnancy, or caesarean section was

associated with subsequent development of wheezing (153). However, in another meta-analysis, caesarean section has been linked to risk of asthma in offspring (113).

1.5.5.7 Maternal microbial exposure

There is a marked gradient in asthma prevalence between urban and rural areas (154). This has been demonstrated most strongly in children who grow up on traditional livestock farms or in families with an anthropomorphic lifestyle (154), and is attributed to the wide range of microbial exposures. Such children are protected from childhood asthma and atopy in proportion to their level of microbial exposure (155). This protective effect against the onset of asthma is stronger if the microbial exposure occurred during the mother's pregnancy (156).

1.5.5.8 Maternal allergen exposure

Allergen exposure can lead to the development of tolerance or sensitization, depending on a complex set of factors (157). This is also likely to be true during the antenatal period. Trials of early allergen avoidance for primary disease prevention have conflicting results (158). Food allergen (egg) avoidance in pregnancy has been shown to reduce levels of maternal ovalbumin specific IgG as well as the levels of specific antibody reaching the foetus (159). In this study, women were randomized to an egg-free or egg containing diet. The effects on this risk for allergic disease in the infants were complex. Children with "mid-range" exposure to egg were at highest risk for atopy at 6 months, whereas low-level or high-level exposure appeared protective. In a Manchester based study of early inhalant allergen (HDM) avoidance (160), this was associated with significantly better lung function in preschool children, but rates of sensitization were significantly higher. In another study, HDM avoidance beginning in the prenatal period was associated with reduced sensitization at 3 years of age (161).

Although these strategies were initiated in pregnancy, it is not possible to separate antenatal and postnatal effects. Currently, there is insufficient evidence to support specific allergen avoidance in pregnancy. Given that asthma is a complex multifactorial disease, it may be impossible to prevent by eliminating only one risk factor. A meta-analysis of multifaceted and monofaceted intervention studies suggested the former will have a much greater chance of being successful, with the latter being unlikely to yield a clinically relevant reduction of asthma(162).

1.5.5.9 Maternal cats and dog exposure

The literature relating pet exposure and risk of development of asthma and allergic diseases is contradictory. A systematic review in 2009 reviewed a total of 17 and 13 birth cohort studies on cat and dog exposure, respectively (102). The authors concluded that cat or dog exposure in early life had no effect on the development of asthma or wheezing symptoms and that dog exposure during infancy was found to protect children from developing sensitization against aeroallergens. A more recent systematic review identified 9 articles relating perinatal pet exposure (from 20 weeks gestation to 4 weeks postnatal life) and subsequent asthma and allergic disease (101). Three articles measured asthma or wheeze as an outcome (163-165). All of these studies reported reduced odds or a reduced hazard ratio associated with a dog (163, 165) or with pets at birth (164). No studies showed an association with cats alone. A major limitation of these reviews is the inability to completely account for the confounding effects of pet-keeping choices made by allergic families.

1.5.5.10 Maternal nutrition

Subjects exposed to the Dutch famine (1944-45) during early and mid gestation had an

increased risk of obstructive airways disease in adult life, not associated with allergy, suggesting an effect on lung and airway development (166). Studies have shown an association between large head circumference at birth and levels of total IgE in childhood (167, 168) and adulthood (169). It is postulated that good nutrient delivery to the foetus in early pregnancy programmes for growth in later gestation. If nutrient demand is not met, there will be continued head growth at the expense of relatively poorer nutrition to the body with consequent effects on rapidly dividing tissues such as those in the immune system (45).

Many dietary components have been proposed as causal in this process, include vitamins (A, C, E and D) trace elements (selenium, zinc), food groups (fruit and vegetables) and adherence to dietary patterns (Mediterranean diet). A recent systematic review and meta-analysis investigated the role of nutrients and foods during pregnancy for the primary prevention of asthma and atopic disorders in children and is summarised in Table 1.3 (170).

Table 1.3 Nutrients and foods for the primary prevention of asthma

Nutrient/ pattern	References	Method of assessment	Outcome and follow up	Effect
Vitamin A	(171, 172)	Dietary intake	Wheeze age 2 years	No association
Vitamin C	(171, 172)	Dietary intake	Wheeze age 2 years	No association
Vitamin E	(171-173)	Dietary intake	Wheeze age 2 years	Higher intake protective
Vitamin D	(174-177)	Dietary intake	Wheeze age 2 to 5 years	Higher intake protective
	(175, 176)	Dietary intake	Asthma age 5 years	No association
Selenium	(178, 179)	Maternal and cord blood levels	Wheeze age 5 years	No association
Zinc	(171, 179, 180)	Cord blood levels	Wheeze age 5 years	No association
Fruit and vegetables	(181)	Fruit and vegetable intake	Asthma age 3 years	Higher intake protective
	(182)	Fruit and vegetable intake	Asthma age 8 years	No effect
	(181)	Apple intake	Wheeze age 5 years	Higher intake protective
	(181)	Apple intake	Asthma age 5 years	Higher intake protective
Mediterranean diet	(183)	Adherence to Mediterranean diet	Atopic wheeze age 6 years	Higher adherence protective
	(183)	Adherence to Mediterranean diet	Persistent wheeze age 6 years	Higher adherence protective

Reproduced from Nurmatov et al 2011 (170).

Overall, the authors found a substantial risk of bias in the studies. However, the results were nonetheless suggestive of potentially beneficial associations of maternal intake of vitamins E, D, fruits and vegetables and adherence to Mediterranean diet during pregnancy for prevention of wheezing or asthma in offspring (170). In the ALSPAC study, no association was found between dietary patterns in pregnancy and respiratory and atopic outcomes in childhood (184). No intervention studies have yet established whether nutrient or dietary supplementation during pregnancy will change the risk of asthma in offspring.

Results of a recent randomized controlled trial of vitamin A supplementation in a population of married women of childbearing age with chronic vitamin A deficiency in rural Nepal are of great relevance to this thesis. At age 9 to 13 years, offspring of mothers who received vitamin A before, during and for 6 months after pregnancy had significantly improved lung function compared with a placebo group (mean increase in FEV1 46mls, 95% CI 6-86mls) (185). All children in the trial received supplemental vitamin A from 6 months of age, suggesting that vitamin A status from preconception through to age 6 months influences long-term lung development. It should be noted that data regarding potential confounders such as later nutritional status were not reported in this trial. The data regarding vitamin A are particularly relevant, due to the common signalling pathway shared by the activated forms of vitamins A and D. The active metabolite of vitamin A, retinoic acid (RA), is a critical signalling molecule in early lung development with maternal deficiency causing profound abnormalities of the infant respiratory system, including tracheoesophageal fistula, lung hypoplasia and lung agenesis (186). Mechanisms include RA effects on TGF-β and Fibroblast growth factor 10 (FGF-10) pathways at prospective sites of lung formation (187). RA also influences alveolarisation (188) so that postnatal treatment with RA increases the number of pulmonary alveoli in rats (189). The study provides evidence that maternal micronutrient status can influence foetal/infant lung development with significant consequences for longer-term respiratory health in humans.

1.5.5.11 Maternal fatty acid intake

There is evidence from epidemiological studies, mechanistic data and results of randomised intervention trials to suggest a protective role for maternal supplementation with omega-3 polyunsaturated fatty acids (n-3 PUFA) during pregnancy on asthma risk in offspring (190, 191). The underlying hypothesis states that Western diets have shifted from a state of balance between anti-inflammatory omega-3 fatty acids (n-3 PUFA) and pro-inflammatory omega-6 fatty acids (n-6 PUFA), to a state where dietary n-6 PUFA are predominant (190). High concentrations of dietary n-6 PUFA favour Th-2 differentiation, and thus atopic sensitization, during immune system development (192). Also, n-3 PUFA supplementation may alter T helper cell balance through suppression of IL-13 production, which has a role in induction of IgE synthesis in B cells and Th-2 type differentiation in T cells (193).

Several intervention trials have been conducted (194-198) and systematically reviewed (190, 191). In the review by Klemens (190), n-3 PUFA supplementation during pregnancy reduced 12-month prevalence of positive egg SPT (two trials) and childhood asthma (two trials) and significantly reduced cord blood IL-13 levels. Supplementation during lactation did not prevent asthma, food allergy or atopy. The authors concluded that large randomized controlled studies are needed before clinical recommendations can be made regarding the benefit of n-3 PUFA supplementation in pregnancy. However other systematic reviews did not confirm this relationship and do not advocate fish oil supplementation for primary prevention of asthma (199).

More recently, a postnatal fish oil supplementation trial has reported and found no effect on childhood allergic disease at 12 months of age (200). A prenatal intervention study of n-3 PUFA's during pregnancy in mothers with a foetus at high hereditary risk of allergic disease found no effect on IgE associated allergies in the first year of life, although atopic eczema and egg sensitisation was lower (201). A prenatal intervention study of n-3 PUFAs in

pregnancy and lactation in women at high risk of having an allergic infant found no difference in prevalence of allergic symptoms between intervention groups. However the cumulative incidence of IgE associated disease was reduced in the intervention group (202).

1.6 Nutritional interventions for Primary Prevention of asthma

1.6.1 Introduction

Asthma has its origins in early life and it is hypothesised that environmental or nutritional manipulation during this period may prevent asthma, known as primary prevention. A number of strategies have been investigated with mixed results. Manipulating early life environmental house dust mite exposure has been unsuccessful, and is associated with increased allergen sensitisation and eczema in offspring in the intervention groups in some trials. Multifaceted environmental interventions combining reduced exposure to environmental and food allergens, along with promotion of breast-feeding and reduced environmental tobacco smoke exposure have shown some clinical benefit in high-risk children. Supplementation with n-3 PUFA's has been effective in reducing asthma in some trials. Results of trials of early life vitamin or food group supplementation are awaited.

1.6.2 n-3 PUFA's

See section 1.5.5.11.

1.6.3 Vitamins and foods

As discussed in section 2.4.4.9, a recent systematic review of nutrition and food for the primary prevention of asthma and allergy found epidemiological and biological evidence suggestive of potentially beneficial associations of maternal intake of vitamins E, D, fruits and vegetables and adherence to a Mediterranean diet during pregnancy on risk of offspring

developing asthma (170). No intervention studies have yet established whether nutrient or dietary supplementation during pregnancy will change the risk of asthma in offspring. One study found that high-dose antenatal vitamin C and E supplementation does not improve infant respiratory outcome and is associated with increased healthcare utilisation and cost of care (203). However, a number of randomised intervention trials are currently in progress. These include a double blind randomised placebo controlled pilot study of a dietary soup intervention during pregnancy to optimise dietary vitamin E (NCT01661530), trials of vitamin D supplementation in pregnancy that will be described later in this thesis, and a randomized trial of the effectiveness of the Mediterranean diet in pregnant women at high risk of having a child with allergy (NCT01634516).

1.7 Vitamin D physiology

1.7.1 Sources

Vitamin D is a pro-hormone made by most living plants and animals. Vitamin D comes in two main forms – vitamin D_2 (ergocalciferol - the major form made by plants), and vitamin D_3 (cholecalciferol - from animals, including humans). With adequate sunlight exposure, the main source for humans is synthesis of vitamin D_3 in the skin by the action of ultraviolet B in the range of 280-320nm on 7-dihydrocholesterol (204). Although humans can only synthesise vitamin D_3 *de novo,* they can also metabolise vitamin D_2. For the purpose of this thesis, vitamin D (without subscript) refers to ergocalciferol and cholecalciferol. Vitamin D is measured in international units (IU) or micrograms. 40 IU equals 1 microgram.

1.7.1.1 Sunlight

Human cutaneous vitamin D_3 synthesis is sunlight dependent and therefore determined by latitude, time of day and season of the year (205). For example, comparing production between different latitudes, in Boston (latitude 42° N) synthesis is possible between March and November, whereas in Bergen (latitude 60° N) synthesis is only possible between April and October, with a difference in peak production between Boston and Bergen of 200% (206). Additional environmental determinants include atmospheric pollutants such as ozone, a major absorber of UVB radiation that is found in the stratosphere, aerosol content, cloud cover, albedo (which describes the amount of radiation reflected by the earth's surface, with snow having 90% albedo) and diffusion, which refers to the scattering of UVB radiation as it passes through the atmosphere (205). How much UVB penetrates through the skin to initiate vitamin D synthesis depends on the concentration of melanin (207). This is genetically determined, and those with darker skin types require longer sun exposure to produce the same amount of vitamin D (208, 209). Clothing is an obvious barrier to vitamin

D production by UV radiation, and women who wear veils are at especially high risk of vitamin D deficiency (207). Proper application of sunscreen will reduce vitamin D synthesis by 99.9% (210). Other factors that determine vitamin D status include adiposity, ethnic and genetic variation, poor renal function, malabsorption, and use of medication such as anticonvulsants and glucocorticoids (211)

1.7.1.2 Diet

Natural dietary sources include oily fish (rich in vitamin D_3), and plant derived sources such as sun dried shitake mushrooms (rich in vitamin D_2) (210). The method of cooking food can have significant effects on the vitamin D content, with frying fish reducing the active vitamin D content by approximately 50% (207). Fortification of foods with vitamin D was introduced as part of a range of public health measures for the prevention of rickets in the 1930's (212). Policies vary considerably between countries, but in the UK, the only statutory requirements are to fortify margarine and infant milk formula (213). In addition, many manufacturers voluntarily fortify breakfast cereals, soya and some dairy products, although this is often a minimal amount (213).

1.7.1.3 Nutritional supplements

A range of vitamin supplements containing vitamin D_3, vitamin D_2, or a combination of the two are available. Although traditionally vitamin D_2 and D_3 have been considered to be equipotent, studies have suggested that supplementation with vitamin D_3 is superior (207).

1.7.1.4 Vitamin D from breast milk and infant formulae

Breast milk is a poor source of vitamin D (207). The average content is 22IU/L (range 15-50IU/L) in a vitamin D-sufficient mother (214), which equates to an intake of approximately

11 to 38IU/day for an infant consuming 750mls of breast milk per day. This compares with infant formulas which in the United Kingdom, as for many countries, are legally obliged to be fortified with 40 to 100IU of vitamin D per 100 kcal per day which provides 300 to 750IU /day for the same estimated intake (215).

1.7.2 Metabolism in the liver and kidneys

Following synthesis in the skin or absorption from the gut, vitamin D can be stored in and then released from fat cells (210). Vitamin D in the circulation travels in the circulation bound to vitamin D binding protein (VDBP). To become metabolically active, vitamin D must undergo two hydroxylations. The first occurs primarily in the liver to form 25-hydroxyvitamin D (25(OH)D), which is biologically inactive but the best marker of an individual's vitamin D status. 25(OH)D is expressed as ng/ml or nmol/L, with 1.0 ng/ml equivalent to 2.5 nmol/L (216). The second hydroxylation occurs in the epithelial cells of the proximal tubules of the kidney and converts 25(OH)D to the biologically active hormone 1,25-dihydroxyvitamin D ($1,25(OH)_2D$). This is driven by the action of CYP27B1, a mitochondrial P450 hydroxylase and is regulated by parathyroid hormone, calcium and phosphorous levels to maintain calcium homeostasis (216). Finally, $1,25(OH)_2D$ induces the expression of the enzyme CYP24, which catabolises 25(OH)D and $1,25(OH)_2D$ to biologically inactive calcitroic acid, which is excreted in bile (216).

1.7.3 Placental metabolism

The vitamin D status of a pregnant woman directly influences that of her foetus/infant (217). During the course of pregnancy, maternal levels of 25(OH)D modestly decline and at delivery, cord 25(OH)D levels are typically ≤ 20% lower than maternal levels (218, 219). The decidua and placenta have large amounts of CYP27B1 enzyme activity resulting in generation of $1,25(OH)_2D$ (220). Methylation of the gene that codes for placental CYP24A1

(the enzyme responsible for catabolism of $1,25(OH)_2D$ to inactive metabolites) results in maternal $1,25(OH)_2D$ levels increasing to as high as two fold in late pregnancy compared to postpartum or non pregnant controls (221). However, $1,25(OH)_2D$ does not readily cross the placenta and combined with foetal suppression of renal CYP27B1 by low parathyroid hormone and phosphorous levels, circulating foetal $1,25(OH)_2D_3$ concentrations are significantly lower than maternal values in humans (218). Postnatally, foetal dependency on maternal vitamin D may manifest in infants of deficient mothers with hypocalcaemia and rickets. Breast milk has a low vitamin D and 25(OH)D content unless the mother takes supplements (218) and so exclusively breastfed infants are largely dependent on their prenatally acquired vitamin D stores or supplementation. For this reason infant formula is supplemented with vitamin D, and many countries have a policy of routine vitamin D supplementation for breastfed infants (213).

1.7.4 Extra-skeletal metabolism

In addition to this classic pathway, the enzyme CYP27B1 is widely expressed in cells throughout the body (210). The extra-skeletal effects of vitamin D were first recognized in 1979 with the observation that patients with sarcoidosis may present with hypercalcemia secondary to elevated circulating levels of $1,25(OH)_2D$ (222). In these patients, the high serum $1,25(OH)_2D$ arises from increased activity of CYP27B1 in disease-associated macrophages (223). Following this discovery, synthesis of $1,25(OH)_2D$ and the ability to elicit intracrine or paracrine responses from immune cells has been described for macrophages, dendritic cells, T-lymphocytes, B-lymphocytes and other cell types from the immune system (224-226). $1,25(OH)_2D$ is now widely recognized as having well defined actions in innate and adaptive immunity including induction of antimicrobial peptide secretion in response to innate immune stimuli (227) and as a potent promoter of tolerogenic dendritic cells and regulatory T cells (228). Importantly for this thesis, extra-renal $1,25(OH)_2D$ is not under feedback control from parathyroid hormone, and it has been argued that global deficiency or excess of 25(OH)D may lead to adverse consequences on immune

development (229). However, it is very important to note that the majority of observations on the extra-skeletal effects of $1,25(OH)_2D$ have been *in-vitro*, and the extent to which extra-renal metabolism occurs in normal health is disputed (230).

1.7.5 Mechanism of action

The actions of $1,25(OH)_2D$ are mediated via two pathways; a slow genomic response and a rapid, non-genomic response (231, 232). Both involve binding to its nuclear receptor, the vitamin D receptor (VDR). In the genomic pathway, VDR functions as a heterodimer with the retinoid X receptor (RXR) (233). After binding, VDR undergoes a conformation change that promotes RXR-VDR heterodimerization. The bound heterodimer translocates to the nucleus where VDR binds to vitamin D responsive elements (VDRE) and ultimately modifies gene transcription (233). The expression of over 200 genes are controlled via $1,25(OH)_2D$/ VDR dependant pathways acting on VDREs (210). In the non-genomic pathway, $1,25(OH)_2D$ binds to VDR present in the caveolae of the plasma membrane, forming ligand bound VDR which activates signalling cascades including protein kinase C and phospholipase C (234). Rapid responses triggered by this pathway include intestinal absorption of calcium and the secretion of insulin by pancreatic β-cells (232).

1.7.6 Optimum levels of vitamin D for health

In the United Kingdom levels of 25(OH)D ≤ 25nmol/L and levels between 25 and 50nmol/L are considered to represent vitamin D deficiency and insufficiency respectively (213). This is based on corresponding markers of bone health such as parathyroid hormone levels, and clear cause and effect clinical outcomes such as rickets in children and osteomalacia in adults (215, 235, 236). Beyond skeletal health, there is a growing body of observational data relating hypovitaminosis D with a range of adverse health outcomes including cardiovascular disease, cancer, autoimmune disease, infection, and all cause mortality (213,

237, 238). For example, studies suggest that levels of 25(OH)D below 50nmol/L are associated with a 30-50% increased risk of incident colon, prostate and breast cancer, along with higher mortality from these cancers (210). Many prospective randomized controlled trials are in progress to answer this question. Many researchers argue that the optimum level of 25(OH)D for health is ≥ 75nmol/L (239). However, the fundamental question is whether vitamin D deficiency is causal in these adverse outcomes, or simply an effect of residual or unrecognized confounding. For example, lower levels of 25(OH)D may simply be a reflection of an unhealthy, sedentary lifestyle, or a type of unhealthy dietary pattern. Also, and of particular relevance to the proposed role for vitamin D in the development of asthma, there is evidence to suggest that plasma concentrations of 25(OH)D decrease after an inflammatory insult(240). This may be a cause of reverse causality, with more severely affected individuals with asthma (inflammation) having lower levels of vitamin D *because* of their disease. It should be noted that the United States Institute of Medicine committee report on dietary reference intakes for calcium and vitamin D concluded that for extra-skeletal outcomes, including cancer, cardiovascular disease, diabetes and autoimmune disorders, evidence is inconsistent, inconclusive as to causality and insufficient to inform nutritional requirements (241). Randomised trials are sparse. The committee concluded that the prevalence of vitamin D inadequacy in North America has been overestimated, and reiterated that 25(OH)D levels above 50nmol/L are clinically sufficient (241).

1.7.7 Prevalence of perinatal vitamin D deficiency

Early life hypovitaminosis D is common and increasingly recognized as a global public health issue (238). Table 1.4 lists recent studies describing the prevalence of hypovitaminosis D amongst pregnant women and their newborn infants in different populations.

Table 1.4 Vitamin D status of pregnant women and their offspring in cross sectional studies worldwide

Setting	Population	Cut off for 'deficiency'	% cord 25(OH)D deficient	% maternal 25(OH)D deficient
US national survey **(242)**	928 unselected pregnant women	<50 nmol/L	-	33%
US antenatal clinic (236)	433 unselected pregnant women 376 newborns	<37.5 nmol/L	38%	23%
US antenatal clinic (237)	80 pregnant Black adolescents	<37.5 nmol/L	-	21%
US antenatal clinic (238)	400 pregnant women: 200 black, 200 white	<37.5 nmol/L	46% Black 10% white	45% Black 2% white
UK antenatal clinic (240)	466 pregnant Caucasian women	<50 nmol/L	-	50%
UK antenatal clinic (241)	263 pregnant adolescents	<25 nmol/L	-	30%
UK antenatal clinic (243)	180 pregnant women 45 Asian, 45 Middle Eastern, 45 Black, 45 Caucasian	<25 nmol/L	-	47% Asian 64% Middle Eastern 58% Black 13% Caucasian
Dutch antenatal clinic (244)	86 pregnant women: 48 'high risk' (dark skin or veiled)	<25 nmol/L	- 63% high risk	- -

	38 ‘controls’ (light skin, not veiled)		16% controls	-
Australian antenatal clinic **(243)**	971 pregnant women and 901 offspring	<25 nmol/L	11%	15% (Veiled women 71%) (Non-veiled women 11%)
NZ primary care clinic (246)	90 unselected pregnant women	<25nmol/L	-	61%

At risk groups include ethnicities with pigmented skin, women who wear concealing clothing (244-248), poor uptake of vitamin D supplementation (243, 249-251) and prolonged breast feeding (213). Hypovitaminosis D is also common in pregnant white women with no additional 'risk factors' (252).

1.7.8 Genetic determination of vitamin D status

Family and twin studies suggest that a substantial portion of the variability in vitamin D can be attributed to genetics, with heritability estimates of 43% to 65% for 25(OH)D, $1,25(OH)_2D$ and VDBP (253, 254). In large, healthy populations, GWAS have shown that genes involved in cholesterol synthesis (DHCR7), vitamin D hydroxylation (CYP2R1), and vitamin D transport (VDBP, or GC) affect vitamin D status, with variation at these loci identifying individuals at substantial increased risk of vitamin D insufficiency (255-257).

1.7.9 Health implications of vitamin D deficiency in pregnant women

The clearest health implications of vitamin D deficiency in pregnant women are on bone health and calcium homeostasis in offspring. There is a strong causal relationship between maternal and foetal cord circulating 25(OH)D levels (204). Severe maternal vitamin D deficiency may manifest as rickets in utero (258). Neonatal hypocalcaemia secondary to vitamin D deficiency is a well-recognized clinical entity. Reduced maternal vitamin D intake is also associated with reduced foetal growth (259) and smaller head circumference (260). Reduced concentration of 25(OH)D in mothers during late pregnancy are associated with reduced whole body and lumbar spine bone mineral content in children aged 9 years (261).

However, observational evidence now links low maternal vitamin D levels during pregnancy with a range of short and long term adverse health outcomes. For the mother, increased risk of pre-eclampsia, gestational diabetes, preterm labour, obstructed labour, caesarean section

and bacterial vaginosis have all been described (262). For offspring, and of most relevance to this thesis, there is accumulating evidence of a role for vitamin D in developmental programming of chronic diseases including type 1 diabetes, multiple sclerosis and schizophrenia (262). The data for schizophrenia are intriguing. It is known that individuals born in winter and spring have increased risk of later developing schizophrenia, and the effect size of this observation is magnified by latitude (263). Individuals with darker skins are at greater risk of developing schizophrenia (263). The VDR is widely expressed in the human brain (264), and in experimental models of developmental vitamin D deficiency in rats, offspring born of deficient mothers have anatomical brain changes and alterations in biological pathways including dopamine metabolism (264). Finally, in a case-control linkage study of the Danish Psychiatric Central register and neonatal dried blood spot analysis, the risk of schizophrenia was significantly associated with neonatal 25(OH)D concentrations (265). In this study, infants with lower and also the highest vitamin D levels had higher risk of schizophrenia - suggesting a U-shaped risk relationship. As will be shown, this has parallels with the data relating prenatal vitamin D status and atopic sensitisation in later life.

1.7.10 Recommendations on Vitamin D intake during pregnancy

In the UK, the National Institute for Clinical Excellence recommends pregnant women and at risk groups take 400IU daily (266). In the United States, the advice is to take 600IU through pregnancy (241). It is acknowledged that current guidance from health care providers to pregnant women is inconsistent and a barrier to effective primary prevention of hypovitaminosis D (252). A recent editorial has called for urgent intervention to address early life hypovitaminosis D (267).

1.8 Evidence of an association between prenatal vitamin D status and child health

1.8.1 Vitamin D hypotheses

Changing early life vitamin D status is one proposed environmental factor for the asthma epidemic. Opposing hypotheses argue that either high or low vitamin D status, at critical times in development have contributed to the asthma epidemic. In the former, Wjst and Dold argue that it was the introduction of vitamin D supplementation in the 1950's to prevent rickets that led to the asthma epidemic (268). Contrary to this, Litonjua argue that as populations have grown more prosperous since the 1960s, more time is spent indoors and there is less exposure to sunlight. This, coupled with inadequate intake from food and supplements, increasing obesity and migration patterns to Western countries have led to an epidemic of vitamin D deficiency, particularly in pregnant women (269). Epidemiological data support both hypotheses.

1.8.2 Ecological epidemiology

Ecological studies provide weak evidence of a relationship between developmental vitamin D deficiency and allergic health outcomes. Limitations include the risk of confounding, and of studying large aggregates of individuals. However, using latitude as a proxy for vitamin D status, rates of prescription of hypoallergenic formulae (270) and epinephrine auto-injectors (271) are significantly higher in those living further south in Australia. In the case of hypoallergenic formulae, there was also a significant East-West effect with more prescriptions in Eastern Australia (270), suggesting a metropolitan effect may be an additional factor. In the United States, the North-East New England region has a higher epinephrine prescription rate compared to Southern regions (272), and rates of emergency department visits for acute allergic reactions are statistically higher in North Eastern vs Southern regions, an association that was stronger when restricted to food related allergic

reactions (273). For epinephrine prescriptions, although a North-South gradient was observed, neither latitude nor longitude were independently analysed for the entire data set. There were clear inconsistencies, with Alaska not having the highest rate (in fact the same as Wisconsin and Tennessee), and South Eastern states (Florida) having higher levels than more Northerly states (272).

Studies using birth month as a surrogate for vitamin D status have shown mixed results with positive, negative and unclear outcomes (274). In one of the largest analyses, which used a validated questionnaire distributed in 54 centres to a representative sample of 20 to 44 year old men and women mainly in Europe, but including North Africa, India, North America, Australia and New Zealand (200,682 participants), allergic rhinitis decreased with geographical latitude, (with many exceptions), and no significant effect of birth month was observed (274). Effect estimates by multivariate analysis of total and specific IgE values in 18,085 individuals excluded major birth month effects and confirmed the independent effect of language grouping (274).

However, fall birth was statistically more common among food allergic subjects in the United States National Health and Nutrition Examination Survey (NHANES) and amongst patients attending a North American allergy clinic patients (n=1514) (275). Also, children less than 5 years of age born in fall/ winter had a 53% higher odds of presenting to a Boston emergency department with food related acute allergic reactions compared to controls in a retrospective review, a relationship independent of suspected trigger and allergic comorbidities (276).

1.8.3 Genetic studies

A number of genes involved in vitamin D metabolism (DHCR7, CYP2R1 and GC) have been identified as conferring substantial risk of vitamin D insufficiency (255-257). These associations were explored in a GWAS of asthmatic patients in three separate cohorts –

Boston, USA, Costa Rica and Puerto Rico (277). The most significant gene, GC, had a strong association in all three cohorts (277) a finding that has been replicated in a cohort of Chinese Han asthmatics (278).

Other genetic studies in asthma populations have shown conflicting results. Variants in the VDR gene have been associated with increased asthma risk (279, 280) but this has not been replicated in all studies (278, 281, 282). Variants in vitamin D pathway genes (283) have also been associated with asthma susceptibility. There is evidence that polymorphisms in the class I MHC-restricted T cell associated molecule gene (CRTAM) are associated with increased rate of asthma exacerbations in the presence of low circulating vitamin D levels, a finding that was replicated in a second independent population (284). CRTAM is highly expressed in activated human $CD8^+$ and natural killer T cells, both of which have been implicated in asthmatic patients (284).

In a study of 649 children (285), vitamin D deficiency increased the risk of food sensitization, but only among individuals with certain polymorphisms [IL-4 (rs2243250), MS4A2 (rs512555), FCER1G (rs2070901) and CYP24A1 (rs2762934)]. This suggests there may be gene–vitamin D interactions determining allergic sensitization(285), a finding that has been replicated in a study on white European adults (286), with the polymorphism MS4A2 (rs512555) and IL-4 (rs2243250) modifying the association between vitamin D deficiency and total IgE concentrations.

1.8.4 Birth cohort studies

15 cohort studies have examined the relationship between early life vitamin D exposure and respiratory or allergic related outcomes (145, 174-176, 285, 287-296). A detailed description of these studies is presented in Table 1.5, followed by a summary of the available evidence for each outcome.

Table 1.5 Epidemiological associations between early life vitamin D status and allergic or respiratory health

Country (Ref)	Follow up	Methodology	Comparison groups	Maternal 25(OH)D (nmol/L) Median (IQR)	Outcome in offspring	Result	Effect of exposure
USA (174)	1194 of 2198 pregnancies to 3 years	Maternal diet FFQ	Highest vs. lowest quartile of intake in mother	-	Recurrent wheeze Respiratory infection Eczema	aOR 0.38 (0.22, 0.65) aOR 0.75 (0.52, 1.09) aOR 0.92 (0.63, 1.35)	Protective No association No association
UK (175)	1212 of 2000 pregnancies to 5 years	Maternal diet FFQ	Highest vs. lowest quintile of intake in mother	-	Ever wheezed Wheeze in last 12m Persistent wheeze Spirometry BDR	aOR 0.48 (0.25, 0.91) aOR 0.35 (0.15, 0.83) aOR 0.33 (0.11, 0.98) No association Lower intake associated with decreased BDR No association	Protective Protective Protective No association Harmful No association

					Skin prick atopy	No association	No association
					eNO		
Finland	1669 of 4193	Maternal	Highest vs. lowest	-	Asthma	aHR 0.80 (0.64, 0.99)	Protective
(176)	pregnancies to	diet FFQ	quartile of intake in		Allergic rhinitis	aHR 0.85 (0.75, 0.97)	Protective
	5 years		mother		Atopic eczema	aHR 0.94 (0.84, 1.04)	No association
UK (287)	178 of 596	Maternal	Mothers with levels	50 (30-75)	Eczema	aOR 1.62 (0.67, 3.89)	No association
	pregnancies to	25(OH)D	>75nmol/L vs.		Eczema	aOR 1.89 (0.51, 6.99)	No association
	9 years		<30nmol/L		Asthma	aOR 5.40 (1.09, 26.65)	Increased risk
Finland	7648 of 12,058	Vitamin D	Supplementation	-	Atopy	aOR 1.33 (1.07, 1.64)	Harmful
(288)	infants born in	supplement	>2000 IU/day vs.		Allergic rhinitis	aOR 1.33 (1.12, 1.58)	Harmful
	1966 to	s during first	no		Asthma	aOR 1.33 (0.97, 1.82)	No association
	31 years	year	supplementation				
Japan	763 of 1002	Maternal	Maternal intake	-	Wheeze	aOR 0.64 (0.43, 0.97)	Protective
(289)	pregnancies to	diet FFQ	≥172IU/day vs.		Eczema	aOR 0.63 (0.41, 0.98)	Protective
	24 months		<172IU/day				

New Zealand (290)	823 of 922 newborns to 5yrs	Cord 25(OH)D	Mothers with levels <25nmol/L vs. ≥75nmol/L	44 (29-78)	RTI at 3 months Any infection Cumulative wheeze Incident asthma	aOR 2.04 (1.13, 3.67) aOR 2.36 (1.17, 4.73) aOR 2.15 (1.39, 3.33) aOR 1.03 (0.97, 1.10)	Protective Protective Protective No association
USA (291)	219 children enrolled at birth to 5 years	Cord 25(OH)D	Comparing mothers with low (<50) and high (≥100) levels to the reference group (50-74.9 nmol/L)	64 (49-81)	Total IgE Specific IgE Skin prick atopy Allergic rhinitis Asthma	Low: aC 0.27 (0.08, 0.47) High: aC 0.27 (-0.00, 0.54) Low: aC 2.8 (1.2, 6.6) High: aC 3.6 (1.2,10.5) Low: aOR 1.2 (0.5, 2.7) High: aOR 3.4 (1.0, 11.4) Low: aOR 1.1 (0.4, 2.8) High: aOR 2.4 (0.8, 7.3) Low: aOR 0.5 (0.2, 1.6) High aOR 1.4 (0.4, 5.4)	Harmful No association Harmful Harmful No association Harmful No association No association No association No association

Spain (292)	1724 pregnancies to 6 years	Maternal 25(OH)D	Highest vs. lowest quartile of intake in mother	73.8 (56-93)	LRTI at 1 year Wheezing at 1 year Wheezing at 4 years Asthma	aOR 0.67 (0.50, 0.90) aOR 0.91 (0.67, 1.23) aOR 0.94 (0.62, 1.43) aOR 0.89 (0.59, 1.32)	Protective No association No association No association
UK (293)	860 mother-child pairs to 6 years	Maternal 25(OH)D	Change in relative risk per 10nmol/litre change in maternal 25(OH)D	59 (41-85)	Any wheeze Current wheeze Current asthma Skin prick atopy eNO at 6 years Spirometry BHR	aRR 1.00 (0.92, 1.02) aRR 0.99 (0.94, 1.05) aRR 0.98 (0.92, 1.04) Not associated Not associated Not associated Not associated	No association No association No association No association No association No association No association
Netherlands (294)	156 neonates followed to 1 year	Cord 25(OH)D	Mothers with levels <50nmol/L vs. ≥75nmol/L	82 (SE 3.5)	RSV	aRR 6.2 (1.6, 24.9)	Protective
Netherland	436 of 2343	Maternal	Mother or child's	Mean ± SD	Spirometry	No association	No association

s (295)	pregnancies to 7 years*	25(OH)D	vitamin D level, or vitamin D supplementation in childhood	46 ±18		Lower intake associated with reduced FEV1 z scores	Inconsistent
USA (285)	649 children enrolled at birth	Cord 25(OH)D	Effect of vitamin D deficiency explored individually and with polymorphisms known regulate IgE and VD	-	Specific IgE to foods	No association when examined alone Strong gene-vitamin D deficiency interaction evident	Deficiency may increase risk of food sensitivity among individuals with certain genotypes
Germany (296)	378 mother-child pairs	Maternal and cord 25(OH)D		Maternal = 55 (36, 78) Cord = 27 (18, 44)	Risk of food allergy Sensitisation against food antigens at age two	Both positively associated Higher maternal levels associated with increased risk of	Harmful Harmful

						sensitisation	
UK (145)	5513 mother-child pairs	Maternal 25(OH)D level	Analysed across quintiles of maternal 25(OH)D level	Maternal = 62 (46, 81)	Wheeze at 7.5 yrs Asthma at 7.5 yrs Atopy at 7.5 yrs Eczema at 7.5 yrs Hayfever at 7.5 yrs IgE at 7 yrs Lung function 8.7 yrs BDR at age 8.7 yrs	No association for any outcomes.	No association

FFQ (Food frequency questionnaire), aOR (adjusted odds ratio), BDR (Bronchodilator response), eNO (exhaled nitric oxide), aHR (adjusted Hazard ratio), RTI (Respiratory tract infection), aC (adjusted coefficient), aRR (adjusted relative risk), SE (standard error), RSV (respiratory syncytial virus) and FEV1 (forced expired volume in 1 second)

*2052 mothers with ‘conventional lifestyle’ and 291 with ‘alternative lifestyle’

1.8.4.1 Wheezing

Three studies based on vitamin D status estimated by maternal dietary intake in pregnancy have suggested a protective effect of a higher intake on wheezing in childhood (175, 289, 297). In studies that measured early life 25(OH)D levels, one study supports this observation with reduced cumulative wheeze by 5 years (290) and three studies found no relationship with wheezing at 1 year, 4 years (292), 6 years (293) and 7.5 years (145).

1.8.4.2 Asthma

One study based on vitamin D status estimated by maternal dietary intake in pregnancy suggested a protective effect of higher intake on asthma diagnosis at 5 years of age (298). In contrast, one study of infant supplementation with 2000 IU of vitamin D in infancy suggested no effect on asthma at 31 years of age (288). Of the five studies that measured early life 25(OH)D levels, none have found an effect on asthma (145, 290-293), and one study found an increased risk of asthma at age 9 years (287).

1.8.4.3 Allergic rhinitis

One study based on vitamin D status estimated by maternal dietary intake in pregnancy suggested a protective effect of higher intake on allergic rhinitis at 5 years (298). In contrast, one study of infant supplementation with 2000 IU in infancy has suggested an increased risk of allergic rhinitis at 31 years of age (288). In the two studies that measured cord 25(OH)D levels, no effect was found (145, 291).

1.8.4.4 Eczema

One study based on vitamin D status estimated by maternal dietary intake in pregnancy

suggested a protective effect of a higher intake on eczema at 24 months (177) and two studies no effect (297, 298). In the two studies that measured maternal 25(OH)D levels in pregnancy, no effect was seen on eczema (145, 287).

1.8.4.5 Respiratory tract infection

In one study based on vitamin D status estimated by maternal dietary intake in pregnancy that reported respiratory tract infection as an outcome, no effect was found (297). However, three studies that measured early life 25(OH)D levels have found a protective effect of higher early life 25(OH)D levels on respiratory tract infections in the first three months (290), or at 1 year of age (292, 294).

1.8.4.6 Skin prick test positivity

One study based on vitamin D status estimated by maternal dietary intake in pregnancy found no association with skin prick test positivity (175), and one study of infant supplementation with 2000 IU in infancy suggested no association at 31 years of age (288). In studies that measured early life 25(OH)D levels, two studies found no association with atopy (145, 293), one study found both low (<50nmol/L) and high (≥100nmol/L) levels of 25(OH)D in cord blood are associated with increased atopy at 5 years compared to a reference group with 25(OH)D levels in the range 50 to 75nmol/L (291), and one study found increased risk of food sensitisation in those with higher early life 25(OH)D levels (296).

1.8.4.7 IgE

In one study that measured 25(OH)D levels in cord blood, both high (>100nmol/L) and low (<50nmol/L) levels were associated with increased specific IgE to 6 aeroallergens at 1,2,3 and 5 years compared to the reference group with levels 50 to 75nmol/L (291). In a second

study that measured cord 25(OH)D levels, vitamin D deficiency was found to increase the risk of raised IgE, but only amongst individuals with polymorphisms known to be involved in regulating IgE and vitamin D (285). In a third study that measured maternal 25(OH)D levels, no association was seen(145).

1.8.4.8 Spirometry

Four studies, one with vitamin D status estimated by maternal dietary intake in pregnancy (175) and three with maternal 25(OH)D levels in pregnancy (145, 293, 299) found no association with lung function measured by spirometry in preschool years.

1.8.4.9 Bronchodilator response

One study with vitamin D status estimated by maternal dietary intake in pregnancy found that lower maternal intake of vitamin D was associated with decreased BDR (175). One study with maternal 25(OH)D levels in pregnancy known found no association (145).

1.8.4.10 Exhaled nitric oxide

Two studies, one with vitamin D status estimated by maternal dietary intake in pregnancy (175) and one with maternal 25(OH)D levels in pregnancy (293) reported on eNO levels in offspring at 5 and 6 years respectively, and found no association.

1.8.4.11 Summary of evidence from birth cohort studies

Early life Vitamin D exposure was assessed in a number of different ways amongst these studies and is an important source of heterogeneity. The gold standard measure of vitamin D status is the blood concentration of 25(OH)D, as it captures vitamin D exposure from diet

and sunlight combined (300). In contrast, dietary intake of vitamin D makes a relatively small contribution to overall vitamin D status, and apparent effects of vitamin D intake may be confounded by other correlated nutrients and socioeconomic factors.

Although early studies suggested higher maternal intake of vitamin D was protective against a range of wheezing and atopic outcomes in offspring (175, 177, 297, 298), well conducted studies with baseline measurements of 25(OH)D levels in pregnancy or cord blood have not replicated these observations (145, 290-293) (287, 291, 297, 298). There is no evidence for an effect on exhaled nitric oxide (175, 293). There is no evidence of an association between early life vitamin D status and lung function measured by spirometry (145, 175, 293, 299), although one study found evidence of an increasing bronchodilator response with higher dietary vitamin D intake in mothers (175).

There is evidence for a protective effect on LRTI in the first year of life (290, 292, 294, 297). There is evidence for a U shaped relationship between 25(OH)D levels and atopic sensitization, with both high and low vitamin D levels associated with increased risk (288, 291), a relationship that has also been found in a recent large British adult cohort (301). However, this relationship was not replicated in two well-conducted birth cohort studies (145, 293).

In summary, it is likely that many of the positive observations found in cohort studies based on estimation of maternal dietary intake of vitamin D are the result of unrecognized confounding from the influence of correlated nutrients or socioeconomic factors. Only through prospective randomised controlled trials of vitamin D supplementation in pregnancy can these observations be clearly determined.

1.8.5 Vitamin D intervention trials

No trials are currently reported in the literature. Five prospective randomized controlled trials of vitamin D supplementation during pregnancy are in progress, and will shed light on the relevance of the mechanisms described above to human health.

In the United States, the 'Maternal Vitamin D Supplementation to Prevent Childhood asthma' (VDAART) study (NCT00920621), will randomise 870 women carrying a foetus at high risk of asthma, to receive 4000IU of cholecalciferol vitamin D daily, or placebo from 10-18 weeks gestation to delivery. The primary outcome measure is asthma or recurrent wheeze at 1 year and 3 years. Also in the united States, Hollis et al are conducting a three arm randomised trial of ether 400, 2000 or 4000IU of vitamin D per day from 12 weeks gestation in healthy women (NCT00292591). Primary outcomes are 25(OH)D3 levels throughout pregnancy and bone mineral density in mother and infant. To our knowledge this trial is not evaluating respiratory or atopic outcomes in the offspring.

In the Copenhagen Vitamin D Supplementation During Pregnancy for Prevention of Asthma in Childhood (ABCvitaminD; ISRCTN NCT00856947), 600 women will be randomised to receive either 2400IU daily of cholecalciferol vitamin D or placebo in addition to 400IU of vitamin D daily from 24 weeks gestation to 1 week after delivery. The primary outcome is recurrent wheeze in the first 3 years. This is a factorial randomised controlled trial with a second intervention of prenatal fish oil or no fish oil. In Southampton the Maternal Vitamin D Osteoporosis Study (MAVIDOS; ISRCTN 82927713) is randomising 1074 pregnant women with a 25(OH)D3 concentration of 25-100nmol/l during pregnancy who receive vitamin D supplementation at a dose determined in pilot work, or placebo from 14 weeks gestation to delivery. Primary outcome measures relate to bone health, but the trial may also yield information on pulmonary and atopic outcomes at follow up.

In our own study (ISRCTN 68645785) 180 women in London with a high prevalence of vitamin D deficiency, were randomised to no additional vitamin D in pregnancy, 200,000IU of calciferol once at 27 weeks gestation or 800IU daily of ergocalciferol from 27 weeks to delivery. The primary outcome measure is history of wheezing during the first 3 years of life, and the results form the main body of work of this thesis.

1.9 Potential mechanisms: Vitamin D and early lung development

1.9.1 Early life nutritional influences on lung development

The respiratory system originates as an outgrowth of the primitive foregut between the 4th and 7th week of human gestation (302). Through timely orchestration of a complex array of transcription factors, growth factors and physical forces (303), epithelial tubules invaginate the surrounding splanchnic mesenchyme to form the bronchial tree. Key processes are branching morphogenesis, angiogenesis, epithelial cell differentiation, sacculation, surfactant production and alveolarisation, the latter being predominantly a postnatal event in humans (188). Epithelial-mesenchyme interactions play a crucial role (304).

Early life influences on this process include both genetic and environmental factors, and minor alterations during foetal and perinatal life may have significant postnatal consequences. For example they may alter responses to early life infections and other airway insults that are implicated in the aetiology of asthma (305, 306). Several long-term cohort studies have established that infant lung function tracks into later life, suggesting that successful modification of early lung development may influence lung function in the long term (75, 307).

Known environmental, and nutritional factors which may influence lung development (117, 308) include cigarette smoking (96), polyunsaturated fatty acids (194, 309) milk fat (310), vitamin E (171), and vitamin A (185).

1.9.2 Animal data

A number of separate observations from animal work suggest that alveolarisation may be influenced in part by a complex growth axis which involves $1,25(OH)_2D$. First, an early study

of the offspring of vitamin D deprived rats showed a significant decrease in lung compliance and compliance to dry lung weight ratio compared with the offspring of vitamin D sufficient rats, suggesting vitamin D deficiency may decrease lung distensibility through an effect on interstitial tissue modelling (311). Second, the VDR is expressed during late intrauterine life in rat pulmonary tissues (312, 313). This is during the period of maturation of alveolar type two (ATII) cells which synthesise and release surfactant (314, 315). $1,25(OH)_2D$ stimulates DNA synthesis in ATII cells (316). The discovery that lung fibroblasts are capable of metabolising 25(OH)D to $1,25(OH)_2D$ identified a paracrine system for vitamin D in the developing rat lung (317).

Other data suggest a role for $1,25(OH)_2D$ in lung fibroblast development, which may in turn influence alveolarisation. $1,25(OH)_2D$ significantly increases proliferation of rat perinatal lung fibroblasts, an effect which is greatest in combination with retinoic acid (RA) on postnatal day 4 (PN4) (318). This effect is dependent on platelet derived growth factor-AB (PDGF-AB). The PDGF null mouse has a bronchopulmonary dysplasia (BPD) like picture with an absence of fibroblasts and abnormal septation, and PDGF appears to be essential for normal alveolarisation of the developing lung (319). PDGF contains a transcriptional control region containing response elements to $1,25(OH)_2D$ (320) and vitamin A (321). Thus a proposed mechanism of action for $1,25(OH)_2D$ in promoting lung development is via a synergistic effect with RA in stimulating growth of immature fibroblasts in the postnatal lung, with PDGF as the critical effector (318). Further experiments have demonstrated a role for the vitamin D analogue EB1089 on fibroblast proliferation but suggested, contrary to other work, that relatively high doses of this vitamin D analogue may disrupt alveolarisation (322). Histological findings in EB1089-treated rats include increased alveolar chord length and prominent regions of fibroblast hypercellularity. These stained strongly for alveolarisation growth factors PDGF-AA and vascular endothelial growth factor (VEGF) and the overall effect was to promote fibroblast proliferation but disrupt alveolarisation (322).

Whether this disruptive effect is dose dependant or related to other effects of the vitamin D analogue in question is not known.

A third mechanism through which $1,25(OH)_2D$ may influence alveolarisation is via the inhibition of apoptosis in lipid laden interstitial lung fibroblasts (323). Lung fibroblasts become highly apoptotic during the first 1-2 weeks of postnatal life in rats, a developmental process that contributes to alveolar wall thinning and alveolarisation (324). Both in vitro treatment (at embryological day 19) and in vivo treatment (at postnatal days 0 to 14) with $1,25(OH)_2D$ resulted in dose-dependent increases in lung fibroblasts and ATII cell proliferation and decreased apoptosis, accompanied by increases in the expression of epithelial mesenchyme differentiation markers (323). These data suggest a role of $1,25(OH)_2D$ in alveolar thinning. $1,25(OH)_2D$ may have a specific role in transcriptional regulation of Lgl1 (late gestation lung 1) during alveolarisation (325). Lgl1 is a mesenchymal protein found in foetal lung that regulates epithelial airway branching and is maximally expressed in late gestation and early postnatal life (326). In LgL1 null mice, absence of Lgl1 is lethal prior to lung formation (14). Heterozygotes also display an abnormal respiratory phenotype. In vitro experiments in rat lung fibroblasts from E18 to PN14 suggest $1,25(OH)_2D$ has a complex interplay with glucocorticoid (GC) and RA in the control of Lgl1. $1,25(OH)_2D$ directly inhibits Lgl1 mRNA production, and inhibits GC induction of Lgl1. When combined with RA there was no effect on GC induction of Lgl1 gene transcription. Lgl1 may be a mesenchymal mediator of $1,25(OH)_2D$ effects on epithelial maturation, with regulation of the target gene promoter by GC and RA as well as $1,25(OH)_2D$ (325).

Further, less direct, evidence of a role for vitamin D status in foetal lung development come from studies of VDUP1 (vitamin D up regulated protein 1) expression in the ovine foetal lung. The basal level of lung expansion is an important determinant of airway epithelial cell

phenotype (327). VDUP1 is an intracellular protein whose expression is up regulated by vitamin D3 administration (328). In sheep lung, VDUP1 is localized to airway epithelium in small bronchioles, AEC's and mesenchymal cells. Expression of VDUP1 mRNA increase significantly during the alveolar stage of lung development (329). Furthermore, VDUP1 mRNA correlate with different levels of lung expansion, with increased levels in under expanded lungs and decreased levels in over expanded lungs compared to normal controls (329). Pressure effects in the airways induced by respiratory movements, contraction and relaxation of smooth muscle in the airway wall and the pressure of amniotic fluid have a potent influence on airway and alveolar development. Reduced amniotic fluid is associated with lung hypoplasia, and reduced foetal respiratory effort (eg opiate-dependent mothers) has a profound effect on post-natal lung function. Thus it is possible that VDUP1 is a vitamin D status dependent moderator of foetal lung growth and development in response to changes in foetal lung expansion(329).

Finally, in mature rats, VDR expression is present in lung fibroblasts and $1,25(OH)_2D$ appears to have a role. Recent work shows that $1,25(OH)_2D$ inhibited TGFβ1-induced fibroblast proliferation, and blunted TGFβ1-induced up regulation of mesenchymal cell markers and abnormal expression of epithelial cell markers. TGFβ is expressed during branching morphogenesis and has an important role in modelling of the airway wall by stimulating fibroblast collagen production (330). Thus vitamin D status may have a homeostatic role in the developing lung, preventing some of the effects of excess TGFβ on the developing epithelial-mesenchymal trophic unit, which are thought to lead to changes such as the airway remodelling commonly seen in asthma.

1.9.3 Human data

Rachitic respiratory distress has been described very early in infancy, for example in small preterm infants (331). This suggests that vitamin D deficiency of prenatal origin can have a

profound acute effect on early lung function. As with the animal studies, there is also evidence in human studies that $1,25(OH)_2D3$ influences different components of lung development including fibroblast proliferation and alveolarisation. The VDR has been detected in human foetal lung fibroblasts from 16 weeks gestation, (332, 333) and in ATII cells from the second trimester (334). In ATII cells, VDR expression is largely dependent on induction by $1,25(OH)_2D$ (334). After incubation of a human ATII cell line with $1,25(OH)_2D$, a number of metabolites are also formed including 1,25-(OH)2-3-epi-VD3, which can increase surfactant phospholipid synthesis, surfactant SP-B mRNA gene expression and surfactant SP-B protein synthesis in pulmonary ATII cells (335). However the effects of vitamin D metabolites on human lung development are likely to be complex since decreased, increased and no effect on surfactant protein synthesis have been observed on treating human fetal lung explants and ATII cells with calcitriol (334).

In the mature human lung, data are now emerging as to the cellular targets that may be influenced by vitamin D status. The VDR is expressed in the epithelia of normal and malignant bronchial tissue (336), in lung epithelial cells treated with vitamin D metabolites (337), and in airway smooth muscle (ASM) cells which demonstrate a large array of changes in gene expression in response to stimulation by $1,25(OH)_2D$ (338). In ASM cells that were passively sensitized with serum from an asthmatic, $1,25(OH)_2D$ suppressed proliferation and significantly down regulated expression of matrix metalloproteinases-9 (MMP-9) and a disintegrin and metalloprotease 33 (ADAM33); both of these being significant promoters of airway remodelling (339-341). Polymorphisms of ADAM 33 are associated with a higher risk of asthma particularly in relation to bronchial hyper-responsiveness. In British Bangladeshi adults, 25(OH)D status has been found to be inversely associated with plasma MMP-9 levels, with supplementation in vitamin D deficient individuals leading to reduced MMP-9 levels in vivo (342). In vitro $1,25(OH)_2D$ inhibits MMP-9 expression by M. Tuberculosis in cell culture (343). Therefore any effects of vitamin D status on lung development may be

mediated through effects on MMP-9 levels in plasma or lung tissue. Finally, in bronchial ASM samples taken during surgery, $1,25(OH)_2D$ decreased PDGF induced cell proliferation (344).

Whilst speculative, if such relationships are present in the developing lung this could explain potential benefits of vitamin D metabolites on future lung health. In light of current concepts that molecular regulators originally associated with developmental processes may be implicated in adult diseases such as chronic obstructive airway disease (188) this is of great interest, and suggests a potential role for maintaining adequate vitamin D repletion to ensure 25(OH)D and 1,25(OH)D are available to the tissues for treatment, or prevention, of airway remodelling in asthma (345).

1.10 Potential mechanisms: Vitamin D and immune development

1.10.1 Introduction

Animal data on the role of $1,25(OH)_2D$ in allergic asthma is contradictory. In human, in-vitro studies, $1,25(OH)_2D$ influences several immune-cell types, including macrophages, dendritic cells and lymphocytes, with an overall immunosuppressive effect. In the innate immune system, $1,25(OH)_2D$ is able to induce cathelicidin production, an antimicrobial peptide, by macrophages and airway epithelial cells, which contributes to defence against bacteria. In dendritic cells, $1,25(OH)_2D$ inhibits up regulation of co-stimulatory molecules necessary for antigen presentation to T cells, thus promoting immune tolerance. In T cells, $1,25(OH)_2D$ decreases the production of IL-2, IL-17 and interferon-γ (IFN-γ) and attenuates the cytotoxic activity and proliferation of CD4+ and CD8+ T cells. $1,25(OH)_2D$ also promotes the development of regulatory T cells that promote a balanced immune response, and blocks B-cell proliferation, plasma-cell differentiation and immunoglobulin production.

1.10.2 Animal knockout models

Despite the large body of evidence suggesting an important role for 1,25(OH)D in immune homeostasis, VDR knockout mice have a normal composition of immune cell populations and reject allogenic and xenogenic transplants at the same rate as wild type mice (233). VDR knockout mice do not develop experimental allergic airway disease suggesting that VDR expression may be necessary for lung inflammation to occur (346, 347).

1.10.3 Animal models of airway inflammation

In mouse models of asthma, $1,25(OH)_2D$ inhibits airway inflammation and decreases IL-4 levels in bronchoalveolar lavage (BAL) fluid and impairs T cell migration to lymph nodes

(348). Irradiation of mice with UVB light prior to sensitization with antigen blunts airway hyper-responsiveness and inflammation, suggesting 1,25$(OH)_2$D may ameliorate airway inflammation (349). In one mouse asthma model, duel effects of 1,25$(OH)_2$D were seen, with promotion of Th-2 cytokines (IL-4 and IL-13) and increased IgE production, but impaired recruitment of eosinophil's and inferior levels of IL-5 seen in BAL fluid (350).

These data illustrate the complexity of the role for vitamin D in inflammatory responses involved in the pathogenesis of asthma. Their contradictory nature may relate to the differences between mouse models of allergic asthma and the human disease, or to the critical importance of dose, nature of vitamin D supplement and timing of administration for its immune effects.

1.10.4 Other animal models of disease

Beneficial immunomodulatory effects of 1,25$(OH)_2$D have been seen in several experimental animal models of Th-1 mediated disease including uveitis and colitis, inflammatory bowel disease, multiple sclerosis, systemic lupus erythematous, allergic encephalitis and diabetes (reviewed in (351)). 1,25$(OH)_2$D prolongs survival in mouse cardiac allografts (233).

Promotion of IL-10+ Treg cell function is thought to be a major mechanism for allergen specific immunotherapy (352). In mouse models of sublingual immunotherapy, calcitriol and dexamethasone both increased the efficacy of immunotherapy through the promotion of Treg function via increased foxp3+ T cell numbers (353, 354).

1.10.5 Enhanced production of antimicrobial peptides

The innate immune system plays a key role in early defence against pathogens and antigen presentation. Cells of the innate immune system include macrophages, neutrophils and

dendritic cells which are important for phagocytosis and antigen presentation, and airway epithelial cells that are the first line of defence against respiratory infection.

The most widely studied effect of $1,25(OH)_2D$ on the innate immune system is the ability to enhance the production of naturally occurring antimicrobial peptides, such as cathelicidin. Cathelicidin has antibiotic properties and is naturally expressed in human cells including neutrophils, alveolar macrophages, epithelial cells and keratinocytes (355). In-vitro stimulation of TLR2 in macrophages results in increased expression of CYP27B1, local $1,25(OH)_2D$ production and increased expression of cathelicidin that enhances killing of intracellular Mycobacterium tuberculosis (227). This effect is blunted by lack of 25(OH)D (356). AEC's also express CYP27B1, and produce cathelicidin in response to $1,25(OH)_2D$, but using a mechanism independent of TLR2 activation (357). Other peptides regulated by vitamin D include Defensin-B 2 and 4 (358).

$1,25(OH)_2D$ decreases respiratory syncytial virus induction of NF-kappaB-linked chemokines and cytokines in airway epithelium while maintaining the antiviral state, suggesting that adequate vitamin D levels could contribute to reduced inflammation and less severe disease in RSV-infected individuals (337),

These effects have been proposed to explain the increased susceptibility to tuberculosis in patients with vitamin D deficiency (359) and to confer protection against viral infection (337).

1.10.6 Inhibition of lymphocyte proliferation

1.10.6.1 Animal data

In-vitro studies demonstrate a duel effect on Th-1/Th-2 cytokine expression (350). In one study, $1,25(OH)_2D$ inhibited IL-4 synthesis during polarisation of naïve T cells suggestive of

Th-2 suppression (360) whereas in another 1,25$(OH)_2$D appeared to promote IL-4, IL-5 and IL-10 secreting cells suggestive of Th-2 phenotype development (361).

1.10.6.2 Human data

The VDR is expressed on activated T lymphocytes (224, 225) with greater expression in effector and memory T cells than naïve T cells (233, 362). There are conflicting reports of the expression of VDR on B lymphocytes, and it is unclear whether 1,25$(OH)_2$D can act directly on B cells (233).

The overall effect of 1,25$(OH)_2$D is a marked inhibitory effect on adaptive immune cells (363-367). For example, inhibiting T cell proliferation and the expression of IL-2 and INF-γ by T cells (363, 368-370), and decreasing CD8 T cell mediated cytotoxicity (233), and inhibiting production of IL-17 (371, 372). Inhibition of T cell proliferation is partly mediated through IL-2 inhibition, and may be partially rescued by exogenous IL-2 (362, 363, 368). Inhibition of IL-2 and IFN-γ is mediated by a genomic mechanism of action, with the VDR-RXR complex binding to VDRE in the promoters of genes encoding IL-2 and INF-γ (373, 374). Overall the effect is to block the induction of Th-1 cytokines, particularly IFN-γ, while promoting Th-2 cell responses (IL-4) (233). The effects on T cell mediated cytotoxicity are similar to those observed with cyclosporine (375).

For B cells, 1,25$(OH)_2$D suppresses proliferation, plasma cell differentiation and IgG secretion (368, 376-378). B cells can secrete significant amounts of 1,25$(OH)_2$D that inhibits IgE production by a direct action on the immunoglobulin class switch (379).

1,25$(OH)_2$D is able to induce CD200 expression by peripheral and respiratory tract T cells, suggesting an additional pathway by which vitamin D can prevent inflammation in the airways to maintain respiratory health (380). CD200 is a cell-surface immunoglobulin-like

molecule expressed by immune and stromal cells, which dampens the pro-inflammatory activity of tissue-resident innate cells via its receptor, CD200R. This interaction is important for promoting peripheral immune tolerance.

1.10.7 Duel effects on Th1/Th2 balance

In terms of the balance between Th-1 and Th-2 cells, the picture is less clear-cut. In adult human in-vitro studies, $1,25(OH)_2D$ reduces IFN-γ (364, 365, 381, 382) and IL-2 production (363, 364, 370, 382-384) characteristic of Th-1 responses, suggesting a favouring of Th-2 responses, which would promote allergic responses. Other human studies have found $1,25(OH)_2D$ to reduce numbers of IL-4 secreting cells, and demonstrated no favouring of the Th-2 phenotype (385). Concentration in vitro may account for this dual effect on Th-2 cell differentiation, with experiments using high concentrations (1x10-6M) of $1,25(OH)_2D$ favouring enhanced IL-5 and IL-13 secretion, and lower concentrations (1x10-7 to 10-9) suppression (386).

Recent in-vivo data from a cross-sectional survey of over seven thousand white British 45 year olds reflect these observations, with a non-linear relationship between 25-hydroxyvitamin D status and total IgE levels described. Both low (<25nmol/L) and excess (>135nmol/L) levels were associated with elevated IgE. (301). This suggests there may be a U shaped dose relationship (301).

1.10.8 Influencing DC's and Tregs to promote immune tolerance

1.10.8.1 Human data

Tregs are important for exerting a suppressive influence on effector T cell activity such as IL-

4, IL-5 and IL-13 secretion characteristic of Th-2 responses (387). In asthma and allergy, the numbers or function of Tregs may be deficient (387). Key suppressive signalling molecules involved in this process include TGF-β and IL-10 (387). Human in-vitro studies show the effect of 1,25$(OH)_2$D on antigen presentation by dendritic cells, and on T cell regulatory function is to promote a more balanced, or tolerogenic phenotype (387).

DC's express the VDR and CYP27B1 once stimulated (388, 389). 1,25$(OH)_2$D suppresses APC's capacity to act as efficient antigen presenting cells which in turn leads to T cell hypo-responsiveness (390, 391). This involves reduced differentiation of dendritic cells, reduced up regulation of co-stimulatory molecules CD40/CD80/CD86, reduced expression of class II MHC molecules, (390) reduced secretion of the pro-inflammatory cytokine IL-12 and increased production of the anti-inflammatory cytokine IL-10 (392, 393). 1,25$(OH)_2$D promotes the induction of CD4+FoxP3+ Treg cells by dendritic cells (392), and also the induction of naïve T cells into IL-10 secreting regulatory T cells when incubated with dexamethasone (394-396).

Ingestion of 1,25$(OH)_2$D by human volunteers led to an increase of both IL-10 and TLR9 expression by CD3+CD4+ T cells (395). In steroid resistant asthmatics, CD4+ T cells do not show steroid induction of IL-10 secretion in vitro. In a landmark paper, this impaired induction of IL-10 by glucocorticoids from steroid resistant patients was reversed by addition of calcitriol in-vitro (396).

1.10.8.2 Human in-vitro and in-vivo data

In steroid resistant patients who received oral administration of vitamin D3, enhanced IL-10 secretion in response to stimulation in vitro with glucocorticoids was seen (396). There is evidence that vitamin D deficiency may modify the glucocorticoid response in children with asthma. In one study, vitamin D enhanced the glucocorticoid action in peripheral blood

mononuclear cells from asthmatic patients and enhanced the immunosuppressive function of dexamethasone in vitro (397).

In rheumatoid arthritis, 25(OH)D levels are inversely correlated with disease activity (233). In multiple sclerosis, vitamin D supplementation has been shown to increase the serum levels of immune modulating TGF-β and IL-10 (398). In cystic fibrosis patients with allergic bronchopulmonary aspergillosis, heightened Th-2 reactivity correlated with lower mean serum vitamin D levels. The co-stimulatory molecule OX40 ligand was critical in driving Th-2 responses to A. fumigatus, and in-vitro addition of $1,25(OH)_2D$ substantially reduced DC expression of OX40 Ligand and increased DC expression of TGF-β (399).

1.10.9 Effect of developmental vitamin D deficiency and prenatal vitamin D supplementation

1.10.9.1 Animal data

In animal models the principle that vitamin D deficiency at a critical time in development may adversely affect immune development has been supported. Developmental deficiency of vitamin D in rats induces alterations in immune organ morphology and function in adult offspring, with an increase in central organ size and a pro-inflammatory IL-2 response by lymphocytes, consistent with a role for vitamin D in promoting immune regulation (400).

1.10.9.2 Human data

$1,25(OH)_2D$ has been shown to inhibit both Th-1 and Th-2 differentiation in cord blood in vitro (385). A recent in-vivo study lends support to the role of vitamin D in inducing tolerogenic dendritic cells, with offspring of mothers who took vitamin D supplements during pregnancy having increased expression of immunoglobulin like transcripts 3 and 4 in cord

blood, inhibitor receptors that are up regulated in tolerogenic dendritic cells (401).

1.10.10 Conclusions

Although 1,25$(OH)_2$D clearly exerts immunomodulatory activity in-vitro and in-vivo, its relative physiological role in maintaining immune tolerance and in shaping immune responses is still unclear (233).

1.11 Public health importance

The economic burden of allergic disease is an increasing problem, especially in developed countries within northern latitudes. In the United Kingdom, allergic conditions account for up to 6% of primary care utilization, 70,000 hospital admissions a year and 10% of the primary care prescribing costs, representing a direct burden of over a billion pounds per anum (402). Primary prevention of asthma and allergic disorders is a high priority.

Since early life vitamin D deficiency is common (403), and supplementation during pregnancy is safe in higher doses in studies with short term follow up (404), and inexpensive, this represents an attractive public health intervention for asthma prevention.

One important measure of any potential impact is healthcare utilization. This has been defined as the outcome of the interaction between health professionals and patients, and in economic terms corresponds to the volume of health services used (405). In the United Kingdom, one such measure for all children registered with a general practice is the electronic health record (e-HR). This is a contemporaneous record of all health care utilisation from that practice. Use of primary out of hours services (OOH) or secondary healthcare is also recorded. In this way the e-HR forms a complete record of a child's healthcare utilisation from birth.

Determinants of a child's overall healthcare utilisation are their underlying health status (406-410), parent mental health and family functioning (411-413), and socioeconomic predictors (age and ethnicity of the child, family income, area of residence, parental educational attainment and social class) (406, 410, 414, 415), although some studies found no effect of social class (407, 408). For hospital utilisation, predictors include socioeconomic position at the time of birth, mother's age, mother's age on leaving education, gestational age, ethnicity, parity, maternal BMI, maternal smoking during pregnancy and mode of delivery (415).

The high prevalence of vitamin D deficiency in pregnancy and infancy in the populations of many countries may be of critical public health importance if the associations between Vitamin D status and disease are confirmed in prospective randomized controlled trials. It is increasingly recognized that many chronic diseases of childhood and adulthood are influenced by early life developmental events, so the potential adverse effects of widespread deficiency of vitamin D at these time points on future population health may be serious. Measuring healthcare utilization in offspring is one important means of assessing the potential impact of vitamin D supplementation in pregnancy.

1.12 Conclusions

The precise relationship between vitamin D status and asthma, and the impact of dietary vitamin D supplementation on this risk, are unclear. Potential mechanisms through which perinatal vitamin D may influence asthma or lung development include direct effects on lung growth and development particularly on alveolarisation, fibroblast proliferation and airway smooth muscle. Interactions with correlated nutrients such as vitamin A, which interacts with vitamin D metabolites in essential ways but can antagonise in excess, may complicate this relationship (416, 417). Other potential mechanisms include increased microbial killing capacity limiting the host consequences of viral infections, and increased immune regulation preventing atopic immune responses.

Current intervention trials evaluating the effects of early life vitamin D supplementation at a range of doses, in a range of populations and using a range of treatment schedules will play a critical role in establishing the possible role of vitamin D.

1.13 Aims of the thesis

The thesis consists of three main bodies of work. First, a pilot study to evaluate the feasibility of performing IOS in preschool children, and its ability to detect differences between children with and without clinical disease. Second, a follow up study of a randomised controlled trial of vitamin D supplementation during pregnancy to evaluate it's effect on parentally reported respiratory and allergic health, objective measures of allergic inflammation and lung function in childhood. Third, an evaluation of the effects of prenatal vitamin D supplementation on healthcare utilization in the first three years of life using the same established randomised trial.

2 General Methods

2.1 Pilot study to evaluate the feasibility of impulse oscillometry in preschool children

This was a cross-sectional survey of 3 to 5 year old children recruited from St. Mary's Hospital Paediatric Outpatient department, to identify success rate, repeatability, and ability of IOS to discriminate between health and disease.

2.1.1 Study subjects

St. Mary's Hospital Research Ethics Committee approved the study (REC reference 10/H0712/13). Children aged 3 to 5 years of age attending St Mary's hospital paediatric outpatient department were eligible to participate. Exclusion criteria were acute illness at the time of the study, known chronic respiratory disease apart from recurrent wheezing, and gestational age <37 weeks. Informed, written, parental consent was obtained for all subjects (See appendix for information leaflet and consent form).

2.1.2 Protocol and questionnaire

Children had their standing height and weight measured. Parents completed a general health (Appendix 10.1) and validated allergy questionnaire (Appendix 10.2) to identify risk factors for abnormal lung function, including previous history of wheezing, wheeze in the year prior to assessment, eczema ever and eczema in the year prior to assessment as defined by the International Study of Asthma and Allergies in Childhood (ISAAC) (8). Other variables recorded included recurrent wheezing (≥2 episodes of reported wheezing since birth), allergic rhinitis, history of doctor diagnosed food allergy and history of >4 episodes of upper respiratory tract infections (frequent URTI) per year (defined as a positive answer to

the question ‘how often does your child have an upper respiratory tract infection, with at least two of the following symptoms, cough, runny nose and fever?’)

2.1.3 IOS measurements

Impulse oscillometry (IOS) was performed in accordance with ERS/ATS task force guidelines (49) using the Jaeger IOS system (Jaeger, Wurzburg, Germany), before and 15 minutes after inhalation of 400mcg salbutamol sulphate via a large volume spacer (Volumatic, Allen and Hanbury, Middlesex, UK). Total impedance (Z), resonant frequency (Fres), area under the reactance curve (AX), reactance at 5Hz (Xrs5) and resistance (R) at 5, 10, 15, 20 and 25 Hz were chosen for data analysis. The IOS system was calibrated twice daily using a 3L volume syringe and a reference resistance device (0.2kPa/L).

Training in this technique was obtained from a tertiary paediatric respiratory team with experience in the use of IOS in young children (Professor Janet Stocks, Dr Jane Kirby; Portex Respiratory Unit, Institute of Child Health, London). In addition, the following online quality assurance protocol was adopted in collaboration with Dr Jane Kirby. Support of the cheeks to ensure no movement of the mouth (chewing, talking etc.) to minimise upper-airway compliance; observation of the tidal volume time-based trace to ensure it was stable, free from drift with no hyper- or hypoventilation; observation of the impedance at 5Hz to ensure consistency throughout the measurement; ensuring no obvious spikes in impedance due to swallowing, glottic closure or cough were present. If these criteria were not met, the measurement was stopped and the technique reviewed.

On completion of all data collection for the study, two trained operators (STG, JCK) assessed all readings. Essential acceptance criteria for a reading to be included in analyses were as follows: at least 10 seconds in duration with a minimum of four tidal breaths; stable, regular tidal breaths, free from drift; no obvious abnormalities, looks physiological (i.e.

identifying physiological implausible data); coherence >0.4 at 5Hz and >0.7 at 10 Hz. Results were rejected if there was evidence of tongue position artefact, (noted by a parallel increase in resistance at all frequencies). For each child, the reported result was the mean of 3 to 5 “acceptable” (as defined above) measurements (49).

2.1.4 Allergic sensitisation

Allergic sensitisation was defined as a skin prick test wheal at least 3mm greater than the negative control (glycerine) at 15 minutes, in the context of an appropriate response to the positive control (histamine 10%). House dust mite, cat, dog, grass pollen, silver birch pollen and alternaria pollen were tested (Stallergenes, Antony, France).

2.1.5 Statistical analysis

The within-occasion, within-test repeatability, or coefficient of variation (CV), was calculated as the standard deviation of each set of measurements as a percentage of the mean. Bronchodilator response was measured in two different ways - as the absolute value of the difference between measurements (Δabs), and as a percentage of the initial value (Δ%Init). IOS data was explored using histograms and were normally distributed. Unadjusted analysis of the difference between groups used the unpaired t-test. Analysis of variance was performed with age, height and pre or postnatal environmental tobacco smoke (ETS) exposure as covariates. All analyses were performed using SPSS version 19.0 (IBM, Chicago USA). A value of $p<0.05$ was considered statistically significant.

2.2 Follow up study of a randomised controlled trial of prenatal vitamin D supplementation to evaluate its effect on respiratory and allergic outcomes in childhood.

2.2.1 Study subjects

This was a follow up study of the offspring of women who took part in an ethnically stratified, parallel group, randomised controlled trial of vitamin D supplementation in pregnancy at St Mary's Hospital London, a university-affiliated hospital antenatal clinic, between April and November 2007. The original trial was conducted to determine the effects of supplementation on the 25(OH)D status of mothers and babies at delivery (418). St. Mary's Hospital Research Ethics Committee approved the study (REC reference 10/H0712/13) and all women gave informed consent to participation for themselves and their child. Eligible participants were women presenting at 27 weeks gestation for routine glucose challenge test from the following ethnic groups: Indian Asian, Middle Eastern, Afro-Caribbean and Caucasian. Exclusion criteria were known sarcoidosis, osteomalacia, renal dysfunction or tuberculosis. We assessed the offspring of study participants when they reached 3 years age, to evaluate subjective and objective measures of health.

2.2.2 Study intervention and randomisation procedure

Women were randomised at 27 weeks gestation to no treatment (control), 800 IU ergocalciferol until delivery (daily), or a single oral dose of 200,000 IU calciferol (bolus). The randomisation sequence was generated by an independent person using computer generated random number lists in blocks of 15, stratified by ethnicity in a 1:1:1:1 ratio. The researcher gave participants a study number on entry to the trial, and treatment was allocated from the hospital pharmacy using the randomization list. Women were given instructions to swallow the tablets whole and to avoid other multivitamin supplements

containing vitamin D. This trial was conducted before national guidance on routinely providing advice on vitamin D intake during pregnancy was introduced in March 2008 (266). No adverse effects were reported.

2.2.3 Weaknesses of original study

Significant weaknesses of the original supplementation trial include the absence of a placebo control, which may have led to biased parental reporting and also control women taking vitamin D supplements of their own volition. Although the authors report that telephone calls were made during the course of the pregnancy to check for compliance in all women on the daily treatment regime, this did not apply to the women in the bolus group, and the results of this assessment were not published. As such adherence was not assessed adequately. These weaknesses would have reduced the magnitude of any effect of vitamin D supplementation.

Also, the process of randomization was not gold standard, such as by an independent randomisation service.

2.2.4 Assessment of maternal and childhood vitamin D status, and their determinants

25(OH)D was measured in mothers prior to randomisation, and in offspring at birth (cord blood) and at 3 years using a radio-immunoassay (DiaSorin, Stilwater, MN) in a clinical biochemistry laboratory that participates in the international Vitamin D external quality assessment program (DEQAS). Vitamin D deficiency was defined as 25(OH)D <25nmol/L, and insufficiency as 25(OH)D ≥25 but <50nmol/L (213).

Dietary and lifestyle determinants of Vitamin D status were assessed by health questionnaire (Appendix 10.3) for mothers and offspring, and by skin colorimeter

assessment on mother and child at the 3-year outcome assessment. Although based on known determinants in the literature, validated questionnaires were not used as they were assessed as being too long to complete, and thus may have led to questionnaire fatigue for the parents. Determinants assessed included for diet - intake from nutritional supplements, mode of infant feeding, duration of breast feeding, age of weaning, observation of a restricted diet (vegan or vegetarian), frequency of consumption of certain foods containing vitamin D (oily fish, margarine, eggs), and for lifestyle - average length of time spent outdoors in daylight hours each day in the last month, use of sunscreen or clothing to protect from the sun in sunny weather, length of time watching TV or using a computer each day, access to a garden, access to a terrace or balcony and Fitzpatrick score of skin type (236, 252, 419). In mothers, choice of clothing (no concealed clothing vs all body covered except hands and face when outdoors vs all body covered including hands and face when outdoors) and use of a sunbed in the last year (252) were assessed.

Skin colour was assessed using reflectance spectroscopy (DSM II Colormeter, Cortex Technology, Hadsund, Denmark). In children a single measurement was taken in each of the following areas: forehead, volar aspect of the forearm and the abdomen just superior to the umbilicus. In mothers a single measurement was taken on the forehead and volar aspect of the forearm only.

2.2.5 Assessment of determinants of child health

Children were characterised by birth weight (using the child's red book which parents were asked to bring with them), gestation, admission to a neonatal unit at birth, immunisation status, presence of congenital abnormalities, presence of other health problems and use of regular medications. The following inherited and environmental determinants of child health were recorded: ethnic background, age mother and father left full time education, number of adults and children in the household, attendance at nursery, number of older and younger

siblings, exposure to household pets since birth, exposure to prenatal and postnatal tobacco smoke and history of doctor diagnosed eczema, rhinitis or asthma in the mother, father or siblings.

2.2.6 Outcome assessment

2.2.6.1 Subjective outcome measurements reported by parent

Investigators were blind to original treatment allocation. To ensure this, parents were instructed on their invitation letter not to disclose their treatment group to the research team (See appendix xxx). There were no cases of accidental unblinding. Children were assessed at three years of age using a health questionnaire, which included a number of validated assessments. The primary outcome was prevalence of 'wheeze ever' as defined by the International Study of Asthma and Allergies in Childhood (ISAAC)(8). Secondary outcomes were recurrent wheezing (≥2 episodes of reported wheezing since birth), wheeze in the year prior to assessment (ISAAC), wheeze with a positive asthma predictive index (loose criteria),(5) reported history of bronchodilator use, eczema ever (ISAAC), eczema in the year prior to assessment (ISAAC), allergic rhinitis, history of doctor diagnosed food allergy, history of lower respiratory tract infection (LRTI) (any of bronchiolitis, bronchitis, croup, pneumonia or an otherwise unspecified chest infection) and history of >4 episodes of upper respiratory tract infections (URTI) per year (defined as a positive answer to the question 'how often does your child have an upper respiratory tract infection, with at least two of the following symptoms: cough, runny nose and fever?')

2.2.6.2 Objective assessment of lung function

IOS was performed as described in section 2.1.3, before and 15 minutes after inhalation of 400mcg salbutamol sulphate via a large volume spacer (Volumatic, Allen and Hanbury,

Middlesex, UK). Resonant frequency, area under the reactance curve, and resistance at 10 and 20 Hz were chosen for primary data analysis. The IOS system was calibrated twice daily using a 3L volume syringe and a reference resistance device (0.2kPa/L).

2.2.6.3 Objective assessment of allergic sensitisation and inflammation

Fully trained personnel undertook allergy skin prick testing in accordance with international guidelines. Individual allergens were placed on the forearm of children at the 3-year outcome assessment, and disposable lancets were used to undertake the test (ALK-Abello, Denmark). House dust mite, alternaria, cladosporium, cat, dog, grass pollen, silver birch pollen, peanut, milk and egg were tested; histamine 10mg/ml and glycerine were used as positive and negative controls respectively (Stallergenes, Antony, France). Skin prick tests were read at 15 minutes, as the mean of the largest wheal diameter and the perpendicular diameter. Allergic sensitisation (atopy) was defined as a skin prick test wheal at least 3mm greater than the negative control to one or more of the aeroallergens tested, in the context of an appropriate response to the positive control.

Exhaled nitric oxide was measured using an offline, tidal breathing technique in accordance with the principles recommended by the ATS/ERS (420): measurements were made before other lung function tests and before administration of salbutamol; subjects breathed through a two-way non-rebreathing mask with partitioned nose and mouth chambers (Series 7975, PED SM M/F Mask Yshp, Han Rudolph, Kansas City, USA); subjects breathed normally from a source of nitric oxide free air (<5ppb) (NO scrubber, Sievers reorder P/N: AFL 01410-01, NORTH part no. N7500-2, USA, 40700842 rev E) and exhaled against 5cm H_20 resistance (Model 7100R, flow range 0 to 2 L/sec, SN 710-1227, Han Rudolph, Kansas City, USA) to prevent contamination of the sample with nasal NO; the initial 10 exhalations were

discarded to allow for washout of dead space of the lungs (421). Five exhalations were collected into a NO-inert bag (Maximum pressure 12 cmH20, Series 6000, P/N CR1735, 10x10 outside measurement, 1L capacity, Hans Rudolph, Kansas City, USA) connected via a three way valve (2100 series 3 way stopcock, Han Rudolph, Kansas City, USA); additional samples were collected after 30 seconds of tidal breathing in ambient air; 3 samples were collected, with the median value of at least two measurements used for data analysis; samples were analysed within 1 hour of collection using the NIOX flex (Aerocrine, Sweden) with the following settings - 7 seconds collection time, flow rate of 50mls/s (421), calculation segment 50% to 100% of measurement.

Serum total IgE (ImmunoCAP, Phadia, Uppsala, Sweden) and eosinophil count were determined on the day of the child's 3 year assessment.

2.2.6.4 SCORAD assessment

A SCORAD assessment (422) was performed in children with eczema identified by the health questionnaire. Training in SCORAD assessment was by using the online training module, which can be found at http://adserver.sante.univ-nantes.fr/Scorad_Course/. A single investigator made all SCORAD assessments.

2.2.6.5 Assessment of health outcomes via Primary Healthcare record analysis

Primary health care records were obtained from participants' general practitioners and reviewed by a single investigator (RJB), blinded to treatment allocation. Children were categorized as having 'recurrent wheezing' where ≥2 episodes of either wheezing, or respiratory distress treated with bronchodilator were recorded; 'eczema' if they had ≥2

attendances separated by ≥6 months where either topical corticosteroids were prescribed for treatment of an itchy skin rash, or a doctor's diagnosis of eczema was made; and 'food allergy' if this diagnosis was recorded in any part of the primary healthcare record.

2.2.6.6 Blood collection

10mls of blood was collected from each child via venous puncture using local anaesthetic cream and appropriate distraction techniques, with a play specialist as needed. Samples were processed as follows: 1ml in EDTA for DNA stored at -80C within one hour of collection, 1mls in EDTA for eosinophil count was sent to Imperial College NHS Trust Hospital haematology laboratory for automated analysis and 8mls in a 10ml polypropylene tube containing 100IU preservative-free heparin collected within one hour and processed for flow cytometry and cell culture. In addition an aliquot of plasma was separated, stored at -20°C and transported to Northwick Park hospital laboratory for analysis of vitamin D levels, and an aliquot was analysed for total IgE level at TDL laboratories (ImmunoCap, Phadia, Sweden).

2.2.7 Statistical analysis

2.2.7.1 Power calculation

The primary outcome measure was presence of at least one wheezing episode in the first 3 years of life, which has been reported as occurring in 34% of children (5). With 180 participants and 80% successful follow up, this study had 80% power with 2-sided alpha of 0.05 to detect a reduction in wheeze from 34% in the children of non-supplemented mothers, (5) to 13% in the supplemented group. This level of risk reduction would be consistent with observational studies of maternal vitamin D intake and childhood wheezing (5, 297).

2.2.7.2 Data analysis

All data were double entered onto a database and underwent range checks, data cleaning and checking with source documents before database lock. A statistical analysis plan was agreed between all the investigators prior to unblinding. Where necessary data were natural log transformed for analysis. All analyses presented here were specified in the statistical analysis plan, and no post-hoc analyses were undertaken. For primary analyses, bolus and daily vitamin D groups were compared with the control group. We also report subgroup analyses of the combined groups versus control, and of children born to mothers with vitamin D deficiency (25(OH)D <25nmol/L) at enrolment. Binary outcomes were analysed using logistic regression, and continuous outcomes using linear regression. For clinical outcomes at age three years, binary outcomes were also analysed using risk ratios for unadjusted analysis. For adjusted analyses, treatment effects were adjusted for mother's ethnic group, presence of household smokers, maternal smoking in pregnancy, exclusive breast-feeding to four months, any parental allergic history, any child vitamin supplementation, number of children in the household, age mother left full-time education, baseline concentration of 25(OH)D in maternal blood, baseline maternal BMI and sex of the child. Analyses are based on all participants with complete data. All analyses were performed using SPSS version 19.0 (IBM, Chicago USA). A value of $p < 0.05$ was considered statistically significant.

Adjustment for multiple testing was performed using the Benjamini and Hochberg method (423). This works by evaluating all the p-values from multiple analyses in order of significance, and determining whether each one can truly be counted as significant or not. For our analyses, there were 28 secondary analyses (14 for the daily group, and 14 for the bolus group) and the false discovery rate was controlled at 20%.

Since not all offspring were followed up, sensitivity analyses were performed to determine if

this influenced the result for the primary outcome. Two scenarios were considered – the first where no drop-outs wheezed and the second where all drop-outs wheezed.

2.3 An evaluation of the effects of prenatal vitamin D supplementation on healthcare utilization in the first three years of life

We asked for consent from the parents of offspring participating in the follow up study of prenatal vitamin D supplementation to obtain a copy of their child's electronic health record (e-HR). Consent was obtained in person or over the telephone, in which case a second researcher verified consent. A complete record was defined as having data for at least 11 months out 12 for each year, and including all three years since birth.

2.3.1 Primary and secondary outcomes

The primary outcome was the total cost of unscheduled NHS healthcare utilization across primary and secondary care recorded in the e-HR from birth until the child's third birthday, including both visit costs and prescription costs. Scheduled care such as routine developmental checks and vaccinations was not included. Secondary outcomes were as follows: total costs for the first, second and third year of life separately; total primary care visit costs, total secondary care visit costs, respiratory costs i.e. healthcare utilisation attributed to wheeze, cough, upper respiratory tract infection, lower respiratory tract infection and bronchiolitis; food allergy costs, eczema costs; prescription costs attributable to wheezing and eczema (separately).

Primary healthcare consultations were classified according to the professional providing the service (general practitioner, out of hours service or nurse) and location (surgery, telephone or home visit). Entries without details were classed as 'primary care; unknown'. Secondary healthcare utilization was classified as new outpatient appointment, follow-up outpatient appointment, accident and emergency attendance, ward admission or intensive care unit admission. For admissions, the number of nights in hospital was recorded. Entries regarding administrative tasks were not included. Coding definitions are shown in Table 2.1.

Table 2.1 Diagnostic codes for electronic health records

Outcome	Definition. Any record of the following in the e-HR.
Wheeze	Wheeze or asthma excluding wheeze in the context of bronchiolitis
Cough	Cough excluding cough in the context of URTI or LRTI
URTI	URTI, pharyngitis, rhinorrhoea, laryngitis, tonsillitis, sinusitis or otitis media OR At least two of fever, sore throat, earache, sticky eye or cough
LRTI	LRTI, chest infection, respiratory infection, bronchitis or pneumonia. OR Cough with a record of signs of infection such as crackles on auscultation
Bronchiolitis	Bronchiolitis
Food allergy	Doctor diagnosed food allergy
Eczema	Eczema or eczematous skin

URTI (Upper respiratory tract infection), LRTI (Lower respiratory tract infection).

2.3.1.1 Visit costs

Costs were calculated according to standard NHS tariffs for 2009 that include training and, where applicable, travel costs and direct care staff costs. As no unit cost was given for telephone calls with a practice nurse, this was estimated as follows: a telephone consultation with an advanced nurse is six minutes long on average, when applied to the hourly rate for client consultation with a GP practice nurse (£34) this gives an average cost of £3.40 per call. A factor of 1.44 was applied to in hours GP utilization to give the estimated out of hours cost. This is based on the mean cost per hour for out of hours GP services being £82.03 (Inflation adjusted average, (424) which is 44% more than the mean cost per hour for in hours GP services of £57.03 (425). For secondary care new and follow-up outpatient attendances, a weighted mean of the cost of all types of paediatric referrals for consultant led outpatient care was taken, giving an overall mean of £198.60 for a first appointment and £155.36 for a follow-up appointment. Similar methods were used for accident and emergency attendances, such that the weighted mean for an A&E attendance leading to admission was £129.11, and an A&E attendance without admission £94.93. This cost excludes the use of minor injury services and walk-in centres.

2.3.1.2 Prescription costs

Prescription costs were calculated from the online version of the British National Formulary for children 2011 (426). These reflect the cost of drugs issued and not the cost of administration associated with delivery to patients. The price of the pharmaceutical brand specified in the record was taken. If no brand was specified the cheapest form of that medication with the same mode of delivery was used. Where costs were itemized as a net price per measured unit of drug (for example per 100ml), the price was applied proportional to the amount prescribed. Where the drug was priced per set volume (for example per tube in the case of creams) then the number of tubes required to cover the volume prescribed

was calculated. The summation of the medication costs in each record was then subjected to deflation calculations using inflation indices (427). As no inflation indices for 2010/11 were available at the time of calculation, the indices to deflate 2010 to 2009 were used twice, thereby assuming a similar rate of inflation across that time. Deflating prices to 2008/09 brought them to the same base year as the healthcare utilization costs. The price of Anusol cream and Dentinox oral solution were not available within the BNFC 2010 and were priced from Boots pharmacy; each of these were prescribed once. Whether or not a child was prescribed drugs relevant to eczema (emollient creams and bath oils, topical corticosteroids with or without antibiotics or antiseptic and calcineurin inhibitors), asthma (inhaled corticosteroids, B2 agonists, leukotriene inhibitors and delivery devices) or food allergy (adrenaline autoinjectors and antihistamines) was separately recorded.

2.3.2 Accuracy of data analysis

Two assessors (RJB and MG) independently examined the first three e-HRs (84 consultations). The kappa score for their assessment of total healthcare utilisation costs was 0.972, showing excellent agreement. The remaining records were all analysed by MG.

2.3.3 Statistical analyses

A database was assembled using Microsoft Excel. Data were explored using histograms and the following became normally distributed after natural log transformation: Total healthcare utilization, total visit costs, total prescription costs, total primary care costs, total year 1 costs and overall respiratory costs. Levels of 25(OH)D at baseline in the mother, in cord blood and at age three years in the child were all normally distributed after natural log transformation. 0.1 was added to all zero values to allow natural log transformation. The following data remained non-parametrically distributed despite natural log transformation: Total secondary care costs, year 2 and 3 costs, respiratory costs, food allergy costs, eczema costs and

prescription costs for eczema and wheezing medications.

For primary analyses, bolus and daily vitamin D groups were compared with the control group. Secondary analysis included bolus and daily vitamin D groups combined (combined vitamin D) compared with the control group and the relationship between 25(OH)D levels measured in cord blood at delivery and in the child at age three years. Differences between groups were analysed using the Independent student t-test or Mann-Whitney U test according to whether the data were normally distributed or not. Analysis of variance was performed using potential covariates that have been associated with healthcare utilisation in the literature including mother's age, mother's age on leaving education, gestational age, ethnicity, parity, body mass index (BMI), smoking during pregnancy and mode of delivery (415). All analyses were performed using SPSS version 19.0 (IBM, Chicago USA). A value of $p<0.05$ was considered statistically significant.

3 Assessment of lung function in 3 to 5 year old children attending a paediatric outpatient department using impulse oscillometry

3.1 Introduction

Objective measures of lung function in preschool children are important for evaluating the evolution of disease processes and response to interventions during this crucial developmental stage (49). However, practical evaluation in young children represents a major challenge (49). The ideal pulmonary function test should be applicable at any age, simple to perform, safe, reproducible, and sensitive and specific enough to detect changes with growth and distinguish clearly between health and disease (49). Impulse oscillometry is one technique that simply requires a child to breathe tidally through a mouthpiece for up to 30 seconds whilst measurements are taken (49). The technique superimposes a small external pressure sine wave of a known frequency to the respiratory system, and the resulting pressure flow relationship is measured (52). The ratio of the amplitude of the pressure wave, to the amplitude of the resulting flow wave, gives the respiratory impedance (Zrs), which represents the overall impediment to flow within the lung (49). Impedance is the complex sum of lung resistance (Rrs) and reactance (Xrs), where Rrs represents frictional losses in the proximal and distal airways and lung parenchyma, of which airway resistance (Raw) is the most significant (49), and Xrs represents elastic and inertive properties within the lung.

Although IOS reference ranges for young children have being published (49, 52, 60), there is limited data on the success rate, reproducibility and ability to distinguish between health and disease in children aged four years or less. Reported success rate for IOS in 4-year-old children range from 21% to 82% (56, 65) in the research setting. The CV for resistance ranges from 6.0% to 11% (61), however, the majority of children included in these studies

were aged over 4 years. There is evidence that bronchodilator responses measured by IOS are greater in children with preschool wheezing compared to controls (59, 68-70, 72). Differences in IOS response have been found in preschool children with wheezing enrolled in prospective randomised controlled trials of pharmacotherapy (50, 73).

The aims of this study were to determine the success rate of IOS for measuring lung function in 3 to 5 year old children, the repeatability (CV) in this age group and the ability of IOS to discriminate between children with and without a history of wheezing and/or atopy.

3.2 Methods

Participants were recruited from a hospital based paediatric outpatient clinic, where they completed a questionnaire and had IOS assessment. Full methods are described in section 2.1.

3.3 Results

3.3.1 Subjects

66 children aged three to five years participated in the study. 64 were recruited from St Mary's paediatric outpatient department and two from the community. Flow of children in the study is shown in Figure 3-1, and the demographics of children who provided acceptable IOS data, with and without a history of wheeze are shown in Table 3.1.

Figure 3-1 Children in the pilot study

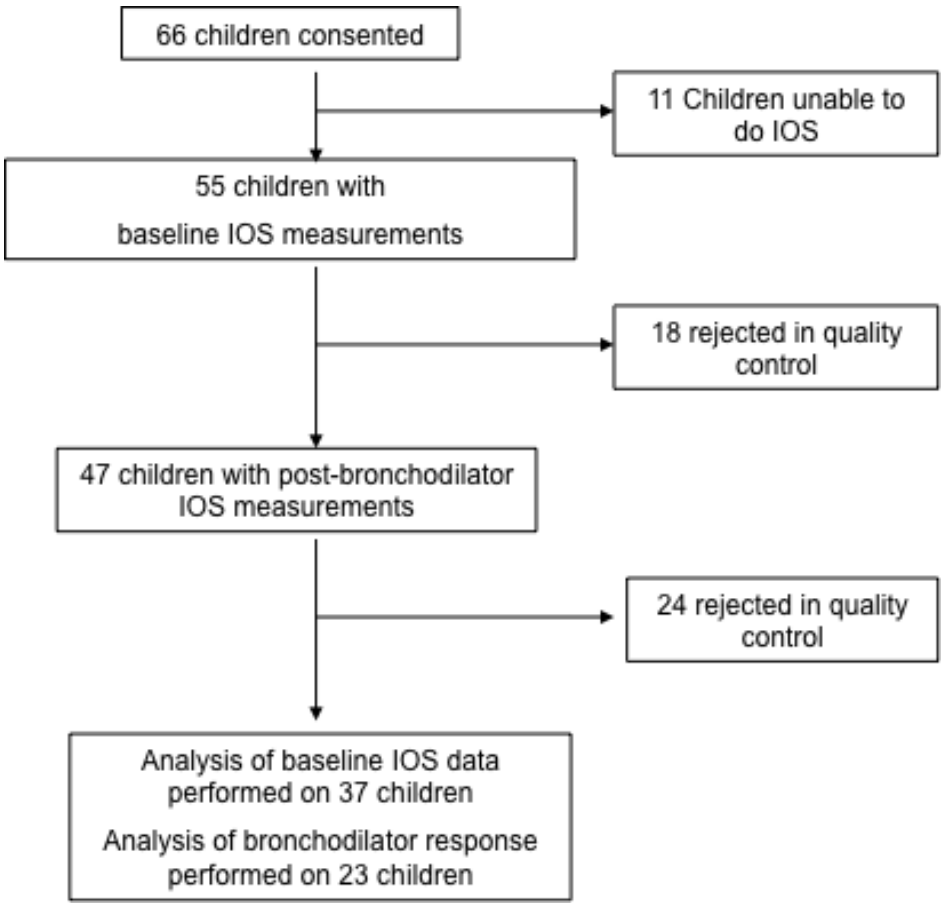


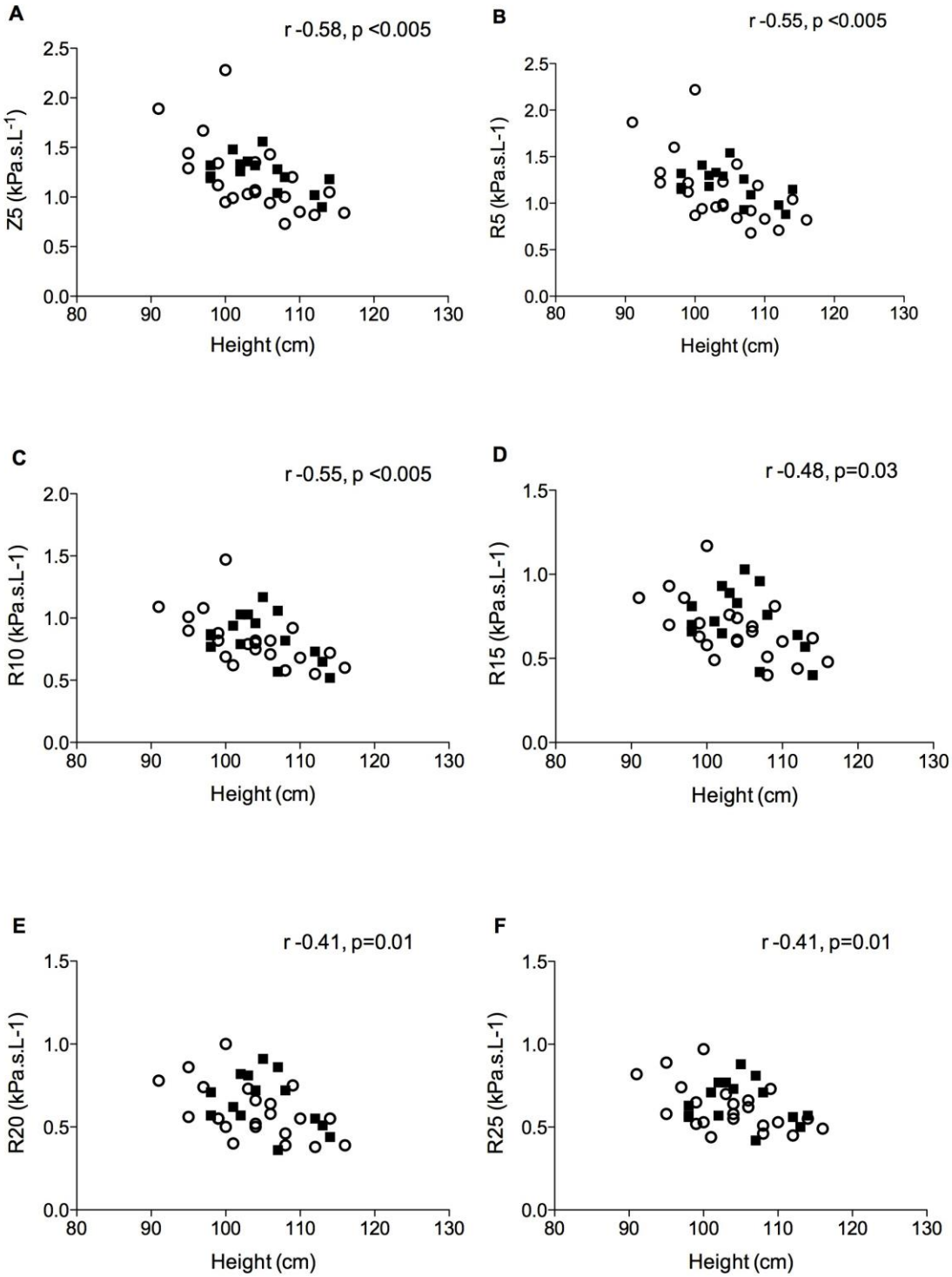
Table 3.1 Children with and without a history of wheezing

	No wheeze	Wheeze
Male sex, n (%)	11/22 (50.0)	8/15 (53.3)
Birth weight (g), mean (SD)	3458 (784)	3569 (527)
Ethnicity, n (%)		
Asian	5 (22.7)	5 (33.3)
Middle Eastern	3 (13.6)	1 (6.7)
Black	3 (13.6)	4 (26.7)
Other	1 (4.5)	2 (13.3)
White	10 (45.5)	3 (20.0)
Age at time of assessment (months), mean (SD)	49 (7)	50 (6)
Height (cm), mean (SD)	104 (7)	104 (5)
Maternal smoking during pregnancy, n (%)	0/22 (0)	1/15 (6.7)
Presence of household smokers, n (%)	3/22 (13.6)	2/15 (13.3)
>4 URTI/ year, n (%)	3/22 (13.6)	4/15 (26.7)
Has your child ever had asthma? N (%)	0/22 (0.0)	7/15 (46.7)
Has your child ever had hayfever? N (%)	4/22 (18.2)	4/15 (26.7)
Has your child ever had eczema? N (%)	11/22 (50.0)	8/15 (53.3)
Doctor diagnosed food allergy? N (%)	6/22 (27.3)	8/15 (53.3)
Atopy, n (%)	7/22 (31.8)	7/15 (46.7)
Acceptable Pre IOS, n (%)	22/22 (100)	15/15 (100)
Acceptable post IOS, n (%)	14/22 (63.6)	9/15 (60.0)

URTI (Upper respiratory tract infection), IOS (Impulse oscillometry).

When baseline measurements from all children were combined for analysis, Z5, R5, R10, R15, R20, R25, Fres and AX were all significantly inversely correlated with height (Figure 3-2). The relationship between X5 and height did not reach significance.

Figure 3-2 Baseline IOS parameters and height



Correlation of A) Impedance at 5Hz (Z5), B) Resistance at 5Hz (R5), C) R10, D) R15, E) R20 and F) R25 at baseline with height in children with (black squares) and without (open circles) a history of wheezing. Pearson's correlation coefficient (r).

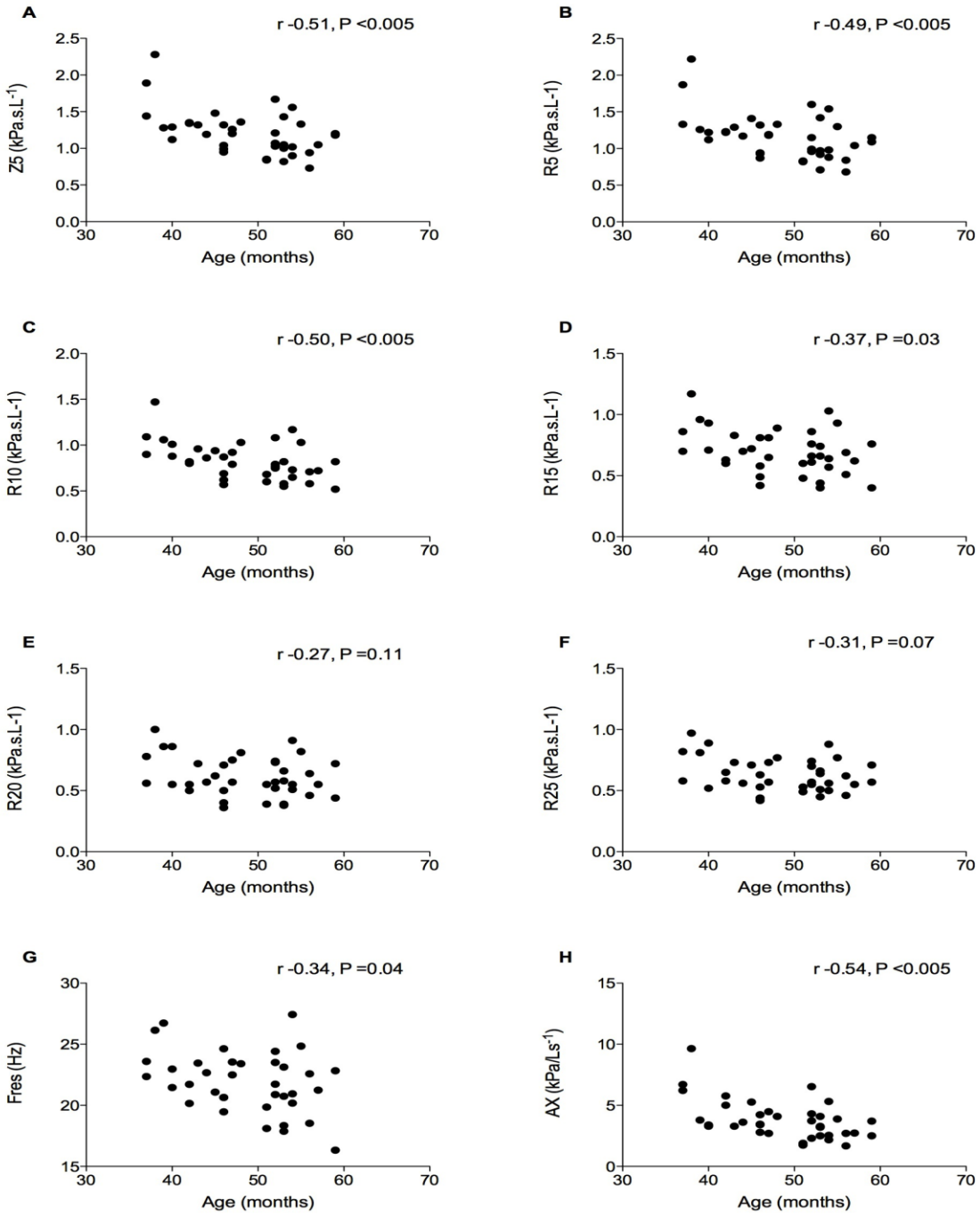
When baseline measurements from all children were combined for analysis, Z5, R5, R10, R15, Fres and AX were all significantly inversely correlated with age in months (Table 3.2 and Figure 3-3).

Table 3.2 Baseline IOS parameters (pilot study) with age in months

	Pearson correlation	p
Z5	-0.51	0.00
R5	-0.49	0.00
R10	-0.50	0.00
R15	-0.37	0.03
R20	-0.27	0.11
R25	-0.31	0.07
X5	0.13	0.45
Fres	-0.34	0.04
AX	-0.54	0.00

Z5 (Impedance at 5Hz), R5 to R25 (resistance at 5 to 25Hz), X5 (Reactance at 5Hz), Fres (Resonant frequency) and AX (Area under the reactance curve).

Figure 3-3 Baseline IOS with age in months



Correlation of A) Impedance at 5Hz (Z5), B) Resistance at 5Hz (R5), C) R10, D) R15, E) R20 and F) R25 at baseline with age in months. Pearson's correlation coefficient (r).

When baseline measurements from all children were combined for analysis, no significant differences in any IOS parameter was found between boys and girls (Table 3.3).

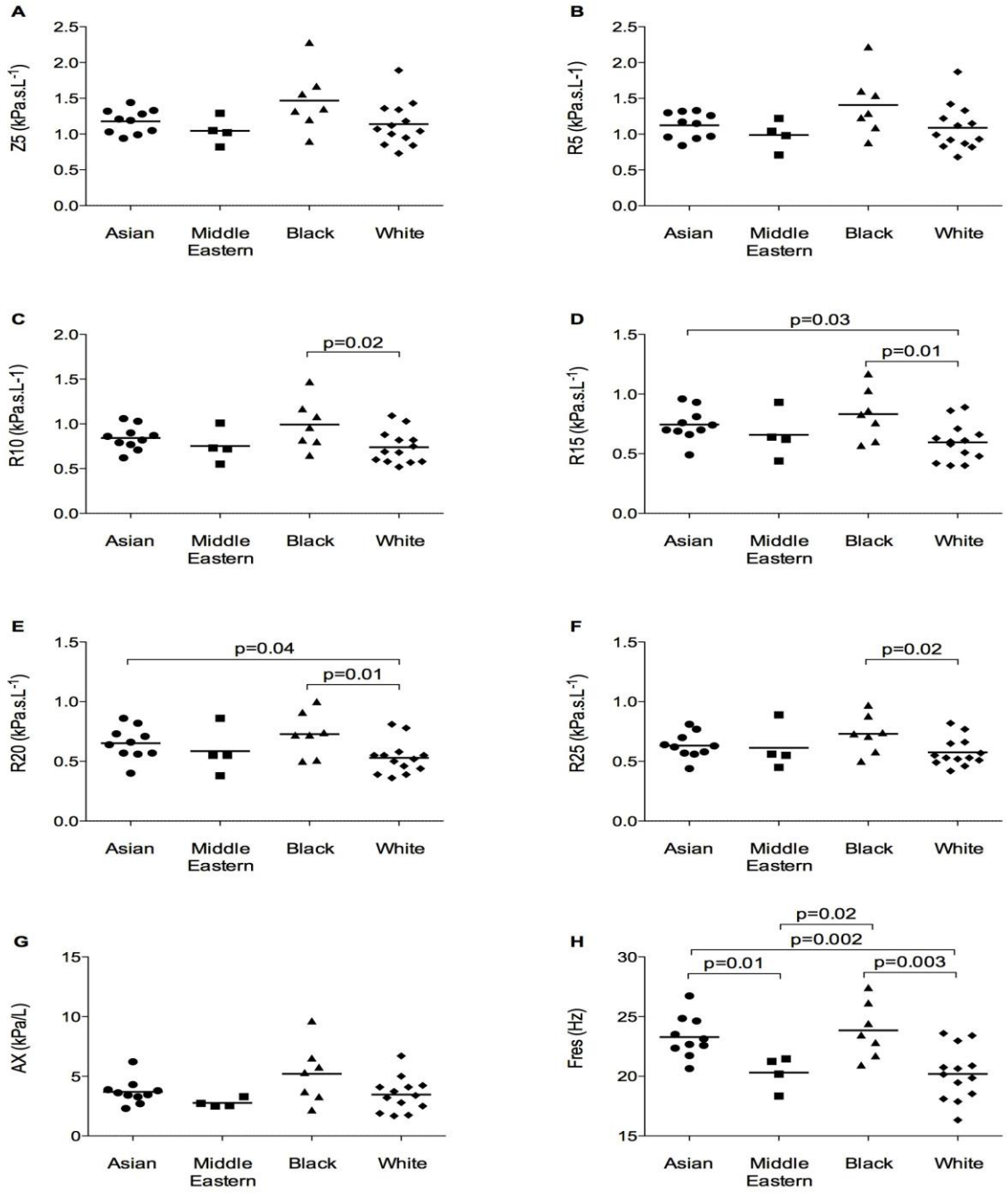
Table 3.3 Baseline IOS parameters (pilot study) in boys vs girls

IOS Parameter	n	Males Mean ± SD	n	Females Mean ± SD	Mean difference (95% CI)	p
Z5	19	1.26 ± 0.35	18	1.17 ± 0.26	0.09 (-0.12, 0.29)	0.41
R5	19	1.21 ± 0.35	18	1.11 ± 0.26	0.09 (-0.12, 0.30)	0.37
R10	19	0.83 ± 0.22	18	0.83 ± 0.18	0.00 (-0.13, 0.14)	0.96
R15	19	0.69 ± 0.19	18	0.71 ± 0.18	-0.02 (-0.14, 0.11)	0.80
R20	19	0.61 ± 0.16	18	0.62 ± 0.17	-0.02 (-0.13, 0.09)	0.77
R25	19	0.63 ± 0.14	18	0.63 ± 0.13	-0.01 (-0.10, 0.09)	0.90
RF	19	21.85 ± 2.26	18	21.97 ± 2.76	-0.11 (-1.79, 1.57)	0.89
AX	19	4.02 ± 1.82	18	3.64 ± 1.41	0.38 (-0.71, 1.47)	0.49

Z5 (Impedance at 5Hz), R5 to R25 (resistance at 5 to 25Hz), X5 (Reactance at 5Hz), Fres (Resonant frequency) and AX (Area under the reactance curve).

When baseline measurements from all children were combined for analysis, significant differences were found between children of different ethnic groups as follows: White and Black children for R10, R15, R20, R25 and Fres; White and Asian children for R15, R20 and Fres; Asian and Middle Eastern children for Fres; Black and Middle Eastern children for Fres (Figure 3-4).

Figure 3-4 Baseline IOS parameters (pilot study) by different ethnic groups



Baseline IOS in children of different ethnic origins. A) Impedance at 5Hz (Z5), B) Resistance at 5Hz (R5), C) R10, D) R15, E) R20, F) R25, G) AX and H) Fres.

3.3.2 Success rate of IOS in preschool children

Acceptable baseline IOS data was acquired for 37/66 (56%) of participating children. Bronchodilator response was measured in 23/66 (35%). Boys and girls were equally able to perform IOS, with 18/31 females (58.1%) and 19/35 males (54.3%) providing baseline data of acceptable quality. Success rates were 18/36 (50%) for three year olds and 19/30 (63%) for 4 year olds. Using a cut off of 42 months, success rates were higher for older children with 8/21 (38.1%) successful aged ≤ 42 months and 29/45 (64.4%) successful >42 months (Pearson Chi Squared, p=0.045). However, there was no difference in median age in months between children who were and were not successful in providing acceptable quality data (Mann-Whitney U Test, p=0.19)

3.3.3 Repeatability of measurements

CV was acceptable for most parameters, but over 20% for X5 and AX (Table 3.4). There were no significant differences between wheezers and non-wheezers for CV (Table 3.5).

Table 3.4 Coefficient of variation for impulse oscillometry measurements

Parameter	Baseline Median (IQR) (%)	Post bronchodilator Median (IQR) (%)
Z5	8.84 (5.81, 10.86)	7.60 (5.44, 9.17)
R5	8.50 (6.21, 11.82)	8.38 (6.36, 9.70)
R10	6.93 (4.31, 8.44)	6.45 (4.52, 8.84)
R15	7.31 (4.27, 9.93)	6.82 (5.67, 9.82)
R20	7.92 (5.56, 10.11)	7.54 (5.25, 11.59)
R25	6.24 (4.97, 10.63)	6.46 (4.32, 11.30)
X5	20.59 (64.27, 8.83)	14.47 (32.33, 9.62)
Fres	4.31 (2.59, 6.21)	3.50 (1.78, 6.09)
AX	12.90 (8.04, 19.69)	12.10 (8.33, 19.56)

Z5 (Impedance at 5Hz), R5 to R25 (resistance at 5 to 25Hz), X5 (Reactance at 5Hz), Fres (Resonant frequency) and AX (Area under the reactance curve).

Table 3.5 Coefficient of variation for impulse oscillometry in children with and without wheezing

Parameter	Non-wheezers Median (IQR) (%)	Wheezers Median (IQR) (%)	p
Z5	8.88 (5.53, 11.89)	8.34 (6.22, 10.60)	0.64
R5	9.77 (6.21, 13.18)	8.16 (5.39, 11.42)	0.35
R10	6.81(4.31, 8.44)	6.93 (3.63, 11.20)	0.80
R15	8.33 (4.08, 9.93)	7.22 (4.92, 11.24)	0.70
R20	9.03 (4.08, 9.62)	7.60 (5.61, 10.35)	0.92
R25	6.09 (4.06, 9.62)	7.76 (5.33, 11.02)	0.30
X5	16 (8.58, 64.27)	31.13 (15.68, 77.89)	0.45
Fres	4.99 (2.59, 6.34)	3.21 (2.55, 5.40)	0.29
AX	15.45 (9.55, 23.92)	11.13 (6.61, 19.69)	0.30

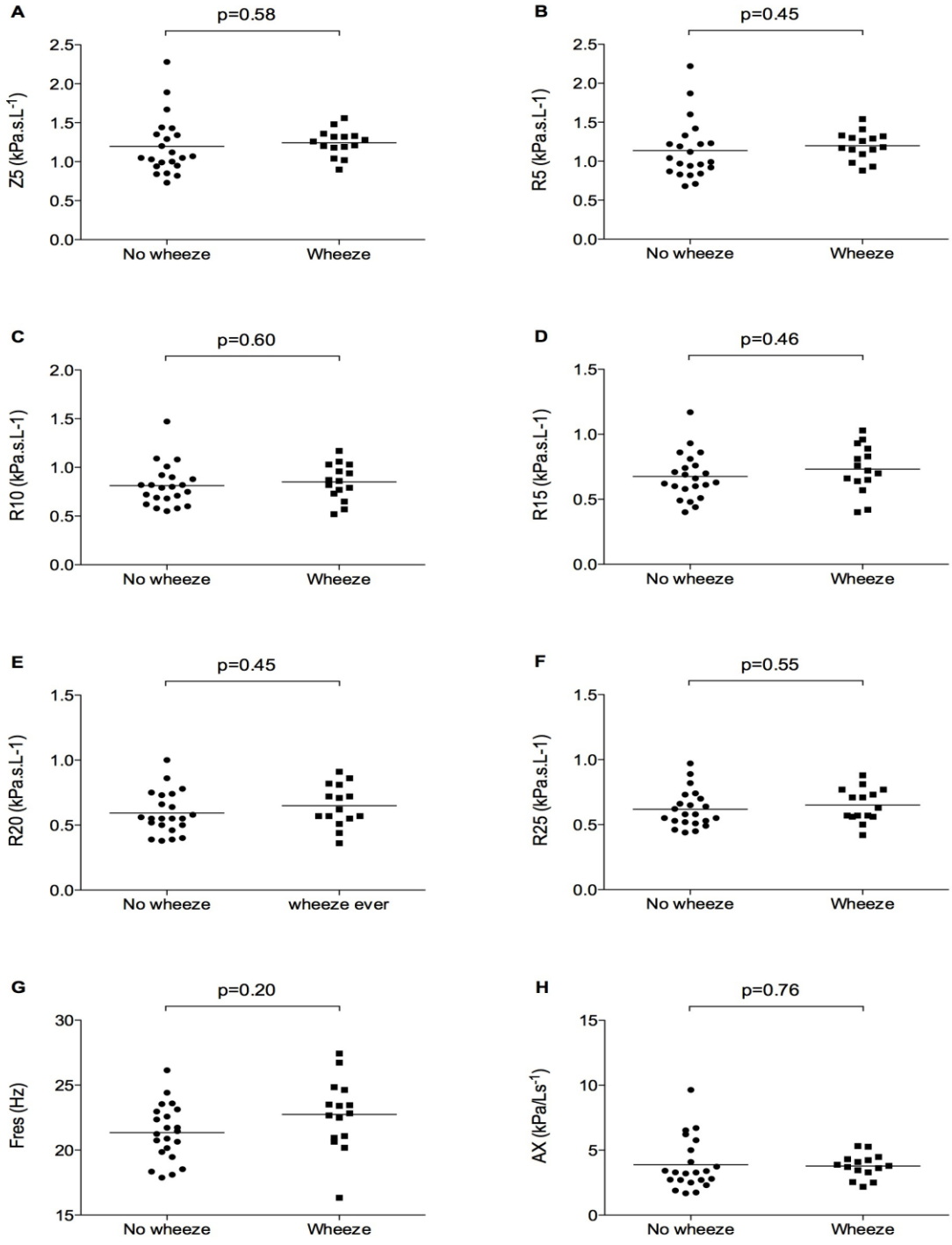
Z5 (Impedance at 5Hz), R5 to R25 (resistance at 5 to 25Hz), X5 (Reactance at 5Hz), Fres (Resonant frequency) and AX (Area under the reactance curve).

3.3.4 Ability to discriminate between groups

3.3.4.1 Differences in baseline lung function

Data for baseline IOS parameters in children with and without history of wheeze are presented in Figure 3-5 and Table 3.6, and for atopic sensitisation, atopic wheeze, any ETS exposure and frequent URTI in Tables 3.7 to 3.10. Children with wheezing had significantly higher baseline Fres than non-wheezers in adjusted analyses only. There were no differences in baseline lung function between groups for atopy, atopic wheezing, any ETS exposure or history of frequent URTI.

Figure 3-5 Baseline lung function in children with and without a history of wheezing



Baseline A) Impedance at 5Hz (Z5), B) Resistance at 5Hz (R5), C) R10, D) R15, E) R20, F) R25, G) Resonant frequency (Fres) and H) Area under the reactance curve (AX) in children with (black squares) and without (black circles) a history of wheezing. Horizontal bars represent means and p values are for adjusted analyses.

Table 3.6 Baseline lung function in preschool children with and without a history of wheeze

IOS Parameter	n	No wheeze Mean ± SD	n	Wheeze Mean ± SD	Mean difference (95% CI)	p	n	[1]Adjusted mean difference (95% CI)	p
Z5 (kPa.s.L)	22	1.20 ± 0.38	15	1.24 ± 0.17	0.05 (-0.16, 0.26)	0.66	37	0.05 (-0.12, 0.22)	0.58
R5 (kPa/Ls^{-1})	22	1.13 ± 0.38	15	1.20 ± 0.18	0.07 (-0.15, 0.28)	0.53	37	0.07 (-0.11, 0.25)	0.45
R10 (kPa/Ls^{-1})	22	0.81 ± 0.21	15	0.85 ± 0.19	0.04 (-0.10, 0.18)	0.58	37	0.03 (-0.09, 0.15)	0.60
R15 (kPa/Ls^{-1})	22	0.67 ± 0.18	15	0.73 ± 0.18	0.06 (-0.07, 0.18)	0.35	37	0.04 (-0.07, 0.15)	0.46
R20 (kPa/Ls^{-1})	22	0.59 ± 0.16	15	0.65 ± 0.16	0.06 (-0.05, 0.17)	0.30	37	0.04 (-0.07, 0.15)	0.45
R25 (kPa/Ls^{-1})	22	0.62 ± 0.14	15	0.65 ± 0.13	0.03 (-0.06, 0.13)	0.48	37	0.03 (-0.07, 0.12)	0.55
Fres (Hz)	22	21.34 ± 2.17	15	22.74 ± 2.75	1.41 (-0.24, 3.05)	0.09	37	0.87 (-0.50, 2.24)	0.20
AX (kPa/L)	22	3.88 ± 1.99	15	3.78 ± 0.92	-0.10 (-1.22, 1.02)	0.86	37	-0.13 (-0.99, 0.73)	0.76

Z5 (Impedance at 5Hz), R5 to R25 (Resistance at 5-25Hz), Fres (Resonant frequency), AX (Area under the reactance curve). [1]Adjusted for age, height, sex and ethnicity.

Table 3.7 Baseline lung function in preschool children with and without atopic skin sensitisation

IOS Parameter	n	Non-atopic Mean ± SD	n	Atopic Mean ± SD	Mean difference (95% CI)	p	n	[1]Adjusted mean difference (95% CI)	p
Z5 (kPa.s.L)	23	1.25 ± 0.33	14	1.15 ± 0.25	-0.10 (-0.31, 0.11)	0.34	37	-0.01 (-0.18, 0.17)	0.96
R5 (kPa/Ls^{-1})	23	1.20 ± 0.34	14	1.10 ± 0.26	-0.10 (-0.32, 0.11)	0.33	37	-0.01 (-0.20, 0.18)	0.90
R10 (kPa/Ls^{-1})	23	0.83 ± 0.22	14	0.82 ± 0.18	-0.01 (-0.15, 0.13)	0.88	37	0.06 (-0.06, 0.18)	0.30
R15 (kPa/Ls^{-1})	23	0.69 ± 0.19	14	0.71 ± 0.16	0.02 (-0.11, 0.15)	0.75	37	0.06 (-0.06, 0.178)	0.29
R20 (kPa/Ls^{-1})	23	0.61 ± 0.17	14	0.63 ± 0.15	0.03 (-0.09, 0.14)	0.65	37	0.05 (-0.06, 0.17)	0.34
R25 (kPa/Ls^{-1})	23	0.63 ± 0.15	14	0.63 ± 0.12	-0.00 (-0.10, 0.09)	0.95	37	0.03 (-0.07, 0.13)	0.51
Fres (Hz)	23	21.61 ± 2.68	14	22.40 ± 2.11	0.80 (-0.91, 2.51)	0.35	37	1.01 (-0.43, 2.44)	0.16
AX (kPa/L)	23	3.97 ± 1.81	14	3.62 ± 1.29	-0.35 (-1.47, 0.78)	0.54	37	0.25 (-0.65, 1.16)	0.57

Z5 (Impedance at 5Hz), R5 to R25 (Resistance at 5-25Hz), Fres (Resonant frequency), AX (Area under the reactance curve). [1]Adjusted for age, height and sex and ethnicity.

Table 3.8 Baseline lung function in preschool children with and without atopic wheeze

IOS Parameter	n	Non atopic wheeze Mean ± SD	n	Atopic wheeze Mean ± SD	Mean difference (95% CI)	p	n	[1]Adjusted mean difference (95% CI)	p
Z5 (kPa.s.L)	30	1.21 ± 0.33	7	1.23 ±0.20	0.02 (-0.25, 0.28)	0.90	37	0.05 (-0.15, 0.25)	0.62
R5 (kPa/Ls^{-1})	30	1.15 ± 0.33	7	1.18 ± 0.20	0.03 (-0.24, 0.30)	0.84	37	0.06 (-0.16, 0.27)	0.59
R10 (kPa/Ls^{-1})	30	0.82 ± 0.20	7	0.87 ± 0.20	0.05 (-0.13, 0.22)	0.60	37	0.06 (-0.08, 0.20)	0.38
R15 (kPa/Ls^{-1})	30	0.69 ± 0.18	7	0.74 ± 0.20	0.05 (-0.11, 0.20)	0.54	37	0.05 (-0.08, 0.19)	0.42
R20 (kPa/Ls^{-1})	30	0.61 ± 0.16	7	0.65 ± 0.19	0.04 (-0.10, 0.18)	0.54	37	0.05 (-0.08, 0.18)	0.45
R25 (kPa/Ls^{-1})	30	0.63 ± 0.13	7	0.65 ± 0.15	0.02 (-0.09, 0.14)	0.69	37	0.04 (-0.07, 0.15)	0.50
Fres (Hz)	30	21.65 ± 2.49	7	23.02 ± 2.28	1.37(-0.72, 3.46)	0.19	37	1.11 (-0.53, 2.75)	0.18
AX (kPa/L)	30	3.81 ± 1.76	7	3.97 ± 0.93	0.16 (-1.25, 1.56)	0.82	37	0.32 (-0.71, 1.35)	0.53

Z5 (Impedance at 5Hz), R5 to R25 (Resistance at 5-25Hz), Fres (Resonant frequency), AX (Area under the reactance curve). [1]Adjusted for height and age, sex and ethnicity

Table 3.9 Baseline lung function in preschool children with and without any ETS exposure

IOS Parameter	n	No ETS Mean ± SD	n	Any ETS Mean ± SD	Mean difference (95% CI)	p	n	[1]Adjusted mean difference (95% CI)	p
Z5 (kPa.s.L)	32	1.24 ± 0.31	5	1.04 ± 0.25	-0.20 (-0.49, 0.10)	0.88	37	-0.07 (-0.32, 0.18)	0.59
R5 (kPa/Ls^{-1})	32	1.19 ± 0.31	5	1.00 ± 0.25	-0.19 (-0.49, 0.12)	0.18	37	-0.05 (-0.32, 0.21)	0.68
R10 (kPa/Ls^{-1})	32	0.83 ± 0.20	5	0.80 ± 0.22	-0.04 (-0.24, 0.16)	0.22	37	0.02 (-0.15, 0.20)	0.78
R15 (kPa/Ls^{-1})	32	0.70 ± 0.18	5	0.68 ± 0.21	-0.02 (-0.19, 0.16)	0.86	37	0.02 (-0.15, 0.19)	0.80
R20 (kPa/Ls^{-1})	32	0.62 ± 0.16	5	0.61 ± 0.20	-0.01 (-0.17, 0.15)	0.87	37	0.02 (-0.14, 0.19)	0.80
R25 (kPa/Ls^{-1})	32	0.64 ± 0.13	5	0.60 ± 0.16	-0.03 (-0.17, 0.10)	0.60	37	-0.00 (-0.14, 0.14)	0.98
Fres (Hz)	32	21.90 ± 2.35	5	21.96 ± 3.56	0.06 (-2.40, 2.52)	0.96	37	0.55 (-1.55, 2.65)	0.60
AX (kPa/L)	32	3.99 ± 1.67	5	2.86 ± 0.87	-1.13 (-2.69, 0.43)	0.15	37	-0.44 (-1.72, 0.84)	0.49

ETS (Pre or postnatal environmental tobacco smoke exposure), Z5 (Impedance at 5Hz),

R5 to R25 (Resistance at 5-25Hz), Fres (Resonant frequency), AX (Area under the reactance curve).

[1]Adjusted for height, age, sex and ethnicity.

Table 3.10 Baseline lung function in preschool children with and without frequent URTI

IOS Parameter	n	≤4 URTI/yr Mean ± SD	n	>4 URTI/yr Mean ± SD	Mean difference (95% CI)	p	n	[1]Adjusted mean difference (95% CI)	p
Z5 (kPa.s.L)	30	1.18 ±0.27	7	1.38 ± 0.42	0.21 (-0.05, 0.46)	0.11	37	0.05 (-0.17, 0.27)	0.67
R5 (kPa/Ls^{-1})	30	1.12 ± 0.28	7	1.32 ± 0.42	0.19 (-0.07, 0.45)	0.14	37	0.04 (-0.20, 0.27)	0.75
R10 (kPa/Ls^{-1})	30	0.80 ± 0.18	7	0.95 ± 0.24	0.15 (-0.02, 0.32)	0.07	37	0.03 (-0.12, 0.18)	0.67
R15 (kPa/Ls^{-1})	30	0.67 ± 0.17	7	0.81 ± 0.18	0.14 (-0.004, 0.29)	0.06	37	0.03 (-0.12, 0.18)	0.65
R20 (kPa/Ls^{-1})	30	0.59 ± 0.15	7	0.73 ± 0.16	0.14 (0.004, 0.27)	0.04	37	0.05 (-0.09, 0.19)	0.49
R25 (kPa/Ls^{-1})	30	0.61 ± 0.12	7	0.73 ± 0.15	0.12 (0.01, 0.23)	0.03	37	0.06 (-0.06, 0.18)	0.30
Fres (Hz)	30	21.71 ± 2.61	7	22.77 ± 1.71	1.06 (-1.05, 3.18)	0.31	37	-0.83 (-2.64, 0.98)	0.36
AX (kPa/L)	30	3.66 ± 1.38	7	4.59 ± 2.40	0.93 (-0.43, 2.30)	0.18	37	0.07 (-1.06, 1.19)	0.91

URTI (Upper respiratory tract infection), Yr (Year), Z5 (Impedance at 5Hz),

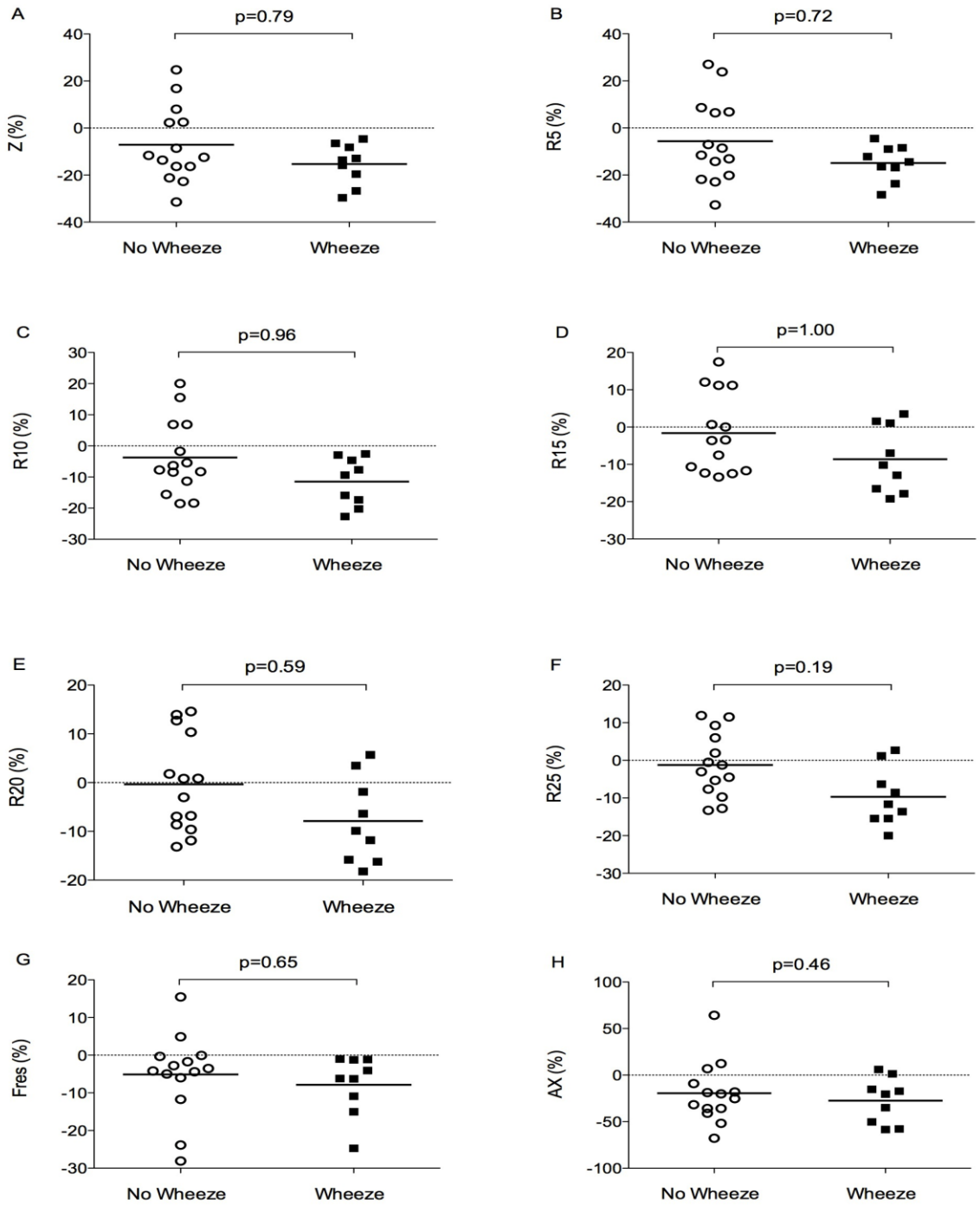
R5 to R25 (Resistance at 5-25Hz), Fres (Resonant frequency), AX (Area under the reactance curve).

[1]Adjusted for height, age, sex and ethnicity.

3.3.4.2 Bronchodilator response

The response to administration of 400mcg of salbutamol in children with and without a history of wheeze is shown in Figure 3-6 and Table 3.11. Results for atopy, atopic wheezing, any ETS exposure and frequent URTI are given in Tables 3.12 to 3.15. In adjusted and unadjusted analyses, children with a history of wheezing had significantly greater bronchodilator response in R25 compared to children with no history of wheezing. There were no significant differences in bronchodilator responses when data was dichotomized by atopic status, atopic wheezing, any ETS exposure or frequent URTI.

Figure 3-6 Bronchodilator responses in children with and without a history of wheezing



Percentage change in lung function measured before and 15 minutes after inhalation of 400mcg salbutamol for A) Impedance at 5Hz (Z5), B) Resistance at 5Hz (R5), C) R10, D) R15, E) R20, F) R25, G) Resonant frequency (Fres) and H) Area under the reactance curve (AX) in children with (black squares) and without (open circles) a history of wheezing. Horizontal lines represent means and p values are for adjusted analyses.

Table 3.11 Bronchodilator response in children with and without a history of wheezing

IOS Parameter	n	No wheeze Mean ± SD	n	Wheeze Mean ± SD	Mean difference (95% CI)	p	n	[1]Adjusted mean difference (95% CI)	p
Z5 %Init	14	-7.13 ± 15.92	9	-15.28 ± 8.69	-8.15 (-20.26, 3.95)	0.18	23	2.47 (-16.79, 21.71)	0.79
R5 %Init	14	-5.66 ± 17.80	9	-14.87 ± 7.56	-9.21 (-22.33, 3.91)	0.16	23	3.53 (-17.17, 24.23)	0.72
R10 %Init	14	-3.74 ± 11.97	9	-11.48 ± 7.69	-7.74 (-17.10, 1.63)	0.10	23	0.37 (-13.98, 14.71)	0.96
R15 %Init	14	-1.61 ± 10.68	9	-8.61 ± 8.87	-7.00 (-15.91, 1.91)	0.12	23	0.00 (-13.95, 13.96)	1.00
R20 %Init	14	-0.36 ± 9.86	9	-7.89 ± 8.73	-7.53 (-15.93, 0.86)	0.08	23	-3.50 (-17.26, 10.25)	0.59
R25 %Init	14	-1.24 ± 8.46	9	-9.67 ± 7.71	-8.43 (-15.70, -1.16)	0.03	23	-7.19 (-18.37, -4.00)	0.19
Fres %Init	14	-5.09 ± 10.81	9	-7.84 ± 7.91	-2.75 (-11.46, 5.97)	0.52	23	3.20 (-11.62, 18.01)	0.65
AX %Init	14	-19.39 ± 32.08	9	-27.45 ± 24.26	-8.06 (-34.13, 18.02)	0.53	23	15.69 (-28.77, 60.16)	0.46

Z5 (Impedance at 5Hz), R5 to R25 (Resistance at 5-25Hz), Fres (Resonant frequency),

AX (Area under the reactance curve), %Init (percentage change in parameter 15 minutes after 400 mcg inhaled salbutamol).

[1]Adjusted for height, age, sex and ethnicity.

Table 3.12 Bronchodilator response in children with and without a history of atopy

IOS Parameter	n	Non atopic Mean ± SD	n	Atopic Mean ± SD	Mean difference (95% CI)	p	n	[1]Adjusted mean difference (95% CI)	p
Z5 %Init	17	-9.02 ± 14.77	6	-13.99 ± 11.49	-4.97 (-18.86, 8.91)	0.47	23	-4.86 (-21.78, 12.07)	0.55
R5 %Init	17	-7.69 ± 15.95	6	-13.74 ± 12.73	-6.05 (-21.10, 9.01)	0.41	23	-3.38 (-21.78, 15.01)	0.70
R10 %Init	17	-6.48 ± 11.72	6	-7.60 ± 9.59	-1.12 (-12.23, 9.99)	0.84	23	1.35 (-11.38, 14.07)	0.82
R15 %Init	17	-4.76 ± 10.81	6	-3.18 ± 10.01	1.58 (-8.92, 12.07)	0.76	23	4.61, (-7.50, 16.73)	0.43
R20 %Init	17	-3.80 ± 9.93	6	-1.91 ± 10.86	1.90 (-8.14, 11.93)	0.70	23	3.29 (-8.92, 15.50)	0.57
R25 %Init	17	-4.66 ± 8.82	6	-4.20 ± 10.49	0.47 (-8.66, 9.59)	0.92	23	0.63 (-9.96, 11.21)	0.90
Fres %Init	17	-5.12 ± 10.06	6	-9.14 ± 8.60	-4.02 (-13.63, 5.59)	0.39	23	0.98 (-14.23, 12.28)	0.88
AX %Init	17	-18.65 ± 29.36	6	-33.58 ± 27.06	-14.92 (-43.39, 13.55)	0.29	23	-15.71 (-55.01, 23.59)	0.41

Z5 (Impedance at 5Hz), R5 to R25 (Resistance at 5-25Hz), Fres (Resonant frequency),

AX (Area under the reactance curve), %Init (percentage change in parameter 15 minutes after 400 mcg inhaled salbutamol).

[1]Adjusted for height, age, sex and ethnicity.

Table 3.13 Bronchodilator response in children with and without a history of atopic wheezing

IOS Parameter	n	No atopy or wheeze Mean ± SD	n	Atopic wheeze Mean ± SD	Mean difference (95% CI)	p	n	[1]Adjusted mean difference (95% CI)	p
Z5 %Init	19	-9.61 ± 15.12	4	-13.71 ± 5.50	4.10 (-12.09, 20.29)	0.60	23	-2.11 (-21.27, 17.05)	0.82
R5 %Init	19	-8.26 ± 16.48	4	-14.03 ± 3.87	5.77 (-11.76, 23.30)	0.50	23	-1.49 (-22.17, 19.20)	0.88
R10 %Init	19	-6.41 ± 11.83	4	-8.49 ± 6.54	2.07 (-10.77, 14.92)	0.74	23	0.46 (-13.81, 14.74)	0.95
R15 %Init	19	-4.32 ± 11.00	4	-4.49 ± 8.25	0.18 (-12.00, 12.36)	0.98	23	1.82 (-12.03, 15.66)	0.78
R20 %Init	19	-2.79 ± 10.27	4	-5.75 ± 9.24	2.96 (-8.63, 14.55)	0.60	23	-3.59 (-17.27, 10.09)	0.58
R25 %Init	19	-3.73 ± 9.10	4	-8.42 ± 8.77	4.69 (-5.67, 15.05)	0.36	23	-5.83 (-17.21, 5.55)	0.29
Fres %Init	19	-6.07 ± 10.42	4	-6.64 ± 5.95	0.58 (-10.75, 11.91)	0.92	23	1.60 (-13.23, 16.42)	0.82
AX %Init	19	-21.93 ± 29.97	4	-25.48 ± 27.27	3.55 (-30.31, 37.42)	0.83	23	1.38 (-43.76, 46.51)	0.95

Z5 (Impedance at 5Hz), R5 to R25 (Resistance at 5-25Hz), Fres (Resonant frequency), AX (Area under the reactance curve), %Init (percentage change in parameter 15 minutes after 400 mcg inhaled salbutamol).

[1]Adjusted for height, age, sex and ethnicity.

Table 3.14 Bronchodilator response in children with and without a history of any ETS exposure

IOS Parameter	n	No ETS exposure Mean ± SD	n	Any ETS exposure Mean ± SD	Mean difference (95% CI)	p	n	[1]Adjusted mean difference (95% CI)	p
Z5 %Init	20	-11.32 ± 14.59	3	-3.63 ± 5.49	7.69 (-10.31, 25.70)	0.38	23	6.75 (-11.87, 25.37)	0.45
R5 %Init	20	-10.40 ± 15.80	3	-1.72 ± 7.14	8.68 (-10.88, 28.24)	0.37	23	8.46 (-11.46, 28.38)	0.39
R10 %Init	20	-7.56 ± 11.35	3	-1.50 ± 7.74	6.07 (-8.17, 20.30)	0.39	23	6.00 (-7.72, 19.71)	0.36
R15 %Init	20	-5.24 ± 10.47	3	1.58 ± 9.36	6.82 (-6.54, 20.17)	0.30	23	7.00 (-6.15, 20.16)	0.27
R20 %Init	20	-4.47 ± 9.58	3	4.44 ± 10.83	8.91 (-3.59, 21.40)	0.15	23	9.68 (-2.85, 22.51)	0.12
R25 %Init	20	-5.71 ± 8.76	3	3.24 ± 8.01	8.95 (-2.24, 20.14)	0.11	23	10.38 (0.26, 20.58)	0.06
Fres %Init	20	-6.61 ± 10.32	3	-3.25 ± 1.84	3.36 (-9.30, 16.02)	0.59	23	4.37 (-10.13, 18.87)	0.53
AX %Init	20	-22.57 ± 31.04	3	-22.41 ± 8.15	0.16 (-38.00, 38.32)	0.99	23	-3.37 (-48.40, 40.93)	0.86

ETS (Pre or postnatal environmental tobacco smoke exposure), Z5 (Impedance at 5Hz),

R5 to R25 (Resistance at 5-25Hz), Fres (Resonant frequency), AX (Area under the reactance curve),

%Init (percentage change in parameter 15 minutes after 400 mcg inhaled salbutamol).

[1]Adjusted for height, age, sex and ethnicity.

Table 3.15 Bronchodilator response in children with and without a history of frequent URTI

IOS Parameter	n	≤4 URTI/yr Mean ± SD	n	>4 URTI/yr Mean ± SD	Mean difference (95% CI)	p	n	[1]Adjusted mean difference (95% CI)	p
Z5 %Init	18	-8.66 ± 14.64	5	-16.30 ± 9.85	-7.65 (-22.22, 6.92)	0.29	23	0.09 (-19.40, 19.57)	0.99
R5 %Init	18	-7.22 ± 16.02	5	-16.62 ± 9.18	-9.39 (-25.12, 6.33)	0.23	23	-0.10 (-21.11, 20.91)	0.99
R10 %Init	18	-6.00 ± 11.74	5	-9.56 ± 8.20	-3.57 (-15.29, 8.16)	0.53	23	4.18 (-10.10, 18.47)	0.54
R15 %Init	18	-3.95 ± 10.92	5	-5.76 ± 9.25	-1.81 (-12.97, 9.35)	0.74	23	6.41 (-7.19, 20.01)	0.33
R20 %Init	18	-3.27 ± 10.27	5	-3.42 ± 9.91	-0.15 (-10.87, 10.57)	0.98	23	7.06 (-6.38, 20.50)	0.28
R25 %Init	18	-4.95 ± 9.24	5	-3.06 ± 9.08	1.89 (-7.79, 11.57)	0.69	23	6.23 (-5.26, 17.72)	0.26
RF %Init	18	-5.76 ± 9.89	5	-7.62 ± 9.82	-1.85 (-12.23, 8.53)	0.71	23	-0.83 (-15.89, 14.24)	0.91
AX %Init	18	-20.45 ± 31.29	5	-30.11 ± 18.93	-9.66 (-40.50, 21.18)	0.52	23	2.65 (-43.14, 48.43)	0.90

URTI (Upper respiratory tract infection), Yr (year), Z5 (Impedance at 5Hz), R5 to R25 (Resistance at 5-25Hz), Fres (Resonant frequency), AX (Area under the reactance curve), %Init (percentage change in parameter 15 minutes after 400 mcg inhaled salbutamol).

[1]Adjusted for pre and postnatal environmental tobacco smoke exposure, height and age.

3.4 Discussion

We have found that in a convenience sample of children aged three and four years attending outpatient clinic, acceptable IOS data could be acquired in just over half. When acceptable quality readings are taken, the technique is repeatable with low CV for most measurements. We found significant differences in IOS parameters between children from different ethnic groups, particularly between White and Black children. No significant differences in lung function were found between children with and without a history of wheezing, atopy, environmental tobacco smoke exposure and frequent URTI.

Our success rates are not as high as Klug et al who reported reporting 65% and 82% for children aged 3 and 4 years respectively (56), but much greater than Guilbert et al who reported a 21% success rate for children aged 4 years (65). A number of factors could account for the difference. Klug et al used a specially designed facemask to minimise upper airway artefact. Our quality criteria are more stringent. If we had included all readings, regardless of quality, our success would have increased to 71%. Although IOS is described as a simple, non-invasive test, children get bored, stick their tongues in the mouthpiece, swallow, and chew and make an incomplete seal. All these manoeuvres lead to data corruption. Whilst these issues are identified in ATS/ERS guidelines on IOS, there are no uniformly agreed criteria for acceptance of an individual reading. It is possible that poor operator technique led to poor quality data. This is hard to quantify but in undertaking this study training was undertaken with an experienced IOS practitioner. The setting may have been important. Children were recruited from a hospital outpatient clinic and a high proportion were attending the asthma/ allergy clinic. This may bias towards children familiar with using mouthpieces and respiratory devices such as spacers leading to increased compliance compared to the general population, however we found no difference in the repeatability between children with and without a history of wheezing, suggesting this is not

the case. Alternatively, because children and families where attending outpatients they may have been tired or less cooperative than usual leading to reduced compliance compared to children attending for research purposes only (56).

Our CV for resistance compares favourably with other published studies (61). As found by others (58, 66), CV for X5 and AX was much higher, suggesting it may not be possible to reliably measure these two parameters in preschool IOS assessment.

Our finding of ethnic variation in impulse oscillometry measurements is of great interest. Whilst such differences have been described for spirometry, (428, 429), to our knowledge only one study has described such differences for impulse oscillomtery in preschool children by comparing reference ranges in Caucasians and Mexicans(430). However, this finding should be viewed with caution, as it is possible that confounding factors such socio-economic background, which may differ between ethnic groups in this convenience sample, could explain the difference.

In keeping with other studies (65, 67, 68, 70), we found no differences in baseline respiratory resistance between children with and without a history of wheezing. However unlike the majority of other studies, we found no differences respiratory resistance between children with and without a history of wheezing (59, 68-71) (Table 3.16).

Table 3.16 Bronchodilator responses for asthmatic/wheezy children vs controls

Study	R5	R10	R20	R25	R35	X5	Fres	AX
Nielsen **(59)**	✓	×	-	-	-	✓	-	-
Marotta **(68)**	✓	✓	-	-	-	×	×	-
Song **(69)**	✓	✓	✓	-	✓	×	×	-
Komarov **(70)**	✓	✓	×	-	-	✓	-	✓
Shin **(72)**	✓	×	-	-	-	✓	-	✓
This study	×	×	x	x	-	×	×	×

✓= significantly greater response in asthmatics/ wheezy children than controls;

× = no difference; - = not measured

The explanation for this finding is not clear. Perhaps the heterogeneity of the groups, including the variation of ethnicity, meant that true differences in lung function between children with wheeze and no wheeze, were masked. This study may have been underpowered to detect a significant difference.

4 Effect of prenatal vitamin D supplementation on parentally reported outcomes

4.1 Introduction

Several observational studies have suggested a protective effect of higher maternal vitamin D intake during pregnancy on risk of wheezing (175, 272, 289), asthma and allergic rhinitis (298), and eczema (289) in offspring. However, dietary intake of vitamin D makes a relatively small contribution to overall vitamin D status, and apparent effects of vitamin D intake may be confounded by other correlated nutrients. These studies did not report outcomes in relation to maternal serum 25-hydroxyvitamin D (25(OH)D) concentration, the gold standard measure of vitamin D status (210, 431), which takes account of skin synthesis, our primary source of vitamin D. Observational studies based on early life 25(OH)D levels have conflicting results; for wheezing, one study found reduced cumulative wheeze by 5 years in offspring of vitamin D deficient mothers (290) but three studies found no relationship (145, 292, 293); For asthma, five studies found no effect by 6 years of age (145, 290-293) but one study an increased risk by age 9 years (287); For allergic rhinitis and eczema no relationship has been found (145, 287, 291). The case for protection against early respiratory infection is more convincing, with observational studies showing a protective effect of higher levels against infection in the first three months of life (290), or by 1 year of age (292, 294).

There are plausible biological mechanisms for these associations. Developmental programming of lung function and immune responses occurs during pregnancy (75, 432). Developmental deficiency of vitamin D in rats induces alterations in immune and early lung development (400, 433). In human *in-vitro* studies, 1,25(OH)D has potent immunomodulatory effects (434). 1,25(OH)D has the ability to enhance the production of naturally occurring antimicrobial peptides, such as cathelicidin, in macrophages and airway

epithelial cells which may have a role in protection against respiratory infections (227, 357).

Current recommendations in the United Kingdom are for provision of 10 μg/day (400 IU/day) to all pregnant women (266). Vitamin D insufficiency is nevertheless very common during pregnancy (242, 252, 435), and it has been proposed that prenatal vitamin D supplementation may prevent childhood wheezing and asthma (269). We assessed children whose mothers had taken part in a randomised controlled trial of prenatal vitamin D supplementation, to determine whether supplementation prevented wheezing, allergic disease or respiratory infection in the first 3 years of life (418).

4.2 Methods

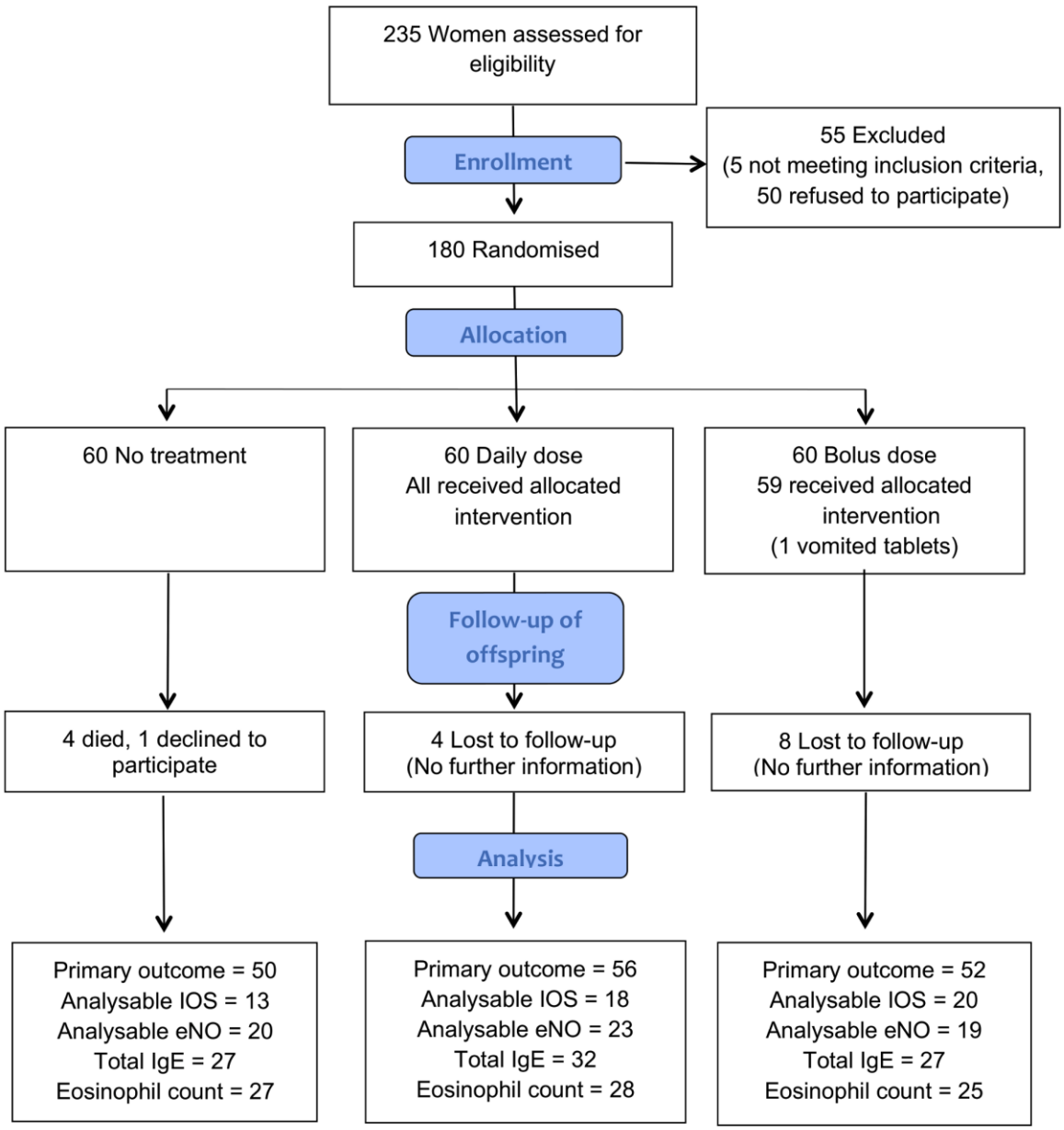
This was a follow up study of the offspring of women who took part in an ethnically stratified, parallel group, randomised controlled trial of vitamin D supplementation in pregnancy at St Mary's Hospital London. Full methods are described in section 2.2.

4.3 Results

4.3.1 Study participants

In the original trial 180 women were recruited who delivered 181 infants. There was one twin pregnancy of whom the first-born was included in analysis. There were 4 deaths, all in the control group: One stillbirth at 41 weeks, one death aged two days (no further details available), one death aged 16 hours from meconium aspiration and one child born with congenital abnormalities who died post-operatively aged 17 months. We successfully followed up 158/180 (88%) of infants at age 3. 129/180 (72%) agreed to a review of their primary care record. Participant flow is shown in Figure 4-1.

Figure 4-1 Participant flow through the study



The characteristics of the children and their mothers in each randomisation group were similar at baseline (Table 4.1).

Table 4.1 Study participants

	Control (n=50)	Daily vitamin D (n=56)	Bolus vitamin D (n=52)	Combined vitamin D (n=108)
Baseline maternal 25(OH)D < 25nmol/L, n/N (%)	24/50 (48)	25/56 (45)	22/52 (42)	47/108 (44)
Male sex, n/N (%)	27/50 (54)	32/56 (57)	26/52 (50)	58/108 (55)
Birth weight (g), mean (SD)	3268 (585)	3321 (525)	3290 (467)	3307 (497)
Ethnicity, n (%)				
Asian	12 (24)	15 (27)	13 (25)	28 (26)
Middle Eastern	13 (26)	14 (25)	14 (26)	28 (26)
Black	12 (24)	14 (25)	13 (25)	27 (25)
White	13 (26)	13 (23)	12 (24)	25 (23)
GA at delivery (weeks), mean (SD)	40 (1)	39 (2)	40 (1)	39 (2)
Nulliparous mother, n/N (%)	23/50 (46)	20/56 (36)	21/52 (40)	41/108 (38)
Vaginal delivery, n/N (%)	29/50 (58)	35/56 (63)	28/52 (54)	63/108 (58)
Maternal smoking during pregnancy, n/N (%)	2/48 (4)	3/55 (5)	0/46 (0)	3/101 (3)
Presence of household smokers, n/N (%)	16/48 (33)	15/55 (27)	17/46 (37)	32/101 (32)
Number of children in household, mean (SD)	2 (1)	2 (1)	2 (1)	2 (1)

Age mother left full time education, mean (SD)	20 (3)	20 (4)	21 (4)	21 (4)
Child in nursery, n/N (%)	27/48 (56)	36/55 (65)	20/45 (44)	57/101 (56)
Cat or dog in household, n/N (%)	4/48 (8)	5/54 (9)	4/46 (9)	9/100 (9)
Maternal Fitzpatrick skin score grade 3-6, n/N (%)	28/44 (64)	33/49 (67)	28/43 (65)	61/92 (66)
Maternal regular vitamin intake at three years, n/N (%)	9/46 (20)	16/53 (30)	16/45 (36)	32/98 (33)
At least one parent with allergic disease, n/N (%)	23/45 (51)	35/53 (66)	21/45 (47)	56/98 (57)
Exclusively breast-fed for 4 months, n/N (%)	22/48 (46)	20/54 (37)	23/44 (52)	43/98 (44)
Any child vitamin supplementation, n/N (%)	30/48 (63)	34/55 (62)	30/46 (65)	64/101 (63)
Completed immunizations to date, n/N (%)	48/49 (98)	54/55 (98)	43/47 (91)	97/102 (95)
Age at time of assessment (months), median (IQR)	37.9 (36.9, 39.9)	37.1 (36.5, 38.8)	37.4 (36.5, 39.5)	37.3 (36.5, 39.0)
Child Fitzpatrick skin score grade 3-6, n (%)	29/42 (69)	34/48 (71)	29/43 (67)	63/91 (69)
Child BMI Z score age 3, mean (SD)	0.51 (1.48)	0.35 (1.15)	0.62 (1.15)	0.47 (1.15)
Child outdoors > 1 hour/day, n/N (%)	32/48 (67)	34/53 (64)	33/45 (73)	67/98 (68)
Child TV/ computer > 2 hours/day, n/N (%)	24/48 (50)	18/53 (34)	17/45 (38)	35/98 (36)
Child 25(OH)D (nmol/L) age 3, median (IQR)	42 (27, 68)	35 (21, 66)	42 (30, 93)	41 (26, 72)

As previously reported (418), median cord 25(OH)D levels at delivery were significantly higher in supplemented children compared to the control group [control 17nmol/l (interquartile range (IQR) 14-22); daily dose 26nmol/l (IQR 17-45); p<0.001; bolus dose 25nmol/l (IQR 18-34); p<0.001)].

4.3.2 Effect of prenatal vitamin D supplementation on parent reported wheezing, allergic disease and respiratory infections.

We found no significant difference in the primary outcome of 'wheeze ever' between treatment groups (daily vs. control Table 4.2, bolus vs. control Table 4.3).

Table 4.2 Parentally reported outcomes at age three years. Daily vitamin D versus control

	Control (%)	Daily Vitamin D (%)	RR (95% CI)	p	OR (95% CI)	p	aOR† (95% CI)	p
Wheeze ever	14/50 (28)	11/56 (20)	0.70 (0.35, 1.40)	0.36	0.63 (0.26, 1.55)	0.31	0.61 (0.20, 1.85)	0.39
Recurrent wheezing	7/50 (14)	8/56 (14)	1.02 (0.40, 2.61)	1.00	1.02 (0.34, 3.06)	0.97	1.35 (0.35, 5.22)	0.66
Wheezing in the last year	8/50 (16)	8/56 (14)	0.89 (0.36, 2.20)	1.00	0.88 (0.30, 2.54)	0.81	1.14 (0.32, 4.13)	0.84
Wheeze with positive API	7/50 (14)	6/56 (11)	0.77 (0.28, 2.13)	0.77	0.74 (0.23, 2.36)	0.61	0.63 (0.13, 3.20)	0.58
Any bronchodilator use	4/49 (8)	10/56 (18)	2.23 (0.75, 6.67)	0.16	2.45 (0.72, 8.37)	0.15	2.03 (0.43, 9.55)	0.37
Eczema ever	15/49 (31)	15/54 (28)	0.91 (0.50, 1.66)	0.83	0.87 (0.37, 2.04)	0.75	0.59 (0.20, 1.77)	0.35
Eczema in the last year	7/49 (14)	11/55 (20)	1.40 (0.59, 3.33)	0.60	1.50 (0.53, 4.23)	0.44	1.79 (0.45, 7.09)	0.41
Atopy	7/27 (26)	4/36 (11)	0.41 (0.13, 1.27)	0.18	0.36 (0.09, 1.38)	0.13	0.43 (0.05, 3.82)	0.45
Allergic rhinitis	7/49 (14)	7/55 (13)	0.89 (0.34, 2.36)	1.00	0.88 (0.28, 2.70)	0.82	0.25 (0.03, 2.09)	0.20
Food allergy diagnosis	3/49 (6)	8/55 (15)	2.38 (0.67, 8.46)	0.21	2.61 (0.65, 10.45)	0.16	16.42 (0.74, 362.37)	0.08
> 4 URTI/year	7/50 (14)	11/55 (20)	1.43 (0.60, 3.40)	0.45	1.54 (0.55, 4.33)	0.42	1.52 (0.40, 5.81)	0.54
LRTI ever	11/50 (22)	14/54 (26)	1.18 (0.59, 2.35)	0.65	1.24 (0.50, 3.07)	0.64	0.95 (0.31, 2.94)	0.93
Primary health care records								
Recurrent wheeze	3/31 (10)	4/36 (11)	1.15 (0.28, 4.74)	1.00	1.17 (0.24, 5.67)	0.85	0.29 (0.03, 4.27)	0.40

Eczema	6/31 (19)	5/36 (14)	0.72 (0.24, 2.12)	0.74	0.67 (0.18, 2.46)	0.55	0.63 (0.10, 4.05)	0.63
Food allergy	2/31 (7)	2/36 (6)	0.86 (0.13, 5.76)	1.00	0.85 (0.11, 6.44)	0.88	-	-

RR=Risk ratio, aOR (adjusted odds ratio), API (Asthma predictive index), URTI (upper respiratory tract infection), LRTI (lower respiratory tract infection)

[1]Adjusted for mother's ethnic group, presence of household smokers, maternal smoking in pregnancy, exclusive breast-feeding to four months, any parental allergic history, any child vitamin supplementation, number of children in the household, age mother left full-time education, baseline concentration of 25(OH)D in maternal blood, maternal baseline BMI and sex of child.

Table 4.3 Parentally reported outcomes at age three years. Bolus vitamin D versus control

	Control (%)	Bolus Vitamin D (%)	RR (95% CI)	p	OR (95% CI)	p	aOR[†] (95% CI)	p
Wheeze ever	14/50 (28)	15/52 (29)	1.03 (0.56, 1.91)	1.00	1.04 (0.44, 2.47)	0.93	1.88 (0.61, 5.83)	0.27
Recurrent wheezing	7/50 (14)	9/52 (17)	1.24 (0.50 (3.07)	0.65	1.29 (0.44, 3.77)	0.65	5.88 (0.99, 34.79)	0.05
Wheezing in the last year	8/50 (16)	9/52 (17)	1.08 (0.45, 2.58)	0.86	1.10 (0.39, 3.12)	0.86	2.34 (0.57, 9.72)	0.24
Wheeze with positive API	7/50 (14)	9/51 (18)	1.26 (0.51, 3.12)	0.79	1.32 (0.45, 3.86)	0.62	3.77 (0.86, 16.56)	0.08
Any bronchodilator use	4/49 (8)	14/48 (29)	3.57 (1.27, 10.09)	0.008[*]	4.63 (1.40, 15.34)	0.008[*]	7.89 (1.67, 37.22)	0.01
Eczema ever	15/49 (31)	15/48 (31)	1.02 (0.56, 1.85)	1.00	1.03 (0.44, 2.44)	0.95	0.98 (0.35, 2.80)	0.98
Eczema in the last year	7/49 (14)	9/48 (19)	1.31 (0.53, 3.24)	0.59	1.39 (0.47, 4.08)	0.55	2.24 (0.51, 9.86)	0.29
Atopy	7/27 (26)	7/32 (22)	0.84 (0.34, 2.11)	0.77	0.80 (0.24, 2.66)	0.72	0.34 (0.06, 2.04)	0.24
Allergic rhinitis	7/49 (14)	4/46 (9)	0.61 (0.19, 1.94)	0.53	0.57 (0.16, 2.10)	0.40	-	-
Food allergy diagnosed	3/49 (6)	4/47 (9)	1.36 (0.32, 5.78)	0.72	1.43 (0.30, 6.75)	0.65	2.25 (0.29, 17.29)	0.44
> 4 URTI/year	7/50 (14)	9/48 (19)	1.34 (0.54, 3.31)	0.59	1.42 (0.48, 4.17)	0.53	2.15 (0.57, 8.07)	0.26
LRTI ever	11/50 (22)	17/47 (36)	1.64 (0.86, 3.14)	0.18	2.01 (0.82, 4.92)	0.12	5.19 (1.55, 17.40)	0.01
Primary health care record								
Recurrent wheeze	3/31 (10)	6/34 (18)	1.82 (0.50, 6.68)	0.48	2.00 (0.46, 8.80)	0.35	2.06 (0.37, 11.67)	0.41

Eczema	6/31 (19)	5/34 (15)	0.76 (0.26, 2.24)	0.74	0.72 (0.20, 2.64)	0.62	0.62 (0.11, 3.57)	0.59
Food allergy	2/31 (7)	0/34 (0)	0.0 (-, -)	0.22	-	-	-	-

RR=Risk ratio, aOR (adjusted odds ratio), API (Asthma predictive index), URTI (upper respiratory tract infection), LRTI (lower respiratory tract infection)

[1]Adjusted for mother's ethnic group, presence of household smokers, maternal smoking in pregnancy, exclusive breast-feeding to four months, any parental allergic history, any child vitamin supplementation, number of children in the household, age mother left full-time education, baseline concentration of 25(OH)D in maternal blood, maternal baseline BMI and sex of child.

There was also no difference between groups in prevalence of eczema. We found significantly increased bronchodilator use and higher prevalence of LRTI's in the offspring of the bolus group, but this did not remain significant after adjusting for multiple testing at a 20% threshold. Also shown are complete unadjusted and adjusted analyses of these outcomes for offspring of mothers with baseline vitamin D deficiency (daily vs. control Table 4.4; bolus vs control, Table 4.5).

Table 4.4 Parentally reported outcomes at age three years in offspring of mothers with baseline vitamin D deficiency. Daily vitamin D versus control.

	Control (%)	Daily vitamin D (%)	RR (95% CI)	OR (95% CI)	p	aOR† (95% CI)	p
Wheeze ever	7/24 (29.2)	4/25 (16)	0.60 (0.20, 1.77)	0.46 (0.12, 1.85)	0.27	0.23 (0.03, 2.08)	0.19
Recurrent wheezing	4/24 (16.7)	3/25 (12.0)	0.72 (0.18, 2.89)	0.68 (0.14, 3.43)	0.64	3.48 (0.12, 103.5)	0.47
Wheezing in the last year	5/24 (20.8)	3/25 (12.0)	0.58 (0.15, 2.15)	0.52 (0.11, 2.46)	0.40	0.55 (0.06, 5.01)	0.60
Wheeze with positive API	4/24 (16.7)	3/25 (12.0)	0.72 (0.18, 2.89)	0.68 (0.14, 3.43)	0.64	3.40 (0.04, 308.9)	0.59
Any bronchodilator use	2/24 (8.3)	4/25 (16.0)	1.92 (0.39, 9.54)	2.10 (0.35, 12.67)	0.42	-	-
Eczema ever	8/24 (33.3)	7/24 (29.2)	0.88 (0.38, 2.03)	0.82 (0.24, 2.80)	0.76	0.22 (0.02, 2.71)	0.24
Eczema in the last year	4/24 (16.7)	5/25 (20.0)	1.20 (0.37, 3.94)	1.25 (0.29, 5.35)	0.76	0.29 (0.02, 4.48)	0.38
Atopy	4/13 (30.8)	1/14 (7.1)	0.23 (0.03, 1.82)	0.17 (0.02, 1.82)	0.11	-	-
Allergic rhinitis	3/24 (12.5)	3/25 (12.0)	0.96 (0.21, 4.30)	0.96 (0.17, 5.27)	0.96	-	0.77
Food allergy diagnosis	2/24 (0.0)	3/25 (8.0)	1.44 (0.26, 7.88)	1.50 (0.23, 9.87)	0.67	-	-
> 4 URTI/year	3/24 (12.5)	6/25 (24.0)	1.92 (0.54, 6.82)	2.21 (0.48, 10.09)	0.30	-	-
LRTI ever	7/24 (29.2)	6/24 (25.0)	0.86 (0.34, 2.18)	0.81 (0.23, 2.90)	0.75	0.06 (0.00, 1.19)	0.07
Primary health care record							
Recurrent wheeze	1/17 (5.9)	2/16 (12.5)	2.13 (0.21, 21.24)	2.29 (0.19, 28.00)	0.51	-	-

Eczema	3/17 (17.6)	2/16 (12.5)	0.71 (0.14, 3.70)	0.67 (0.10, 4.62)	0.68	-	-
Food allergy	0/17 (0.0)	1/16 (6.3)	-	-	-	-	-

RR=Risk ratio, aOR (adjusted odds ratio), API (Asthma predictive index), URTI (upper respiratory tract infection), LRTI (lower respiratory tract infection)

[1]Adjusted for mother's ethnic group, presence of household smokers, maternal smoking in pregnancy, exclusive breast-feeding to four months, any parental allergic history, any child vitamin supplementation, number of children in the household, age mother left full-time education, baseline concentration of 25(OH)D in maternal blood, maternal baseline BMI and sex of child

Table 4.5 Parentally reported outcomes at age three years in offspring of mothers with baseline vitamin D deficiency. Bolus vitamin D versus control

	Control (%)	Bolus vitamin D (%)	RR (95% CI)	OR (95% CI)	p	aOR† (95% CI)	p
Wheeze ever	7/24 (29.2)	7/22 (31.8)	1.09 (0.46, 2.61)	1.13 (0.32, 3.98)	0.85	3.83 (0.37, 39.56)	0.26
Recurrent wheezing	4/24 (16.7)	5/22 (22.7)	1.36 (0.42, 4.44)	1.47 (0.34, 6.37)	0.61	43.36 (0.72, 2610)	0.07
Wheezing in the last year	5/24 (20.8)	5/22 (22.7)	1.09 (0.36, 3.27)	1.12 (0.28, 4.54)	0.88	3.15 (0.29, 34.70)	0.35
Wheeze with positive API	4/24 (16.7)	3/21 (14.3)	0.86 (0.22, 3.40)	0.83 (0.16, 4.24)	0.83	-	-
Any bronchodilator use	2/24 (8.3)	5/19 (26.3)	3.16 (0.69, 14.52)	3.93 (0.67, 23.10)	0.11	-	-
Eczema ever	8/24 (33.3)	7/19 (36.8)	1.11 (0.49, 2.50)	1.17 (0.33, 4.12)	0.81	1.60 (0.12, 20.86)	0.72
Eczema in the last year	4/24 (16.7)	5/19 (26.3)	1.58 (0.49, 5.08)	1.79 (0.41, 7.86)	0.44	-	-
Atopy	4/13 (30.8)	3/13 (23.1)	0.75 (0.21, 2.71)	0.68 (0.12, 3.87)	0.66	-	-
Allergic rhinitis	3/24 (12.5)	1/19 (5.3)	0.42 (0.05, 3.73)	0.39 (0.04, 4.07)	0.42	-	-
Food allergy diagnosis	2/24	2/19	1.26 (0.20, 8.16)	1.29 (0.17, 10.15)	0.81	-	-
> 4 URTI/year	3/24 (12.5)	6/19 (31.6)	2.53 (0.72, 8.81)	3.23 (0.69, 15.20)	0.13	-	-
LRTI ever	7/24 (29.2)	7/19 (36.8)	1.26 (0.54, 2.98)	1.42 (0.39, 5.11)	0.59	5.95 (0.71, 49.76)	0.10
Primary health care record							
Recurrent wheeze	1/17 (5.9)	3/13 (23.1)	3.92 (0.46, 33.52)	4.80 (0.44, 52.76)	0.17	-	-

Eczema	3/17 (17.6)	4/13 (30.8)	1.74 (0.47, 6.47)	2.07 (0.37, 11.53)	0.40	-	-
Food allergy	0/17	0/13	-	-	-	-	-

RR=Risk ratio, aOR (adjusted odds ratio), API (Asthma predictive index), URTI (upper respiratory tract infection), LRTI (lower respiratory tract infection).

[1]Adjusted for mother's ethnic group, presence of household smokers, maternal smoking in pregnancy, exclusive breast-feeding to four months, any parental allergic history, any child vitamin supplementation, number of children in the household, age mother left full-time education, baseline concentration of 25(OH)D in maternal blood, maternal baseline BMI and sex of child.

We found no significant difference in the primary outcome of ‘wheeze ever’, or for any secondary outcomes.

Also shown are unadjusted and adjusted analyses of these outcomes for the two groups combined (all offspring, Table 4.6; offspring of mothers with baseline vitamin D deficiency, Table 4.7).

Table 4.6 Parentally reported outcomes at age three years. Combined vitamin D groups versus control

	Control (%)	Combined vitamin D (%)	RR (95% CI)	p	OR (95% CI)	p	aOR[†] (95% CI)	p
Wheeze ever	14/50 (28)	26/108 (24)	0.86 (0.49, 1.50)	0.69	0.82 (0.38, 1.74)	0.60	0.90 (0.38, 2.15)	0.82
Recurrent wheezing	7/50 (14)	17/108 (16)	1.12 (0.50, 2.54)	1.00	1.15 (0.44, 2.97)	0.77	1.53 (0.52, 4.55)	0.44
Wheezing in the last year	8/50 (16)	17/108 (16)	0.98 (0.46, 2.13)	1.00	0.98 (0.39, 2.45)	0.97	1.23 (0.43, 3.50)	0.70
Wheeze with positive API	7/50 (15)	15/107 (14)	1.00 (0.44, 2.30)	1.00	1.00 (0.38, 2.64)	1.00	1.46 (0.47, 4.56)	0.51
Any bronchodilator use	4/49 (8)	24/104 (23)	2.83 (1.04, 7.71)	0.03[*]	3.37 (1.10, 10.34)	0.03[*]	3.36 (0.98, 11.53)	0.06
Eczema ever	15/49 (31)	30/102 (29)	0.96 (0.57, 1.61)	1.00	0.75 (0.35, 1.62)	0.47	0.82 (0.35, 1.91)	0.64
Eczema in the last year	7/49 (14)	20/103 (19)	1.36 (0.62, 3.00)	0.50	1.34 (0.52, 3.47)	0.55	1.98 (0.66, 5.92)	0.22
Atopy	7/27(26)	11/68 (16)	0.62 (0.27, 1.44)	0.38	0.56 (0.19, 1.66)	0.30	0.33 (0.08, 1.42)	0.14
Allergic rhinitis	7/49 (14)	11/101 (12)	0.76 (0.31, 1.85)	0.60	0.63 (0.22, 1.78)	0.38	0.17 (0.02, 1.23)	0.08
Food allergy diagnosis	3/49 (6)	12/102 (12)	1.92 (0.57, 6.50)	0.39	1.81 (0.48, 6.85)	0.38	2.88 (0.56, 14.12)	0.19
> 4 URTI/year	7/50 (14)	20/103 (19)	1.39 (0.63, 3.06)	0.50	1.43 (0.55, 3.68)	0.46	1.54 (0.53, 4.42)	0.43
LRTI ever	11/50 (22)	31/101 (31)	1.40 (0.77, 2.54)	0.34	1.47 (0.64, 3.37)	0.37	1.95 (0.77, 4.94)	0.16
Primary health care records								
Recurrent wheeze	3/31 (10)	10/70 (14)	1.48 (0.44, 5.00)	0.75	1.56 (0.40, 6.10)	0.52	1.30 (0.28, 6.07)	0.74

Eczema	6/31 (19)	10/70 (14)	0.74 (0.29, 1.85)	0.56	0.46 (0.23, 2.12)	0.52	0.70 (0.19, 2.61)	0.59
Food allergy	2/31 (6)	2/70 (3)	0.44 (0.07, 3.00)	0.58	0.43 (0.06, 3.18)	0.58	0.06 (0.00, 35.17)	0.38

RR=Risk ratio, aOR (adjusted odds ratio), API (Asthma predictive index), URTI (upper respiratory tract infection), LRTI (lower respiratory tract infection).

[1]Adjusted for mother's ethnic group, presence of household smokers, maternal smoking in pregnancy, exclusive breast-feeding to four months, any parental allergic history, any child vitamin supplementation, number of children in the household, age mother left full-time education, baseline concentration of 25(OH)D in maternal blood, maternal baseline BMI and sex of child.

Table 4.7 Parentally reported outcomes at age three years in offspring of mothers with baseline vitamin D deficiency. Combined vitamin D versus control

	Control (%)	Combined vitamin D (%)	RR (95% CI)	OR (95% CI)	p	aOR† (95% CI)	p
Wheeze ever	7/24 (29)	11/47 (23)	0.80 (0.36, 1.80)	0.74 (0.25, 2.25)	0.60	0.96 (0.22, 4.32)	0.96
Recurrent wheezing	4/24 (17)	8/47 (17)	1.02 (0.34, 3.05)	1.03 (0.28, 3.82)	0.97	2.16 (0.39, 12.13)	0.38
Wheezing in the last year	5/24 (21)	8/47 (17)	0.82 (0.30, 2.23)	0.78 (0.23, 2.71)	0.69	1.10 (0.22, 5.42)	0.91
Wheeze with positive API	4/24 (17)	6/46 (13)	0.78 (0.24, 2.51)	0.75 (0.19, 2.96)	0.68	3.10 (0.38, 25.52)	0.29
Any bronchodilator use	2/24 (8)	9/44 (21)	2.46 (0.58, 10.46)	2.83 (0.56, 14.33)	0.20	24.49 (0.97, 616.5)	0.05
Eczema ever	8/24 (33)	14/43 (33)	0.98 (0.48, 1.99)	0.97 (0.33, 2.79)	0.95	1.05 (0.22, 5.10)	0.95
Eczema in the last year	4/24 (17)	10/44 (23)	1.36 (0.48, 3.89)	1.47 (0.41, 5.31)	0.56	1.75 (0.32, 9.66)	0.52
Atopy	4/13 (31)	4/27 (15)	0.48 (0.14, 1.63)	0.39 (0.08, 1.91)	0.24	-	-
Allergic rhinitis	3/24 (13)	4/44 (9)	0.73 (0.18, 2.99)	0.70 (0.14, 3.42)	0.66	3.34 (0.06, 187.41)	0.56
Food allergy diagnosis	2/24 (8.3)	5/44 (11.4)	1.36 (0.29, 6.51)	1.41 (0.25, 7.88)	0.69	0.32 (0.00, 26.52)	0.62
> 4 URTI/year	3/24 (13)	12/44 (27)	2.18 (0.68, 6.99)	2.63 (0.66, 10.43)	0.16	7.01 (0.95, 151.93)	0.06
LRTI ever	7/24 (29)	13/43 (30)	1.04 (0.48, 2.24)	1.05 (0.35, 3.15)	0.93	1,72 (0.35, 8.32)	0.50
Primary health care record							
Recurrent wheeze	1/17 (6)	5/29 (17)	2.93 (0.37, 23.05)	3.33 (0.36, 31.26)	0.27	-	-

Eczema	3/17 (18)	6/29 (21)	1.17 (0.34, 4.09)	1.22 (0.26, 5.67)	0.80	0.68 (0.06, 7.78)	0.76
Food allergy	0/17 (0)	1/29 (3)	-	-	-	-	-

RR=Risk ratio, aOR (adjusted odds ratio), API (Asthma predictive index), URTI (upper respiratory tract infection), LRTI (lower respiratory tract infection).

[1]Adjusted for mother's ethnic group, presence of household smokers, maternal smoking in pregnancy, exclusive breast-feeding to four months, any parental allergic history, any child vitamin supplementation, number of children in the household, age mother left full-time education, baseline concentration of 25(OH)D in maternal blood, maternal baseline BMI and sex of child.

We found no significant difference in the primary outcome of ‘wheeze ever’, or for eczema or any of the secondary outcomes.

Since 22 offspring were not followed up, sensitivity analyses were performed to determine if this influenced the result for the primary outcome. Two scenarios were considered – the first where no drop-outs wheezed and the second where all drop-outs wheezed. There was no significant difference between groups in wheezing prevalence for either of these scenarios (Table 4.8).

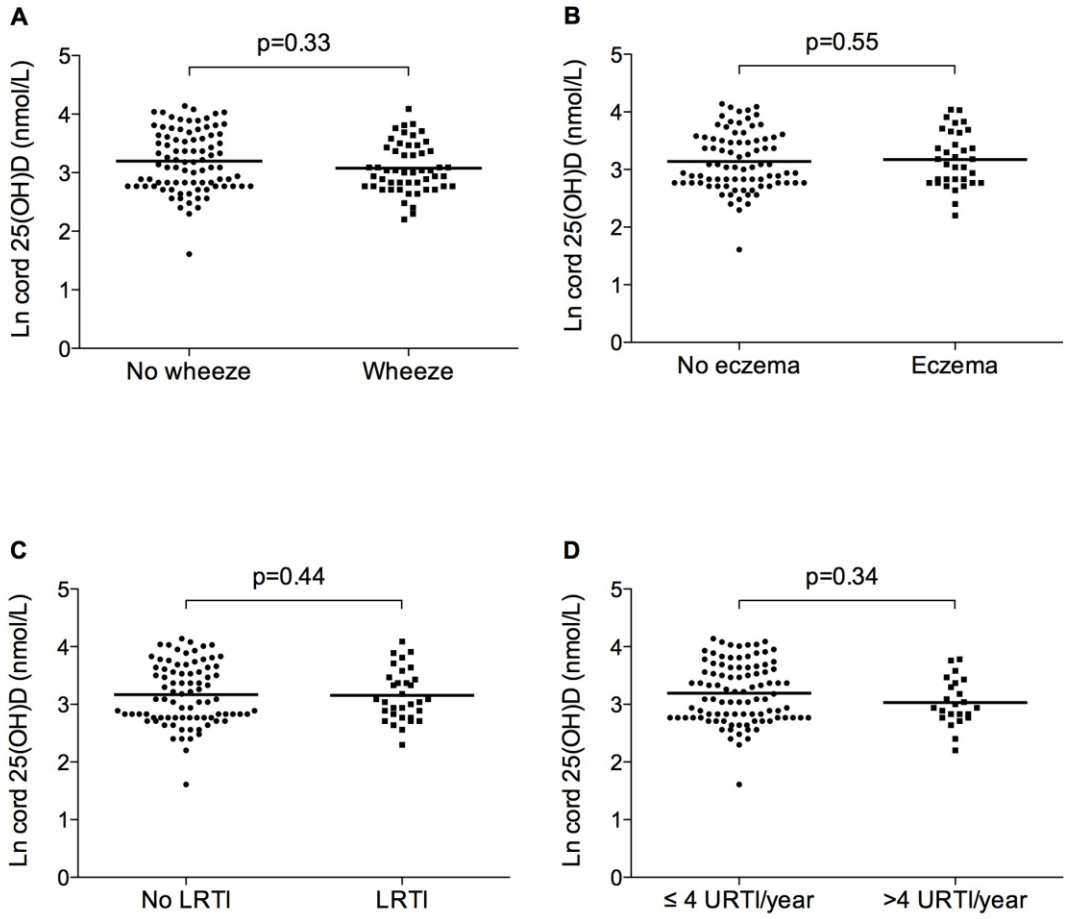
Table 4.8 Sensitivity analysis for primary outcome 'wheeze ever' using imputation for missing data

	Control (% wheeze)	Treatment group (% wheeze)	RR (95% CI)	OR (95% CI)	p
Scenario assuming no drop-outs wheezed					
Control vs Combined	14/60 (23)	26/120 (22)	0.93 (0.52, 1.64)	0.91 (0.43, 1.90)	0.80
Control vs Daily	14/60 (23)	11/60 (18)	0.79 (0.39, 1.59)	0.74 (0.30, 1.79)	0.50
Control vs Bolus	14/60 (23)	15/60 (25)	1.07 (0.57, 2.02)	1.10 (0.48, 2.53)	0.83
Scenario assuming all drop-outs wheezed					
Control vs Combined	24/60 (40)	38/120 (32)	0.79 (0.53, 1.19)	0.70 (0.37, 1.32)	0.27
Control vs Daily	24/60 (40)	15/60 (25)	0.63 (0.37, 1.07)	0.50 (0.23, 1.09)	0.08
Control vs Bolus	24/60 (40)	23/60 (38)	0.96 (0.61, 1.50)	0.93 (0.45, 1.94)	0.85

RR=Risk ratio, OR=Odds ratio, CI= Confidence intervals

For 123 children assessed at 3 years, cord 25(OH)D levels were available. After natural log transformation, we found no difference between cord blood 25(OH)D levels in children with and without a history of wheeze, eczema, any LRTI or >4 episodes of URTI per year (Figure 4-2 and Table 4.9). There was also no correlation between Ln cord blood 25(OH)D level and Ln Total IgE at age three years for 68 children (Beta coefficient = -0.05, p=0.72).

Figure 4-2 Cord vitamin D levels and parentally reported outcomes at age three years



Ln Cord 25(OH)D at birth and A) Wheeze ever, B) Eczema ever, C) Lower respiratory tract infection (LRTI) ever and D) Frequency of upper respiratory tract infection (URTI) per year in offspring at age three years. Horizontal bars represent means and p values adjusted analyses.

Table 4.9 Ln Cord vitamin D levels and parentally reported outcomes at age 3 years

Outcome	n	Outcome absent Mean ± SD	n	Outcome present Mean ± SD	Unadjusted Mean Difference (95% CI)	p	Adjusted[1] mean Difference (95% CI)	p
Wheeze	88	3.20 ± 0.52	34	3.07 ± 0.43	-0.13 (-0.33, 0.07)	0.19	-0.07 (-0.26, 0.11)	0.43
Atopy	59	3.19 ± 0.49	14	3.28 ± 0.44	0.08 (-0.20, 0.37)	0.56	0.10 (-0.17, 0.37)	0.47
Eczema	82	3.14 ± 0.51	34	3.17 ± 0.48	0.03 (-0.23, 0.17)	0.74	0.05 (-0.13, 0.23)	0.56
Any LRTI	84	3.17 ± 0.52	31	3.16 ± 0.45	-0.007 (-0.20, 0.22)	0.95	-0.10 (-0.29, 0.09)	0.30
> 4 URTI/year	95	3.19 ± 0.52	22	3.03 ± 0.41	-0.16 (-0.40, 0.07)	0.17	-0.11 (-0.32, 0.11)	0.33

LRTI (Lower respiratory tract infection), URTI (Upper respiratory tract infection).

[1]Adjusted for treatment group, mother's ethnic group, presence of household smokers, maternal smoking in pregnancy, exclusive breast-feeding to four months, any parental allergic history, any child vitamin supplementation, number of children in the household, age mother left full-time education and baseline concentration of 25(OH)D in maternal blood.

4.4 Discussion

In this randomised controlled trial of vitamin D supplementation in late pregnancy in a relatively deficient mixed race population, daily supplementation with 800 IU ergocalciferol or a single bolus dose of 200,000 IU cholecalciferol from 27 weeks gestation did not prevent parentally reported wheezing, allergic disease or respiratory tract infections in the first three years of life. We were unable to confidently exclude an effect on some secondary outcome measures such as atopy at 3 years, where statistical power was limited. Our results suggest that the most likely explanation for the finding of decreased wheezing risk in offspring of mothers with a higher vitamin D intake during pregnancy in observational studies (175, 289, 297, 298), is confounding by other dietary or lifestyle factors associated with vitamin D intake.

Our study has a number of limitations. First, we relied on a subjective primary outcome measure and participants were not blind to treatment allocation, which introduces a risk of reporter bias. Investigators were, however, kept blind to treatment allocation until database lock. Moreover the results of subjective primary outcome measures are supported by the results of both our secondary objective outcome assessments and a blinded assessment of prospectively recorded healthcare records. Second, this was a small study with limited statistical power, particularly for detecting differences in objective outcomes. Our power calculation was based on the study by Camargo et al (297), which estimated a risk reduction for recurrent wheezing from 34% to 13% comparing mothers with the highest quartile of daily vitamin D intake (median 724 IU) to those in the lowest quartile of intake (median 356 IU). However the prevalence of wheeze in our control group was 28% thus reducing the power of the study. Third, we did not assess vitamin D receptor polymorphisms or other potentially important genotypic variations. It is possible that vitamin D supplementation is effective for preventing wheezing or atopy in the presence of specific maternal or infant genotypes, as suggested by others (285). Fourth, women from four ethnic groups were enrolled in this

study, with significant differences in baseline vitamin D levels between Caucasian women and all other ethnic groups (data not shown). Previous observational studies have predominantly studied Caucasians (297). Although randomisation in this trial was stratified by ethnicity, and we included baseline vitamin D in our adjusted model, it is possible that this source of heterogeneity could have masked positive findings. Fifth, supplementation with vitamin D only started at 27 weeks of gestation. It is known that immune cells develop much earlier in fetal life (436), and that airway development to the respiratory bronchioles is complete by 16 weeks gestation, (437). It may be that supplementation earlier in pregnancy, or indeed pre-conception, is necessary for protection against childhood wheezing.

We are also unable to exclude the possibility that vitamin D supplementation at a higher dose, might protect against early childhood wheezing. Although the vitamin D doses used in this trial were greater than the current recommended intake for pregnant women of 400 IU/day during pregnancy in the United Kingdom (266, 403), and 600 IU in the United States (438), only a small percentage of offspring had cord 25(OH)D levels in the sufficient range (13% daily group, 3% bolus group). It has recently been shown that doses of 4000 IU of vitamin D daily are safe and effective for short term treatment of vitamin D deficiency during pregnancy (439), although the long term effects on child health have not been studied. Two on-going trials, the Vitamin D Prenatal Asthma Reduction Trial (NCT00920621), and ABCvitaminD (NCT00856947) are specifically exploring the effects of earlier and higher dose prenatal vitamin D supplementation on child health.

Although our findings of increased bronchodilator use and increased LRTI in offspring of mothers who received a bolus dose of vitamin D became non-significant after adjusting for multiple testing, this is an important adverse outcome to consider. The principle that non-physiological dosing regimens of vitamin D may be harmful is established in other settings, with two randomised controlled trials in adults finding high dose bolus vitamin D is associated with increased risk of fractures (440, 441) and/or falls (440). With evidence of a U

shaped, or reverse J shaped relationship between 25(OH)D levels and health outcomes in adults suggesting both lower and upper limits for healthy 25(OH)D levels (438, 442), these findings should sound a note of caution in relation to high dose bolus vitamin D treatment in pregnancy. These outcomes should be carefully assessed in the ongoing randomised controlled trials of vitamin D supplementation in pregnancy.

Two recent observational studies that documented prenatal vitamin D status by measuring cord blood 25(OH)D concentration, found relationships with allergic sensitisation and/or wheezing in early childhood (290, 291). When we analysed our own data on cord blood 25(OH)D concentration and wheezing, atopy and frequency of respiratory infection in the first three years, no significant relationship was found. While our interventions did result in at least 50% higher cord blood 25(OH)D concentrations compared with no treatment, cord blood levels were still significantly lower in the intervention groups than in these studies. The results of our randomised controlled trial do not support a strong causal relationship between low prenatal vitamin D status within the deficient/insufficient range and increased risk of allergic sensitization or wheezing in early childhood. We are unable to confidently exclude a similar relationship in more vitamin D sufficient populations (290).

The findings of our study are specific to a population of vitamin D deficient women (half had baseline 25(OH)D levels below 25nmol/L) and two specific forms of prenatal vitamin D supplementation. The doses of supplementation were chosen pragmatically, were not stratified based on individual vitamin D status or genotype, and response to treatment was not monitored before delivery. The study population was not selected for allergy or asthma risk and, as a mixed race urban population, is representative of many populations of pregnant women worldwide (443). Although these findings cannot automatically be generalised to other populations of pregnant women, one might expect prenatal vitamin D supplementation to show its clearest clinical effects in a deficient population such as this.

In summary we found no evidence for a protective effect of prenatal vitamin D supplementation from 27 weeks gestation on parentally reported childhood wheezing and allergic disease in the first 3 years of life. Given the modest effects on cord blood vitamin D achieved by these interventions, the safety and efficacy of higher dose prenatal vitamin D supplementation strategies need to be explored. Given our finding of increased bronchodilator use and increased LRTI in offspring of mothers who received a bolus dose of vitamin D, (in this case a single dose of 200,000 units at 27 weeks gestation), the use of such regimens should be viewed with caution.

5 Effect of prenatal vitamin D on atopy, inflammation and lung function

5.1 Introduction

Both vitamin D deficiency (269) and supplementation (268) have been proposed as a cause of the asthma epidemic. Epidemiological evidence based on long term follow up of birth cohort studies can be found to support both hypotheses, with studies showing protective (174, 175, 177, 298), no effect (290-293) and harmful effects (287) of higher early life vitamin D status on childhood wheezing. For allergic sensitisation, although three studies have found no association with skin prick test sensitivity (175, 288, 293), in another study both high (≥100nmol/L) and low (<50nmol/L) cord levels of 25(OH)D were associated with increased skin prick test sensitivity and IgE levels at 5 years (291), an effect that may be genetically determined (285). Two studies have investigated spirometry and exhaled nitric oxide as outcomes, and found no association (175, 293).

There is plausible biological data to support a relationship between early life vitamin D status and atopic sensitisation, allergic inflammation and lung function in early childhood. Developmental deficiency of vitamin D in rats induces alterations in immune and early lung development (400, 433). Failure of VDR knockout mice to develop experimental allergic airway disease suggests vitamin D is necessary for Th-2 driven lung inflammation to occur (346, 347). In mouse models, $1,25(OH)_2D$ inhibits airway inflammation (348), and irradiation with UVB light prior to sensitization blunts airway hyper-responsiveness and inflammation (349). Animal data suggest that alveolarisation may be influenced in part by a complex growth axis which involves $1,25(OH)_2D3$. The VDR is expressed during late intrauterine life in rate pulmonary tissues (312, 313). Rat lung fibroblasts are capable of metabolising 25(OH)D to $1,25(OH)_2D$ suggesting a paracrine system for vitamin D (316, 317). $1,25(OH)_2D$

significantly increases proliferation of rat perinatal lung fibroblasts (318) and inhibits apoptosis of lipid laden interstitial lung fibroblasts (323).

In human in-vitro work, the VDR has been detected in human foetal lung fibroblasts from 16 weeks gestation, (332, 333) and in alveolar type two cells from the second trimester (334). 1,25(OH)D has a marked inhibitory effect on adaptive immune cells (233). In cord blood, 1,25(OH)D inhibits differentiation of naïve T cells into Th1 and Th2 cells (385). 1,25(OH)D is a potent promoter of tolerogenic dendritic cells and regulatory T cells (386). In-vivo data from a cross-sectional survey of over seven thousand white British 45 year olds suggest a non-linear relationship between 25(OH)D status and total IgE levels, with both low (<25nmol/L) and excess (>135nmol/L) levels associated with elevated IgE (301). Data from adult studies suggest $1,25(OH)_2D$ may have a role in protection against airway remodelling (345).

The aim of this study was to determine if prenatal vitamin D supplementation influenced objective markers of atopic sensitisation and inflammation (skin prick test reactivity, total IgE, exhaled nitric oxide, peripheral eosinophil count) and lung function in offspring at three years of age.

5.2 Methods

This was a follow up study of the offspring of women who took part in a randomised controlled trial of vitamin D supplementation in pregnancy at St Mary's Hospital London. Full methods are described in section 2.2.

5.3 Results

For patient selection see section 2.2.1. Of the 180 offspring, IOS data of acceptable quality for analysis was available for 51 (28%), IgE results for 86 (48%), eNO levels for 62 (34%) and eosinophil count for 80 (44%).

5.3.1 Effect of prenatal vitamin D supplementation on atopy

We found no significant difference in atopy between treatment groups, but confidence intervals were wide (Offspring of all mothers, Table 5.1; Offspring of mothers with baseline 25(OH)D <25nmol/L, Table 5.2).

Table 5.1 Atopy at age three years. Daily, bolus and combined groups vs control

	Control (%)	Intervention group (%)	Unadjusted OR (95% CI)	p	n	aOR[1] (95% CI)	p
Daily vs control	7/27 (26)	4/36 (11)	0.36 (0.09, 1.38)	0.13	60	0.36 (0.05, 2.94)	0.34
Bolus vs control	7/27 (26)	7/32 (22)	0.80 (0.24, 2.66)	0.72	56	0.31 (0.05, 1.83)	0.20
Combined vs control	7/27(26)	11/68 (16)	0.55 (0.18, 1.62)	0.27	90	0.29 (0.07, 1.16)	0.08

aOR (adjusted odds ratio).

[1]Adjusted for mother's ethnic group, presence of household smokers, maternal smoking in pregnancy, exclusive breast-feeding to four months, any parental allergic history, any child vitamin supplementation, number of children in the household, age mother left full-time education and baseline concentration of 25(OH)D in maternal blood.

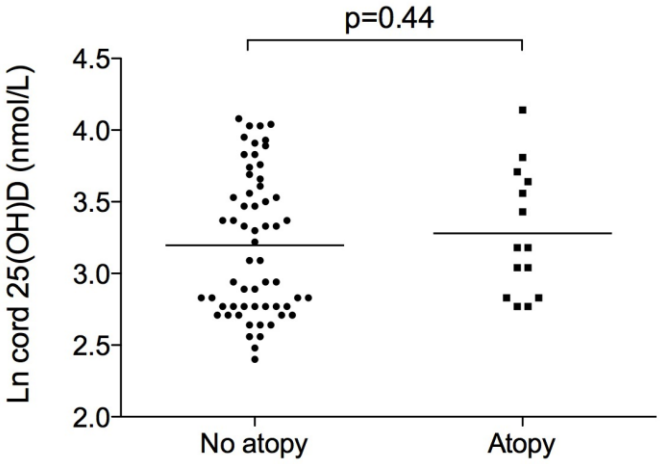
Table 5.2 Atopy at age three years in offspring of mothers with baseline vitamin D deficiency. Daily, bolus and combined groups vs control

	Control (%)	Intervention group (%)	Unadjusted OR (95% CI)	p	n	aOR[1] (95% CI)	p
Daily vs control	4/13 (30.8)	1/14 (7.1)	0.17 (0.02, 1.82)	0.11	-	-	-
Bolus vs control	4/13 (30.8)	3/13 (23.1)	0.68 (0.12, 3.87)	0.66	-	-	-
Combined vs control	4/13 (31)	4/27 (15)	0.39 (0.08, 1.91)	0.24	-	-	-

aOR (adjusted odds ratio) not done as numbers insufficient.

When results for atopy were analysed by Ln cord 25(OH)D levels, no association was found in unadjusted and adjusted analyses (mean ± SD Ln cord 25(OH)D, 3.19 ± 0.49 in 59 non-atopic children vs 3.28 ± 0.44 in 14 atopic children, (adjusted mean difference 0.10; 95% CI, -0.16,0.37; p=0.44) (Fig 5-1). Represented as the geometric mean gives 24.29 ± 1.64 nmol/L in non-atopic children vs 26.58 ± 1.55 nmol/L in atopic children, (multiplicative mean difference 1.11; 95% CI, 1.17-1.15; p = 0.44).

Figure 5-1 Ln cord 25(OH)D levels and atopic status at age three years

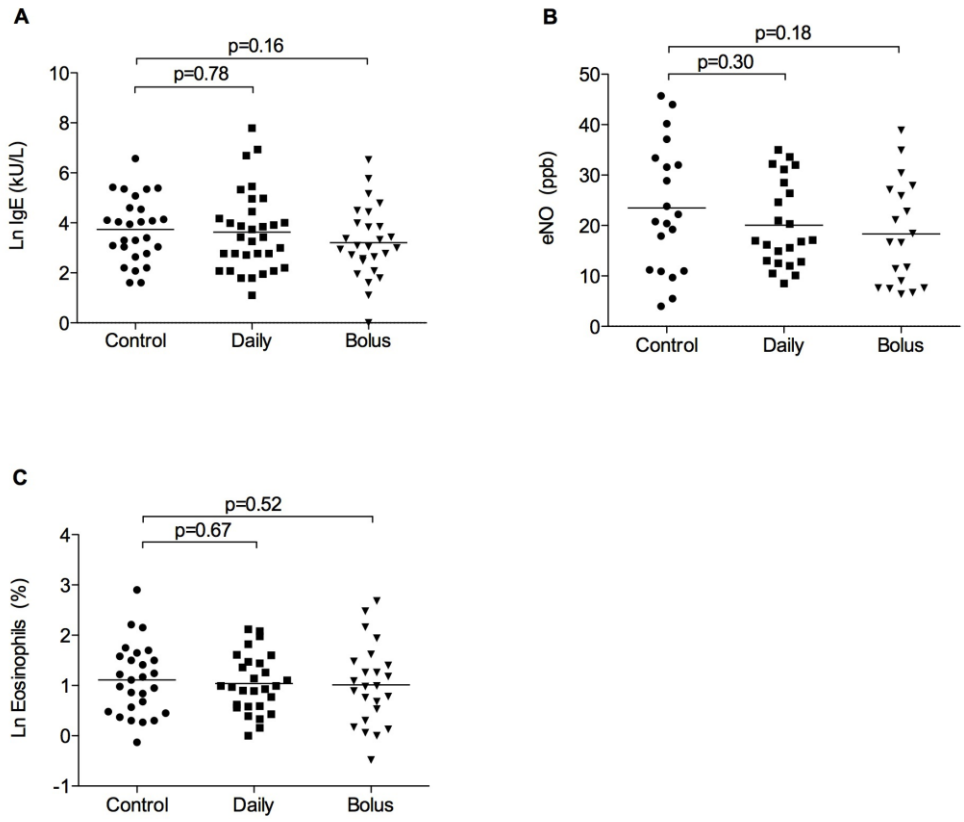


Horizontal bars represent means and p values adjusted analyses.

5.3.2 Effect of parental vitamin D supplementation on allergic inflammation

We found no significant difference in adjusted and unadjusted analysis between groups in total IgE, exhaled nitric oxide or eosinophil count assessed at age 3 years (Figure 5-2).

Figure 5-2 Allergic inflammation at age three years. Daily and bolus vitamin D vs control.



A) Ln IgE, B) exhaled nitric oxide (eNO) and C) Ln % eosinophil count. Control versus daily vitamin D or bolus vitamin D. Horizontal bars represent medians, and p values represent adjusted analysis.

When data were analysed for the two forms of prenatal vitamin D supplementation combined, there was no significant difference between groups (Table 5.3).

Table 5.3 Measures of allergic inflammation at age three years. Combined vitamin D groups versus control

Outcome	n	Control Mean ± SD	n	Combined vitamin D Mean ± SD	Unadjusted mean difference (95% CI)	p	n	Adjusted[1] mean difference (95% CI)	p
Ln IgE	27	3.74 ± 1.29	59	3.44 ± 1.52	-0.30 (-0.97, 0.37)	0.38	82	-0.39 (-1.15, 0.36)	0.30
IgE[‡] (kU/L)	27	42.1 ± 3.62	59	31.20 ± 4.57	0.74 (0.38, 1.45)	0.38	82	0.68 (0.32, 1.43)	0.30
eNO (ppb)	20	23.50 ± 12.71	42	19.30 ± 9.28	-4.18 (-9.88, 1.52)	0.15	58	-3.34 (-9.44, 2.75)	0.28
Ln Eos	27	1.11 ± 0.70	53	1.01 ± 0.69	-0.10 (-0.43, 0.22)	0.53	76	-0.25 (-0.61, 0.11)	0.18
Eos[§] (%)	27	3.03 ± 2.01	53	2.75 ± 1.99	0.90 (0.65, 1.25)	0.53	76	0.78 (0.54, 1.12)	0.18

eNO (exhaled nitric oxide), Eos (eosinophil count as a % of total white cell count)

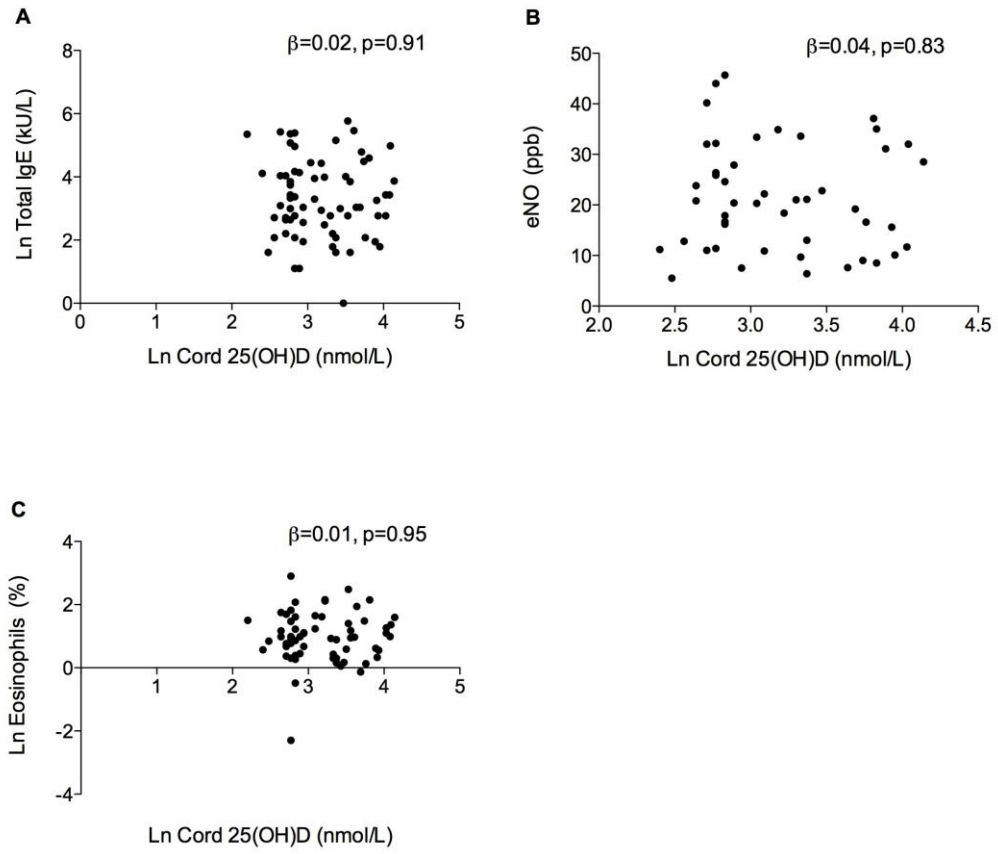
[1]Adjusted for mother's ethnic group, presence of household smokers, maternal smoking in pregnancy, exclusive breast-feeding to four months, any parental allergic history, any child vitamin supplementation, number of children in the household, age mother left full-time education and baseline concentration of 25(OH)D in maternal blood.

[‡]Ln IgE result transformed into geometric mean ± SD and multiplicative mean difference with 95% CI

[§]Ln Eos result transformed into geometric mean ± SD and multiplicative mean difference with 95% CI

When results for Total IgE, eNO and eosinophil counts were analysed by Ln cord 25(OH)D levels, no associations were found (Figure 5-4; Table 5.4).

Figure 5-3 Ln Cord vitamin D levels and markers of inflammation at age three years



Adjusted correlation (Beta coefficient, **β**) between Ln cord 25(OH)D levels and A) Ln Total IgE, B) exhaled nitric oxide (eNO) and C) Ln eosinophil % at age three years.

Table 5.4 Ln Cord 25(OH)D levels and markers of inflammation at age three years.

Outcome	n	β	p	n	Adjusted[1] β	p
Ln Total IgE (kU/L)	68	-0.05	0.72	65	0.02	0.91
eNO (ppb)	47	-0.05	0.73	44	0.04	0.83
Ln Eosinophils (%)	62	0.03	0.81	59	0.01	0.95

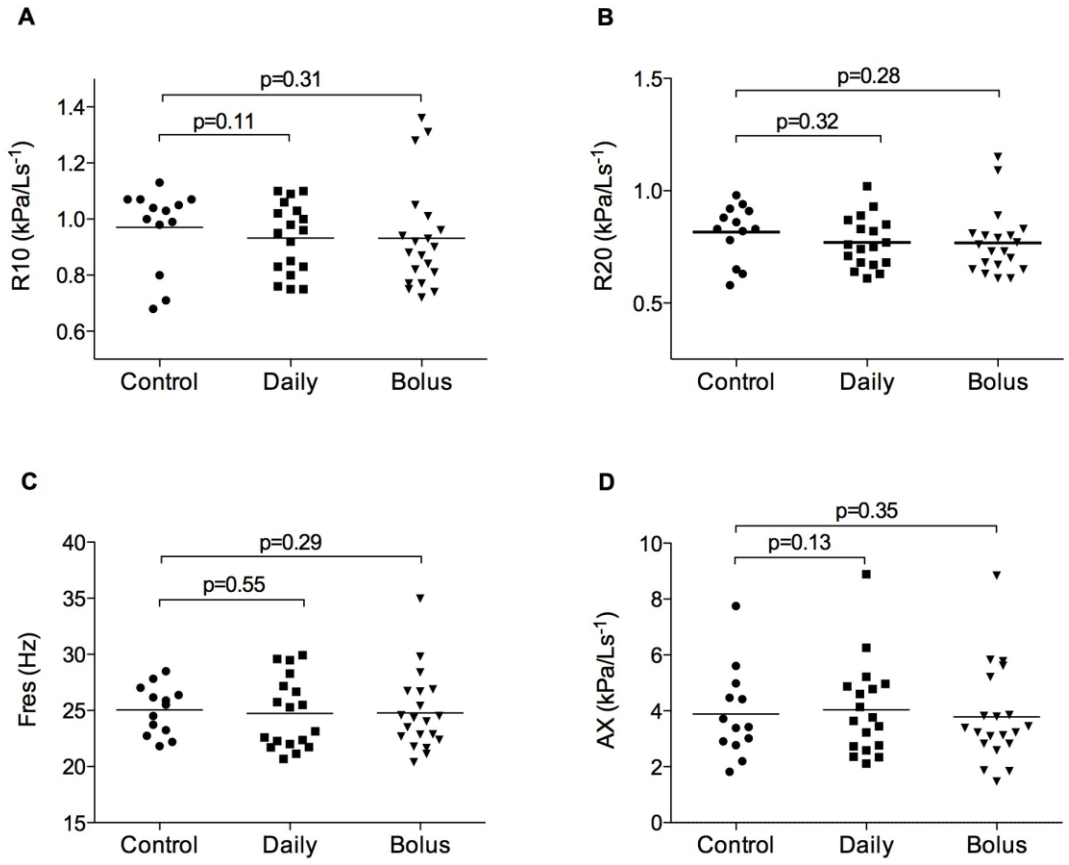
β (Beta coefficient)

[1]Adjusted for treatment group, mother's ethnic group, presence of household smokers, maternal smoking in pregnancy, exclusive breast-feeding to four months, any parental allergic history, any child vitamin supplementation, number of children in the household, age mother left full-time education and baseline concentration of 25(OH)D in maternal blood.

5.3.3 Effect of prenatal vitamin D supplementation on lung function and bronchodilator responsiveness

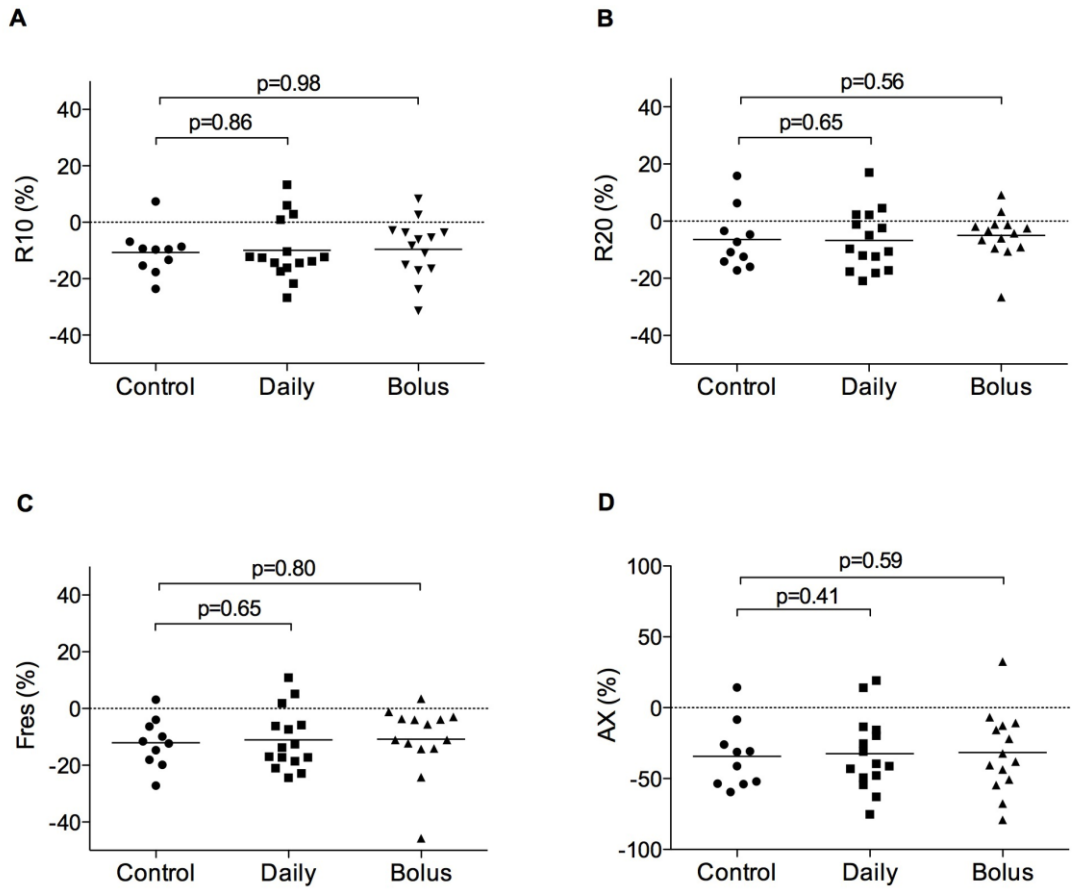
We found no significant difference between groups in either baseline respiratory resistance or BDR for R10, R20, Fres or AX (Baseline lung function, Figure 5-5; BDR, Figure 5-6).

Figure 5-4 Baseline lung function at age three years. Daily and bolus groups vs control.



Baseline A) Resistance at 10Hz (R10), B) R20, C) Resonant frequency (Fres) and D) Area under the reactance curve (AX). Control versus daily vitamin D or bolus vitamin D. Horizontal bars represent medians, p values are for adjusted analysis.

Figure 5-5 Bronchodilator response at age three years. Daily and bolus groups vs control.



Percentage change in lung function measured before and 15 minutes after inhalation of 400 mcg salbutamol for A) resistance at 10Hz (R10), B) R20, D) Resonant frequency (Fres) and D) Area under the reactance curve (AX). Control versus daily vitamin D or bolus vitamin D. Horizontal bars represent medians and p value are for adjusted analyses.

When data were analysed for the two forms of prenatal vitamin D supplementation combined, there was no significant difference between groups (Table 5.5).

Table 5.5 Baseline lung function and bronchodilator response at age three years. Combined vitamin D groups versus control.

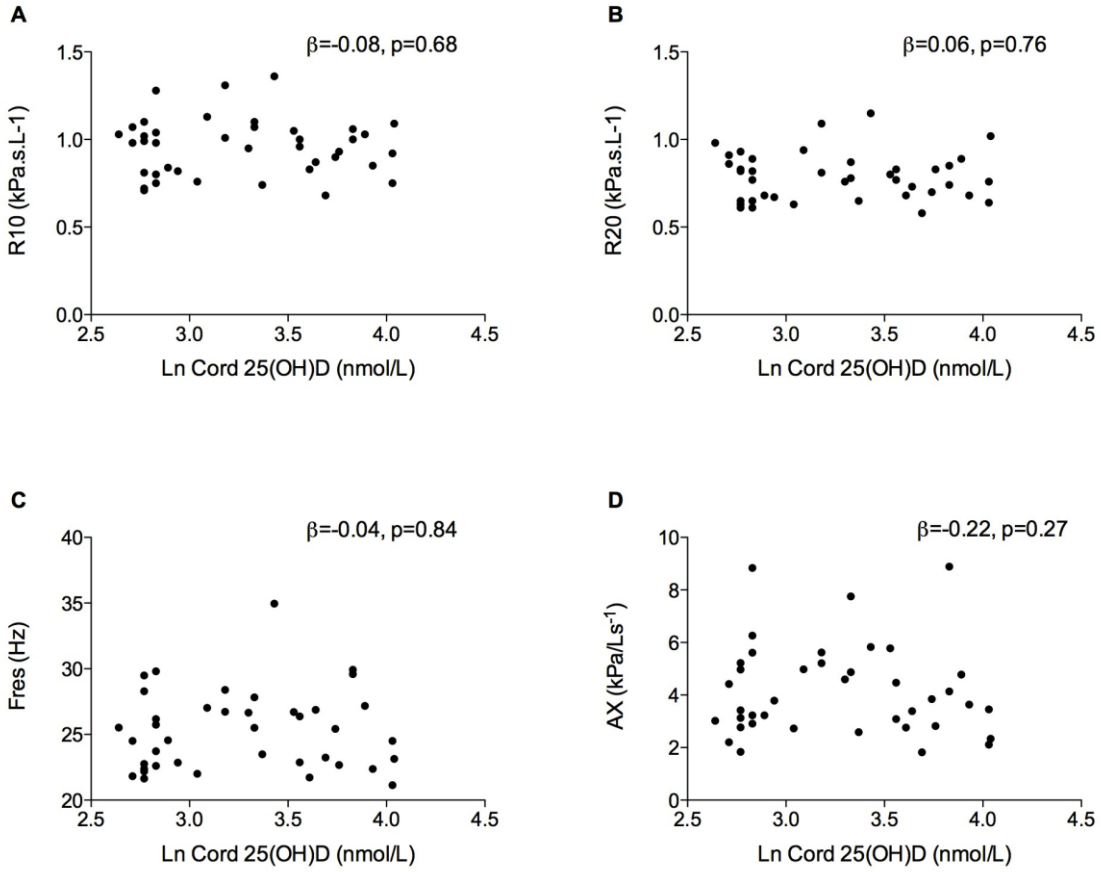
IOS parameter	n	Control Mean ± SD	n	Combined vitamin D Mean ± SD	Unadjusted mean difference (95% CI)	p	n	Adjusted[1] mean difference (95% CI)	p
R10 (kPa/L.s^{-1})	13	0.97 ± 0.14	38	0.93 ± 0.16	-0.04 (-0.14, 0.06)	0.44	49	-0.06 (-0.18, 0.07)	0.35
R20 (kPa/L.s^{-1})	13	0.82 ± 0.12	38	0.77 ± 0.13	-0.05 (-0.13, 0.04)	0.26	49	-0.05 (-0.15, 0.05)	0.36
Fres (Hz)	13	25.0 ± 2.17	38	24.8 ± 3.28	-0.28 (-2.25, 1.68)	0.77	49	-0.58 (-2.95, 1.79)	0.62
AX (kPa/L)	13	3.89 ± 1.59	38	3.90 ± 1.69	0.02 (-1.06, 1.10)	0.97	49	-0.43 (-1.69, 0.80)	0.48
R10 %Init	10	-10.66 ± 8.12	29	-9.74 ± 10.50	-0.91 (-8.33, 6.50)	0.80	37	-0.12 (-6.90, 6.67)	0.97
R20 %Init	10	-6.37 ± 10.52	29	-5.89 ± 9.31	-0.48 (-7.62, 6.67)	0.89	37	-1.26 (-8.69, 6.16)	0.73
Fres %Init	10	-12.07 ± 8.59	29	-10.92 ± 11.22	-1.15 (-9.05, 6.76)	0.77	37	0.59 (-8.41, 9.59)	0.89
AX% Init	10	-34.20 ± 23.30	29	-31.94 ± 26.92	-2.26 (-21.64, 17.12)	0.81	37	4.02 (-19.76, 27.81)	0.73

Baseline R10 and R20 (Resistance at 10 and 20 Hz), Fres (Resonant frequency) and AX (Area under the reactance curve). Parameters labelled %Init represent the percentage change from baseline, 15 minutes after inhalation of 400 mcg of salbutamol.

[1]Adjusted for mother's ethnic group, presence of household smokers, maternal smoking in pregnancy, exclusive breast-feeding to four months, any parental allergic history, any child vitamin supplementation, number of children in the household, age mother left full-time education, baseline concentration of 25(OH)D in maternal blood and sex, age and height of offpsring

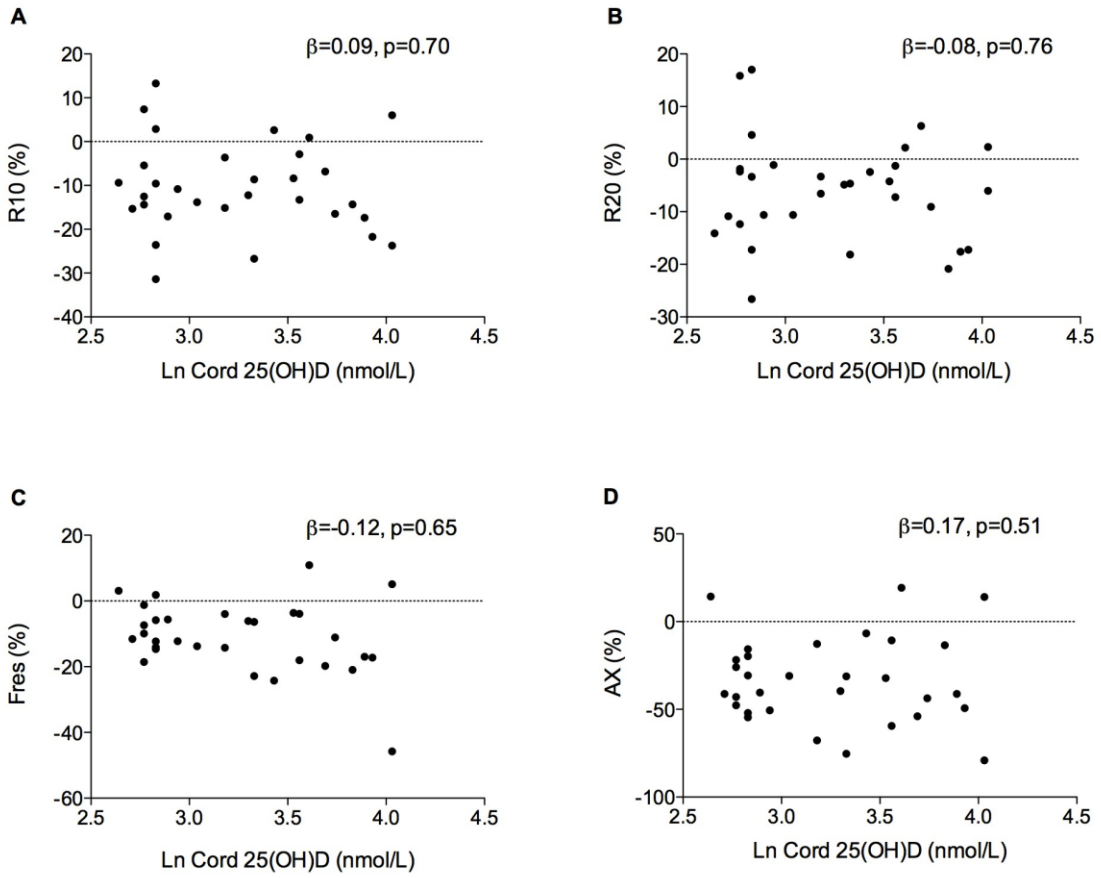
When results for lung function were analysed by Ln cord 25(OH)D levels, no associations were found (Baseline lung function, Figure 5-7; BDR Figure 5-8; All data Table 5.6).

Figure 5-6 Ln Cord 25(OH)D levels and baseline lung function at age three years.



Adjusted correlation (Beta coefficient, **β**) between Ln cord 25(OH)D levels and A) Resistance at 10Hz (R10), B) R20, C) Resonant frequency (Fres) and D) Area under the reactance curve (AX) at age three years.

Figure 5-7 Ln Cord 25(OH)D levels and bronchodilator response at age three years.



Percentage change in lung function measured before and 15 minutes after inhalation of 400 mcg of salbutamol for A) Resistance at 10Hz (R10), B) R20, C) Resonant frequency (Fres) and D) Area under the reactance curve (AX).

Table 5.6 Ln cord 25(OH)D levels and lung function at age three years.

Parameter	n	β	p	n	Adjusted[1] β	p
R10 (kPa/(L.s^{-1})	40	-0.02	0.91	38	-0.08	0.68
R20 (kPa/(L.s^{-1})	40	-0.01	0.97	38	0.06	0.76
Fres (Hz)	40	0.05	0.75	38	-0.04	0.84
AX (kPa/L)	40	-0.05	0.76	38	-0.22	0.27
R10 %Init	31	-0.08	0.68	29	0.09	0.70
R20 %Init	31	-0.10	0.58	29	-0.08	0.76
Fres %Init	31	-0.30	0.11	29	-0.12	0.65
AX %Init	31	-0.03	0.88	29	0.17	0.51

β (Beta coefficient). Baseline R10 and R20 (Resistance at 10 and 20 Hz), Fres (Resonant frequency) and AX (Area under the reactance curve). Parameters labelled %Init represent the percentage change from baseline 15 minutes after inhalation of 400 mcg of salbutamol.

[1]Adjusted for treatment group, mother's ethnic group, presence of household smokers, maternal smoking in pregnancy, exclusive breast-feeding to four months, any parental allergic history, any child vitamin supplementation, number of children in the household, age mother left full-time education, baseline concentration of 25(OH)D in maternal blood and age, sex and height of the child.

5.4 Discussion

In this randomised controlled trial of vitamin D supplementation in late pregnancy in a relatively deficient mixed race population, daily supplementation with 800 IU ergocalciferol or a single bolus dose of 200,000 IU cholecalciferol from 27 weeks gestation did not influence atopic sensitisation, IgE levels, exhaled nitric oxide or lung function in offspring at age three years.

These results are in agreement with observational data from most birth cohort studies (175, 288, 293). However, in one study, both low (<50nmol/L) and high (≥100nmol/L) levels cord 25(OH)D levels were associated with increased atopic sensitisation and IgE levels at 5 years (291), an effect that may be genetically determined (285). When we analysed our own data on cord blood 25(OH)D concentration and objective markers of atopy, inflammation and lung function at age first three years, no significant relationships were found. However, while our interventions did result in at least 50% higher cord blood 25(OH)D concentrations compared with no treatment, cord blood levels were still significantly lower in the intervention groups than in this study (291).

The results of our randomised controlled trial do not support a causal relationship between low prenatal vitamin D status within the deficient/insufficient range and increased risk of allergic sensitization or inflammation, or improved lung function in early childhood. We are unable to confidently exclude a similar relationship in more vitamin D sufficient populations.

6 Effect of prenatal vitamin D on healthcare utilisation in offspring

6.1 Introduction

The economic burden of allergic disease is an increasing problem, especially in developed countries within northern latitudes. In the United Kingdom, allergic conditions account for up to 6% of primary care utilization, 70,000 hospital admissions a year and 10% of the primary care prescribing costs, representing a direct burden of over a billion pounds per anum (402). Primary prevention of asthma and allergic disorders is a high priority.

Early life vitamin D deficiency is common (403), and has been proposed as a cause of the asthma epidemic (269), based on observational studies finding an increased risk of wheezing in the offspring of mothers with lower intake of vitamin D during pregnancy (175, 176, 289, 297). Vitamin D supplementation during pregnancy is safe in higher doses in studies with short term follow up (404) and inexpensive, and thus represents an attractive public health intervention for asthma prevention.

In the United Kingdom, all children registered with a general practice have an electronic health record (e-HR). This is a contemporaneous record of all health care utilisation from that practice. Use of primary out of hours services (OOH) or secondary healthcare is also recorded, providing a discharge summary is sent to the child's general practice, and the data electronically entered. When children move practices, the e-HR follows the child via the local health authority. In this way the e-HR forms a complete record of a child's healthcare utilisation from birth.

Determinants of a child's overall healthcare utilisation are their underlying health status (406-410), parent mental health and family functioning (411-413), and socioeconomic predictors (age and ethnicity of the child, family income, area of residence, parental educational attainment and social class) (406, 410, 414, 415), although some studies found no effect of

social class (407, 408). For hospital utilisation, predictors include socioeconomic position at the time of birth, mother's age, mother's age on leaving education, gestational age, ethnicity, parity, maternal BMI, maternal smoking during pregnancy and mode of delivery (415).

We hypothesized that prenatal vitamin D supplementation during pregnancy would reduce a child's healthcare utilisation during the first three years of life.

6.2 Methods

We analysed the child health records of the offspring of mothers who participated in the prenatal vitamin D supplementation study. Full methods are described in section 2.3.

6.3 Results

Of the 180 offspring from the original trial, 159 participated in the questionnaire and we successfully obtained 130 e-HRs from their GP's. 31 had incomplete data and could not be used, leaving 99/180 (55%) infants with complete e-HR data for analysis. An additional 12 records were incomplete but had at least one year's health data for analysis. Incomplete records were due to inadequate GP summaries or where the patient had moved GP practice over the last three years and we were unable to obtain the old or new records. Only records with complete data were used for primary analysis. The characteristics of children with complete records in each randomization group are shown in Table 6.1.

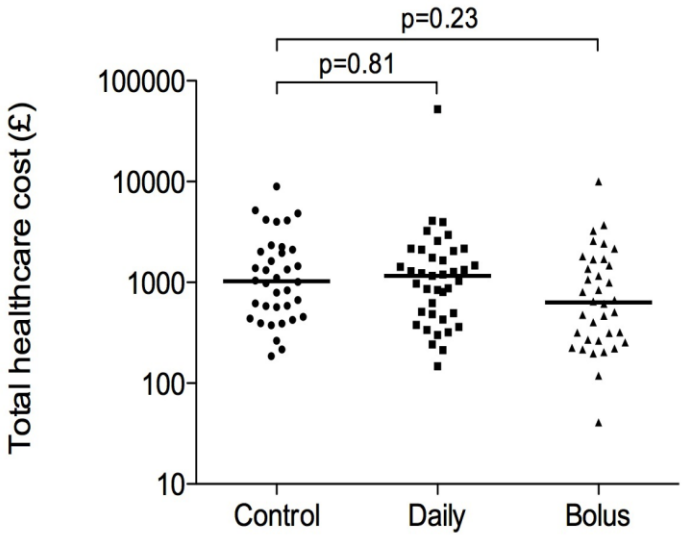
Table 6.1 Characteristics of children with complete e-HR data

	Control (n=31)	Daily (n=36)	Bolus (n=32)
Male sex, n (%)	14/31 (45)	22/36 (61)	16/32 (50)
Gestational age, weeks (SD)	39.92 (1.28)	39.34 (1.69)	39.72 (1.66)
Delivery by caesarean section, n (%)	10/31 (32)	10/36 (28)	10/32 (31)
Maternal Ethnicity, n (%)			
Asian	6/31 (19)	10/36 (28)	9/32 (28)
Middle Eastern	11/31 (35)	8/36 (22)	6/32 (19)
Black	5/31 (16)	8/36 (22)	8/32 (25)
Caucasian	9/31 (29)	10/36 (28)	9/32 (28)
Maternal age at booking, years (SD)	30 (7)	32 (6)	32 (6)
Maternal highest education, years (SD)	20 (3)	20 (4)	20 (3)
Family history of atopy, n (%)	13/29 (45)	23/35 (63)	15/31 (48)
Maternal smoking in pregnancy, n (%)	1/31 (3)	3/36 (8)	0/31 (0)
Number children in household, n (SD)	2 (1)	2 (1)	3 (1)
Multiparous, n (%)	19/31 (61)	27/36 (75)	20/32 (63)
Maternal BMI > 30 at booking, n (%)	9/31 (29)	4/36 (11)	4/32 (12.5)

6.3.1 Effect of prenatal vitamin D supplementation on health care utilization

There was no significant difference in the primary outcome measure, total healthcare utilisation, between treatment groups (Figure 6-1).

Figure 6-1 Effect of prenatal vitamin D randomisation on total healthcare utilisation in the first three years of life.



Horizontal bars represent medians and p values are for adjusted analyses.

We found no significant difference in secondary outcomes between offspring in the daily treatment group compared to controls (Table 6.2). There was weak evidence that secondary care health utilisation costs (p=0.048) and year 3 costs (p=0.045) were reduced in the bolus group (Table 6.3). When treatment groups were combined for analysis, no difference was found from controls (Table 6.4).

Table 6.2.Healthcare utilisation in the first three years of life. Daily vitamin D versus control.

	n	Control Mean ± SD or Median (IQR)	n	Daily Mean ± SD or Median (IQR)	Mean difference (95% CI)	p	n	Adjusted[1] mean difference (95% CI	p
Ln total cost	31	7.07 ± 0.99	36	7.08 ± 0.99	0.02 (-0.47, 0.50)	0.95	53	0.07 (-0.48, 0.62)	0.81
Ln all Visits	31	7.03 ± 0.99	36	7.02 ± 1.01	-0.01 (-0.50, 0.47)	0.95	53	0.02 (-0.53, 0.58)	0.93
Ln prescription cost	31	3.23 ± 1.83	36	3.57 ± 1.12	0.34 (-0.40, 1.08)	0.36	53	0.50 (-0.36, 1.36)	0.25
Ln year 1 cost	31	5.66 ± 2.02	36	5.95 ± 1.49	0.29 (-0.56, 1.15)	0.50	53	0.51 (-0.46, 1.48)	0.30
Year 2 cost	31	247 (103, 607)	36	248 (123, 487)	-	0.92	-	-	-
Year 3 cost	31	239 (103, 552)	36	212 (96, 463)	-	0.57	-	-	-
Ln primary care cost	31	5.96 ± 0.98	36	6.11 ± 0.62	0.14 (-0.25, 0.54)	0.47	53	0.29 (-0.15, 0.72)	0.20
Secondary care cost	31	632 (206, 1853)	36	368 (155, 1502)	-	0.42	-	-	-
Wheeze cost	31	0 (0, 0)	36	0 (0, 0)	-	0.71	-	-	-
Cough cost	31	0 (0, 36)	36	0 (0, 36)	-	0.26	-	-	-
URTI cost	31	180 (36, 324)	36	150 (54, 234)	-	0.78	-	-	-
LRTI cost	31	0 (0, 0)	36	0 (0, 0)	-	0.75	-	-	-
Bronchiolitis cost	31	0 (0, 0)	36	0 (0, 0)	-	0.89	-	-	-
Ln overall resp cost	31	5.14 ± 1.83	36	4.95 ± 1.65	-0.19 (-1.15, 0.76)	0.69	53	0.10 (-1.06, 1.26)	0.86
Food allergy cost	31	0 (0, 0)	36	0 (0, 0)	-	0.35	-	-	-

Eczema cost	31	0 (0, 88)	36	0 (0, 36)	-	0.62	-	-	-

URTI (Upper respiratory tract infection), LRTI (Lower respiratory tract infection).

[1]Adjusted for mother's age, mother's age on leaving education and gestational age as potential covariates, and ethnicity (Caucasian, Asian, Middle Eastern, Black), parity, BMI>30, smoking during pregnancy, mode of delivery (vaginal, instrumental or caesarean) as potential cofactors.

Table 6.3 Healthcare utilization in the first three years of life. Bolus vitamin D versus control.

	n	Control Mean ± SD or Median (IQR)	n	Bolus Mean ± SD or Median (IQR)	Mean difference	p	n	Adjusted[1] mean difference	p
Ln total cost*	31	7.07 ± 0.99	31	6.61 ± 1.06	-0.46 (-0.97, 0.06)	0.08	49	-0.34 (-0.90, 0.22)	0.23
Ln all visit costs	31	7.03 ± 0.99	32	6.56 ±1.08	0.47(-0.99, 0.05)	0.08	49	-0.36 (-0.93, 0.21)	0.21
Ln all prescription cost	31	3.23 ± 1.82	32	3.11 ±1.53	0.11 (-0.96, 0.73)	0.79	49	0.21 (-0.67, 1.10)	0.63
Ln year 1 cost	31	5.66 ± 2.02	32	5.41 ±1.27	0.25 (-1.09, 0.60)	0.56	49	0.02 (-0.86, 0.90)	0.96
Year 2 cost	31	247 (103, 607)	32	202 (101, 603)	-	0.75	-	-	-
Year 3 cost	31	239 (103, 552)	32	142 (36, 333)	-	0.04	-	-	-
Ln primary care cost	31	5.96 ± 0.98	32	5.95 ±0.82	0.02 (-0.47, 0.44)	0.95	49	0.01 (-0.45, 0.47)	0.98
Secondary care cost	31	632 (206, 1853)	32	313 (0, 848)	-	0.04	-	-	-
Wheeze visit cost	31	0 (0, 0)	32	0 (0, 0)	-	0.22	-	-	-
Cough visit cost	31	0 (0, 36)	32	0 (0, 36)	-	0.59	-	-	-
URTI visit cost	31	180 (36, 324)	32	108 (72, 255)	-	0.91	-	-	-
LRTI visit cost	31	0 (0, 0)	32	0 (0, 36)	-	0.31	-	-	-
Bronchiolitis visit cost	31	0 (0, 0)	32	0 (0, 0)	-	0.59	-	-	-
Ln overall resp cost	31	5.14 ± 1.84	32	4.92 ±2.15	-0.22 (-1.23, 0.79)	0.66	49	-0.17 (-1.31, 0.97)	0.77
Food allergy cost	31	0 (0, 0)	32	0 (0, 0)	-	1.00	-	-	-

Eczema cost	31	0 (0, 88)	32	0 (0, 36)	-	0.77	-	-	-

URTI (Upper respiratory tract infection), LRTI (Lower respiratory tract infection).

[1]Adjusted for mother's age, mother's age on leaving education and gestational age as potential covariates, and ethnicity (Caucasian, Asian, Middle Eastern, Black), parity, BMI>30, smoking during pregnancy, mode of delivery (vaginal, instrumental or caesarean) as potential cofactors.

Table 6.4 Healthcare utilization costs in the first three years of life. Combined vitamin D versus control.

	n	Control Mean ± SD or Median (IQR)	n	Combined Mean ± SD or Median (IQR)	Mean difference	p	n	Adjusted* mean difference	p
Ln total cost*	31	7.07 ± 0.99	68	6.86 ± 1.05	-0.21 (-0.65, 0.24)	0.36	84	-0.16 (-0.64, 0.33)	0.52
Ln all visit cost	31	7.03 ± 0.99	68	6.80 ± 1.06	-0.24 (-0.67, 0.22)	0.31	84	-0.19 (-0.68, 0.30)	0.45
Ln all prescription cost	31	3.23 ± 1.82	68	3.35 ± 1.36	0.13 (-0.53, 0.78)	0.70	84	0.29 (-0.40, 0.98)	0.41
Ln year 1 total cost	31	5.66 ± 2.02	68	5.70 ± 1.39	0.04 (-0.66, 0.73)	0.91	84	0.22 (-0.53, 0.97)	0.56
Year 2 total cost	31	247 (103, 607)	68	244 (108, 558)	-	0.81	-	-	-
Year 3 total cost	31	239 (103, 552)	68	168 (60, 430)	-	0.14	-	-	-
Ln primary care visit cost	31	5.96 ± 0.98	68	6.03 ± 0.72	0.07 (-0.28, 0.42)	0.70	84	0.15 (-0.22, 0.51)	0.42
Secondary care visit cost	31	632 (206, 1853)	68	316 (103, 1050)	-	0.11	-	-	-
Wheeze visit cost	31	0 (0,0)	68	0 (0, 0)	-	0.37	-	-	-
Cough visit cost	31	0 (0, 36)	68	0 (0, 36)	-	0.33	-	-	-
URTI visit cost	31	180 (36, 324)	68	130 (72, 252)	-	0.82	-	-	-
LRTI visit cost	31	0 (0, 0)	68	0 (0, 24)	-	0.68	-	-	-
Bronchiolitis visit cost	31	0 (0, 0)	68	0 (0, 0)	-	0.70	-	-	-
Ln overall resp cost	31	5.14 ± 1.84	68	4.93 ± 2.08	-0.21 (-1.07, 0.66)	0.64	84	-0.02 (-0.99, 0.95)	0.97
Food allergy cost	31	0 (0, 0)	68	0 (0, 0)	-	0.50	-	-	-

Eczema cost	31	0 (0, 88)	68	0 (0, 36)	-	0.90	-	-	-

URTI (Upper respiratory tract infection), LRTI (Lower respiratory tract infection).

[1]Adjusted for mother's age, mother's age on leaving education and gestational age as potential covariates, and ethnicity (Caucasian, Asian, Middle Eastern, Black), parity, BMI>30, smoking during pregnancy, mode of delivery (vaginal, instrumental or caesarean) as potential cofactors.

6.3.2 Effect of randomisation on number of prescriptions for eczema and asthma

There was no difference in the number of prescriptions issued for medicated skin creams [Median 1.0 IQR (0.0, 2.0) combined vitamin D versus 0.0 (0.0, 2.0) controls; p=0.27] or wheezing medications [Median 0.0 IQR (0.0, 1.0) combined vitamin D versus 0.0 (0.0, 0.0) controls; p=0.42].

Prescribing data were dichotomized according to whether any prescription was made in the first three years of life. There was weak evidence that offspring of mothers who received bolus vitamin D were more likely to have been prescribed a wheezing medication (p=0.05), and that offspring of mothers who received daily vitamin D were more likely to have been prescribed an eczema medication (p=0.04) (Table 6.5).

Table 6.5 Any prescription for wheezing or eczema. Daily, bolus and combined groups vs controls.

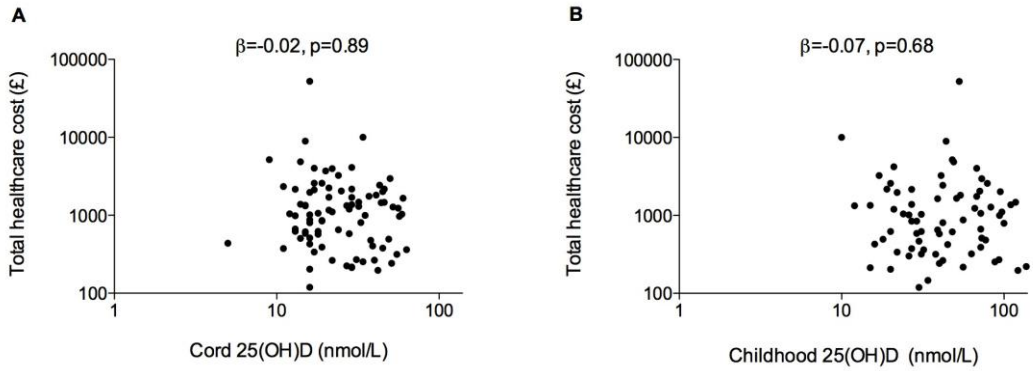
	Control n (%)	Treatment group, n (%)	Unadjusted OR (95%CI)	p	n	aOR[1] (95% CI)	p
Wheezing medications							
Daily vs controls	4/31 (12.9)	8/36 (22.2)	1.93 (0.66, 1.97)	0.32	66	3.19 (0.57, 17.77)	0.19
Bolus vs controls	4/31 (12.9)	11/32 (34.4)	3.54 (0.98, 12.70)	0.05	62	4.07 (0.98, 16.84)	0.05
Combined vs controls	4/31 (12.9)	19/68 (27.9)	2.62 (0.81, 8.49)	0.11	96	3.08 (0.85, 11.24)	0.09
Eczema medications							
Daily vs controls	14/31 (45.2)	22/36 (61.1)	1.91 (0.72, 5.06)	0.19	66	3.60 (1.05, 12.34)	0.04
Bolus vs controls	14/31 (45.2)	15/32 (46.9)	1.07 (0.40, 2.89)	0.89	62	2.13 (0.60, 7.53)	0.24
Combined vs controls	14/31 (45.2)	37/68 (54.4)	1.45 (0.62, 3.40)	0.39	96	2.22 (0.82, 6.02)	0.12

aOR (Adjusted odds ratio). [1]Adjusted for mother's age, mother's age on leaving education and gestational age as potential covariates, and ethnicity (Caucasian, Asian, Middle Eastern, Black), parity, BMI>30, smoking during pregnancy and mode of delivery (vaginal, instrumental or caesarean) as potential cofactors.

6.3.3 Effect of 25(OH)D level on health care utilization

Of the 99 offspring with complete e-HR data, cord levels of 25(OH)D_2 were available for 79, and levels at age three years for 65. There was no correlation between either of these measures with any healthcare utilisation outcome (Both analyses Figure 6-2; Ln Cord 25(OH)D, Table 6.6; Ln Childhood 25(OH)D, Table 6.7).

Figure 6-2 Cord and childhood 25(OH)D levels and total healthcare utilisation in the first three years of life.



Adjusted correlation (Beta coefficient, β) between 25(OH)D levels measured at A) Birth and B) Age three years, and total health care utilisation by age three years.

Table 6.6 Cord 25(OH)D levels and healthcare utilisation in the first three years of life.

Outcome	n	β or ρ	p	n	Adjusted[1] β	p
Ln total healthcare	79	-0.06	0.58	76	-0.02	0.89
Ln all visit cost	79	-0.07	0.54	76	-0.01	0.93
Ln all prescription cost	79	-0.03	0.78	76	0.12	0.36
Ln year 1 cost	79	-0.07	0.57	76	0.01	0.94
Year 2 cost	79	-0.04	0.76	-	-	-
Year 3 cost	79	0.07	0.57	-	-	-
Ln primary care cost	79	-0.01	0.96	76	0.12	0.36
Secondary care cost	79	-0.06	0.62	-	-	-
Wheeze cost	79	0.01	0.95	-	-	-
Cough cost	79	-0.003	0.98	-	-	-
UTRI cost	79	-0.09	0.42	-	-	-
LRTI cost	79	0.12	0.28	-	-	-
Bronchiolitis cost	79	-0.07	0.55	-	-	-
Ln Overall Resp cost	79	-0.10	0.35	76	-0.05	0.74
Food allergy cost	79	-0.02	0.86	-	-	-
Eczema cost	79	0.18	0.12	-	-	-

β (Beta coefficient), ρ (Spearmans correlation coefficient), URTI (Upper respiratory tract infection, LRTI (Lower respiratory tract infection).

[1]Adjusted for treatment group, mother's age, mother's age on leaving education and gestational age as potential covariates, and ethnicity (Caucasian, Asian, Middle Eastern, Black), parity, BMI>30, smoking during pregnancy, mode of delivery (vaginal, instrumental or caesarean) as potential cofactors.

Table 6.7 Childhood 25(OH)D levels and healthcare utilisation in the first three years of life.

Outcome	n	β or ρ	p	n	Adjusted[1] β	p
Ln total healthcare	65	-0.08	0.52	63	-0.07	0.68
Ln all visit cost	65	-0.09	0.49	63	-0.08	0.66
Ln all prescription cost	65	0.02	0.88	63	-0.007	0.97
Ln year 1 cost	65	-0.06	0.63	63	-0.05	0.76
Year 2 cost	65	-0.08	0.52	-	-	-
Year 3 cost	65	-0.15	0.24	-	-	-
Ln primary care cost	65	0.02	0.86	63	0.12	0.93
Secondary care cost	65	-0.05	0.70	-	-	-
Wheeze cost	65	0.05	0.72	-	-	-
Cough cost	65	0.07	0.56	-	-	-
UTRI cost	65	-0.16	0.21	-	-	-
LRTI cost	65	-0.09	0.50	-	-	-
Bronchiolitis cost	65	-0.01	0.94	-	-	-
Ln overall Resp cost	65	-0.20	0.11	63	-0.14	0.45
Food allergy cost	65	-	-	-	-	-
Eczema cost	65	0.02	0.86	-	-	-

β (Beta coefficient), ρ (Spearmans correlation coefficient), URTI (Upper respiratory tract infection, LRTI (Lower respiratory tract infection).

[1]Adjusted for treatment group, mother's age, mother's age on leaving education and gestational age as potential covariates, and ethnicity (Caucasian, Asian, Middle Eastern, Black), parity, BMI>30, smoking during pregnancy, mode of delivery (vaginal, instrumental or caesarean) as potential cofactors.

6.4 Discussion

To our knowledge, this is the first randomized controlled trial of prenatal vitamin D supplementation to have assessed healthcare utilisation in offspring. We evaluated the overall evidence that prenatal vitamin D supplementation, at two specific doses, can reduce health care utilisation in the first three years of life. We found no consistent evidence for a treatment effect. Furthermore, we found no evidence that prenatal vitamin D status is inversely associated with healthcare utilization, which suggests that the very high prevalence of prenatal vitamin D deficiency seen in many populations across the world may not have major health implications for children in the short term.

These data contrast with studies in other populations, which have suggested an inverse relationship between cord blood vitamin D level and child health outcomes (290). There are a number of possible explanations for this. First, healthcare utilization as an outcome is rather crude, with large health effects required to influence global healthcare utilisation. However, the national framework for health provision in the UK provides a powerful resource of objective information, the merit of which has been validated on systematic review (444, 445). When prevalence based on diagnosis and prescribing patterns are taken together, as has been done here, the reliability of this resource further increases (446). Second, this is a relatively small study. However, the high nature of the p-values and narrow confidence intervals taken with the lack of effect from confounders on multivariate analysis lends strength to our conclusions from this relatively small data set. Overall these data suggest that a clinically meaningful relationship between vitamin D in cord-blood or at three years of age and healthcare utilization is unlikely. Third, the timing and dose of vitamin D in our study. While our interventions did result in at least 50% higher cord blood 25(OH)D concentrations compared with no treatment, cord blood levels were significantly lower in our intervention groups [daily dose 26nmol/l (IQR 17-45); bolus dose 25nmol/l (IQR 18-34)] than in studies that have found protective effects of higher early life 25(OH)D levels on wheezing

or respiratory tract infections in early childhood [Camargo 44nmol/L (IQR 29-78) (290); Morales 73.75nmol/L (IQR 56.3-92.8) (292); Belderbos 82nmol/L (SE 3.5nmol/L) (294)].

Other limitations of this study include the following. The follow up rate of only 55% of the original offspring could have led to selection bias. Analyses with imputation for missing data were not undertaken due to the relatively small sample size. This was a diverse ethnic group. Variation in healthcare use has been reported between ethnic minorities in the UK, with lower levels of asthma diagnosis and treatment in those from the Indian subcontinent (447, 448) but higher frequency of consultation (449, 450). However, multivariate analysis of our data showed that although maternal ethnicity correlated to vitamin D level there was no relationship between maternal ethnicity and our measures of healthcare utilization.

Interestingly we found weak evidence that secondary care health utilisation costs and year 3 costs were reduced in the bolus group only. This contrasts with our earlier finding of increased LRTI and bronchodilator use in the bolus group as a parentally reported outcome when children attended for research assessment. This disparity may be a consequence of the different follow up rates for the outcomes (55% for healthcare utilisation and 88% for parentally reported outcomes), and may also reflect underlying differences between the sample population who attended for assessment at three years, and the sample population we had for electronic health record data.

7 Assessment of lung function in 3 year old children whose mothers participated in a vitamin D supplementation trial during pregnancy using impulse oscillometry

7.1 Introduction

Like other investigators (59, 68-70, 72), our pilot study found that IOS could determine differences in lung function between children with and without a history of respiratory symptoms. We found that children with a history wheeze had significantly higher Fres and greater BDR. However, this study was limited by the relatively small sample size. As such the lung function results for children in the prenatal vitamin D follow up study were analysed to determine if the findings were replicable.

7.2 Methods

As for the pilot study (section 2.1). In addition, treatment allocation (daily, bolus or combined) was added as a cofactor in adjusted analyses.

7.3 Results

7.3.1 Success rate of IOS in preschool children

Acceptable baseline IOS data was acquired for 51/105 (48.6%) of children who attended for physical assessment, and a bronchodilator response in 39/105 (37.1%). The mean age of children able to perform successful IOS was 37.5 (SD 1.5) months.

7.3.2 Repeatability of measurements

CV was acceptable for most parameters, but commonly over 17% for X5 and AX (Table 7.1).

Table 7.1 Coefficient of variation for impulse oscillometry measurements

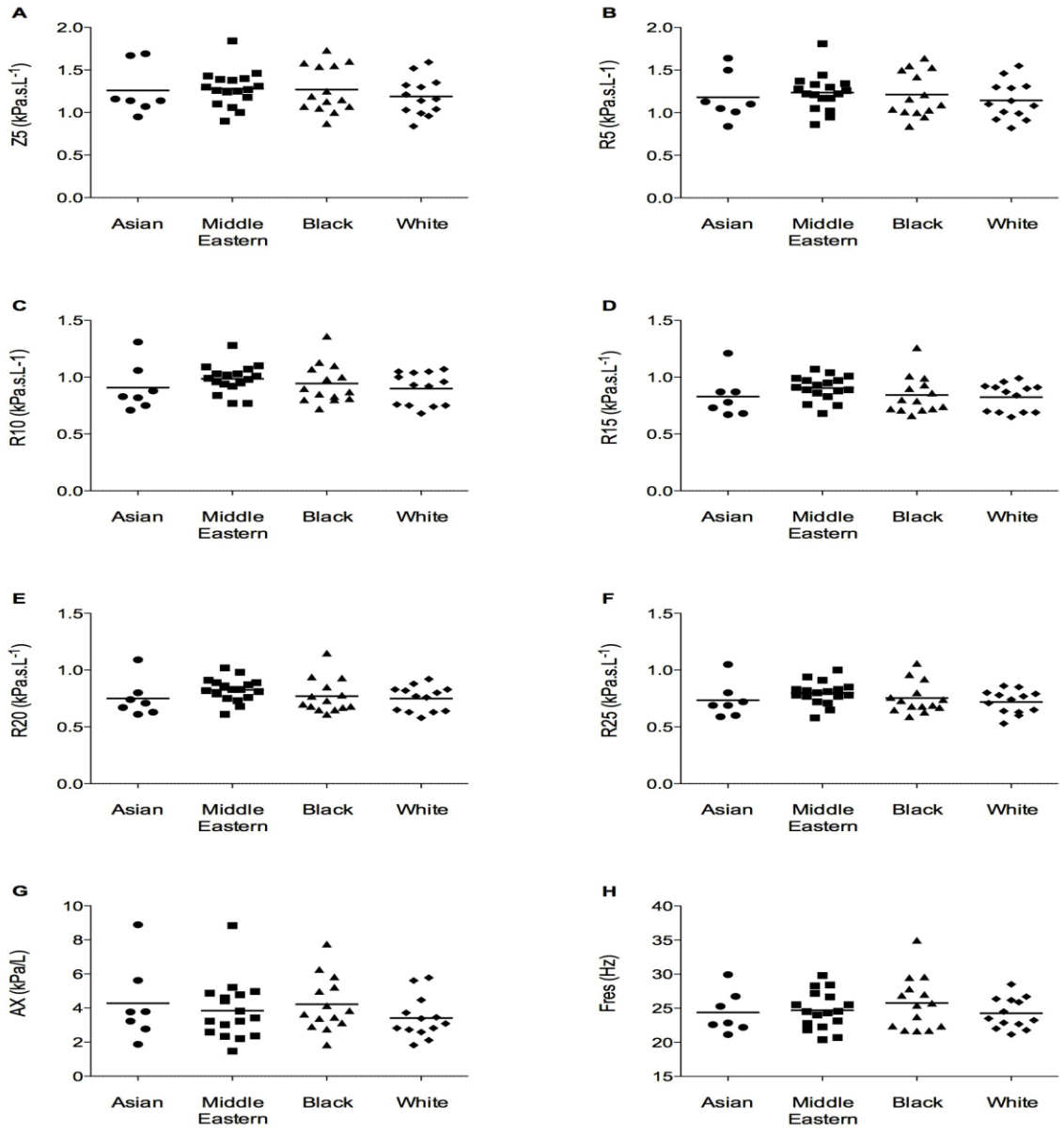
Parameter	Pre Median (IQR) (%)	Post Median (IQR) (%)
Z5	7.56 (4.43, 11.85)	8.23 (5.50, 10.75)
R5	5.72 (3.94, 8.46)	8.53 (6.27, 11.08)
R10	5.72 (3.94, 8.46)	6.30 (4.38, 9.17)
R15	6.01 (3.53, 9.05)	6.09 (4.32, 8.88)
R20	6.19 (3.76, 9.78)	6.42 (4.61, 9.40)
R25	6.78 (4.55, 9.73)	7.09 (4.59, 9.36)
X5	23.52 (15.04, 37.74)	28.72 (19.44, 57.14)
Fres	4.70 (3.31, 8.50)	6.54 (4.65, 8.99)
AX	17.44 (9.91, 26.83)	19.46 (13.23, 25.22)

Z5 (Impedance at 5Hz), R5 to R25 (resistance at 5 to 25Hz), X5 (Reactance at 5Hz), Fres (Resonant frequency) and AX (Area under the reactance curve).

7.3.2.1 Differences between ethnic groups

When baseline measurements from all children were combined for analysis, no significant differences were found between children of different ethnic groups as follows (Figure 7-1).

Figure 7-1 Baseline IOS parameters (main study) by different ethnic groups



Baseline IOS in children of different ethnic origins. A) Impedance at 5Hz (Z5), B) Resistance at 5Hz (R5), C) R10, D) R15, E) R20, F) R25, G) AX and H) Fres.

7.3.2.2 Differences in baseline lung function

There were no group differences between children with and without wheeze, atopic skin sensitisation, atopic wheezing, pre or postnatal ETS exposure or frequent URTI for baseline lung function (Tables 7.2 to 7.6).

Table 7.2 Baseline lung function in children with and without a history of wheeze (offspring of prenatal vitamin D study).

IOS Parameter	n	No wheeze Mean ± SD	n	Wheeze Mean ± SD	Mean difference (95% CI)	p	n	Adjusted[1] mean difference (95% CI)	p
Z5 (kPa.s.L)	36	1.26 ± 0.23	15	1.23 ± 0.28	-0.03 (-0.18, 0.12)	0.65	51	-0.05 (-0.21, 0.10)	0.50
R5 (kPa/Ls^{-1})	36	1.21 ± 0.23	15	1.16 ± 0.25	-0.05 (-0.19, 0.10)	0.51	51	-0.07 (-0.22, 0.09)	0.39
R10 (kPa/Ls^{-1})	36	0.96 ± 0.16	15	0.90 ± 0.13	-0.06 (-0.15, 0.04)	0.25	51	-0.07 (-0.17, 0.03)	0.18
R15 (kPa/Ls^{-1})	36	0.88 ± 0.15	15	0.81 ± 0.11	-0.06 (-0.15, 0.02)	0.15	51	-0.08 (-0.17, 0.01)	0.08
R20 (kPa/Ls^{-1})	36	0.80 ± 0.13	15	0.74 ± 0.10	-0.06 (-0.14, 0.02)	0.11	51	-0.08 (-0.16, 0.00)	0.06
R25 (kPa/Ls^{-1})	36	0.77 ± 0.13	15	0.71 ± 0.10	-0.06 (-0.13, 0.01)	0.11	51	-0.07 (-0.15, 0.00)	0.06
Fres (Hz)	36	24.72 ± 3.18	15	25.09 ± 2.67	0.37 (-1.51, 2.25)	0.69	51	0.17 (-1.83, 2.18)	0.86
AX (kPa/L)	36	3.79 ± 1.44	15	4.15 ± 2.11	0.36 (-0.66, 1.39)	0.48	51	0.36 (-0.73, 1.44)	0.51

Z5 (Impedance at 5Hz), R5 to R25 (Resistance at 5-25Hz), Fres (Resonant frequency) and AX (Area under the reactance curve).

[1]Adjusted for age, height, sex, ethnicity and randomisation.

Table 7.3 Baseline lung function in children with and without atopic skin sensitisation (offspring of prenatal vitamin D study).

IOS Parameter	n	Non-atopic Mean ± SD	n	Atopic Mean ± SD	Mean difference (95% CI)	p	n	Adjusted[1] mean difference (95% CI)	p
Z5 (kPa.s.L)	38	1.24 ± 0.24	12	1.30 ± 0.26	0.06 (-0.10, 0.22)	0.49	50	0.05 (-0.11, 0.22)	0.53
R5 (kPa/Ls^{-1})	38	1.19 ± 0.23	12	1.24 ± 0.26	0.04 (-0.11, 0.20)	0.59	50	0.04 (-0.12, 0.21)	0.59
R10 (kPa/Ls^{-1})	38	0.94 ± 0.14	12	0.97 ± 0.20	0.03 (-0.07, 0.14)	0.52	50	0.04 (-0.07, 0.15)	0.48
R15 (kPa/Ls^{-1})	38	0.85 ± 0.12	12	0.88 ± 0.19	0.03 (-0.07, 0.12)	0.55	50	0.06 (-0.03, 0.14)	0.20
R20 (kPa/Ls^{-1})	38	0.78 ± 0.11	12	0.80 ± 0.17	0.02 (-0.06, 0.11)	0.59	50	0.03 (-0.06, 0.12)	0.52
R25 (kPa/Ls^{-1})	38	0.76 ± 0.11	12	0.77 ± 0.16	0.01 (-0.07, 0.10)	0.72	50	0.02 (-0.06, 0.10)	0.64
Fres (Hz)	38	24.50 ± 2.85	12	25.98 ± 3.46	1.49 (-0.51, 3.49)	0.14	50	1.46 (-0.65, 3.57)	0.17
AX (kPa/L)	38	3.78 ± 1.76	12	4.36 ± 1.29	0.58 (-0.53, 1.68)	0.30	50	0.49 (-0.68, 1.65)	0.40

Z5 (Impedance at 5Hz), R5 to R25 (Resistance at 5-25Hz), Fres (Resonant frequency) and AX (Area under the reactance curve).

[1]Adjusted for age, height, sex, ethnicity and randomisation.

Table 7.4 Baseline lung function in children with and without atopic wheeze (offspring of prenatal vitamin D study).

IOS Parameter	n	No atopy or wheeze Mean ± SD	n	Atopy and wheeze Mean ± SD	Mean difference (95% CI)	p	n	Adjusted[1] mean difference (95% CI)	p
Z5 (kPa.s.L)	46	1.25 ± 0.24	4	1.29 ± 0.29	0.03 (-0.22, 0.29)	0.80	50	-0.02 (-0.29, 0.25)	0.89
R5 (kPa/Ls^{-1})	46	1.20 ± 0.24	4	1.22 ± 0.26	0.02 (-0.23, 0.26)	0.90	50	-0.04 (-0.31, 0.23)	0.79
R10 (kPa/Ls^{-1})	46	0.95 ± 0.16	4	0.91 ± 0.13	-0.03 (-0.20, 0.13)	0.68	50	-0.05 (-0.23, 0.13)	0.55
R15 (kPa/Ls^{-1})	46	0.86 ± 0.14	4	0.82 ± 0.10	-0.05 (-0.20, 0.10)	0.51	50	-0.06 (-0.22, 0.10)	0.44
R20 (kPa/Ls^{-1})	46	0.79 ± 0.13	4	0.74 ± 0.08	-0.05 (-0.19, 0.08)	0.46	50	-0.06 (-0.20, 0.08)	0.39
R25 (kPa/Ls^{-1})	46	0.76 ± 0.12	4	0.71 ± 0.07	-0.05 (-0.18, 0.08)	0.44	50	-0.06 (-0.20, 0.07)	0.35
Fres (Hz)	46	24.82 ± 3.12	4	25.20 ± 2.18	0.38 (-2.84, 3.60)	0.81	50	0.50 (-4.01 3.02)	0.78
AX (kPa/L)	46	3.87 ± 1.67	4	4.50 ± 1.70	0.63 (-1.12, 2.38)	0.47	50	0.35 (-1.56, 2.26)	0.72

Z5 (Impedance at 5Hz), R5 to R25 (Resistance at 5-25Hz), Fres (Resonant frequency) and AX (Area under the reactance curve).

[1]Adjusted for age, height, sex, ethnicity and randomisation.

Table 7.5 Baseline lung function in children with and without any ETS exposure (offspring of prenatal vitamin D study).

IOS Parameter	n	No ETS Mean ± SD	n	Any ETS Mean ± SD	Mean difference (95% CI)	p	n	Adjusted[1] mean difference (95% CI)	p
Z5 (kPa.s.L)	34	1.28 ± 0.25	17	1.19 ± 0.22	-0.09 (-0.23, 0.05)	0.20	51	-0.09 (-0.23, 0.06)	0.23
R5 (kPa/Ls^{-1})	34	1.22 ± 0.24	17	1.15 ± 0.23	-0.08 (-0.22, 0.06)	0.27	51	-0.07 (-0.21, 0.07)	0.32
R10 (kPa/Ls^{-1})	34	0.96 ± 0.16	17	0.90 ± 0.14	-0.06 (-0.15, 0.03)	0.20	51	-0.06 (-0.15, 0.04)	0.22
R15 (kPa/Ls^{-1})	34	0.88 ± 0.15	17	0.82 ± 0.12	-0.06 (-0.14, 0.02)	0.16	51	-0.06 (-0.14, 0.02)	0.16
R20 (kPa/Ls^{-1})	34	0.80 ± 0.13	17	0.74 ± 0.11	-0.06 (-0.13, 0.02)	0.12	51	-0.06 (-0.13, 0.02)	0.12
R25 (kPa/Ls^{-1})	34	0.78 ± 0.12	17	0.72 ± 0.11	-0.06 (-0.13, 0.02)	0.12	51	-0.05 (-0.13, 0.02)	0.13
Fres (Hz)	34	25.22 ± 3.21	17	24.06 ± 2.49	-1.15 (-2.94, 0.63)	0.20	51	-1.07 (-2.90, 0.76)	0.24
AX (kPa/L)	34	4.06 ± 1.64	17	3.58 ± 1.67	-0.47 (-1.46, 0.51)	0.34	51	-0.45 (-1.45, 0.55)	0.37

ETS (Pre or postnatal environmental tobacco smoke exposure), Z5 (Impedance at 5Hz), R5 to R25 (Resistance at 5-25Hz), Fres (Resonant frequency) and AX (Area under the reactance curve).

[1]Adjusted for age, height, sex, ethnicity and randomisation.

Table 7.6 Baseline lung function in children with and without frequent URTI (offspring of prenatal vitamin D study).

IOS Parameter	n	≤4 URTI/yr Mean ± SD	n	>4 URTI/yr Mean ± SD	Mean difference (95% CI)	p	n	Adjusted[1] mean difference (95% CI)	p
Z5 (kPa.s.L)	39	1.25 ± 0.24	12	1.26 ± 0.24	0.01 (-0.15, 0.17)	0.93	51	-0.02 (-0.18, 0.14)	0.79
R5 (kPa/Ls^{-1})	39	1.20 ± 0.24	12	1.20 ± 0.23	0.00 (-0.15, 0.16)	0.96	51	-0.02 (-0.18, 0.14)	0.79
R10 (kPa/Ls^{-1})	39	0.94 ± 0.14	12	0.95 ± 0.20	0.00 (-0.10, 0.11)	0.93	51	-0.01 (-0.12 0.09)	0.80
R15 (kPa/Ls^{-1})	39	0.85 ± 0.12	12	0.87 ± 0.19	0.02 (-0.07, 0.11)	0.68	51	-0.00 (-0.10 0.09)	0.99
R20 (kPa/Ls^{-1})	39	0.78 ± 0.11	12	0.79 ± 0.18	0.02 (-0.07, 0.10)	0.69	51	-0.00 (-0.09, 0.08)	0.96
R25 (kPa/Ls^{-1})	39	0.75 ± 0.11	12	0.77 ± 0.16	0.02 (-0.06, 0.10)	0.69	51	0.00 (-0.08, 0.08)	0.98
Fres (Hz)	39	24.80 ± 2.87	12	24.91 ± 3.58	0.11 (-1.91, 2.13)	0.91	51	-0.20 (-2.31, 1.90)	0.85
AX (kPa/L)	39	3.92 ± 1.74	12	3.82 ± 1.40	-0.10 (-1.21, 1.00)	0.85	51	0.22 (-1.37, 0.92)	0.70

URTI (Upper respiratory tract infection), Z5 (Impedance at 5Hz), R5 to R25 (Resistance at 5-25Hz), Fres (Resonant frequency) and AX (Area under the reactance curve).

[1]Adjusted for age, height, sex, ethnicity and randomisation.

7.3.2.3 Bronchodilator response

The response to administration of 400mcg of salbutamol in children with and without a history of wheezing, atopy, atopic wheezing, pre or postnatal ETS exposure and frequent URTI are given in Tables 7.7 to 7.11. There was no evidence for differences in BDR between these groups, other than weak evidence that atopic children had a smaller BDR for R25 compared to non-atopic children (p=0.05).

Table 7.7 Bronchodilator response in children with and without a history of wheezing (offspring of prenatal vitamin D study).

IOS Parameter	n	No wheeze Mean ± SD	n	Wheeze Mean ± SD	Mean difference (95% CI)	p	n	Adjusted[1] mean difference (95% CI)	p
Z5 %Init	26	-11.85 ± 13.99	13	-11.10 ± 7.95	0.75 (-7.75, 9.26)	0.86	39	-0.12 (-8.18, 7.94)	0.98
R5 %Init	26	-10.25 ± 14.85	13	-9.94 ± 8.25	0.31 (-8.69, 9.31)	0.94	39	-0.59 (-8.59, 7.77)	0.89
R10 %Init	26	-9.65 ± 11.24	13	-10.63 ± 6.60	-0.99 (-7.85, 5.88)	0.77	39	-1.80 (-7.07, 3.47)	0.49
R15 %Init	26	-8.11 ± 10.66	13	-9.05 ± 6.54	-0.94 (-7.49, 5.62)	0.77	39	-1.18 (-6.16, 3.79)	0.63
R20 %Init	26	-5.96 ± 10.62	13	-6.11 ± 7.09	-0.15 (-6.77, 6.47)	0.96	39	0.19 (-5.28, 5.66)	0.95
R25 %Init	26	-3.69 ± 10.11	13	-3.71 ± 7.68	-0.02 (-6.48, 6.45)	1.00	39	-0.00 (-5.45, 5.45)	1.00
Fres %Init	26	-12.26 ± 11.30	13	-9.12 ± 8.76	3.14 (-4.12, 10.39)	0.39	39	3.01 (-4.84, 10.86)	0.44
AX %Init	26	-34.26 ± 25.84	13	-29.05 ± 26.27	5.20 (-12.68, 23.08)	0.56	39	3.61 (-15.16, 22.38)	0.70

Z5 (Impedance at 5Hz), R5 to R25 (Resistance at 5-25Hz), Fres (Resonant frequency), AX (Area under the reactance curve), %Init (percentage change in parameter 15 minutes after 400 mcg inhaled salbutamol).

[1]Adjusted for age, height, sex, ethnicity and randomisation.

Table 7.8 Bronchodilator responses in children with and without a history of atopy (offspring of prenatal vitamin D study).

IOS Parameter	n	Non-atopic Mean ± SD	n	Atopic Mean ± SD	Mean difference (95% CI)	p	n	Adjusted[1] mean difference (95% CI)	p
Z5 %Init	29	-11.93 ± 12.42	9	-9.06 ± 11.69	2.87 (-6.62, 12.36)	0.54	38	-2.19 (-11.37, 6.99)	0.63
R5 %Init	29	-10.66 ± 12.95	9	-6.98 ± 13.01	3.68 (-6.35, 13.72)	0.46	38	-1.88 (-11.42, 7.67)	0.69
R10 %Init	29	-10.78 ± 9.94	9	-5.88 ± 8.42	4.90 (-2.55, 12.35)	0.19	38	0.09 (-5.98, 6.16)	0.98
R15 %Init	29	-9.23 ± 9.49	9	-4.50 ± 8.20	4.73 (-2.40, 11.87)	0.19	38	-0.30 (-6.01, 5.40)	0.91
R20 %Init	29	-7.00 ± 9.65	9	-1.58 ± 7.81	5.42 (-1.75, 12.60)	0.13	38	0.67 (-5.58, 6.91)	0.83
R25 %Init	29	-5.09 ± 8.82	9	2.01 ± 8.77	7.09 (0.28, 13.91)	0.04	38	2.92 (-3.20, 9.05)	0.34
Fres %Init	29	-10.21 ± 11.44	9	-14.07 ± 7.21	-3.87 (-12.11, 4.37)	0.35	38	-5.69 (-14.46, 3.08)	0.20
AX %Init	29	-31.32 ± 27.43	9	-34.22 ± 21.47	-2.90 (-23.19, 17.40)	0.77	38	-9.19 (-30.47, 12.09)	0.39

Z5 (Impedance at 5Hz), R5 to R25 (Resistance at 5-25Hz), Fres (Resonant frequency), AX (Area under the reactance curve), %Init (percentage change in parameter 15 minutes after 400 mcg inhaled salbutamol).

[1]Adjusted for age, height, sex, ethnicity and randomisation.

Table 7.9 Bronchodilator responses in children with and without a history of atopic wheezing (offspring of prenatal vitamin D study).

IOS Parameter	n	No atopy or wheeze Mean ± SD	n	Atopic wheeze Mean ± SD	Mean difference (95% CI)	p	n	Adjusted[1] mean difference (95% CI)	p
Z5 %Init	35	-11.54 ± 12.58	3	-7.87 ± 4.96	3.67 (-11.32, 18.66)	0.62	38	-5.47 (-20.64, 9.70)	0.47
R5 %Init	35	-10.09 ± 13.29	3	-6.33 ± 7.05	3.76 (-12.13, 19.65)	0.63	38	-6.02 (-21.76, 9.71)	0.44
R10 %Init	35	-9.86 ± 9.89	3	-6.85 ± 8.67	3.01 (-8.98, 14.99)	0.61	38	-4.59 (-14.52, 5.34)	0.35
R15 %Init	35	-8.30 ± 9.43	3	-5.86 ± 9.28	2.44 (-9.06, 13.94)	0.67	38	-3.41 (-12.80, 5.98)	0.46
R20 %Init	35	-5.94 ± 9.64	3	-3.13 ± 7.61	2.81 (-8.83, 14.45)	0.63	38	-1.76 (-12.13, 8.60)	0.73
R25 %Init	35	-3.79 ± 9.27	3	1.10 ± 8.59	4.89 (-6.38, 16.16)	0.38	38	1.50 (-8.80, 11.81)	0.77
Fres %Init	35	-11.16 ± 11.04	3	-10.62 ± 4.23	0.54 (-12.61, 13.69)	0.93	38	-3.64 (-18.56, 11.28)	0.62
AX %Init	35	-32.43 ± 26.93	3	-27.08 ± 6.41	5.35 (-26.64, 37.33)	0.74	38	-9.88 (-45.48, 25.72)	0.58

Z5 (Impedance at 5Hz), R5 to R25 (Resistance at 5-25Hz), Fres (Resonant frequency), AX (Area under the reactance curve), %Init (percentage change in parameter 15 minutes after 400 mcg inhaled salbutamol).

[1]Adjusted for age, height, sex, ethnicity and randomisation.

Table 7.10 Bronchodilator responses in children with and without a history of environmental tobacco exposure (offspring of prenatal vitamin D study).

IOS Parameter	n	No ETS exposure Mean ± SD	n	ETS exposure Mean ± SD	Mean difference (95% CI)	p	n	Adjusted[1] mean difference (95% CI)	p
Z5 %Init	26	-11.77 ± 11.50	13	-11.28 ± 13.98	0.49 (-8.02, 8.99)	0.91	39	1.02 (-6.64, 8.68)	0.79
R5 %Init	26	-10.55 ± 12.05	13	-9.34 ± 14.96	1.21 (-7.78, 10.20)	0.79	39	1.71 (-6.22, 9.65)	0.66
R10 %Init	26	-10.21 ± 8.46	13	-9.50 ± 12.56	0.71 (-6.16, 7.58)	0.84	39	0.66 (-4.38, 5.71)	0.79
R15 %Init	26	-8.72 ± 7.97	13	-7.83 ± 12.13	0.89 (-5.67, 7.44)	0.79	39	0.47 (-4.27, 5.22)	0.84
R20 %Init	26	-6.54 ± 8.78	13	-4.96 ± 11.09	1.58 (-5.02, 8.18)	0.63	39	0.56 (-4.23, 6.15)	0.71
R25 %Init	26	-4.10 ± 9.33	13	-2.88 ± 9.46	1.22 (-5.23, 7.67)	0.70	39	0.51 (-4.67, 5.69)	0.84
Fres %Init	26	-12.37 ± 11.70	13	-8.89 ± 7.46	3.48 (-3.75, 10.72)	0.34	39	4.08 (-3.32, 11.47)	0.27
AX %Init	26	-31.17 ± 27.74	13	-35.22 ± 22.04	-4.04 (-21.96, 13.87)	0.65	39	-1.93 (-19.82, 15.96)	0.83

ETS (Pre or postnatal environmental tobacco smoke exposure), Z5 (Impedance at 5Hz), R5 to R25 (Resistance at 5-25Hz), Fres (Resonant frequency), AX (Area under the reactance curve), %Init (percentage change in parameter 15 minutes after 400 mcg inhaled salbutamol).

[1]Adjusted for age, height, sex, ethnicity and randomisation.

Table 7.11 Bronchodilator response in children with and without a history of frequent URTI (offspring of prenatal vitamin D study).

IOS Parameter	n	≤4 URTI/year Mean ± SD	n	>4 URTI/year Mean ± SD	Mean difference (95% CI)	p	n	Adjusted[1] mean difference (95% CI)	p
Z5 %Init	31	-12.22 ± 12.59	8	-9.22 ± 10.94	3.00 (-6.88, 12.88)	0.54	39	-0.84 (-10.30, 8.61)	0.86
R5 %Init	31	-10.73 ± 13.22	8	-7.87 ± 12.16	2.87 (-7.60, 13.33)	0.58	39	-1.46 (-11.26, 8.35)	0.76
R10 %Init	31	-10.89 ± 9.13	8	-6.44 ± 12.31	4.45 (-3.43, 12.34)	0.26	39	0.61 (-5.62, 6.83)	0.84
R15 %Init	31	-9.28 ± 8.59	8	-5.11 ± 12.16	4.17 (-3.36, 11.70)	0.27	39	0.46 (-5.40, 6.32)	0.87
R20 %Init	31	-6.68 ± 9.27	8	-3.43 ± 10.55	3.25 (-4.41, 10.90)	0.40	39	0.60 (-5.82, 7.01)	0.85
R25 %Init	31	-4.34 ± 9.14	8	-1.19 ± 9.96	3.15 (-4.32, 10.62)	0.40	39	0.67 (-5.73, 7.06)	0.83
Fres %Init	31	-11.77 ± 11.09	8	-9.04 ± 8.09	2.74 (-5.77, 11.24)	0.52	39	0.57 (-8.73, 9.87)	0.90
AX %Init	31	-33.89 ± 27.78	8	-27.22 ± 15.95	6.67 (-14.18, 27.53)	0.52	39	0.09 (-22.00, 22.17)	0.99

URTI (Upper respiratory tract infection), Z5 (Impedance at 5Hz), R5 to R25 (Resistance at 5-25Hz), Fres (Resonant frequency), AX (Area under the reactance curve), %Init (percentage change in parameter 15 minutes after 400 mcg inhaled salbutamol).

[1]Adjusted for age, height, sex, ethnicity and randomisation.

7.4 Discussion

We have found that in a study of preschool children aged three years, measurement of baseline lung function or response to bronchodilator with IOS was unable to discriminate between children with and without a history of wheezing. Furthermore, unlike in our pilot study, no differences in impulse oscillometry were found between ethnic groups.

There are a number of possibilities as to why no differences between ethnic groups were found in this analysis. The pilot study was a convenience sample from outpatients, and so there may have been many sources of bias accentuating ethnic differences in lung function which were not present in the offspring of the randomized trial.

For baseline lung function, this is in keeping with other studies (65, 67, 68, 70). For BDR, only one other study has similar findings (67), with the majority finding increased BDR in wheezy children compared to controls (59, 68-71). Our pilot study found increased BDR for R25 in wheezy children. There are a number of possible explanations for these differences. First, offspring of mothers who received a bolus of 200,000 IU of vitamin D in pregnancy had increased bronchodilator use compared to controls, and thus it is possible the intervention itself influenced the result. Second, the intervention selected mothers from four ethnic groups, which may have made it harder to discern small differences between groups (429). Third, in the pilot study, and positive studies in the literature, children with wheeze were recruited from hospital based asthma clinics. This may have resulted in selecting cases with a more severe wheezing phenotype. We note that the one study finding no difference in BDR between children with and without asthma recruited from a Kindergarten (451). Fourth, we looked for differences between children with and without any episode of wheeze as opposed to 'asthma'. Although diagnosing asthma in children under six years of age is of limited utility (13), using more stringent criteria may have selected out children with more severe lung impairment that would have enabled us to discern differences. However, we did

not find any differences when evaluating atopic wheezers separately. Fifth, the mean age of children in this study was 37 months, considerably lower than published studies which range from 4.1 to 7.7 years. Finally, although a larger group than the pilot study, this was still a relatively small sample compared to the majority of published studies.

Our study used consistent methodology. However, lung function was performed by two different operators in the pilot (UB) and main study (SG). For bronchodilator responses, the number of study subjects is relatively small compared to other studies. We used a stringent process to check and maintain quality of data. Whilst this led to the analysis of high quality data, which is reflected in our low CVs for most parameters, this also meant a significant proportion of IOS data was not analysed. At present there are no standards for post acquisition IOS quality control in preschool children.

In conclusion, in this study of preschool children of mixed ethnicity who are the offspring of an intervention trial of prenatal vitamin D supplementation, IOS was unable to discriminate between children with and without a history of wheezing. Further research should evaluate the role of IOS in assessing longitudinal changes to therapy in individual children.

8 Discussion

8.1 Assessment of lung function in preschool children using impulse oscillometry

8.1.1 Summary of findings

1. Acceptable IOS data acquired in 56% of children (pilot study) and 49% (follow up study). In the pilot study, IOS was more successful in children aged over 42 months. The CVs were acceptable, ranging from 3.5 to 8.8%, except for AX and X5 which were considerably higher.
2. No significant differences in baseline impulse oscillometry measurements between children with and without wheeze, atopy, ETS exposure or frequent URTI.
3. In the pilot study, ethnic differences in lung function were found that were not replicated in the main study population.

8.1.2 Consistency with other studies

The majority of studies have found increased BDR in wheezy children compared to controls (59, 68-71). The most likely explanation for us not consistently observing this difference are that unlike studies in the literature, children in both the pilot study and vitamin D follow up study came from a wide range of ethnicities, which may have made it harder to discern small differences between groups (429). Second, studies that found a difference in BDR in children with and without asthma all recruited from hospital based clinics, which may have lead to selecting wheezy children with a more 'severe' phenotype. In the study that found no difference in BDR, children were recruited from a community based Kindergarten (451), to which the vitamin D follow up offspring may be more similar to.

8.1.3 Strengths and weaknesses of the data

Our study used consistent methodology. However, lung function was performed by two different operators in the pilot (UB) and main study (SG). For bronchodilator responses, the number of study subjects is relatively small compared to other studies. We used a stringent process to check and maintain quality of data. Whilst this led to the analysis of high quality data, which is reflected in our low CVs for most parameters, this also meant a significant proportion of IOS data was not analysed. At present there are no standards for post acquisition IOS quality control in preschool children.

8.1.4 Implications for future research and clinical practice

We have shown that IOS can be performed reliably in children as young as 3 years, and is acceptable in terms of repeatability. However, more research is required to understand the correlation between measured parameters and clinical outcomes in preschool children.

8.2 Effect of prenatal vitamin D on child health

8.2.1 Summary of findings

1. No effect of prenatal vitamin D supplementation on parentally reported wheezing.
2. Increased bronchodilator use and increased LRTI in offspring of mothers who received a bolus dose of vitamin D (although this result became non-significant after adjusting for multiple testing)
3. No effect of prenatal vitamin D supplementation on atopic sensitisation, IgE levels, exhaled nitric oxide or lung function measured by IOS in offspring at age three years
4. No consistent evidence for a treatment effect on healthcare utilisation.
5. No correlation between cord 25(OH)D levels and wheezing or any other outcome.

8.2.2 Consistency with other studies

Our results are in agreement with observational studies that have found no relationship between cord or maternal gestational 25(OH)D levels and these outcomes (145, 175, 288, 290-293).

When we analysed our own data on cord blood 25(OH)D concentration and parentally reported child health and objective markers of atopy, inflammation and lung function at age first three years, no significant relationships were found. However, while our interventions did result in at least 50% higher cord blood 25(OH)D concentrations compared with no treatment, cord blood levels were still significantly lower in the intervention groups than in studies that found significant relationships (291). If prenatal supplementation with vitamin D is to make a difference, one might expect to more clearly see an effect in such a deficient population.

8.2.3 Strengths and weaknesses of the study

The key strength of this study is that we have comprehensively assessed child health using a range of overlapping techniques including validated parental questionnaire, objective assessment of biological parameters and analysis of prospectively recorded primary healthcare records for clinical outcomes and total healthcare utilisation. Reassuringly, results are consistent across these fields. However, our study has a number of limitations, as have been described previously. Perhaps the most important limitations are first, this was a small study with limited statistical power apart from for the primary outcome of wheezing. Second, we did not assess vitamin D receptor polymorphisms or other potentially important genotypic variations. It is possible that vitamin D supplementation is effective for preventing wheezing in the presence of specific maternal or infant genotypes, as suggested by others (285). Third, the effectiveness of the intervention. It is possible that vitamin D supplementation at a higher dose, or earlier in pregnancy might protect against early childhood wheezing. Finally, the ethnic variability may have led to more 'noise' between groups making small differences in outcomes harder to discern.

8.2.4 Implications for future research and clinical practice

Although our data excludes a large effect on preschool wheezing, given the modest effects on cord blood vitamin D achieved by these interventions, the safety and efficacy of higher dose prenatal vitamin D supplementation strategies need to be explored. Two on-going trials, the Vitamin D Prenatal Asthma Reduction Trial (NCT00920621), and ABCvitaminD (NCT00856947) are specifically exploring the effects of earlier and higher dose prenatal vitamin D supplementation on child health. Given our finding of increased bronchodilator use and increased LRTI in offspring of mothers who received a bolus dose of vitamin D, (in this case a single dose of 200,000 units at 27 weeks gestation), the use of such regimens should be viewed with caution.

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10 Appendices

10.1 Pilot study participant information sheet

Participant Information Sheet

The Effects of Prenatal Vitamin D Supplementation on Child Health: Pilot study

Protocol version 1.0 31/12/09, Pilot study Patient information sheet version 2.0, 03/03/10)

We would like to invite you to take part in a research study. Before deciding you need to understand why the research is being done and what it involves. Please take time to read the following information carefully. Talk to others about the study if you wish. Ask us if there is anything that is not clear or if you would like more information.

Part I tells you about the purpose of the study and what will happen if you take part.
Part II gives you more detailed information about the conduct of the study.

Part I

What is the study about?

In the UK, one in three children become wheezy before they are three. These illnesses reduce quality of life for children and families, and our understanding of these illnesses is quite limited. Recently a new method has been developed for testing young children's lung function, and we would like to develop this method to help us understand why wheezing in young children happens. We wish to use this method to test lung function in 60 young children and compare this with information about any illnesses they have had.

Why has my child been invited to take part?

We are inviting pre-school children who are currently not suffering from any infection or acute illness, and who are attending St Mary's hospital as a patient, relative or visitor to take part in this study.

Do we have to take part?

It is up to you to decide. We will answer any questions that you have, and give you time to decide. We will then ask you to sign a consent form to show that you have agreed to take part. You are free to withdraw from the study at any time, without giving a reason. If you withdraw from the study it will not affect your usual medical care in any way.

What will happen if we agree to take part?

We will ask you some questions about your child's health, and examine your child's skin and chest. We will ask your child to breathe in and out of a tube attached to a machine for 1 minute, to measure their lung function. We will then give them a medicine to inhale called salbutamol (ventolin) and repeat the breathing test 15 minutes later. Salbutamol is a medicine which helps children with asthma breathe more easily, and is very safe. To give the medicine, your child simply breathes in and out of a plastic box filled with salbutamol spray for a few seconds.

Your child will also have:

1. An allergy skin test. This is a test for allergy where small drops of liquid are placed on the child's forearm or back, and a painless scratch is made through each drop into the surface of the skin. 15 minutes later the appearance of the skin is observed for signs of an allergic reaction. We will discuss the results with you, and of course let your GP know and ask them to arrange appropriate follow up where necessary.
2. Nose test. This is a test for allergy in the nose – we will place a small piece of paper in your child's nose and leave it there for 2 minutes. After removing the paper we will look at the fluid which the paper has absorbed for signs of allergy.

The whole assessment will take approximately 30 minutes.

What else will we have to do?
Nothing – once the above assessment is completed no further tests or participation is needed.

Are there any disadvantages to taking part in this study?
The main disadvantage is the time taken. Allergy skin tests and the nose test can cause some itchiness at the time of the test which settles within a few minutes.

Are there any benefits to taking part in this study?
We cannot promise the study will help you, but we hope the information we get from the study will help lead to an intervention that can prevent some children from developing asthma.
If we discover any health problems during the assessment, such as allergies, we will give you the written results so that you can contact your general practitioner and arrange appropriate medical treatment or assessment.

What if there is a problem?
Any complaint about the way you have been dealt with during the study or any possible harm you or your child might have suffered will be addressed. The detailed information on this is given in part 2.

Will taking part in the study be kept confidential?
Yes. We will follow ethical and legal practice and all information about you and your child will be handled in confidence. The details are included in part 2.

Part 2

What will happen if I don't want to carry on with the study?
If you withdraw from the study, or change your mind, any stored tissue samples that can be identified as yours will be destroyed if you wish.

What if there is a problem?
If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions. If you remain unhappy and wish to complain formally, you can do this through the NHS complaints procedure which is called PALS (Patient Advice and Liaison Service.)

What if something goes wrong?
Imperial College London holds insurance policies which apply to this study. If you experience serious and enduring harm or injury as a result of taking part in this study, you may be eligible to claim compensation without having to prove that Imperial College is at fault. This does not affect your legal rights to seek compensation.

If you are harmed due to someone's negligence, then you may have grounds for a legal action. Regardless of this, if you wish to complain, or have any concerns about any aspect

of the way you have been treated during the course of this study then you should immediately inform the Investigator (Dr Stephen Goldring, contact details at end of this leaflet). The normal National Health Service complaint complaints mechanisms are also available to you. If you are still not satisfied with the response, you may contact the Imperial AHSC Joint Research Office.

Will my taking part in this study be kept confidential?
All information which is collected about you during the course of the research will be kept strictly confidential, and any information about you which leaves the hospital will have your name and address removed so that you cannot be recognised.

Involvement of your General Practitioner
We will inform your General Practitioner of your child's involvement in this brief study, and if we identify any important health problem we will give you and your GP a written copy of the results so that you can discuss them with your GP.

What will happen to the nose samples?
All samples will be labelled with a code with no identifying information about you.
Some tests will be done straight away. We know that samples from children are a precious resource. We try and collect as little as possible, and also use as little as possible, so that when this study is finished we can store what's left and use the samples for future ethically approved research. At any time you wanted, you could ask for these samples to be destroyed.

Will any genetic (DNA analysis) tests be done?
No.

What will happen to the results of the research study?
Results of this study will be published in the scientific peer-reviewed literature, and presented at national and international congresses relevant to asthma and allergic disease. Results will also be disseminated to asthma patients via the funder Asthma UK. Patients will not be identifiable in any report, publication or presentation unless you have given your consent.

Who is organising and funding the research?
The research is co-ordinated by Imperial College London and has been funded by Asthma UK, a national organisation that supports research into the causes of asthma.
It has also been reviewed and given a favourable opinion by the St Mary's Hospital ethics committee. None of the investigators performing the research or participants taking part will benefit financially from the study.

Who has reviewed the study?
Service users were involved in the design of this research. The funders, Asthma UK, have peer reviewed the study, and it has also been reviewed by the St Mary's hospital research and ethics committee.

Further information and contact details
If you would like more information or to discuss anything in this leaflet, please contact the study coordinator Dr Stephen Goldring, who will be happy to discuss any aspect of this study.

Dr Stephen Goldring
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This information can be made available in other languages and formats if requested.

10.2 Pilot study consent form

Consent Form for Parents/ Guardian

The Effects of Prenatal Vitamin D Supplementation on Child Health: Pilot study

Protocol Version 1.0 31/12/09. Consent form version 2, 03/03/10

		Please initial
1.	I confirm that I have read and understand the information Sheet dated 03/03/10, for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	☐
2.	I understand that my child's participation is voluntary, and that I am free to withdraw consent for my child at any time, without giving any reason and without my child's medical care or legal rights being affected.	☐
3.	I understand that relevant sections of any of my child's medical notes and data collected during the study, may be looked at by responsible individuals from Imperial College London, from Imperial College Healthcare NHS Trust and from regulatory authorities, where it is relevant to my taking part in this research. I give permission for these individuals to access my child's records.	☐
4.	I agree to my child's general practitioner being informed of my child's participation in the study	☐
5.	I agree to the use of my child's nasal samples as described in the patient information sheet	☐
7.	I agree to the use of my child's samples in any future ethically-approved studies.	☐
8.	I agree to my child taking part in the above study.	☐

_____	_____	_____
Name of Parent/ Guardian	Date	Signature

_____
Name of Child

_____	_____	_____
Name of Person taking consent	Date	Signature

When completed: 1 for parent, 1 for researcher site file, 1 to be posted to GP (if consent given)

10.3 Pilot study – general health questionnaire

1.Patient Name:

2. Hospital No: ☐☐☐☐☐☐☐☐☐

3. Patient Code: ☐☐☐

4. Contact Details (preferable telephone number details):

Address:……

……..

……

……

……

Telephone……

Email…….

5. DOB: ☐☐/☐☐/☐☐☐☐

☐ ☐

6. Sex: Male / Female

7. Ethnicity: Middle Eastern ☐

Asian ☐

Caucasian ☐

Afrocarribean ☐

Other ☐

8. Height (cm): ☐ ☐ ☐cm

9. Weight (kg): ☐ ☐ ☐g

Medical History Questions

10. How often does your child get a cold or chest infection?

Every two weeks ☐

Every month ☐

☐

Every 3-4 Months

Every 6 months ☐

Once a year ☐

Less than once a year ☐

Never ☐

11. Has your child ever had pneumonia? Yes ☐ No ☐

12. Has your child ever been admitted to hospital? Yes ☐ No ☐

If yes, what for?

13. Does your child have any other medical conditions? Yes ☐ No ☐

If yes, what are they?

14. What was the weight of your child when they were born?

☐☐.☐Kg

15. Did the doctors tell you your child had had growth problems in utero?

Yes ☐ No ☐

16. Did the mother smoke during pregnancy? Yes ☐ No ☐

17. Was the mother exposed to smoke during the pregnancy – e.g. father smoking at home?

Yes ☐ No ☐

18. From being born to now, is the child exposed to cigarette smoke?

Yes ☐ No ☐

Now complete Wheezing Questionnaire.

10.4 Pilot study – wheezing questionnaire

Questionnaire Before Testing – Wheezing Questionnaire – Modified from ISAAC study for 6-7 year olds.

Patient Code: ☐ ☐ ☐

Date: ☐ ☐ / ☐ ☐ ☐ ☐ ☐ ☐

Interviewers Initials: ☐ ☐

Has your child ever had wheezing or whistling in the chest at any time in the past?

Yes ☐ No ☐

If 'NO' go to question 6.

At what age did your child first wheeze?

Before 1st Birthday ☐

When they were one ☐

When they were two ☐

At age three years old or more ☐

Has your child had wheezing or whistling in the chest in the last 12 months?

Yes ☐ No ☐

If 'NO' go to question 6

How many attacks of wheezing has your child had in the last 12 months?

None ☐

1- 3 ☐

4-12 ☐

More than 12 ☐

In the last 12 months, how often, on average, has your child's sleep been affected due to wheezing?

Never woken due to wheezing ☐

Less than one night per week ☐

One or more night per week ☐

In the last 12 months has the wheezing ever been severe enough to limit your child's speech to only one or two words at a time between breaths?

Yes ☐ No ☐

Has your child ever had asthma?

Yes ☐ No ☐

In the last 12 months has your child's chest sounded wheezy during or after exercise?

Yes ☐ No ☐

Has your child ever had a runny nose, blocked nose, sneezing when he/she does not have a cold?

☐ ☐

Yes No

If 'NO' go to question 11

In the last 12 months has your child had a runny nose, blocked nose, sneezing when he/she does not have a cold?

Yes ☐ No ☐

If 'NO' go to question 11

When does this occur?

January ☐

February ☐

March ☐

April ☐

May ☐

June ☐

July ☐

August

September ☐

October ☐

November ☐

December ☐

Has your child ever had hayfever?

Yes ☐ No ☐

Does your child ever have an itchy rash that comes and goes?

Yes ☐ No ☐

If ‘NO’ go to question 15

In the last 12 months has your child had this itchy rash that comes and goes?

Yes ☐ No ☐

If ‘NO’ go to question 15

Has this itchy rash, at any time, affected any of the following places: the folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears or eyes?

Yes ☐ No ☐

Has your child ever has eczema?

Yes ☐ No ☐

Does your child have any food allergies?

Yes ☐ No ☐

If yes, what?

Has it been medically diagnosed?

Yes ☐ No ☐

Skin Prick Test Results [positive = ≥3mm wheal at 15 minutes]

Skin Prick Test Completed? Yes ☐ No ☐

Skin Prick Test Problem? 0 Nil ☐

1 No clear skin ☐

☐

2 Antihistamine taken ☐

3 Dermatographism

4 Other ☐

Details……

…….

Skin Prick Test Site: Back ☐ Forearm ☐

Results

Dust Mite ____.____mm Neg ☐ Pos ☐

Cat ____.____mm Neg ☐ Pos ☐

Dog ____.____mm Neg ☐ Pos ☐

Grass Pollen ____.____mm Neg ☐ Pos ☐

Tree Pollen ____.____mm Neg ☐ Pos ☐

Mould - alternaria ____.____mm Neg ☐ Pos ☐

Neg Control ____.____mm

Histamine ____.____mm

Others (e.g. if child is reported to have food allergy):

_____ ____.____m Neg ☐ Pos ☐

_____ ____.____mm Neg ☐ Pos ☐

Patient Code:

Date: ☐☐/☐☐/☐☐☐☐

Results of the Nasal Secretion test

Nasal Secretion test competed?

Yes ☐ No ☐

Details:……

……

……

……

……

Weight Before: ☐☐☐.☐☐mg

Weight After: ☐☐☐.☐☐mg

Difference: ☐☐☐.☐☐mg

Lab Results:

10.5 Main study - participant information leaflet

Participant Information Sheet

The Effects of Prenatal Vitamin D Supplementation on Child Health

(Protocol Version 2.0_ 21/06/10, Patient information sheet version 3.0_21/06/10)

We would like to invite you and your child to take part in a research study. Before deciding you need to understand why the research is being done and what it involves. Please take time to read the following information carefully. Talk to others about the study if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Part I tells you about the purpose of the study and what will happen if you take part.
Part II gives you more detailed information about the conduct of the study.

Part I

What is the study about?

In the UK, one in three children have a 'wheezing illness' before they are three, and one in five children develop asthma by age six. We don't know why so many children have wheezing illnesses, but new research has found a link between vitamin D intake during pregnancy and a child's lungs development and risk of having wheezing illnesses. Vitamin D intake during pregnancy may also change how well a child's bones and immune system develop.

Why have we been invited to take part in this study?

You have been contacted because you took part in a trial of different vitamin D supplements when you were pregnant a few years ago. We would like to see whether the treatment that you took during pregnancy has had any effect on your child's health – particularly the development of their lungs, their bones and their immune system.

Do we have to take part?

It is up to you to decide. We will answer any questions that you have, and give you time to decide. We will then ask you to sign a consent form to show that you have agreed to take part. You are free to withdraw from the study at any time, without giving a reason. If you withdraw from the study it will not affect your usual medical care in any way.

What will happen if we agree to take part?

We would like you to bring your child, when they are aged three years, for a one-off health assessment at the Paediatric Research Unit, St Mary's Hospital. We expect each study visit to take less than two hours, but it would be sensible to allow a morning, or an afternoon to complete the assessment. This will involve the following:

1. A short questionnaire about you and your child's health.
2. An examination of your child by a children's doctor or nurse.
3. Two simple breathing tests were we ask your child to breathe in and out of a machine that measures how he/she breathes, and what is in their breath. During one of the breathing tests we will ask your child to breathe in a medicine called salbutamol (or Ventolin), and then repeat the breathing test. Salbutamol is a medicine which helps children with asthma to breathe more effectively, and is very safe. To give the medicine, your child simply breathes in and out of a plastic box filled with salbutamol spray for 6-10 breaths.

4. Allergy skin test. This is a test for allergy where small drops of liquid are placed on the child's forearm or back, and a painless scratch is made through each drop into the surface of the skin. 15 minutes later the appearance of the skin is observed for signs of an allergic reaction. We will discuss the results with you, and of course let your GP know and ask them to arrange appropriate follow up where necessary.
5. Nose test. This is a test for allergy in the nose – we will place a small piece of paper in your child's nose and leave it there for 2 minutes. After removing the paper we will look at the fluid which the paper has absorbed for signs of allergy.
6. A blood test (about 10mls or 2 teaspoons) from both you and your child. From this sample we would like to measure you and your child's vitamin D levels. If either of you have low levels of vitamin D, we would recommend a vitamin supplement that your GP can prescribe.
7. A test of you and your child's skin darkness using a gentle probe placed on the skin.
8. A test using a cotton bud to gently rub the inside of you and/or your child's mouth for a minute. This allows us to collect cells which can be used for laboratory tests, and does not cause any discomfort.
9. We will also write to your GP to ask for a copy of your child's GP health records, so that we can see what breathing illnesses they have had.

That seems like a lot of tests – do I need to do them all?
We prefer that you and your child do all of the tests, but if you wish to only do some of them then you are still able to join the study. If it is very difficult for you to visit the hospital then we may be able to arrange an interview over the telephone.

Will the tests hurt?
The blood test can cause some discomfort. For both you and your child we will use a local anaesthetic cream to numb the skin, and distraction techniques as appropriate. All blood tests will be taken by fully trained staff.

Expenses and payments
We will reimburse your travel or parking expenses if you travel to the hospital for your health check by public transport or in your own vehicle.

What else will we have to do?
Once you and your child have completed the tests there is no other commitment. However depending on the results of this study we may contact you again in the future to ask your permission to undertake a further health check for your child when they are older.

Are there any disadvantages to taking part in this study?
The main disadvantages are the time taken, and any discomfort from collecting the blood sample. Both allergy skin tests and the nose test may cause some slight itching during the test, which settles within a few minutes.

Are there any benefits to taking part in this study?

Your child will have a health check supervised by Dr Goldring who is a fully trained children's specialist. This will include testing the health of their lungs, immune system and bones. We will provide you (and your GP if you agree) with a report of any test results that are important for your child's healthcare. The information we get from the study may also help us to prevent other children from developing asthma, and to advise women on what vitamins to take during pregnancy.

What if there is a problem?

Any complaint about the way you have been dealt with during the study or any possible harm you or your child might have suffered will be addressed. The detailed information on this is given in part 2.

Will taking part in the study be kept confidential?

Yes. We will follow ethical and legal practice and all information we have about you and your child will be handled in confidence. The details are included in part 2.

Part 2

What if relevant new information becomes available?
Sometimes we get new information about the subject being studied. If this happens the research doctor will contact you with the new information and the implications for the study, and for you and your child.

What will happen if I don't want to carry on with the study?
If you withdraw from the study, or change your mind, any stored blood or tissue samples that can be identified as yours will be destroyed if you wish.

What if there is a problem?
If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions. If you remain unhappy and wish to complain formally, you can do this through the NHS complaints procedure which is called PALS (Patient Advice and Liaison Service.)

What if something goes wrong?
Imperial College London holds insurance policies which apply to this study. If you experience serious and enduring harm or injury as a result of taking part in this study, you may be eligible to claim compensation without having to prove that Imperial College is at fault. This does not affect your legal rights to seek compensation.
If you are harmed due to someone's negligence, then you may have grounds for a legal action. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been treated during the course of this study then you should immediately inform the Investigator (Dr Stephen Goldring, contact details at end of this leaflet). The normal National Health Service complaint complaints mechanisms are also available to you. If you are still not satisfied with the response, you may contact the Imperial AHSC Joint Research Office.

Will my taking part in this study be kept confidential?
All information which is collected about you during the course of the research will be kept strictly confidential, and any information about you which leaves the hospital will have your name and address removed so that you cannot be recognised.

Involvement of your General Practitioner
If you decide to take part in the study, we will ask for your consent to send a letter to you and your child's general practitioner to let them know of your participation. We would also like to include in that letter any relevant health information that we discover during the assessment that your GP should know about.

What will happen to the blood and nose samples?
These samples will be used to find out how our immune system responds to vitamin D during pregnancy. The blood samples will also be used to check you and your child's vitamin D level. All samples will be labelled with a code with no identifying information about you.
Some tests will be done straight away. We know that samples from children are a precious resource. We try and collect as little as possible, and also use as little as possible, so that when this study is finished we can store what's left and use the samples for future ethically approved research. At any time you want, you can ask for these samples to be destroyed.

Will any DNA analysis or genetic tests be done?
We would like to collect either a blood sample or cells from the surface of the mouth from you and your child for genetic tests which may be done at the time of the study, or at a later date in other ethically approved studies. The aim of these tests is to understand how vitamin D during pregnancy affects wheezing in children.
Although unlikely, if we discover a genetic result that may be important for you or your child's health, we will write to your GP who can make contact with you and arrange for a full explanation of the result, what it means for you and your child, and whether any further action is necessary.

What will happen to the results of the research study?
Results of this study will be published in scientific peer-reviewed literature, and presented at national and international congresses relevant to asthma and allergic disease. Results will also be disseminated to asthma patients via the funder Asthma UK. Patients will not be identifiable in any report, publication or presentation.

Who is organising and funding the research?
The research is co-ordinated by Imperial College London and has been funded by Asthma UK, a national organisation that supports research into the causes of asthma. It has also been reviewed and given a favourable opinion by the St Mary's Hospital ethics committee. None of the investigators performing the research will benefit financially from the study.

Who has reviewed the study?
Patient representatives were consulted during the design of this research. Experts in vitamin D and asthma have reviewed the study, together with the charity Asthma UK. It has also been reviewed by the St Mary's hospital research and ethics committee.

Who to contact for further information
If you would like more information or to discuss anything in this leaflet, please contact the study coordinator Dr Stephen Goldring, who will be happy to discuss any aspect of this study.

Dr Stephen Goldring
Clinical Research Fellow and Paediatrician
Department of Paediatrics, Imperial College London, London, W2 1PG
Dedicated contact number: 07590 250650
Email: sgoldring@nhs.net
This information can be made available in other languages and formats if requested.

10.6 Main study - consent form

Child's name:

DOB:

Study number:

Consent Form for Parents/ Guardians

The Effects of Prenatal Vitamin D Supplementation on Child Health

Protocol Version 2.0 21/06/10. Consent form version 3.0 21/06/10

		Please initial
1.	I confirm that I have read and understand the participant information sheet dated 03/03/10 for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	☐
2.	I understand that my child's participation is voluntary, and that I am free to withdraw consent for my child at any time, without giving any reason and without my child's medical care or legal rights being affected.	☐
3.	I understand that relevant sections of any of my child's medical notes and data collected during the study may be looked at by responsible individuals from Imperial College London, from Imperial College Healthcare NHS Trust and from regulatory authorities, where it is relevant to my child taking part in this research. I give permission for these individuals to access my child's records.	☐
4.	I agree to my child's general practitioner being informed of my child's participation in the study	☐
5.	I agree to the use of my child's blood as described in the participant information sheet	☐
6.	I agree to the use of my child's blood or mouth swab for genetic studies as described in the participant information sheet	☐
7.	I agree to the use of my child's samples in any future ethically-approved studies.	☐
8.	I agree to my child taking part in the above study.	☐

_____	_____	_____
Name of Parent/ Guardian	Date	Signature

_____
Name of Child

_____	_____	_____
Name of Person taking consent	Date	Signature

When completed: 1 for parent, 1 for researcher site file, 1 to be posted to GP (if consent given)

10.7 Main study questionnaire

Interviewer ID: ☐ (Steve-1 Bob-2 Heather-3 Other:……-4)

Date of questionnaire __ __/__ __/__ __ __ __

Date of assessment __ __/__ __/__ __ __ __

Mother's Name……D.O.B__ __/__ __/__ __

Surname First Name

Child's Name…..……

……D.O.B__ __/__ __/__ __

Surname First Name

Sex: □ 1 Male □ 2 Female

Current Address……

……..

……

Current phone……

Home Work Mobile

Current email……..@……..

Place of interview □ 1 Phone □ 2 PRU □ 3 Home visit

Interviewee: □ 1 Mother □ 2 Father □ 3 Other……..

Translator required: □ 1Yes □ 2 No Language……

Checklist (please tick when completed)

Pre-assessment □ Check no exclusion criteria (severe congenital respiratory abnormality)

□ Appointment letter sent (+Map+PIL)

□ Reminded to bring red book

□ Translater booked if required

□ Booked on PRU google diary

□ Phone before appointment and offer to do questionnaire over phone

On the day (Child) □ Consent □copy to mother □copy for GP □Original copy for research file □ Ametop applied

□ Questionnaire

□ Height

□ Weight

□ Colorimeter reading

□ SCORAD score

□ ENO

□ IOS (pre)

□ Salbutamol

□ Check no antihistamines

□ Skin prick test □ Read at 10/15 mins

□ IOS (post)

□ Blood test □Sheree sample □DNA sample in -80 Freezer on PRU

□FBC + form labeled for eosinophils taken to haem reception

□ Nasal sample □ Weight recorded □Stored in -80 freezer on PRU

□ Buccal swab □Stored in -4 fridge on PRU

On the day (Mother) □ Consent form

□ Colorimeter

□ Buccal swab □Stored in -4 fridge on PRU

Post assessment □ eHR from GP □ Phone/ fax GP with request (Letter/ consent form/ PIL)

□ Received eHR back from GP

□ Data entry □ Questionnaire □ eHR data

□ Chase eosinophil result

CHILD HEALTH

Q1. Birth weight? g

Q2. Gestational age? ☐☐ weeks

Q3. SCBU? 1Yes□ 0No□ 99N/A□

Details.....

.....

Q4. The child's immunisation data is taken from:

□ 1 Red book □ 2 Verbal □ 3 GP electronic records □ 99 NA

Q5. Has the child completed the immunization schedule?

8 week	DTaP/IPV/Hib	□ 1Yes □ 0No □ 99NA
	PCV	□ 1Yes □ 0No □ 99NA
12 week	DTaP/IPV/Hib	□ 1Yes □ 0No □ 99NA
	Men C	□ 1Yes □ 0No □ 99NA
16 week	DTaP/IPV/Hib	□ 1Yes □ 0No □ 99NA
	Men C	□ 1Yes □ 0No □ 99NA
PCV	□ 1Yes □ 0No □ 99NA	
12 month	Hib/MenC	□ 1Yes □ 0No □ 99NA
13 month	MMR	□ 1Yes □ 0No □ 99NA
PCV	□ 1Yes □ 0No □ 99NA	
3 years 4 months:	MMR	□ 1Yes □ 0No □ 99NA
DTaP/ IPV	□ 1Yes □ 0No □ 99NA	
dTaP/IPV	□ 1Yes □ 0No □ 99NA	

Q6. Any congenital abnormalities? □ 1Yes □ 0No □ 99NA

Details.....

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Q7. Is your child on any regular medications? □ 1Yes □ 0No □ 99NA

Details.....

.....

Q8. Does your child have any health problems? □ 1Yes □ 0No □ 99NA

Details.....

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RESPIRATORY TRACT INFECTIONS

Q9. Since your child was born have you ever been told by a health professional that your child has bronchiolitis, bronchitis, croup, pneumonia or a chest infection?

□ 1Yes □ 0No □ 99NA

Q10. Since your child was born have they ever had to be admitted overnight in hospital because of bronchiolitis, bronchitis, croup, pneumonia or a chest infection?

□ 1Yes □ 0No □ 99NA

Q11. Details of all episodes:

Age Months 99 = N/A	Parents report of diagnosis 1=bronchitis 2=bronchiolitis 3=croup 4=Pneumonia 5=Chest infection 6=Other (describe) 99=N/A	Antibiotics 1=Yes 0=No 99=N/A	Management 1=Managed at home 2=Attended A+E but discharged 3=Admitted to hospital overnight 99 = N/A

Q12. How often does your child have an upper respiratory tract infection, with at least 2 of

the following symptoms: cough, runny nose, fever?

□ 1 Never

□ 2 1-4 per year

□ 3 5-8 per year

□ 4 9-12 per year

□ 5 >12 per year

□ 99 NA

WHEEZING

Q13. Has your child ever had wheezing or whistling in the chest at any time in the past? □ 1Yes □ 0No □ 99NA

Q14. Has your child's breathing ever been like this (show video)? □ 1Yes □ 0No □ 99NA

If you have answered 'No' to both these questions please skip to q21

Q15. At what age did your child first wheeze?

□ 1 Before 1st Birthday
□ 2 When they were one
□ 3 When they were two
□ 4 At age three or more

Q16. Has your child had wheezing or whistling in the chest in the past 12 months? □ 1Yes □ 0No □ 99NA

If you have answered 'No' please skip to question 21

Q17. How many attacks of wheezing has your child had since birth? □□ Episodes □ 99NA

Q18. How many attacks of wheezing has your child had in the past 12 months? □□ Episodes □ 99NA

Q19. In the last 12 months, how often, on average, has your child's sleep been

disturbed due to wheezing?

□ 1 Never woken with wheezing

□ 2 Less than one night per week

□ 3 One or more nights per week

□ 99 N/A

Q20. 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?

□ 1Yes □ 0No □ 99NA

Q21. Has your child ever had asthma? □ 1Yes □ 0No □ 99NA

Q22. In the past 12 months, has your child's chest sounded wheezy during or after exertion?

□ 1Yes □ 0No □ 99NA

Q23. In the past 12 months, has your child had a dry cough at night, apart from a cough associated with a cold or chest infection?

□ 1Yes □ 0No □ 99NA

Q24. Since your child was born have they had wheezing without a cold? (API)

□ 1Yes □ 0No □ 99NA

WHEEZING MEDICATION

Q25. Has any health professional ever prescribed a blue (salbutamol or reliever)

inhaler for your child? (show inhaler) □ 1Yes □ 0No □ 99NA

Q26. If yes to Q25, on average, how often have you used this inhaler for your child during the following time periods?

	Q 0-12 months	Q 12-24 months	Q 24-36 months	Q Age 36m+
1 Daily				
2 Most weeks				
3 Most months				
4 Occasionally				
5 Never				
99 NA				

Q27. Has any health professional ever prescribed a brown or purple or white/red (steroid or preventer) inhaler for your child? (Show inhaler) □ 1Yes □ 0No □ 99NA

Q28. If yes to Q27, on average, how often have you used a steroid/preventer) inhaler for your child during the following time periods?

	Q 0-12 months	Q 12-24 months	Q 24-36 months	Q Age 36+
1 Daily				
2 Most weeks				
3 Most months				
4 Occasionally				
5 Never				
99 NA				

Q29. Has any health professional ever prescribed a course of oral steroids (pink tablets/ liquid) for your child? (Show) □ 1Yes □ 0No □ 99NA

Q30. If yes to Q29, How many courses of oral steroids as your child ever had?

	Q 0-12 months	Q 12-24 months	Q 24-36 months	Q 36+months
Number of courses 99 NA				

Q31. Has any health professional ever prescribed monteleukast (Singulair) for your child? (Show packet) □ 1Yes □ 0No □ 99NA

Q32. If yes to Q31, On average, how often have you used monteleukast (Singulair/ granules) for your child?

	Q 0-12 months	Q 12-24 months	Q 24-36 months	Q 36+months
1 Daily				
2 Most weeks				
3 Most months				
4 Occasionally				
5 Never				
99 NA				

RHINITIS

Q33. Has your child ever had a problem with sneezing, or a runny, or blocked nose when he/she DID NOT have a cold or the flu? □ 1Yes □ 0No □ 99NA

If you have answered 'No' please skip to question 38

Q34. In the last 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? □ 1Yes □ 0No □ 99NA

If you have answered 'No' please skip to question 38

Q35. In the past 12 months, has this nose problem been accompanied by itchy-watery eyes? □ 1Yes □ 0No □ 99NA

Q36. In which of the past 12 months did this nose problem occur? (Please tick any which apply)

□ 1 January	□ 5 May	□ 9 September
□ 2 February	□ 6 June	□ 10 October
□ 3 March	□ 7 July	□ 11 November
□ 4 April	□ 8 August	□ 12 December

Q37. In the past 12 months, how much did this nose problem interfere with your child's daily activities? □ 1 Not at all

□ 2 A little □ 4 A lot

□ 3 Moderate amount □ 99 NA

Q38. Has your child ever had hayfever?

□ 1Yes □ 0No □ 99NA

Q39. Since your child was born have they had doctor diagnosed allergic rhinitis? (API) □ 1Yes □ 0No □ 99NA

ECZEMA

Q40. Has your child ever had an itchy skin rash that was coming and going for at least six months? □ 1Yes □ 0No □ 99NA

If you have answered 'No' please skip to question 46

Q41. Has your child had this itchy rash at any time in the past 12 months? □ 1Yes □ 0No □ 99NA

If you have answered 'No' please skip to question 46

Q42. Has this itchy rash, at any time, affected any of the following places:

The folds of the elbows, behind the knees, In front of the ankles, under the buttocks, or around the neck, ears or eyes? □ 1Yes □ 0No □ 99NA

Q43. At what age did this itchy rash first occur?

□ 1 First 6 months
□ 2 Before 1st birthday
□ 3 Age one
□ 4 Age two
□ 5 Age three or more
□ 99 NA

Q44. Has this rash cleared completely at any time during the past 12 months?

□ 1Yes □ 0No □ 99NA

Q45. In the past 12 months, how often, on average, has your child been kept awake at night by this itchy rash?

□ 1 Never in the past 12 months
□ 2 Less than one night per week
□ 3 One or more nights per week
□ 99 NA

Q46. Has your child ever had eczema?

□ 1Yes □ 0No □ 99NA

Q47. Since your child was born have they had doctor diagnosed eczema? (API)

□ 1Yes □ 0No □ 99NA

FOOD ALLERGY

History of possible food allergies?

Q48a. Has your child ever had a possible food allergy?	□ 1Yes □ 0No □ 99NA
Q48. History of possible egg allergy	□ 1Yes □ 0No □ 99NA
Q49. History of possible cow milk allergy	□ 1Yes □ 0No □ 99NA
Q50. History of possible peanut allergy	□ 1Yes □ 0No □ 99NA
Q51. History of possible tree nut allergy	□ 1Yes □ 0No □ 99NA
Q52. History of possible fish allergy	□ 1Yes □ 0No □ 99NA
Q53. History of possible seafood allergy	□ 1Yes □ 0No □ 99NA
Q54. History of possible soy allergy	□ 1Yes □ 0No □ 99NA
Q55. History of possible wheat allergy	□ 1Yes □ 0No □ 99NA
Q56. History of possible sesame allergy	□ 1Yes □ 0No □ 99NA
Q57. History of other possible food allergy	□ 1Yes □ 0No □ 99NA

History of doctor diagnosed food allergies?

Q58. History of doctor diagnosed egg allergy	□ 1Yes □ 0No □ 99NA
Q59. History of doctor diagnosed cow milk allergy	□ 1Yes □ 0No □ 99NA
Q60. History of doctor diagnosed peanut allergy	□ 1Yes □ 0No □ 99NA
Q61. History of doctor diagnosed tree nut allergy	□ 1Yes □ 0No □ 99NA
Q62. History of doctor diagnosed fish allergy	□ 1Yes □ 0No □ 99NA
Q63. History of doctor diagnosed seafood allergy	□ 1Yes □ 0No □ 99NA
Q64. History of doctor diagnosed soy allergy	□ 1Yes □ 0No □ 99NA
Q65. History of doctor diagnosed wheat allergy	□ 1Yes □ 0No □ 99NA
Q66. History of doctor diagnosed sesame	□ 1Yes □ 0No □ 99NA
Q67. History of other doctor diagnosed food allergy	□ 1Yes □ 0No □ 99NA

Details.....

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Q68. What is the ethnic group of the child's father?

□ 1 White (British/ Irish/ Other white)

□ 2 Mixed (White + Black Caribbean/White +Black African/ White +Asian/ Other_____)

□ 3 Asian (Indian/ Pakistani/ Bangladeshi/ Other_____)

□ 4 Black (Caribbean/ African/ Other_____)

□ 5 East Asian

□ 6 Other ethnic group _____

□ 99 NA

Q69. What is the ethnic group of the child's mother?

□ 1 White (British/ Irish/ Other white)

□ 2 Mixed (White +Black Caribbean/White +Black African/ White +Asian/ Other_____)

□ 3 Asian (Indian/ Pakistani/ Bangladeshi/ Other_____)

□ 4 Black (Caribbean/ African/ Other_____)

□ 5 East Asian

□ 6 Other ethnic group _____

□ 99 NA

Q70. Age mother left full time education? □□ years □ 99NA

Q71. Age father left full time education? □□ years □ 99NA

Q72. How many adults live in your household? □□ adults □ 99NA

Q73. How many children live in your household? □□ children □ 99NA

Q74. Does your child attend nursery? □ 1Yes □ 0No □ 99NA

Q75. If attending nursery from what age did they start? ☐☐ months □ 99NA

Q76. How many older brothers and sisters does your child have?

older □ 99NA ☐☐

Q77. How many younger brothers and sisters does your child have?

☐☐ younger □ 99NA

Q78. Have you ever had a household pet? Cat □ 1Yes □ 0No □ 99NA

Dog □ 1Yes □ 0No □ 99NA

Other □ 1Yes □ 0No □ 99NA

Details.....

Q79. Do any household member smoke cigarettes? □ 1Yes □ 0No □ 99NA

Q80. Did the child's mother (female guardian) smoke cigarettes during this pregnancy?

□ 1Yes □ 0No □ 99NA

Q81. Have you or a family member ever had doctor diagnosed asthma? (API)

Mother □ 1Yes □ 0No □ 99NA

Father □ 1Yes □ 0No □ 99NA

Sibling □ 1Yes □ 0No □ 99NA

Q82. Have you or a family member ever had doctor diagnosed Rhinitis?

Mother □ 1Yes □ 0No □ 99NA

Father □ 1Yes □ 0No □ 99NA

Sibling □ 1Yes □ 0No □ 99NA

Q83. Have you or a family member ever had doctor diagnosed Eczema?

Mother □ 1Yes □ 0No □ 99NA

Father □ 1Yes □ 0No □ 99NA

Sibling □ 1Yes □ 0No □ 99NA

CHILD VITAMIN D SUPPLEMENTATION

Q84. Have you ever given your child vitamin supplements? □ 1Yes □ 0No □ 99NA

	Q84 0-12 months	Q84 12-24 months	Q84 24-36 months	Q84 36+months
Multivitamins Eg abidec/ dalivit/ Healthy Start vitamins	□ 1Yes □ 0No □ 99NA Brand name	□ 1Yes □ 0No □ 99NA Brand name	□ 1Yes □ 0No □ 99NA Brand name	□ 1Yes □ 0No □ 99NA Brand name
Specific Vitamin D supplements Eg vitamin D, cod liver oil	□ 1Yes □ 0No □ 99NA Brand name	□ 1Yes □ 0No □ 99NA Brand name	□ 1Yes □ 0No □ 99NA Brand name	□ 1Yes □ 0No □ 99NA Brand name
Other Vitamin supplements	□ 1Yes □ 0No □ 99NA Brand name	□ 1Yes □ 0No □ 99NA Brand name	□ 1Yes □ 0No □ 99NA Brand name	□ 1Yes □ 0No □ 99NA Brand name

Q85. Why did you give them? □ 1 HV recommended

□ 2 GP recommended

□ 3 Other health professional recommended

□ 4 Personal choice

□ 5 Other (Describe) _____

□ 6 Recommended during antenatal care

□ 99 NA

CHILD DIET QUESTIONNAIRE

Q86. Was your child breastfed? □ 1Yes □ 0No □ 99NA

Q87. For how long did you breast feed for? ☐☐ Months □ 99NA

Q88. At what age did your child start a formula? ☐☐ Months □0never given □99NA

Q89. At what age did your child start solids? ☐☐ Months □ 99NA

Q90. Do you observe any of the following diets for your child?

□ 1 Vegan (No animal products)

□ 2 Vegetarian (No meat)

□ 3 Vegetarian (No meat or fish)

□ 4 Other_____

□ 5 No

□ 99 NA

Q91. How often does your child eat oily fish (Salmon/ trout/ mackerel/ sardines)?

□ 1 Weekly

□ 2 Less than weekly

□ 3 Never

□ 99 NA

Q92. How often does your child eat margarine? □ 1 Daily

□ 2 Weekly

□ 3 Less than weekly

□ 4 Never

□ 99 NA

Q93. How often does your child eat eggs?

□ 1 Daily

□ 2 Weekly

□ 3 Less than weekly

□ 4 Never

□ 99 NA

CHILD SUNLIGHT EXPOSURE

Q94. How much time, on average, did your child spend outdoors in daylight hours each day in the last month?

□ 1 <15mins

□ 2 15-30mins

□ 3 30-60mins

□ 4 1-2 hours

□ 5 3-4 hours

□ 6 >4hours

□ 99 NA

Q95. During sunny weather in the United Kingdom or abroad, how often would you apply sunscreen or clothing to your child?

□ 1 Often

□ 2 Sometimes

□ 3 Rarely

□ 4 Never

□ 99 NA

Q96. How much time does your child spend watching TV or using a personal computer each

day?

□ 2 <1hr

□ 3 1-2hr

□ 4 2-3hr

□ 5 3-4hr

□ 6 >4hr

□ 1 None

□ 99 NA

Q97. Does your home have access to a garden? □ 1Yes □ 0No □ 99NA

Q98.Does your home have access to a terrace or balcony? □ 1Yes □ 0No □ 99NA

Q99. Which one of the 6 categories below best describe your child's skin type and your child's skins response to the midday sun in summer months?

□ 1 My child has extremely fair skin. He/she always burns and never tans.

□ 2 My child has fair skin. He/she always burn and sometimes tans.

□ 3 My child has pale coloured skin. He/she sometime burns and always tans.

□ 4 My child has an olive skin. He/she rarely burns and always tans.

□ 5 My child has moderately pigmented brown skin which never burns and always tans

□ 6 My child has markedly pigmented black skin which never burns and always tans.

□ 99 NA

MOTHER VITAMIN D SUPPLEMENTATION

Q100. Are you currently taking regular vitamin D supplements?

□ 1Yes □ 0No □ 99NA Brand.....

Q101. Are you currently taking regular multivitamin supplements?

□ 1Yes □ 0No □ 99NA Brand.....

MOTHER DIET QUESTIONNAIRE

Q102. Do you observe any of the following diets? □ 1 Vegan (No animal products)

□ 2 Vegetarian (No meat)

□ 3 Vegetarian (No meat or fish)

□ 4 Other_____

□ 5 No

□ 99 NA

Q103. How often do you eat oily fish (Salmon/ trout/ mackerel/ sardines)?

□ 1 Weekly

□ 2 Less than weekly

□ 3 Never

□ 99 NA

Q104. How often do you eat margarine? □ 1 Daily

□ 2 Weekly

□ 3 Less than weekly

□ 4 Never

□ 99 NA

Q105. How often do you eat eggs?

□ 1 Daily

□ 2 Weekly

□ 3 Less than weekly

□ 4 Never

□ 99 NA

MOTHER SUNLIGHT QUESTIONNAIRE

Q106. Do you usually wear clothing to cover all skin except face and hands when outdoors?

□ 1Yes □ 0No □ 99NA

Q107. Do you usually wear clothing to cover all skin including the face and hands when outdoors?

□ 1Yes □ 0No □ 99NA

Q108. How much time, on average, did you spend outdoors in daylight hours each day in the last month?

□ 1 <15mins

□ 2 15-30mins

□ 3 30-60mins

□ 4 1-2 hours

□ 5 3-4 hours

□ 6 >4hours

□ 99 NA

Q109. During sunny weather in the United Kingdom or abroad, how often would you apply sunscreen or clothing?

□ 1 Often

□ 2 Sometimes

□ 3 Rarely

□ 4 Never

□ 99 NA

Q110. How much time do you spend watching TV or using a personal computer each day?

(Hyponnen) □ 1 None

□ 2 <1hr

□ 3 1-2hr

□ 4 2-3hr

□ 5 3-4hr

□ 6 >4hr

□ 99 NA

Q111. Have you used a sun bed in the last year? □ 1Yes □ 0No □ 99NA

Q112. Which one of the 6 categories below best describes your skin type and your skins response to the midday sun in summer months?

□ 1 I have extremely fair skin. I always burn and never tan

□ 2 I have fair skin. I always burn and sometimes tan

□ 3 I have pale coloured skin. I sometime burn and always tan

□ 4 I have an olive skin. I rarely burn and always tan.

□ 5 I have moderately pigmented brown skin which never burns and always tans

□ 6 I have markedly pigmented black skin which never burns and always tans

□ 99 NA

PHYSICAL EXAMINATION

Q113. Height □□□.□ cm □ 99 NA

Q114. Weight □□.□kg □ 99 NA

COLORIMETER READINGS:

Q115. Infant colorimeter readings taken? □ 1Yes □ 0No

Q116. Mother colorimeter readings taken? □ 1 Yes □ 0 No

INFANT

Q117. FOREHEAD	Q118. FOREARM	Q119 TUMMY
E= □□.□□	E= □□.□□	E= □□.□□
M= □□.□□	M= □□.□□	M= □□.□□
L= □□.□□	L= □□.□□	L= □□.□□
A= □□.□□	A= □□.□□	A= □□.□□
B= □□.□□	B= □□.□□	B= □□.□□

MOTHER

Q120. FOREHEAD

E= □□.□□

M= □□.□□

L= □□.□□

A= □□.□□

B= □□.□□

Q121. FOREARM

E= □□.□□

M= □□.□□

L= □□.□□

A= □□.□□

B= □□.□□

SCORAD SCORE

Q122. SCORAD score (part A/5) □ □ 99 NA

Q123. SCORAD score (part B*3.5) □ □ 99 NA

Q124. SCORAD score (part C) □ □ 99 NA

Q125. SCORAD score [TOTAL] (0-103) □

ENO SAMPLE COLLECTION:

Q126. ENO sample collected? □ 1Yes □ 0No

Q127. If no, why? □ 1 Equipment problem

□ 2 Subject refused

□ 3 Other_____

□ 99 NA

Q128. Number of samples collected: □□ Samples

Q129 ENO value sample 1 □□ ppb □ 99NA

Q130 ENO value sample 2 □□ ppb □ 99NA

Q131 ENO value sample 3 □□ ppb □ 99NA

IOS MEASURMENT

Q132. Pre IOS measurements taken? □ 1Yes □ 0No

Q133. If no, why? □ 1 Equipment problem

□ 2 Subject refused

□ 3 Other_____

□ 99 NA

Q134 Was salbutamol given? □ 1Yes □ 0No

Q135. If no, why? □ 1 Equipment problem

□ 2 Subject refused

□ 3 Other_____

□ 99 NA

Q136. Post IOS measurements taken? □ 1Yes □ 0No

Q137. If no, why? □ 1 Equipment problem

□ 2 Subject refused

□ 3 Other_____

□ 99 NA

SKIN PRICK TESTS Results [positive = ≥3mm wheal at 15 minutes]

Q138. Skin Prick Test carried out? □ 1Yes □ 0No

Q139. Skin Prick Test Problem? □ 0 Nil

□ 1 No clear skin

□ 2 Antihistamine taken

□ 3 Dermatographism

□ 4 Other

Details……

Q140. Skin Prick Test Site: □ 1 Back □ 2 Forearm □ 99NA

Q141. Dust mite ☐☐ mm □ 0Neg □ 1Pos □ 99NA

Q142. Cat ☐☐ mm □ 0Neg □ 1Pos □ 99NA

Q143. Silver birch ☐☐ mm □ 0Neg □ 1Pos □ 99NA

Q144. Grass ☐☐ mm □ 0Neg □ 1Pos □ 99NA

Q145. Alternaria ☐☐ mm □ 0Neg □ 1Pos □ 99NA

Q146 Cladosporum ☐☐ mm □ 0Neg □ 1Pos □ 99NA

Q147. Peanut ☐☐ mm □ 0Neg □ 1Pos □ 99NA

☐☐

Q148. Egg mm □ 0Neg □ 1Pos □ 99NA

Q149. Cow's Milk □□ mm □ 0Neg □ 1Pos □ 99NA

Q150. Dog □□ mm □ 0Neg □ 1Pos □ 99NA

Q151. Neg Control □□ mm □ 0Neg □ 1Pos □ 99NA

Q152. Histamine □□ mm □ mg/ml □ 0Neg □ 1Pos □ 99NA

Q153. Atopy? (≥1 +ve SPT) □ 0No □ 1Yes □ 99NA

NASAL SAMPLE COLLECTION:

Q154. Nasal sample collected? □ 1 Yes □ 0 No

Q155. No. mcl collected ☐☐☐☐ mcl □ 99 NA

Any collecting issue?

……

……

……

BLOOD TEST COLLECTION

Q156. Blood test collected from child? □ 1 Yes □ 0 No

Q157. Number of mls blood taken ☐☐.☐

□ 99 NA

Q158 and 159 HAVE BEEN REMOVED NOW ONLY TAKING BUCCAL SWAB

DNA SAMPLE COLLECTION

Q160. INFANT DNA sample collected? □ 1 Yes (Blood)

□ 2 Yes (Buccal swab)

□ 0 No

Q161. Mother's Buccal DNA sample collected? □ 1 Yes □ 0 No

e-HR Data

Q162. e-HR received from GP □ 1 Yes □ 0 No

IMMUNISATIONS

Q163. Has the child completed the immunization schedule?

8 week	DTaP/IPV/Hib	□ 1Yes □ 0No □ 99NA
	PCV	□ 1Yes □ 0No □ 99NA
12 week	DTaP/IPV/Hib	□ 1Yes □ 0No □ 99NA
	Men C	□ 1Yes □ 0No □ 99NA
16 week	DTaP/IPV/Hib	□ 1Yes □ 0No □ 99NA
	Men C	□ 1Yes □ 0No □ 99NA
PCV	□ 1Yes □ 0No □ 99NA	
12 month	Hib/MenC	□ 1Yes □ 0No □ 99NA
13 month	MMR	□ 1Yes □ 0No □ 99NA
PCV	□ 1Yes □ 0No □ 99NA	
3 years 4 months:	MMR	□ 1Yes □ 0No □ 99NA
DTaP/ IPV	□ 1Yes □ 0No □ 99NA	
dTaP/IPV	□ 1Yes □ 0No □ 99NA	

e-HR Data: LOWER RESPIRATORY TRACT INFECTIONS

Q164. Do the records describe any episodes of bronchiolitis, bronchitis, croup, pneumonia or a chest infection?

□ 1Yes □ 0No □ 99NA

Q165. Details of all episodes:

Age Months 99 = N/A	GP diagnosis 1=bronchitis 2=bronchiolitis 3=croup 4=Pneumonia 5=Chest infection 6=Other (describe) 99=N/A	Antibiotics 1=Yes 0=No 99=N/A	Management 1=Managed at home 2=Attended A+E but discharged 3=Admitted to hospital overnight 99 = N/A

e-HR Data: UPPER RESPIRATORY TRACT INFECTIONS

Q166. Do the records describe any episodes of upper respiratory tract infection?

□ 1Yes □ 0No □ 99NA

Q167. Details of all episodes:

Age Months 99 = N/A	GP diagnosis 1=URTI 2=Cold 3=Flu 5=Other (describe) 99=N/A	Antibiotics 1=Yes 0=No 99=N/A	Management 1=Managed at home 2=Attended A+E but discharged 3=Admitted to hospital overnight 99 = N/A