Vitamin D and Preschool Children – predictors of status and relationship with allergic and respiratory diseases in New Zealand

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Abstract

Background

The role of vitamin D in allergic and respiratory conditions is increasingly being recognised through an immune-modulatory role. The current evidence is inconsistent, with very limited data in preschool children, a target group with high prevalence of early childhood allergic and respiratory disease. There are little data on the vitamin D status and factors associated with vitamin D deficiency in the preschool age group in New Zealand. Knowledge of these factors can assist prediction of preschool children at risk of vitamin D deficiency, improving health outcomes.

Aims and Objectives

To describe the vitamin D status of a self-selected sample of preschool children and determine predictors of vitamin D deficiency in order to develop a predictive questionnaire to assess vitamin D deficiency in this age group, and to investigate the relationship of vitamin D status and prevalence of allergic diseases - eczema, food allergy, allergic rhinoconjunctivitis and asthma – and respiratory infections.

Method

A cross-sectional sample of 1329 preschool children aged 2 to <5 years from throughout New Zealand enrolled during late-winter to early-spring in 2012. 25-hydroxyvitamin D (25[OH]D) was analysed from dried blood spots collected using capillary sampling. Caregivers completed a survey describing their child's demographics, factors known to affect vitamin D status and medical history of allergic and respiratory diseases. Predictors of vitamin D deficiency (25[OH]D <25nmol/L) were identified using multivariable logistic regression in a randomly selected sub-sample (n=929) for development of a predictive questionnaire, which was then validated by receiver operating characteristics (ROC) analysis (n=400).

Results

Mean (SD) dried blood spot 25(OH)D concentration was 52 (19)nmol/L. Vitamin D deficiency was present in 86 (7%) and vitamin D insufficiency (25[H]D <50nmol/L) in 642 (48%)children. Factors independently associated with the risk of vitamin D deficiency were female gender (OR=1.92, 95%CI 1.17-3.14), children of other non-European ethnicities (not including Maori or Pacific)

(3.51, 1.89-6.50), children whose mothers had less than secondary school qualifications (5.00, 2.44-10.21), who had olive-dark skin colour (4.52, 2.22-9.16), who did not take vitamin D supplements (2.56, 1.06-6.18) and who lived in more deprived households (1.27, 1.06-1.53). There were no children who drank toddler milk with 25(OH)D concentrations <25nmol/L thus these children had a zero risk of vitamin D deficiency. The predictive questionnaire had low sensitivity for the identification of children at risk of vitamin D deficiency (sensitivity 42%, specificity 97%).

Children with 25(OH)D concentrations ≥75nmol/L had a two-fold increased risk for parent reported, doctor diagnosed food allergy (OR=2.21, 95%CI 1.33-3.68). No association was present between 25(OH)D concentration and prevalence of eczema, allergic rhinoconjunctivitis, asthma or respiratory infection.

Conclusion

Dried blood spot methods facilitated the measurement of 25(OH)D concentrations in a large sample of preschool children from throughout New Zealand. Prevalence of deficiency in winter was low (7%). The predictors of deficiency were consistent with those in previous studies of other age groups in New Zealand. The predictive questionnaire identified less than half of the children with vitamin D deficiency, so has limited diagnostic ability. In this sample of preschool children, vitamin D deficiency was not associated with allergic diseases or respiratory infections. In contrast, high vitamin D concentrations were associated with a two-fold increased risk of food allergy. This relationship between vitamin D status and allergic diseases is complex, and needs to be further investigated in the preschool age group.

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List of Abbreviations

$1,25(OH)_2D$	1α,25dihydroxyvitamin D or calcitriol
25(OH)D	25-hydroxyvitamin D
AD	Atopic dermatitis
ALRI	Acute lower respiratory infection
AMP	Antimicrobial protein
APC	Antigen presenting cell
AUC	Area under the curve
BMI	Body mass index
CD28	Cluster of differentiation 28
CD4+	CD4 lymphocyte antigen
CI	Confidence intervals
CYP27A1	Gene member of cytochrome P450 family
DBP	Vitamin D binding protein
DC	Dendritic cell
EASI	Eczema Area and Severity Index
FFQ	Food frequency questionnaire
FGF-23	Fibroblast growth factor
FOX P3+	Regulatory T cell
GINA	Global Initiative for Asthma
IFN-γ	Interferon γ
IgE	Immunoglobulin E
IL-12	Interleukin-12
IU	International unit
ISAAC	International Studies of Asthma and Allergies in Childhood
LC-MS/MS	Liquid chromatography with tandem mass spectrometry detection
LRI	Lower respiratory infection
MED	Minimal erythemal dose
mRNA	Messenger RNA
NESS	Nottingham Eczema Severity Score
NHANES	National Health and Nutrition Examination Survey
NHMRC	National Health and Medical Research Council
NMF	Natural moisturising factor

NPV	Negative predictive value
NZ	New Zealand
OR	Odds ratio
P450C1	1-α-hydroxylase gene
PBMC	Peripheral blood mononuclear cells
PTH	Parathyroid hormone
PPV	Positive predictive value
RCT	Randomised controlled trial
RIA	Radioimmunoassay
ROC	Receiver Operating Characteristics
RSV	Respiratory syncytial virus
RXR	Retinoid X receptor
SCORAD	SCORing Atopic Dermatitis
SMS	Short message service or text
SNP	single nucleotide polymorphism
Th	T Helper cell
TLR	Toll like receptor
TNR-α	Tumor necrosis factor α
Treg	T regulatory cell
URI	Upper respiratory infection
US	United States
UV	Ultraviolet
UVB	Ultraviolet beta radiation
UVR	Ultraviolet radiation
VDR	Vitamin D receptor
WHO	World Health Organization
ZO1	Tight junction protein

Contributions of the Study Team

Study Team Member	Contribution
Carolyn Cairncross School of Food and Nutrition, Massey University, Auckland, New Zealand	Planned and managed the execution of the research, designed study questionnaire, obtained ethics approval, study manager, recruited and co-ordinated pharmacies, trained pharmacy staff, recruited participants, conducted research, analysed data, performed statistical analysis, interpreted the results, author of thesis
Dr Pamela von Hurst School of Food and Nutrition, Massey University, Auckland, New Zealand	Main supervisor of PhD, conceptualised and principal investigator of the research, compiled the study team, obtained HRC funding for the research, supervised development of questionnaire, initial contact and negotiations with Pharmacy Brands, contributed to training of pharmacy staff, revised and approved final thesis.
Associate Professor Welma Stonehouse School of Food and Nutrition, Massey University, Auckland, New Zealand; CSIRO Food and Nutrition Flagship, Adelaide, Australia	Co-supervisor of PhD; contributed to design of research and obtaining of funding, assisted with development of questionnaire, supervised statistical analysis of data, revised and approved final thesis.
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Study Team Member	Contribution
Associate Professor Darryl Eyles Queensland Brain Institute, University of Queensland; Queensland Centre for Mental Health Research, Australia	Contributed to the design of the research, advised and assisted with the development of standard fingerprick procedures, developed and performed the biochemical tests for analysing 25(OH)D in dried blood spots.
Dr Lisa Houghton Nutrition Department, University of Otago, Dunedin, New Zealand	Expertise in vitamin D in paediatric age groups, assisted with the development of questionnaire, recruited pharmacies in the Dunedin area of the South Island, recruited participants, trained pharmacy staff, conducted fingerprick tests.
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Chapter 1: **Introduction**

1.1 Introduction

Worldwide, high rates of childhood vitamin D deficiency are a public health concern (Holick, 2007). Infantile rickets has long been recognised as a clinical manifestation of severe vitamin D deficiency but this deficiency can also prevent a child reaching their full growth and peak bone mass potential (Holick, 2004). This has implications for later life with osteoporotic fracture risk determined by peak bone mass (Holick, 2006a). There is emerging evidence which suggests that vitamin D deficiency in childhood may play have an important function in the pathophysiology not only of rickets, but also of non-skeletal diseases which have an immune system mediated pathogenesis (Huh and Gordon, 2008). Examples include allergic diseases such as eczema and asthma, along with respiratory infections, which are prevalent in young New Zealand children, and cause significant morbidity for children and their families (Kiebert *et al.*, 2002; de Jong *et al.*, 2005; Canonica *et al.*, 2007; Holick and Chen, 2008). There is little known about the vitamin D status and factors associated with vitamin D deficiency in preschool aged children in New Zealand. This may in part be due to the reluctance of parents to subject their child to blood sampling (Grant *et al.*, 2009; Nichols *et al.*, 2015). Newly validated methods allow 25-hydroxyvitamin D (25[OH]D) to be measured from a dried blood spot (Eyles *et al.*, 2009), increasing the opportunity to use minimally invasive methods to determine vitamin D status in research studies of vulnerable populations, such as young children.

1.2 Vitamin D status of preschool children in New Zealand and factors associated with vitamin D deficiency

Vitamin D status has been described in various population groups in New Zealand; newborns and infants (Camargo *et al.*, 2010; Wall *et al.*, 2013), children aged 6 to 23 months (Grant *et al.*, 2009; Houghton *et al.*, 2010) and 5 to 14 years (Rockell *et al.*, 2005), and in adults aged 15 years and above (Rockell *et al.*, 2006) (Ministry of Health and Cancer Society of New Zealand, 2012). The vitamin D status of children aged 2 to <5 years in New Zealand has not been researched to date.

Few studies worldwide have specifically examined the vitamin D status of preschool aged children, with most including either younger or older children. Comparison between studies is complicated by month of testing, multiple definitions of deficiency and analysis technique used (Hilger *et al.*, 2014). The prevalence of vitamin D deficiency (25[OH]D <25nmol/L) reported from studies that have recruited their sample over the complete calendar year is 14% of Inuit preschool children in Arctic communities (El Hayek *et al.*, 2010), 20-24% of Asian preschool children living in

London (Lawson and Thomas, 1999) and 1% of the 1-11 years age group in the United States (US) NHANES 2001-6 survey (Mansbach *et al.*, 2009). The prevalence of 25(OH)D <25nmol/L reported in preschool children in summer in Jordan was 3% (Gharaibeh and Stoecker, 2009) and autumn in Nigeria was 0% (Pfitzner *et al.*, 1998). Of children aged 12-59 months tested in spring in Adelaide, Australia, 4% had 25(OH)D <30nmol/L (Zhou *et al.*, 2014).

There are many factors which determine vitamin D status. Identified risk factors for vitamin D deficiency in New Zealand infants and children include female gender (Grant *et al.*, 2009; Houghton *et al.*, 2010), non-European ethnicity (Rockell *et al.*, 2005; Grant *et al.*, 2009; Houghton *et al.*, 2010), residence in a crowded household (Grant *et al.*, 2009), lower maternal education level (Houghton *et al.*, 2010), increased body mass index (BMI) (Rockell *et al.*, 2005), not taking vitamin D supplements (Grant *et al.*, 2009), not drinking infant milk formula (Grant *et al.*, 2009) and measurement in winter or spring (Rockell *et al.*, 2005; Houghton *et al.*, 2010). The impact of these factors specifically on the vitamin D status of preschool aged children is unknown. One potentially important area yet to be explored is the relationship between attendance at daycare (early childhood or preschool education) centres and 25(OH)D concentrations.

An issue that may have contributed to vitamin D status being less well described in the preschool age group is the challenges faced in obtaining blood samples from this age group (Fradet *et al.*, 1990). Parents of young children are reluctant for their children to undergo venepuncture, adversely affecting participation of this age group in research studies (Shaddy and Denne, 2010; Nichols *et al.*, 2015). Recently, sensitive methods for measuring 25(OH)D concentrations on dried blood spots have been developed with good agreement shown between capillary 25(OH)D concentrations measured on a dried blood spot compared with serum obtained by venepuncture (McNally *et al.*, 2008; Eyles *et al.*, 2009; Newman *et al.*, 2009; Heath *et al.*, 2014). This dried blood spot methodology allows for small volume capillary sampling and thus creates the opportunity for direct measurement of 25(OH)D concentration in a large sample of preschool children, which would be particularly attractive for research studies.

1.3 Predictive questionnaires to assess vitamin D deficiency

Currently in New Zealand, vitamin D status is tested through measurement of serum 25(OH)D concentration. The number of requests for measurement of 25(OH)D concentration by New Zealand clinicians has increased 4-fold over the past decade (Bolland *et al.*, 2012). Routine testing

for serum 25(OH)D is expensive and not publically funded (Bolland *et al.*, 2012; Labtests, 2014). Use of capillary dried blood spot analysis techniques also requires costly laboratory analysis (Eyles *et al.*, 2009). Obtaining blood samples by venepuncture can be an intimidating and painful process for young children (Fassler, 1985; Fradet *et al.*, 1990). Clinicians therefore currently rely on an informal assessment of risk factors to decide whether their patient should be treated with vitamin D supplementation (Ministry of Health and Cancer Society of New Zealand, 2012).

Questionnaires which predict vitamin D deficiency or insufficiency are non-invasive and inexpensive. A small number have been developed using predictors of risk of vitamin D deficiency for specific populations including children and adolescents (Absoud *et al.*, 2011), young adults (Bolek-Berquist *et al.*, 2009) and older adults (Nabak *et al.*, 2013; Lopes *et al.*, 2014; Sohl *et al.*, 2014). To our knowledge there is currently no questionnaire for predicting vitamin D deficiency in preschool aged children in New Zealand.

1.4 The relationship between vitamin D status and allergic diseases and respiratory infection in preschool children

Historically, research on vitamin D deficiency in young children focussed on bone development and rickets. Recent discoveries of an immune modulation role for vitamin D has led to the investigation of associations of vitamin D status with a number of immune mediated childhood illnesses, including auto-immune, atopic and infectious conditions (Hyppönen *et al.*, 2001; Holick, 2007; Bener *et al.*, 2009; Hollams *et al.*, 2011; Lerner *et al.*, 2012).

1.4.1 Vitamin D and eczema

Eczema, or atopic dermatitis, is a recurring chronic inflammatory skin condition present in 10-20% of children and 1-3% of adults worldwide (Mallol *et al.*, 2013). It occurs more frequently in urban, higher socioeconomic families in industrialised nations (Kay *et al.*, 1994; Beasley, 1998; Wong *et al.*, 2001; Mallol *et al.*, 2013). Although the prevalence of eczema in preschool children in New Zealand is unknown, rates in New Zealand children are high as illustrated by 16% of children aged 6-7 years reporting eczema in 2006 compared with a worldwide prevalence of 8% in this age group (Williams *et al.*, 2008).

Morbidity from eczema can be high, particularly for young children. Quality of life is diminished for both the child and their family due to pruritus (itch), recurrent skin infections, diminished educational progress through school absence, daily treatment regimens and parental stress

(Kiebert *et al.*, 2002). Sleep disturbance during eczema exacerbations may mediate increased discipline problems and attention deficit hyperactivity disorder (ADHD) (Reid and Lewis-Jones, 1995; Chamlin *et al.*, 2005; Beattie and Lewis-Jones, 2006). Economic costs to individual families and health providers are substantial through primary care visits and provision of medications for daily treatment and recurrent infections (Emerson *et al.*, 2001).

The aetiology of eczema is complex and is currently not well understood. Contributory factors are believed to include defects in the immune system, antimicrobial defence actions and epidermal barrier integrity (De Benedetto *et al.*, 2009; Bieber, 2010). Risk factors for eczema include atopic predisposition and environmental factors (seasonal climate changes, chemical irritants, bacterial colonisation and psychological stress) (Kusunoki *et al.*, 1999). Eczema has been described as the first step of the 'atopic march'; the progression from eczema in early childhood to development of asthma, allergic rhinitis and food allergy in later life (Zheng *et al.*, 2011).

Vitamin D is potentially involved in eczema aetiology. A regulatory role of vitamin D on immune response has been proposed as a possible therapeutic target for this allergic condition. Skin barrier function and secretion of antimicrobial peptides are inhibited in eczema through the down regulation of the Th_2-driven immune response (Mrabet-Dahbi and Maurer, 2010). The active form of vitamin D, 1-25$(OH)_2D_3$, stimulates keratinocytes to produce antimicrobial peptides in the epidermis, which subsequently reduce the colonisation by bacteria such as *Staphylococcus aureus*, promoting integrity of the permeability barrier (Leung *et al.*, 1993).

The relationship between vitamin D concentration and eczema is unclear. Results from observational studies which include preschool aged children investigating associations between vitamin D and eczema report contradictory findings, with both lower (Oren *et al.*, 2008; Peroni *et al.*, 2011; Lee *et al.*, 2013; Shin *et al.*, 2014; Wang *et al.*, 2014) and higher (Heimbeck *et al.*, 2013) vitamin D concentrations associated with increased eczema prevalence and severity. Conflicting results have also been reported in birth cohort studies of early-life measures of vitamin D and association with childhood eczema prevalence rates (Camargo Jr *et al.*, 2007; Gale *et al.*, 2007; Back *et al.*, 2009; Erkkola *et al.*, 2009; Miyake *et al.*, 2010; Jones *et al.*, 2012; Tolppanen *et al.*, 2013; Baïz *et al.*, 2014).

Vitamin D receptor activation has been shown to improve allergen-triggered eczema in murine studies (Hartmann *et al.*, 2012). However, conflicting results have been seen in clinical trials. Increased expression of cathelicidin in lesional biopsies was seen in a trial of eczema patients

receiving daily supplementation of vitamin D (Hata *et al.*, 2008). Randomised controlled trials (RCTs) have found vitamin D supplementation improved eczema severity scores in children in Boston and Mongolia (Sidbury *et al.*, 2008; Camargo Jr *et al.*, 2014), in Iranian adolescents and adults (Javanbakht *et al.*, 2011) and adults living in Poland and Iran (Amestejani *et al.*, 2012; Samochocki *et al.*, 2013). In contrast, no improvement in eczema severity in those receiving vitamin D supplementation compared to controls was observed in a study of adults in the United States (Hata *et al.*, 2014). Currently, the overall association of vitamin D and eczema is inadequately defined.

Treatment of eczema involves reducing skin inflammation together with improved skin barrier function. Phototherapy has been used in the treatment of severe cases for some time. The mechanism of action remains incompletely understood (Rose *et al.*, 2014). Alternative treatment options are needed as there is often poor adherence by parents to routine application of topical treatment in children together with potential side effects of the topical preparations. It has been proposed that vitamin D has the potential to offer a relatively safe, low-cost and easily administered treatment option to reduce eczema prevalence and severity.

1.4.2 Vitamin D and food allergy

Food allergy is a modern disease of the developed world (Prescott and Allen, 2011). Epidemiological data suggests rates of exaggerated immune responses to certain food proteins and associated anaphylaxis have increased over the past 20 years (Liew *et al.*, 2009) as a component of the allergy epidemic (Allen and Dharmage, 2010; Prescott and Allen, 2011). Rates of hospitalisations of young children due to serious reactions to food are increasing (Mullins, 2007). There is no clear explanation for the rise in food allergy rates, proposed factors include timing of complementary feeding, increased hygienic standards, adoption of western life styles with less outdoor physical activity and poor vitamin D status (West *et al.*, 2011).

Reporting accurate prevalence of food allergy is problematic, as the most reliable indicator of food allergy is the double-blind, placebo-controlled food challenge (Burks *et al.*, 2012). However, use of this method is time-consuming, resource intensive, costly and impractical for prevalence studies (Gaspar-Marques *et al.*, 2014). Overestimation of food allergy prevalence by parental report is a well-recognised issue, illustrated in a UK study which reported a parental report cumulative food allergy incidence of 34% for 3 year old children, however the incidence confirmed by food challenge and detailed medical history was 5% (Venter *et al.*, 2008). The

prevalence of food allergy in New Zealand has not been determined (Crooks *et al.*, 2008) but is estimated to be approximately 10% based on epidemiological studies from neighbouring Australia (Osborne *et al.*, 2011; Allergy New Zealand, 2014). A small study of 110 Auckland children aged 0-5 years attending a well-child clinic found 40% of parents reported an adverse effect to food with only 4% having had clinical evaluation and allergy testing (Crooks *et al.*, 2010).

The immune reaction of a child to a food protein can be IgE mediated, non-IgE mediated or a combination, and vary in both timing and severity (Sackeyfio *et al.*, 2011). Reaction can be immediate or delayed, with symptoms ranging from mild (itching or redness around mouth) to severe and potentially fatal (systemic anaphylactic reaction) (Sackeyfio *et al.*, 2011). The consequences of food allergy in children are serious for the child, family and community. Quality of life is compromised through the stress of worrying about the potential risk of reaction to food and avoidance of offending food (Marklund *et al.*, 2006) combined with disruption and limitation to usual family activities (Sicherer *et al.*, 2001), particularly so for children who are allergic to peanuts (Primeau *et al.*, 2000).

Two alternate hypotheses of a potential role for vitamin D status as a contributing factor in the recent global increase of allergic diseases have been proposed. The hypothesis suggesting that vitamin D deficiency increases the risk of food allergy (Vassallo and Camargo Jr, 2010) is supported by research where proxy indicators of vitamin D status, latitude and season of birth, are indirectly associated with food allergy prevalence. Latitudinal variation has been observed with residence at higher latitudes associated with higher rates of epinephrine auto-injector prescriptions for treatment of anaphylaxis (EpiPen) in children in Australia (Mullins *et al.*, 2009) and the United States (Camargo Jr *et al.*, 2007), anaphylaxis hospital admission rates in Chilean children (Hoyos-Bachiloglu *et al.*, 2014) and infant hypoallergenic formula prescription rates in Australia (Mullins *et al.*, 2010). Children born during autumn/winter when UVB exposure and vitamin D levels are lowest are more likely to be diagnosed with food allergy (Vassallo *et al.*, 2010; Mullins *et al.*, 2011; Keet *et al.*, 2012).

The second hypothesis states that higher vitamin D levels increase the risk of allergic diseases (Wjst and Dold, 1999; Wjst, 2006). This hypothesis is supported by data from birth cohort studies which show an increased risk of allergic conditions and atopy in offspring of mothers with higher 25(OH)D concentrations during pregnancy, or children who were supplemented with vitamin D in infancy (Hypponen *et al.*, 2004; Kull *et al.*, 2006; Gale *et al.*, 2007). Although a large amount of literature exists detailing the mechanistic pathways of vitamin D and food allergy, studies

examining the association between direct measurement of vitamin D status and food allergy have yet to be conducted. To date, only one study has been reported in Australian infants, where infants aged 12 months with 25(OH)D concentrations <50nmol/L were at increased risk of egg or peanut allergy (Allen *et al.*, 2013).

Current treatment of food allergy consists of avoidance of offending foods and anaphylaxis management plans, with immunotherapy treatment still laboratory based. It has been hypothesised vitamin D status has the potential to influence the development of food allergy (Vassallo and Camargo Jr, 2010).

1.4.3 Vitamin D and allergic rhinoconjunctivitis

Allergic rhinoconjunctivitis is the most common allergic airways disease, with symptoms of itchy eyes, runny nose, sneezing and nasal congestion (Asher *et al.*, 2006; Bousquet *et al.*, 2008). There is a high prevalence worldwide, particularly in countries with western lifestyles (Pawankar *et al.*, 2010). In New Zealand this condition affects 11% of children aged 6-7 years (Asher *et al.*, 2006). The rate of allergic rhinoconjunctivitis in this age group increased by 1% from 1994/5 to 2006 while over the same time decreased by 1% in 13-14 year old children (Asher *et al.*, 2008). Although allergic rhinitis is the term most commonly used in scientific literature, allergic rhinoconjunctivitis is preferable as it includes the itchy eye symptoms experienced by children over an extended period of time (Skoner, 2001). Common or seasonal 'hayfever' symptoms are an intermittent response to airborne pollens (Skoner, 2001).

The impact of allergic rhinoconjunctivitis is high in both economic and social terms. Asthmatic children who also have allergic rhinoconjunctivitis have more asthma exacerbations, hospital visits and higher healthcare costs than those with asthma alone. (Solé *et al.*, 2005; Gaugris *et al.*, 2006). Quality of life is impaired for both children with allergic rhinoconjunctivitis and their families, with interrupted sleep, associated fatigue from cough and nasal drip or blockage and the detrimental impact on schooling, with educational progress damaged through missed schooling and decreased concentration in class (de Jong *et al.*, 2005; Canonica *et al.*, 2007; Bousquet *et al.*, 2008). Data from epidemiological studies suggest that rhinitis is an independent risk factor for asthma (Leynaert *et al.*, 2000) in addition to being a manifestation of atopy (Guerra *et al.*, 2002; Peroni *et al.*, 2003).

Allergic rhinoconjunctivitis has a multifactorial aetiology. The specific aspect being considered in this thesis is the role played by vitamin D. The immune-modulatory actions of vitamin D affect both the innate and adaptive components of the immune system, and may offer potential targets for prevention or treatment of allergic diseases such as allergic rhinoconjunctivitis (Mathieu and Adorini, 2002). Animal model studies show evidence for a vitamin D mediated enhanced T helper 2 (Th_2) cell antigen response by two pathways; firstly by shifting the T lymphocyte balance toward Th_2 cells through the inhibition of Th_1 cells and secondly, through increased production of Th_2 cells through the differentiation of naive T-Cells into Th_2 cells (Adorini *et al.*, 2003; Wintergerst *et al.*, 2007). However, clinical studies have found mixed results for a role of vitamin D in the immune pathogenesis of allergic rhinoconjunctivitis, as has also been reported for studies that have sought to define the role of vitamin D in asthma pathogenesis (Litonjua, 2012b; Paul *et al.*, 2012).

A small number of observational studies investigating the relationship between vitamin D and allergic rhinoconjunctivitis have been conducted in Europe, the Middle East, Asia and the United States. These studies report conflicting findings. From birth cohort studies, both inverse and null associations of early-life measures of vitamin D status with childhood allergic rhinitis have been reported (Erkkola *et al.*, 2009; Rothers *et al.*, 2011; Baïz *et al.*, 2014). Higher rates of vitamin D deficiency were reported in Iranian adults diagnosed with allergic rhinitis compared to population controls (Arshi *et al.*, 2012). In contrast for children and adults living in the United States, allergic rhinitis prevalence was shown to increase as serum 25(OH)D concentration increased (Wjst and Hyppönen, 2007) while no association was found in studies of adults in Korea and of children in Qatar (Ehlayel *et al.*, 2011; Cheng *et al.*, 2014). To date, there have been no published studies that have measured serum 25(OH)D concentration and prevalence of allergic rhinoconjunctivitis in preschool aged children, and determined whether or not a relationship exists between these two variables.

Current treatment for allergic rhinoconjunctivitis is by allergen avoidance, pharmacotherapy with antihistamines or corticosteroids and immunotherapy (Bousquet *et al.*, 2008) Each of these options has limitations; allergen avoidance can be difficult, there are side effects of drugs and results of immunotherapy are inconclusive to date for allergic rhinoconjunctivitis. Ongoing investigation into prevention strategies such as ensuring normal vitamin D status has the potential to offer a low-cost, relatively safe and easily-administered treatment for a disease with high morbidity and healthcare costs.

1.4.4 Vitamin D and asthma

Asthma is a major public health issues, affecting more than 300 million individuals worldwide, with significant morbidity (Braman, 2006; Bousquet *et al.*, 2008). Childhood asthma rates in New Zealand are amongst the highest in the world, affecting around 200,000 children, and along with respiratory illness are the most common causes for hospital admissions and absence from schooling in young children (Wald *et al.*, 1991; Asher *et al.*, 1995; Masoli *et al.*, 2004).

Asthma has many phenotypes to which multiple environmental and genetic factors contribute (Borish and Culp, 2008). Non-allergic asthma is characterised by neutrophilic inflammation independent of the Th_2 lymphocyte atopic pathway (Borish and Culp, 2008). Risk factors for non-allergic asthma include viral infection, stress, obesity and environmental factors such as air and workplace pollutants (Kim *et al.*, 2010). Allergic asthma is the most common form of asthma, where the immunological mechanism involves eosinophilic inflammation dependent on a Th_2 lymphocyte response following allergen exposure (Arbes Jr *et al.*, 2007; Kim *et al.*, 2010). Development of atopy, defined as the production of immunoglobulin (IgE) antibodies in response to ordinary exposure to antigens, in children increases the risk of asthma occurrence, persistence and severity (Martinez, 2002; Rasmussen *et al.*, 2002; Holloway and Holgate, 2004). The immune-modulatory role of vitamin D in both innate and adaptive immune response (Mathieu and Badenhoop, 2005; Kim *et al.*, 2010) inhibits production of the Th_{17} cytokines allied with asthma severity and compromised response to steroid treatments (Nanzer *et al.*, 2013).

Epidemiological data that have investigated the relationship between vitamin D status and asthma prevalence and severity in children have produced conflicting results (Brehm *et al.*, 2009; Freishtat *et al.*, 2010; Rajabbik *et al.*, 2014). A low maternal vitamin D intake during pregnancy and low cord blood 25(OH)D concentrations have been associated with increased risk of childhood wheezing and asthma (Camargo *et al.*, 2007; Devereux *et al.*, 2007; Erkkola *et al.*, 2009; Hollams *et al.*, 2011; Baïz *et al.*, 2014). In contrast, higher maternal vitamin D intake during pregnancy and infant vitamin D supplementation has also been associated with increased risk of subsequent asthma (Hypponen *et al.*, 2004; Miyake *et al.*, 2010), while several studies report no association was evident between maternal 25(OH)D concentration nor cord blood 25(OH)D concentrations and childhood asthma (Rothers *et al.*, 2011; Morales *et al.*, 2012; Baïz *et al.*, 2014). Increased responsiveness to corticosteroid medication has been reported in asthmatic patients supplemented with vitamin D (Sutherland *et al.*, 2010).

Current treatment options for children with asthma include beta-antagonists and corticosteroids, both of which are expensive and have significant side effect profiles including growth suppression (Prevention Program, 2007). Children with severe asthma risk potential adverse effects of oral corticosteroid treatment including compromised bone density, and subsequent increased risk of fracture and adrenal suppression (Lipworth, 1999). Vitamin D has the potential to offer a low-cost, relatively safe and easily-administered intervention for a disease with significant morbidity and healthcare costs if associations are confirmed in future research.

1.4.5 Vitamin D and respiratory infections

The World Health Organisation estimates 4 million deaths worldwide in 2004 were due to acute respiratory infections (Jolliffe *et al.*, 2013) with acute lower respiratory infection the leading cause of childhood mortality for approximately 1.8 million children under 5 years of age each year (Williams *et al.*, 2002). In developed countries acute respiratory infections have become an uncommon cause of mortality but remain a significant cause of morbidity. New Zealand is an industrialised nation but has a higher rate of hospitalisation for acute lower respiratory infections such as pneumonia than other developed countries, with rates increasing between 2000-01 and 2010 (Craig *et al.*, 2013).

Respiratory infections can be divided into upper and lower respiratory infections, according to where they occur in the respiratory tract, and cause diseases with a broad illness spectrum (e.g. from the common cold through to complicated pneumonia) (Heikkinen and Järvinen, 2003; Grant *et al.*, 2011). The common cold is the most frequent upper respiratory infection, with the highest rates in children under five years of age, occurring more often in winter and in children who attend daycare or preschool centres (Kværner *et al.*, 2000; Heikkinen and Järvinen, 2003; Carroll *et al.*, 2009). Acute respiratory infections are responsible for initiating between 80-85% of asthma attacks in children (Johnston *et al.*, 1995). Respiratory illnesses caused directly or indirectly by respiratory infections are the most common causes for hospital admissions and absence from schooling in young children (Wald *et al.*, 1991).

Vitamin D has a potential role in respiratory infection through the immune system. Vitamin D deficiency has been shown to be associated with impaired innate immunity and a reduced production of the antimicrobial peptide cathelicidin (Walker and Modlin, 2009; Bhan *et al.*, 2011) and, via these mechanisms, is believed to increase susceptibility to respiratory infections (Ginde *et al.*, 2009a; Ginde *et al.*, 2009b; Camargo *et al.*, 2011). Epidemiological studies have shown that

lower concentrations of 25(OH)D are associated with increased risk of lower respiratory tract and viral infections in young children (Wayse *et al.*, 2004; Ginde *et al.*, 2009b; Jartti *et al.*, 2010). Cohort studies suggest a protective effect, with an increased risk of childhood wheezy lower respiratory tract infection with low maternal vitamin D intake during pregnancy and lower cord blood 25(OH)D concentrations (Camargo *et al.*, 2007; Camargo *et al.*, 2011). Supplementation trials of vitamin D have shown a protective effect for reduced medical visits for respiratory infections in New Zealand infants (Grant *et al.*, 2014) and children living in New York (Linday *et al.*, 2004), reduced incidence of acute respiratory infection in Mongolian school children (Camargo *et al.*, 2012) and influenza risk in Japanese schoolchildren (Urashima *et al.*, 2010).

Current management options for respiratory infection include prevention through immunisation and treatment with antimicrobials (Monto, 2002). Both of these options have limitations. Vaccines are only available for some of the pathogens which cause respiratory infections, and antimicrobials are limited primarily to bacterial infections with their effectiveness reduced by increased rates of antimicrobial resistance (Thomas *et al.*, 2013). Alternative interventions, such as vitamin D, which contribute to the prevention of infection, decrease the duration and severity of infection, the need for antibiotic treatment, and the subsequent progression to asthma exacerbations are needed.

1.5 Study Aims & Objectives

The aim of this research is to determine, in children aged 2 to <5 years in New Zealand, the prevalence and risk factors for low 25(OH)D concentrations, to develop a questionnaire to assess the risk of vitamin D deficiency, and to investigate the relationship of vitamin D and allergic and respiratory diseases.

The objectives of this research are to:

- Describe the vitamin D status of a self-selected sample of preschool children in New Zealand aged 2 to <5 years measured in dried blood spot
- To identify factors associated with vitamin D deficiency in this age group
- Develop and validate a predictive questionnaire to assess risk of vitamin D deficiency in children of preschool age
- Investigate the relationship of vitamin D status with prevalence of allergic diseases - eczema, food allergy, allergic rhinoconjunctivitis and asthma – and respiratory infection in preschool children.

1.6 Study Hypotheses

Hypothesis 1

Collection of data on vitamin D status and known and hypothesised risk factors will enable the creation and validation of a risk assessment questionnaire for vitamin D deficiency in preschool children in New Zealand.

Hypothesis 2

Vitamin D status is inversely associated with the prevalence of allergic and respiratory diseases in preschool children in New Zealand.

1.7 Overview of this thesis

In this thesis I will present the original research undertaken during my PhD candidature. An introduction is provided in Chapter 1, followed by a comprehensive review of the literature around my research questions in Chapter 2. In Chapter 3, the study methodologies are described, along with the definitions of disease investigated in this thesis.

The results of my research are presented in Chapter 4. The order in which the results are presented are; vitamin D status and predictors of vitamin D deficiency, the development of a predictive questionnaire to assess the risk of vitamin D deficiency in preschool children; the relationship of vitamin D status and prevalence of allergic diseases and respiratory infection. The order for allergic diseases follows the progression of the 'atopic march' - eczema, food allergy, allergic rhinoconjunctivitis and asthma. Chapter 5 provides the main findings, followed by a more detailed discussion of each of the topics in the order detailed above. The methodological strengths and limitations are combined at the end of this chapter to aid reading and avoid repetition. This thesis concludes with Chapter 6, outlining the significance of results, recommendations for future research and final conclusion.

Chapter 2: Review of the literature

2.1 Introduction

2.1.1 Structure of this review

This literature review begins with a brief history and overview of the sunshine vitamin, vitamin D. Following this, measurement of vitamin D status is described, firstly contrasting traditional serum testing methods with more recent techniques of dried blood spot analysis of 25(OH)D and secondly, briefly examining the controversy surrounding definitions of vitamin D status.

A review of previous studies which have measured vitamin D status in preschool children aged 2 to <5 years will be made. To date, research measuring this status has yet to be carried out in New Zealand, so studies which have determined vitamin D status in other age groups in New Zealand will also be examined.

There are many factors which may contribute to vitamin D deficiency. An examination of studies in preschool aged children will include evidence about both dietary and non-dietary factors. In order to provide local context, studies which have examined these factors in infants and young children in New Zealand are included.

Identification of vitamin D deficiency in preschool children without need for blood sampling and analysis would be beneficial in order to improve health outcomes. This literature review will focus on predictive questionnaires which have been developed to determine the risk of vitamin D deficiency being present.

Finally, there is a wealth of literature on the potential mechanistic pathways of vitamin D and the immune system, based primarily from in vitro and murine studies. Much of the evidence in humans comes from birth cohort studies, which are reviewed along with studies investigating the relationship between vitamin D and allergic and respiratory diseases in preschool children.

There were various strategies employed to investigate the literature for this review. An initial search of the Web of Science and PubMed databases was conducted using the keywords 'vitamin D', 'status', 'children', 'deficiency'. 'Vitamin D' and 'children' were then combined with 'predictive', 'questionnaire' and subsequently 'eczema', 'food allergy', 'allergic rhinoconjunctivitis', 'asthma' and 'respiratory disease'. References cited in major articles and books were accessed, as were

those which had cited these relevant publications. Incidental methods included references offered by colleagues and keeping up to date with major pertinent journals.

2.1.2 Literature review introduction

New Zealand is an island nation in the South Pacific, with latitude from 34^{o}S to 47^{o}S (Central Intelligence Agency, 2007). The population of 4.2 million reside almost entirely on the two main North and South Islands (77% and 23% respectively). New Zealand's population is diverse with over 200 ethnic groups represented (Statistics New Zealand, 2013). The two largest groups are of European (71%) and indigenous Maori (14%) heritage. Previously people from the Pacific islands comprised the third largest ethnic group (8%), recently this has been overtaken by those of Asian heritage (11%) with other ethnic groups each representing less than one percent of the population (Statistics New Zealand, 2013). New Zealand provides a unique location for vitamin D research – NZ has a sunny climate but due to high rates of skin cancer has a sun avoidance policy, there is no mandatory fortification of the food supply with vitamin D nor routine supplementation with vitamin D recommended in childhood by health authorities. The Ministry of Health recommends supplementation of 10µg/day for babies and infants at risk of vitamin D deficiency (Ministry of Health, 2008). Vitamin D deficiency of sufficient frequency to be of public health concern has been reported in all age groups studied.

Preschool children are characterised as the life stage between infants and children attending school. In a long-held tradition, children in New Zealand start school on the day of their fifth birthday. Thus, preschool children are aged 2 to <5 years in this country.

Vitamin D is routinely described as a fat-soluble vitamin with a primary role in bone metabolism. More accurately, vitamin D consist of a series of steroid hormones. There have been five forms of vitamin D described, the two of greatest consequence for humans are vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol). These are produced by the action of sunlight on different sources; plant surfaces for ergocalciferol (D_2) and the skin of animals for cholecalciferol (D_3) (Godar, 2005).

2.2 History of vitamin D

The pathway of nutritional science through the late 19th and early 20th centuries evolved to recognise that many health conditions were due to nutrient deficiencies (Cannon and Leitzmann, 2005). Many children in industrial England (circa 1880-1920) suffered from weak, bowed legs as a result of abnormal bone mineralisation, subsequently known as rickets. Rickets had been described as early as the 17th century but the aetiology was first theorised in 1822 by Sniadecki in Poland (Sniadecki, 1939). He observed children living in rural areas had a far lower prevalence of rickets compared to those living in industrial cities, and concluded this was due to a lack of sun exposure (Sniadecki, 1939). In 1890, through observation of rickets in Northern Europe compared with Japan and tropical countries, Palm hypothesised that exposure to sunlight prevented rickets (Palm, 1890).

Unfortunately these findings were not embraced by the scientists of this time. Almost one hundred years later Sir Edward Mellanby ran experiments on his own beagle dogs in exploring his theory of rickets being related to diet (Mellanby, 1976). He fed these dogs an oatmeal-prominent diet which was similar to that of children from areas with high rates of rickets. He also inadvertently excluded the dogs from sun exposure by keeping them indoors. This mimicked the conditions children with rickets were living in; polluted atmospheres over cities from new industrial factories which limited sun exposure. The dogs subsequently developed rickets, which was cured by the administration of cod liver oil (Mellanby, 1976).

Simultaneous to Mellanby's experiment, Huldschinsky and Chick reported curing and preventing rickets through exposure of children to artificial sunlight from a UV lamp (Huldschinsky, 1919). These findings were consistent with the observation that all children with rickets showed signs of improvement in their condition from exposure to sunlight on the roof of the New York City hospital (Hess and Unger, 1921). These researchers had thus identified two treatment avenues for the rickets condition - one dietary (absorption of vitamin D from cod liver oil), the second exposure to sun or ultraviolet light (vitamin D produced by action of UVB radiation on skin).

This work lead to public health campaigns in the UK, US and Europe urging sensible sun exposure for children (Hess and Unger, 1921) and the fortification of milk with vitamin D_2 (Holick, 2006b). These strategies proved successful in decreasing rates of rickets through the first half of the twentieth century (Holick, 2006b). However, the process of milk fortification was not closely monitored or regulated, and the high level of vitamin D was blamed for an outbreak of

hypercalcemia in the UK in the 1950's, halting the fortification of milk in most of Europe (Samuel, 1964).

Historically, researchers have been slow to recognise the effects of vitamin D. Initially, the cure of rickets was attributed to the vitamin A component of cod liver oil (McCollum *et al.*, 1916; McCollum *et al.*, 1922). Early investigations into treatment of asthma and allergy around 1930 were focused on the nutrient calcium, with researchers inadvertently using preparations containing vitamin D in the form of cod liver oil or viosterol (irradiated ergosterol) (Rappaport and Reed, 1933). Initial trials with extremely high doses of viosterol were inconclusive, but a 1934 study of hayfever and asthma sufferers found those treated with calcium and viosterol (vitamin D_2) reported decreased symptoms of hayfever while serum calcium levels were unchanged (Rappaport *et al.*, 1934). It is not known exactly why, but subsequent studies on asthma and allergy concentrated on calcium until the 1990's (Litonjua, 2012a).

From the middle to late 20th century, rickets ceased to be a prominent public health concern in many countries. In the last two decades there has been a re-emergence of infantile rickets, not only in countries with low levels of sunshine and nutritional limitations but also in countries considered 'sunny' historically with predominately outdoor lifestyles, such as New Zealand (Blok *et al.*, 2000; Judkins and Eagleton, 2006). Another recent important advance, which has resulted in a broadening of the perspective of the potential roles of vitamin D in health, is the discovery of vitamin D receptors in many cells with the mechanistic potential for vitamin D to be involved in other disease conditions, such as asthma, allergy and respiratory infections, in addition to calcium homeostasis and bone health (Hewison *et al.*, 2000). This has contributed to the rapid rise in the number of research projects investigating vitamin D over the past decades (Figure 2.1).

2.3 Metabolism of vitamin D

There are separate mechanistic pathways followed for the two sources of vitamin D. Dietary sources of vitamin D_2 and D_3 are absorbed within the small intestine, packaged into chylomicrons (lipoproteins) and transported through the lymphatic system to enter the hepatic portal circulation (DeLuca, 2004). Endogenous synthesis of vitamin D involves the absorption of ultraviolet B radiation (UVB, 280-320nm) by 7-dehydrocholesterol to form previtamin D_3, which is rapidly converted via thermal isomerisation to vitamin D (Hewison *et al.*, 2000). Vitamin D diffuses across dermal capillaries and enters the circulation, and binds to vitamin D binding protein (DBP) for transport to the liver (DeLuca, 2004) (Figure 2.2).

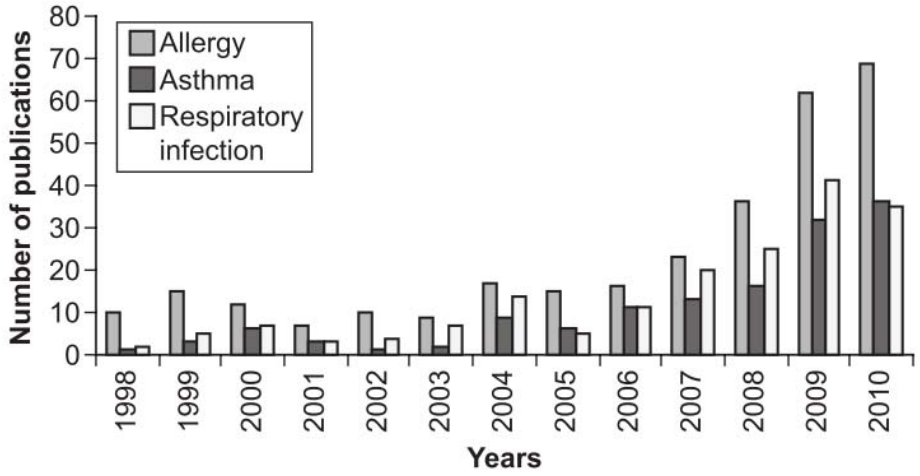


Figure 2.1. Published studies on vitamin D and allergy, asthma and respiratory infections from 1998 to 2010. Reproduced with permission (Bozzetto *et al.*, 2012)

Upon release from DBP, the inert vitamin D is hydroxylated by the enzyme vitamin-D-25-hydroxylase (CYP27A1) to 25-hydroxyvitamin D (25[OH]D) (calcidiol) (Miller and Portale, 2000). A second hydroxylation is required to fully activate 25(OH)D. 25-hydroxyvitamin D is bound to DBP and transported to the renal tubule cell mitochondria for hydroxylation via enzyme 1-α-hydroxylase (also known as P450C1 or CYP27B1) to produce the hormonally active $1,25(OH)_2D$ (calcitriol) and enzyme 24-hydroxylase (P450C24 or CYP24A1) to $1,24,25(OH)_3D$, which has limited biological activity (Holick, 2004). Recent research has shown that the 1-α-hydroxylase enzyme is also present within a range of cell types in addition to the renal tubule cell, including alveolar macrophages, lymph nodes, placenta, colon, osteoblasts, keratinocytes, monocytes and pancreatic beta cells, suggesting an autocrine-paracrine role for the active metabolite (Hewison *et al.*, 2000; Adams and Hewison, 2008). The active $1,25(OH)_2D$ is discharged from the kidney and circulates around the body through the blood stream to the nuclei of target cells by intracellular binding protein (Adams and Hewison, 2010). In these cells, the nuclear vitamin D receptor (VDR) is activated, inducing a hetero-dimerization between a retinoic X receptor (RXR) and the VDR, forming the complex $1,25(OH)_2D$-RXR-VDR. This complex aids interaction between DNA and the VDR, altering mRNA and stimulating de novo protein synthesis (Christakos *et al.*, 2003).

Serum levels of 25(OH)D and $1,25(OH)_2D$ are controlled through feedback mechanisms in the liver and kidney, albeit with the existence of vitamin-D-25-hydroxylase and 1-α-hydroxylase in other tissues such as skin and intestine (DeLuca, 2004). Calcium homeostasis closely regulates $1,25(OH)_2D$ production. Parathyroid hormone (PTH) induces renal synthesis of calcitriol with

reduced serum calcium levels (Jones *et al.*, 1998). Circulating $1,25(OH)_2D$ itself reduces expression of 1-α-hydroxylase in conjunction with an increased expression of 24-hydroxylase, which exists in almost all cells and is responsible for degradation of $1,25(OH)_2D$ to the inactive $1,24,25(OH)_3D$ which is excreted in bile (Jones *et al.*, 1998; Hewison *et al.*, 2000).

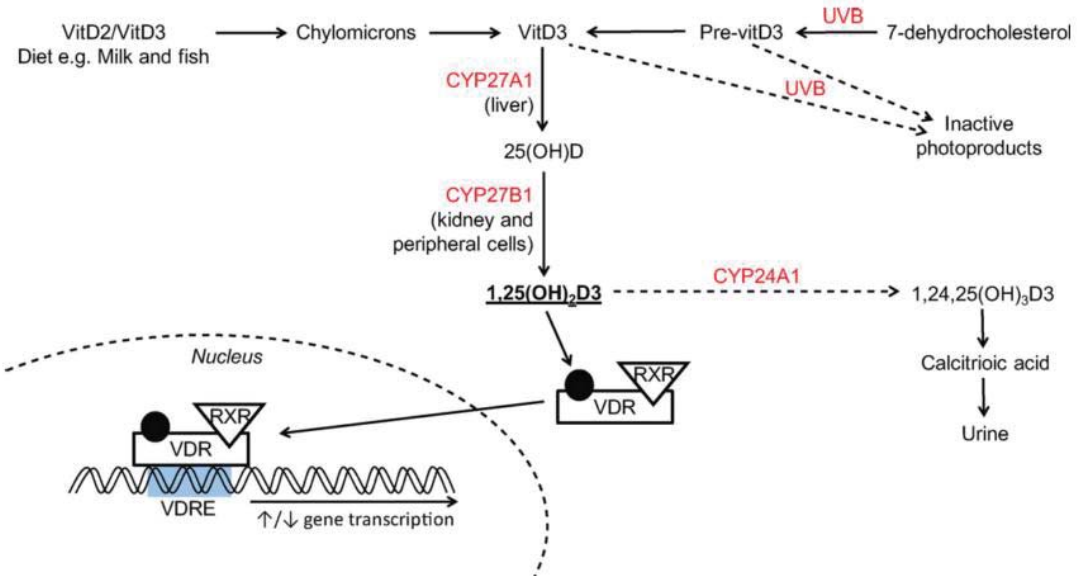


Figure 2.2. Vitamin D metabolism and receptor binding. Reproduced with permission (Mann *et al.*, 2014)

2.4 Vitamin D_2 and D_3

Habitually, vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol) were considered to have equivalent biological effect but recent research has challenged this view (Houghton and Vieth, 2006). Trang *et al* supplemented adults with 4000 IU of either D_2 or D_3 for 14 days in winter months, and reported 25(OH)D concentrations were approximately 2 times higher for those participants consuming D_3 compared to D_2 after a 2 week period (Trang *et al.*, 1998). Whilst both forms increase serum 25(OH)D concentrations initially, Armas *et al* found 25(OH)D concentrations were sustained for a longer time in those who consumed a large dose (50000 IU) of D_3 compared to D_2, with 25(OH)D concentration in D_2 participants decreasing after day 3 whilst those supplemented with D_3, 25(OH)D decreased only after day 14. The authors attributed this to $25(OH)D_3$ having a higher affinity for the DBP transporter (Armas *et al.*, 2004). The lower affinity of $25(OH)D_2$ for DBP means it has a shorter half-life than 25(OH) D_3, lowering its effectiveness (Houghton and Vieth, 2006).

2.5 Sources of vitamin D

Unique among vitamins, the human body has two avenues for obtaining vitamin D - firstly, dietary through the consumption of foods and supplements, and secondly, from the action of sunlight on skin. On average, over 90% of vitamin D is sourced from sun exposure, but the relative contribution from food increases over winter months when sun exposure is limited (Davies *et al.*, 1999; Pasco *et al.*, 2001).

2.5.1 Sun exposure source of vitamin D

There are many factors which affect the extent of cutaneous production of vitamin D, including time of day of sun exposure, seasonality, atmospheric conditions, latitude, age, skin pigmentation and sun protection practices. These factors are discussed in detail in Section 10 later in this review.

2.5.1.1 Sunlight and skin cancer

The situation where sunshine, the primary source of vitamin D, is a known carcinogen creates a dilemma (International Agency for Research on Cancer, 1992). With over 90% of vitamin D sourced through the cutaneous conversion of 7-dehydrocholesterol to vitamin D_3, this situation calls for a balance between sufficient sun exposure to ensure adequate vitamin D production whilst minimising the risk for skin cancer (Nowson and Margerison, 2002; Munns *et al.*, 2006; Webb and Engelsen, 2006; Stalgis-Bilinski *et al.*, 2011). In addition, exposure to sunlight has been implicated in other health problems, such as eye damage including cataracts and decreased skin elasticity (David, 1994; Contet-Audonneau *et al.*, 1999; McCarty and Taylor, 2002).

Episodes of sunburn and high levels of sun exposure, in childhood and adolescence have been linked to development of skin cancer; non-melanocytic skin cancer and melanoma (Armstrong and Kricker, 2001; Kennedy *et al.*, 2003; MacKie, 2006). New Zealand has the highest incidence rates of cutaneous melanoma in the world (Ferlay *et al.*, 2010a; Liang *et al.*, 2010), with skin cancer being the most common form of cancer in this country (O'Dea D, 2009). New Zealand public health campaigns for skin cancer prevention advise avoidance during peak UV periods and protection from the sun ("Slip Slop Slap & Wrap" - protective clothing, sunscreen, wide-brim hat, wrap-around sunglasses), and urge parents of young children to be vigilant in their protection efforts (Cancer Society of New Zealand, 2014).

Currently, there is increasing awareness amongst parents that adequate exposure to sunlight is necessary for adequate vitamin D levels, but they are unsure as to how long, and which times of the day, are safe for their children to be outside (Nowson *et al.*, 2012). The 2012 Consensus Statement on Vitamin D and Sun Exposure in New Zealand provides recommendations for adults while the companion statement for pregnancy and infancy advises these two life stage groups, there remains no specific recommendations for preschool aged children (Ministry of Health and Cancer Society of New Zealand, 2012; Ministry of Health, 2013a).

2.5.2 Dietary sources of vitamin D

2.5.2.1 Food

Dietary intake from food only contributes a small percentage towards serum 25(OH)D concentrations, the biomarker used to measure vitamin D status. Vitamin D is found in small concentrations in a limited range of foods, with the best dietary sources being salmon, herring, sardines, tuna, egg, butter, red meat and liver (Shrapnel and Truswell, 2006). Variation in vitamin D content exists. For example wild salmon contain four times more than farmed salmon due to feed sources (Chen *et al.*, 2007) and fried fish has half the active vitamin D content of baked fish as a result of cooking methods (Misra *et al.*, 2008).

A low dietary intake of vitamin D has been linked with reduced 25(OH)D concentrations in children (Weng *et al.*, 2007). The current NHMRC Nutrient Reference Values for Australia and New Zealand for vitamin D are 5µg (200 IU) daily for both adults and children (Commonwealth Department of Health and Ageing (Australia) Ministry of Health (New Zealand) National Health & Medical Research Council, 2004). In 2006, this was acknowledged to be inadequate and is currently under review (Nowson *et al.*, 2012). The recently revised Recommended Daily Allowance from the Institute of Medicine recommends 15µg (600 IU) daily for ages 1 to 70 years, and 20µg (800 IU) for those more than 70 years old (Institute of Medicine (US), 2010). It is difficult to measure vitamin D intake in New Zealand as the current Food Composition Database does not contain complete information on vitamin D content (New Zealand Institute for Plant and Food Research Limited and the Ministry of Health (New Zealand), 2014). The most recent National Children's and Adult Nutrition Surveys did not analyse vitamin D intake (Ministry of Health, 2003; Ministry of Health, 2012). In the 2008/9 Australian Nutrition Survey it was estimated that the average intake of vitamin D was 1.2 to 2.6µg/day, with children unlikely to be consuming more

vitamin D than adults (Nowson and Margerison, 2002; Commonwealth Department of Health and Ageing (Australia) Ministry of Health (New Zealand) National Health & Medical Research Council, 2004). During winter, dietary intake was found to be a significant contributor to vitamin D status in British children aged 1.5 to 4.5 years and in Australian osteoporotic women (Davies *et al.*, 1999; Pasco *et al.*, 2001). In Canadian children aged between 2 and 16 years, dietary intake was a small determinant of 25(OH)D concentration, but when calculated by kilogram of body weight this intake became significant (Roth *et al.*, 2005). With the small stature and body size of preschool children the contribution of dietary vitamin D may be a significant contributor to vitamin D status in winter months.

Foods which are rich sources of vitamin D are unlikely to be consumed in significant quantities by preschool children. In a 2006 survey of Auckland preschool children aged 3.5 years, 18% ate oily fish one or more times a week, 73% ate red meat two or more times a week and 73% ate eggs one or more times a week (Theodore *et al.*, 2006). With the exception of egg above, all of these foods are protein-rich and would be considered expensive foods by households of high deprivation (Drewnowski, 2010) and may lead to lower intake levels in these families (Whiting *et al.*, 2007). In the Avon Longitudinal Study of Parents and Children, the main dietary sources of vitamin D were fat spreads and milk (Cribb *et al.*, 2014). In Canada, where milk is routinely fortified with vitamin D, a study of preschool children reported the major source of vitamin D for 3-5 year old children is milk, with children drinking 2 or more serves per day having higher 25(OH)D concentrations than those drinking less than this amount (El Hayek *et al.*, 2013). In New Zealand milk is not routinely fortified with vitamin D. An Auckland survey reported 85% of 3.5 year old preschool children consumed one or more servings of milk daily (Theodore *et al.*, 2006).

2.5.2.2 Supplements and fortified foods

Cholecalciferol (D_3) is used in New Zealand to fortify foods and as a supplement. Overseas, D_2 is used in supplement manufacture with these products now available in New Zealand stores or available for purchase over the internet (Thompson's, 2014). Cod liver oil is an historic rich source of vitamin D given to children to prevent rickets, and promoted as a good alternative to eating oily fish (Lu *et al.*, 2007; Porojnicu *et al.*, 2008).

Another dietary form of vitamin D for young children is fortified foods, especially milk formulas. Recent increased parental awareness of iron deficiency in toddlers has many parents electing to feed their toddlers formula past the 12 months recommended by the Ministry of Health (Ministry

of Health, 2008). Infant formula companies now offer specifically designed 'junior milk drinks', or toddler milk, for children over 1 year of age which are fortified with vitamin D_3 (Watties, 2014).

New Zealand does not have mandatory fortification of the food supply with vitamin D. Since 1996, food manufacturers have been able to fortify margarine, fats and dairy products on a voluntary basis (Food Standards Australia New Zealand, 2012). The Australia New Zealand (ANZ) Food Standards Code does not permit addition of vitamins and minerals to full cream liquid milk, with an allowance of a maximum claim of 1.0µg (10%) vitamin D for reduced fat liquid milk (Food Standards Australia New Zealand, 2014). In contrast in Australia, margarine is mandatorily fortified with vitamin D, and is now the major dietary source of vitamin D (Nowson *et al.*, 2012). All milk and margarines are fortified with vitamin D in Canada, while in the US all products fortified with vitamin D are required to be labelled as such. The majority of milks in New Zealand do not have vitamin D added, with dairy companies marketing reduced fat milks fortified with vitamin D for young children (Anchor Mega Milk) and for bone health (Anchor Calci Plus) (Anchor, 2014). These fortified milks are sold at a premium price over standard milk, which may promote inequalities in the vitamin D intake of preschool children.

Consumption patterns of milk have altered in the past decade for reasons including childhood food allergy, lactose intolerance or other parental concerns. Adults and children alike now consume non-dairy milk sources including oat, soy, rice and almond milks (Sanitarium, 2014). In the 2002 National Children's survey, 74% of children aged 5 to 14 years drank standard milk, 13% reduced fat, 7% trim, 4% extra-calcium and 2% soy milks (Ministry of Health, 2003). The survey also reported highest consumption in the 5 to 6 year age group, with more European children drinking milk daily than Maori or Pacific children (Ministry of Health, 2003). In 2006, a survey of Auckland 3.5 year old preschool children reported 86% consuming milk or dairy products two or more times a day, with 85% drinking milk at least once daily and 75% eating dairy products at least once daily (Theodore *et al.*, 2006). Dairy milk is a major source of calcium for children, with bone health adversely affected by drinking lower volumes (Goulding *et al.*, 2004). Nutritional deficiencies including rickets have been reported in toddlers drinking non-dairy milk sources (Carvalho *et al.*, 2001). The non-dairy milks are naturally poor sources of both calcium and vitamin D and only a small percentage are currently fortified with vitamin D (Sanitarium, 2014).

Fortification of foods is a vehicle to increase vitamin D status of individuals and a population (Piirainen *et al.*, 2006). Increases in vitamin D levels have been reported after fortification was introduced in Finland (Laaksi *et al.*, 2006). In elderly adults consumption of 5ug/day, through

drinking 500ml of fortified milk, significantly increased 25(OH)D concentrations (Keane *et al.*, 1998), whilst English elderly adults who reported eating fortified margarine on a daily basis had higher 25(OH)D concentrations than those who ate fortified margarine less often (Scragg *et al.*, 1995). Fortification may improve population vitamin D status but may not be sufficient to correct deficiency states of individuals. In the elderly adults described above, whilst serum 25(OH)D concentrations were increased, this amount was not enough to correct deficiencies (Keane *et al.*, 1998).

2.6 Measurement of vitamin D

The major circulating form of vitamin D (25[OH]D) has long been considered the best indicator of total body vitamin D levels as it reflects the contribution of both $25(OH)D_2$ and $25(OH)D_3$ from dietary and sun exposure sources (Heaney, 2004; Diamond *et al.*, 2005; Munns *et al.*, 2006; Cashman, 2007; Seamans and Cashman, 2009; Lips, 2010). Although 25(OH)D is not biologically active, it is a superior marker to the active $1,25(OH)_2D$ for three reasons; firstly, the longer half-life of 2-3 weeks compared to 4 hours; secondly, it is present at concentrations in serum 100-1000 times greater than $1,25(OH)_2D$, making 25(OH)D an indicator of long term diet intake or sun exposure (Zerwekh, 2008); thirdly, levels of $1,25(OH)_2D$ are tightly bound to PTH. Secretion of PTH is increased with vitamin D deficiency, increasing expression of 1-α-hydroxylase and maintaining $1.25(OH)_2D$ concentrations (Brenza and DeLuca, 2000). As both D_2 and D_3 forms contribute to increasing total serum 25(OH)D concentrations, it is essential both are analysed for accurate determination of 25(OH)D status (Cashman, 2007; Hewavitharana, 2013).

There has been evolution in analysis techniques used for measurement of 25(OH)D concentration over the past decades. In a worldwide systematic review of population vitamin D status, of the 195 studies investigated, over half of the 25(OH)D concentrations reported used radioimmunoassay (RIA) techniques (Yetley *et al.*, 2010). The next most used method was competitive protein-binding followed by high performance liquid chromatography, chemilunimescence immunoassay and other techniques (Hilger *et al.*, 2014). This predominance of RIA assays indicates the historical nature of the studies, currently LC-MS/MS is considered the gold standard and used whenever the technology in available (Gomes *et al.*, 2013; Hilger *et al.*, 2014).

2.6.1 Blood sampling – capillary versus serum

Traditionally, 25(OH)D concentration is measured from blood serum collected during venepuncture. In 1963, Robert Guthrie developed a technique to screen neonates for phenylketonuria which involved dropping capillary whole blood from a heel prick onto a filter paper, resulting in a blood spot which is then dried (Guthrie and Susi, 1963). With recent technological advances, more compounds can now be accurately assessed from dried blood spots, including 25(OH)D (Eyles *et al.*, 2009).

A blood spot sample is obtained from children and adults via a finger prick test of the side of the 2nd or 3rd finger on the non-dominant hand (ZRT Laboratories, 2014). The blood is dropped onto filter paper until a pre-printed circle is full (ZRT Laboratories, 2014). Complete drying of the blood spot is required at room temperature before storage, to enable complete penetration of the paper and equal gradient of vitamin D within the spot (Kvaskoff *et al.*, 2012). This usually takes 1-2 hours, however 3 hours may be necessary to avoid bacterial growth (Keevil, 2011).

Capillary blood obtained through a fingerprick is neither venous nor arterial blood; however it is closer to arterial. Haemoglobin and haematocrit levels are lower in venous blood than capillary blood, meaning dried blood spots will have a lower serum fraction than samples taken from traditional venepuncture (Kayiran *et al.*, 2003). 25(OH)D concentrations are lower in dried blood spots than venous blood samples due to 94.5% of 25(OH)D being located in the sera compartment of whole blood and virtually all being bound to the vitamin D binding protein and excluded from red blood cells (Larkin *et al.*, 2011; Kvaskoff *et al.*, 2012). For this reason, a haematocrit conversion factor is required in order for 25(OH)D concentrations from dried blood spots to correlate with serum 25(OH)D concentrations obtained from whole blood (Bain, 2011).

There has been some concern that 25(OH)D measurements obtained from dried blood spots are not as accurate or robust as those obtained from venous samples (Keevil, 2011). Haematocrit varies with age, gender, season, smoking and exercise so a haematocrit conversion factor may not fully account for haematocrit under all circumstances (Heath *et al.*, 2014). Recent research suggests good agreement of 25(OH)D assay measurements of dried blood spot and serum samples in adults and infants, with researchers reporting that dried blood spot analysis is an accurate and valid alternative to serum methods (Newman *et al.*, 2009; Eyles *et al.*, 2010; Larkin *et al.*, 2011; Heath *et al.*, 2014). Differences between capillary and serum 25(OH)D testing are outlined in Table 2.1.

Table 2.1. Features of capillary versus serum 25(OH)D testing

	Capillary	**Serum**
Blood volume	5µL	5-10ml
Sampling technique	Fingerprick test Non-specialised location Low level of tester training required Minimal pain	Venepuncture Specialised location Phlebotomist required Painful for young children
Blood	Capillary Haematocrit correction factor required	Sera
Sample handling	Drying at room temperature Room temperature storage and transport Long term stability	Immediate processing Refrigeration or freezer storage and transport
Assay	Validated method required Extra extraction step Automation previously limited Currently not available in New Zealand	Validated method required Automated easily Routine analysis Available in New Zealand

(Parker and Cubitt, 1999; Craft *et al.*, 2000; Eyles *et al.*, 2009; Newman *et al.*, 2009; Eyles *et al.*, 2010; Keevil, 2011; Kvaskoff *et al.*, 2012; Fraser and Milan, 2013; Griffiths *et al.*, 2014)

2.7 Definitions of vitamin D status

Vitamin D deficiency is common in adults and children worldwide (Holick, 2007). Health effects such as stunted growth and skeletal abnormalities in children may be caused by vitamin D deficiency affecting calcium homeostasis and bone health, leading to lifelong consequences such as osteoporosis in older adults (Holick, 2006a). The recent identification of vitamin D receptors on a wide range of cells has widened the range of health conditions vitamin D is potentially involved in, including autoimmune and cardiovascular diseases and some cancers (Holick, 2007). The role of vitamin D and health in preschool children is discussed later in this review, in particular the relationship of vitamin D with allergic and respiratory diseases.

There is currently a lack of consensus within the scientific community on the threshold for adequacy of vitamin D. Various 25(OH)D concentrations have been suggested, as described in Table 2.2, based on biomarkers including calcium absorption and PTH (Hollis, 2005).

Table 2.2. Definitions of vitamin D status by serum 25(OH)D concentration

Definition	**American Academy of Paediatrics** (Wagner and Greer, 2008)	**Australia & New Zealand Bone & Mineral Society, Endocrine Society of Australia, Osteoporosis Australia** (Nowson *et al.*, 2012)	**Canadian Paediatric Society** (Canadian Paediatric Society, 2007)	**Institute of Medicine** (Ross *et al.*, 2011)	**New Zealand Consensus Statement Ministry of Health and Cancer Society** (Ministry of Health and Cancer Society of New Zealand, 2012)	**US Endocrine Society** (Holick *et al.*, 2011)
Units	nmol/L (ng/ml)	nmol/L (ng/ml)	nmol/L (ng/ml)	nmol/L (ng/ml)	nmol/L (ng/ml)	nmol/L (ng/ml)
Severe deficiency		<12.5 (5)	<25 (10)			
Moderate deficiency		12.5-29 (5-12)				
Mild deficiency		30-49 (12-20)				
Deficiency	<25 (10)	<50 (20)	<25 (10)	0-50 (0-20)	<25 (10)	0-50 (0-20)
Insufficiency			25-75 (10-30)			52.5-72.5 (21-29)
Sufficiency / adequacy	>50 (20)	>50 (20) (at end of winter)	75-225 (30-90)	>50 (20)	>50 (20)	75-90 (30-100)
Toxic			>500 (200)	>250 (150)	Treatment >125 not recommended	

A detailed description of the studies that formed these recommendations is beyond the scope of this thesis. The Institute of Medicine guidelines were established on evidence related to bone health, citing a lack of evidence from randomised controlled trials for effects of vitamin D on other aspects of health, such as immune-modulatory actions, to recommend an adequate level greater than 50nmol/L (Ross *et al.*, 2011). The US Endocrine Society and many vitamin D researchers recommend a higher 25(OH)D concentration of >75nmol/L for adequacy, based on overall health and impaired calcium absorption and lower bone density at 25(OH)D concentrations lower than this (Vieth, 1999; Heaney *et al.*, 2003; Bischoff-Ferrari *et al.*, 2006; Holick *et al.*, 2011). The American Academy of Paediatrics suggest the adequate 25(OH)D concentration for children be above 50nmol/L, citing well recognised data that this concentration is sufficient to prevent rickets (Greer, 2008; Wagner and Greer, 2008). The optimal concentration for children is higher (above 75nmol/L) according to the Canadian Paediatric Society, based on maximal calcium intestinal absorption and minimizing bone calcium resorption in adults at this concentration (Lips, 2001; Canadian Paediatric Society, 2007). In New Zealand, the Consensus Statement on Vitamin D and Sun Exposure in New Zealand defines vitamin D deficiency as 25(OH)D concentrations below 25nmol/L and an adequate concentration above 50nmol/L (Ministry of Health and Cancer Society of New Zealand, 2012).

2.8 Vitamin D status of preschool children

Only a small number of studies have been published which describe the vitamin D status of preschool children aged between 2 to <5 years of age, either worldwide or in the Asia-Pacific region (Wahl *et al.*, 2012). A recent systematic review and meta-analysis of vitamin D status in population groups worldwide included 195 studies, of which 27 investigated the child and adolescent (1 to 18 years) age group. Only 6 of these studies included preschool children aged 2 to <5 years (Hilger *et al.*, 2014). Table 2.3 describes the studies of vitamin D status in groups including preschool-aged children.

Comparison of studies on vitamin D status in preschool children is complicated by; firstly, inconsistent age group ranges; secondly, vitamin D status affected by latitude, ethnicity and socioeconomic status, dietary intake and mandatory fortification regulations of the country, season of sampling and analytical methodology (Lips, 2010; van Schoor and Lips, 2011; Hilger *et al.*, 2014), thirdly, variation in health status due to 25(OH)D testing conducted within hospital or clinical settings on blood taken for another reason (Dyson *et al.*, 2014), fourthly lack of international consensus on 25(OH)D concentration ranges for defining deficiency and insufficiency (Heaney and Holick, 2011).

In these studies, measurement of vitamin D status has occurred over varying times of the year. Reported mean 25(OH)D concentrations were lowest in Mongolian children (25nmol/L) (Lander *et al.*, 2008) and highest in children from Northern Australia (97nmol/L) (Dyson *et al.*, 2014). In those studies which reported concentrations by season, mean 25(OH)D concentrations in winter were lower than in summer, with the smallest variation (10nmol/L) in Canadian children (El Hayek *et al.*, 2010; El Hayek *et al.*, 2013) and greatest (35nmol/L) in Italian children (Mazzoleni *et al.*, 2014).

Reported rates of vitamin D deficiency (25(OH)D <25nmol/L) range from 0% in Canada (Newhook *et al.*, 2009), Denmark (Madsen *et al.*, 2014) and Nigeria (Pfitzner *et al.*, 1998) to 34% of children of Asian ethnicity residing in the United Kingdom (Lawson and Thomas, 1999), while for 25(OH)D <50nmol/L, studies report ranges of 0 to 2% in US female children (Stein *et al.*, 2006) to 81% in UK children of Asian ethnicity (Lawson and Thomas, 1999). Children at highest risk of vitamin D deficiency are those with darker skin colouring who have immigrant parents, as shown in studied from Germany (Hintzpeter *et al.*, 2008) and the UK (Lawson and Thomas, 1999), while those at lower risk of deficiency live in locations closer to the equator, for example, Nigeria (Pfitzner *et al.*, 1998) and Northern Australia (Dyson *et al.*, 2014).

There were no studies from New Zealand which described the vitamin D status of preschool children aged between 2 to <5 years.

2.9 Vitamin D status in New Zealand

The New Zealand population resides in towns between latitudes 36^{o}S to 46^{o}S. Recent studies have revealed a prevalence of low 25(OH)D concentrations, most notably in infants and older adults (Grant *et al.*, 2009; Houghton *et al.*, 2010; Ministry of Health, 2012). Vitamin D status has been described in studies of newborns, infants, pregnant women, school children, adolescents, adults and older adults. Studies describing the vitamin D status of these age groups are presented in Table 2.4.

To date, no data on the vitamin D status of preschool children aged 2 to <5 years in New Zealand has been published.

Table 2.3. Studies of vitamin D status which include preschool aged children, 2 to <5 years

Study	Country and city/region	Latitude	Age (years)	Number of children (n)	Mean 25(OH)D (nmol/L)	Season of testing	Winter Mean 25(OH)D (nmol/L)	Summer Mean 25(OH)D (nmol/L)	Prevalence vitamin D <50nmol/L	Prevalence vitamin D deficiency/ insufficiency (nmol/L)	Description of participant group
Australia											
Binks 2014 (Binks *et al.*, 2014)	Northern Territory	12°S	<3	74	84	Whole year				~33% (<75nmol/L)	Hospital admissions
Dyson 2014 (Dyson *et al.*, 2014)	Northern Territory	12°S	0.2 - 13	42 indigenous 64 non-indigenous	92 97	August-December (Spring/ summer)			3%		Presentations at hospital
Zhou 2014 (Zhou *et al.*, 2014)	Adelaide	35 °S	1- 5	221	73	Whole year	58	86	20%	4% (<30nmol/L)	Population representative sample of Adelaide preschool children
United States											
Breen (2011) (Breen *et al.*, 2011)	Georgia	34°N	4 - 8	76	88	Whole year			4%		Population national survey
Carpenter 2012 (Carpenter *et al.*, 2012)	New Haven	41°N	0.5 - 3	781	66	Whole year			15%		Well-child clinic visits
Kumar 2009 (Kumar *et al.*, 2009)	NHANES 2001-4	28-48°N	4 - 10	9757	--	Whole year				15% (<37.5nmol/L) 61% 37.5-75nmol/L)	Population national survey
Looker 2008 (Looker, 2011)	NHANES	28-48°N	1 - 5	895	76	Whole year					Population, national survey Tested: summer/higher latitude winter/lower latitude
Mansbach (2009) (Mansbach *et al.*, 2009)	NHANES 2001-6	28-48°N	1 - 11	1799	68	Whole year			18%	1% (<25nmol/L)	Population national survey Tested: summer/higher latitude winter/lower latitude
Stein 2006 (Stein *et al.*, 2006)	Georgia	34°N	4 - 8	168	94	Whole year	74	107	2% winter 0% summer		Female gymnasts

Study	Country and city/region	Latitude	Age (years)	Number of children (n)	Mean 25(OH)D (nmol/L)	Season of testing	Winter Mean 25(OH)D (nmol/L)	Summer Mean 25(OH)D (nmol/L)	Prevalence vitamin D <50nmol/L	Prevalence vitamin D deficiency/ insufficiency (nmol/L)	Description of participant group
Canada											
El Hayek (2010) (El Hayek *et al.*, 2010)	Nunavut	51 - 70°N	3 - 5	282		Whole year	38	48		14% (<25nmol/L) 37% (<37.5nmol/L)	Inuit preschool children
El Hayek (2013) (El Hayek *et al.*, 2013)	Montreal	45°N	2 - 5	508	74.4 [a]	Whole year	70 [a]	80 [a]	12%		Children attending preschool daycare centres
Maguire (2011) (Maguire *et al.*, 2011)	Toronto	43°N	2 - 2.5	91	60 [a]	Winter/ spring	--	--	32%	1% (<25nmol/L)	Children in well-child visits
Newhook (2008) (Newhook *et al.*, 2009)	Newfound-land, Labrador	46° - 53°N	0 - 14	48	--	Summer - winter	53	68	17%	0% (<25nmol/L)	Hospital presentations
Omand (2013) (Omand *et al.*, 2013)	Toronto	44°N	1 - 6	1540	83	Whole year			5%		Children of non-Western immigrant families
Roth (2005) (Roth *et al.*, 2005)	Edmonton	52°N	2 - 16	90	47	April (spring)	--	--		6% (<25nmol/L) 34% (<40nmol/l)	Hospital presentations
United Kingdom											
Absoud (2011) (Absoud *et al.*, 2011)	Great Britain	51 - 56°N	4 - 18	1102	68F 75M 4-8 years	Whole year	51	71	35%		Population national survey 1997/8
Alonso (2015 (Alonso *et al.*, 2015)	Spain	43°N	2 - 6	112	77	Whole year			18%	3% (<25nmol/L)	Hospital emergency department presentations
Carroll (2014) (Carroll *et al.*, 2014)	Ireland	53°N	1 - 16	252	51	Whole year	38	62	55%	22% (<30nmol/l)	Medical or surgery patients
Davies (1999) (Davies *et al.*, 1999)	Great Britain	51 - 56°N	1.5 - 4.5	756	Scotland 66 N England 67 Central 71 London 66	Whole year	52	-			Population national survey 1992/3
Gregory (2000) (Gregory *et al.*, 2000)	United Kingdom	51 -56°N	1.5 - 2.5	213	67	October/ November			17%	1% (<25nmol/L)	Population National survey

Study	Country and city/region	Latitude	Age (years)	Number of children (n)	Mean 25(OH)D (nmol/L)	Season of testing	Winter Mean 25(OH)D (nmol/L)	Summer Mean 25(OH)D (nmol/L)	Prevalence vitamin D <50nmol/L	Prevalence vitamin D deficiency/ insufficiency (nmol/L)	Description of participant group
Lawson (1999) (Lawson and Thomas, 1999)	England, London	51°N	2	618	Bangladeshi 42 Pakistani 36 Indian 42	October/ November (autumn)	--	--	Bangledeshi 69% Pakistani 81% Indian 70%	Bangledeshi 20% Pakistani 34% Indian 25% (<25nmol/L)	Asian children in England
Europe											
Akman (2011) (Oden Akman *et al.*, 2011)	Turkey	40°N	1 - 16	849	95	April-May (end-winter)	--	--	15% (1-7 years)	22% (50-75nmol/L)	Primary school children
Andrian (2012) (Andıran *et al.*, 2012)	Turkey, Ankara	40°N	0 - 16	440	85 0-5y	Whole year			40%	17% (<37.5nmol/L) 10% (37.5-50nmol/L)	Outpatient clinic presentations
Koenig (2000) (Koenig and Elmadfa, 2000)	Austria	48°N	4 - 19	1143	26[a]	Whole year					Population survey
Gharaibeh (2009) (Gharaibeh and Stoecker, 2009)	Jordan	32°N	4 - 5	93	56	Summer	--	56	39%	0.5% (<25nmol/L)	Urban children
Hintzpeter (2007) (Hintzpeter *et al.*, 2008)	Germany	53°N	3 - 17	10,015	--	Whole year			32% non-immigrant boys 41% immigrant boys 36% non-immigrant girls 45% immigrant girls	18% non-immigrant boys 29% immigrant boys 17% non-immigrant girls 31% immigrant girls (<25nmol/L)	Population national survey
Madsen (2014) (Madsen *et al.*, 2014)	Denmark	56°N	4 - 17	340	73	September -October (late summer)	--	73	7%	0% (<25nmol/L)	Population vitmaD study

Study	Country and city/region	Latitude	Age (years)	Number of children (n)	Mean 25(OH)D (nmol/L)	Season of testing	Winter Mean 25(OH)D (nmol/L)	Summer Mean 25(OH)D (nmol/L)	Prevalence vitamin D <50nmol/L	Prevalence vitamin D deficiency/ insufficiency (nmol/L)	Description of participant group
Mallet (2005) (Mallet *et al.*, 2005)	France, Rouen	49°N	1.5 - 6	135	--	Whole year				6% (<25nmol/L)	Hospital presentations
Mazzoleni (2014) (Mazzoleni *et al.*, 2014)	Italy	45°N	1 - 15	113	60	Whole year	50	85		7% (<25nmol/L) 66% (25-75nmol/L)	Outpatient clinic presentations
Nicols (2015) (Nichols *et al.*, 2015)	Jordan	32°N	1 - 5	1077	46 [a]	Spring	--	--	57%	20% (<30nmol/L)	Nationally representative preschool children
Oren (2010) (Oren *et al.*, 2010)	Israel	30 - 33°N	<5	21	71.5	Whole year			52%	19% (<37.5nmol/L)	Israeli children
Nicolaidou (2006) (Nicolaidou *et al.*, 2006)	Greece	39°N	3 - 6	393	70 immigrant 84 Greek	October	--	--		7% (<25nmol/L)	Children attending preschool, daycare
Ohlund (2013) (Öhlund *et al.*, 2013)	Sweden	63°N	4 - 6	90		August/September (late summer) & Jan/Feb (winter)	55	60	25% summer 40% winter		Swedish preschool children
Piirainen (2007) (Piirainen *et al.*, 2006)	Finland	47°N	4	82 prior to and 36 after winter	55 prior to and 65 after winter	Winter	55 prior to and 65 after winter	--		10% prior (<37.5nmol/L) 3% after (<37.5nmol/L)	Prospective study, before and after mandatory fortification
Stellinga-Boelen (2007) (Stellinga-Boelen *et al.*, 2007)	The Netherlands	52°N	2 - 12	112	48	Mid spring			19%	3% (<25nmol/L)	Children of asylum seekers
Vierucci (2013) (Vierucci *et al.*, 2013)	Italy	43 - 44°N	2 - 10.9	283	52[a]	Whole year	41[a]	68 [a]	40%	10% (<25nmol/L)	Italian children
Asia											
Jeong (2013) (Jeong, 2013)	Korea	37°N	0.1 - 16	969	57	Whole year			20%	21% (<37.5nmol/L)	Outpatient clinic presentations
Lander (2008) (Lander *et al.*, 2008)	Mongolia	46°N	0.5 - 3	243	25	November				61% (<25nmol/L)	Mongolian children

Study	Country and city/region	Latitude	Age (years)	Number of children (n)	Mean 25(OH)D (nmol/L)	Season of testing	Winter Mean 25(OH)D (nmol/L)	Summer Mean 25(OH)D (nmol/L)	Prevalence vitamin D <50nmol/L	Prevalence vitamin D deficiency/ insufficiency (nmol/L)	Description of participant group
Zhu (2012) (Zhu *et al.*, 2012)	China, Hangzhou	30°N	2 - 5	2269	70				22%	1% (<25nmol/L)	Hospital presentations
Africa											
Pfitzner (1998) (Pfitzner *et al.*, 1998)	Nigeria, Jos	8°N	0.6 - 4	218	67	March April (autumn)				0% (<25nmol/L) 1% (<75nmol/L)	Nigerian children from Jos, Nigeria

[a] median

Table 2.4. New Zealand studies investigating vitamin D status

Study	Participant Age, Location	Number of participants	Mean 25(OH)D (nmol/L)	Season	Winter Mean 25(OH)D (nmol/L)	Summer Mean 25(OH) (nmol/L)	25(OH)D Deficiency (nmol/L)	25(OH)D Concentration <50nmol/L
Camargo (2011) (Camargo *et al.*, 2011)	Newborn (cord blood) Wellington	929	44[a]	Whole year	32[a]	85[a]	19% (<25nmol/L)	57%
Wall (2013) (Wall *et al.*, 2013)	Breastfed Infants 2-3 months Auckland	94	53[a]	Whole year	21[a]	75[a]	24% (<27.5nmol/L)	--
Grant (2009) (Grant *et al.*, 2009)	Infants 2-23 months Auckland	353	55[a]	Whole year	47[a] *	71[a] *	10% (<27.5nmol/L)	--
Houghton (2010) (Houghton *et al.*, 2010)	Infants 2-23 months Dunedin	193	52.3	Whole year	39	74	16% (<27.5nmol/L)	--
Graham (2009) (Graham *et al.*, 2009)	Year 3 students 7 years Waikato	83 (control group)	44	Winter	44	--	39% (<37.5nmol/L)	--
Rockell (2005) (Rockell *et al.*, 2005)	Children 5-14 years National Children's Nutrition Survey 2002	1585	50	Whole year	43	58	4% (<17.5nmol/L)	
Rockell (2006) (Rockell *et al.*, 2006)	Adults 15+ years Adult Nutrition Survey 1997/8	2946	50	Whole year	F: 44 M:45	F: 67 M: 70	3% (<17.5nmol/L)	48%
Ministry of Health (2012) (Ministry of Health, 2012)	Adults 15+ years Adult Nutrition Survey 2008/9	3099	63	Whole year	*	*	5% (<25nmol/L)	27%

[a] median

* higher deficiency rates reported in winter compared to summer

2.10 Risk factors for vitamin D deficiency in preschool children

Whilst risk factors of vitamin D deficiency have been identified for children overseas, there is a paucity of literature specific to the age group 2 to <5 years, particularly in New Zealand. These factors, and New Zealand studies of risk factors in infants and children, are discussed below.

2.10.1 Seasonality

Year-round, UVB rays are strongest around noon (Diffey *et al.*, 1982). The amount of UVB reaching the earth's surface in winter is up to four times lower due to a lower angle of incidence, increasing travelling distance and the opportunity for atmospheric absorption of rays (Godar, 2005). Cutaneous production of vitamin D in winter is compounded further with more cloud cover, a reduction in amount of skin exposed due to clothing protective of the cold, and insufficient concentration of UVB rays at higher latitudes (>37^{o}) (Webb *et al.*, 1988). In New Zealand, winter brings shorter days, reduced sunshine hours and UV radiation strength only 10% of summer levels (McKenzie *et al.*, 1999). Guidelines for sun exposure can be confusing for consumers. The New Zealand Ministry of Health and Cancer Society recommend sun protection between September and April, especially between 10am and 4pm (Ministry of Health and Cancer Society of New Zealand, 2012; Cancer Society of New Zealand, 2014). Conversely, recommendations for adequate vitamin D and bone health are to expose hands, arms and face to sunlight three to six times per week for short periods (Nowson and Margerison, 2002).

Vitamin D status is influenced by the season in which testing occurs. With less sunshine exposure in winter, vitamin D stored in adipose tissues over the sunny, warmer months is slowly used up, leading to lower 25(OH)D concentrations and a higher prevalence of deficiency in the winter months (Bolland *et al.*, 2008). Lower vitamin D status, and a higher prevalence of deficiency, have been reported in winter compared to summer months in studies of children around the world including; Australia (Zhou *et al.*, 2014), Canada (Roth *et al.*, 2005; Newhook *et al.*, 2009; Maguire *et al.*, 2011; Omand *et al.*, 2013), Asia (Du *et al.*, 2001; Zhu *et al.*, 2012; Jeong, 2013), US (Stein *et al.*, 2006; Weng *et al.*, 2007), Great Britain (Davies *et al.*, 1999; Absoud *et al.*, 2011; Carroll *et al.*, 2014) and Europe (Vierucci *et al.*, 2013; Mazzoleni *et al.*, 2014). In a cross-sectional study of Italian preschool children, the prevalence of vitamin D deficiency (25[OH]D <25nmol/L) in winter was 15% compared with 5% in summer (Vierucci *et al.*, 2013). The prevalence of vitamin D deficiency

(25[OH]D <30nmol/L) was also higher in winter compared with summer, 4% and 0% respectively, in preschool children living in Adelaide, Australia (Zhou *et al.*, 2014).

A seasonal influence has been reported in New Zealand studies, where nadir 25(OH)D concentrations occur in late winter/early spring (Bolland *et al.*, 2008; Ministry of Health, 2012). The season of sampling was found to be the strongest determinant of vitamin D status for children aged 5-14 years, with a 30nmol/L lower concentration in August (late winter) compared with March (early autumn) (Rockell *et al.*, 2005). In Dunedin (latitude 46^{o}S), mean 25(OH)D concentrations in 2 to 23 month old infants were almost double in summer than in winter, 74nmol/L versus 39nmol/L (Houghton *et al.*, 2010). A seven-fold increase in risk of vitamin D deficiency was found for infants aged 2 to 23 months in Auckland when sampled in winter or spring rather than summer (Grant *et al.*, 2009). Rates of vitamin D deficiency were higher in newborns with a winter season of birth compared to those born in summer (Camargo *et al.*, 2010).

2.10.2 Latitude

The UV dose received by humans decreases moving further from the equator, or with increasing latitude (Godar, 2005). The UV reaching earth is insufficient for cutaneous production of vitamin D above 51^{o} during certain months of the year (Engelsen *et al.*, 2005).

The atmosphere, and ozone in particular, provides a protective barrier for the earth's surface by absorbing UV radiation, especially UVB (de Gruijl and Van der Leun, 1993). The sun is closest to earth in the southern hemisphere summer, and together with lower levels of ozone in this hemisphere, results in higher levels of UV radiation reaching the earth’s surface in New Zealand and Australia compared with similar latitudes in the northern hemisphere summer (McKenzie *et al.*, 1997). These countries have the highest melanoma rates in the world, hence well promoted advice on sun protection (O'Dea D, 2009; Ferlay *et al.*, 2010b; Cancer Society of New Zealand, 2014). Particular matter and atmospheric pollution produced by modern and industrialised technologies absorb, scatter and deflect the shorter wavelength UVB, reducing transmission through the atmosphere (Godar, 2005). The negative implications for endogenous synthesis of vitamin D are illustrated in the lower 25(OH)D concentrations measured in Indian infants living in areas with high haze readings compared with those with lower haze readings, suggesting the

atmospheric pollution impeded the UV rays from reaching the surface of the earth (Agarwal *et al.*, 2002).

Studies from northern United States (Stein *et al.*, 2006; Breen *et al.*, 2011) and Canada (Maguire *et al.*, 2011; El Hayek *et al.*, 2013; Omand *et al.*, 2013) report higher mean values than other countries of similar latitude. This is consistent with the effect of latitude on 25(OH)D concentrations being less in countries which have routine fortification of the food supply (Kimlin, 2008; Prentice, 2008). Studies in Mediterranean countries (39-40^{o}N) report mean 25(OH)D concentrations over a whole year between 52 and 85nmol/L (Andıran *et al.*, 2012; Mazzoleni *et al.*, 2014) and 58nmol/L for children up to 16 years of age in Korea (37^{o}N) (Jeong, 2013).

The effects of latitude on vitamin D status within New Zealand vary. In the most recent Adult Nutrition Survey, mean 25(OH)D concentrations were lower for those living in the southern regions compared to the north but the effect of latitude on serum 25(OH0D was modest (northern 65nmol/L, central 61nmol/L and south 61nmol/L) (Ministry of Health, 2012). The prevalence of vitamin D deficiency was only greater in southern regions during the winter season (Ministry of Health, 2012). Winter 25(OH)D concentrations of infants living in Dunedin (45^{o}S) had a median concentration 10nmol/L lower than those living in Auckland (34^{o}S) (Grant *et al.*, 2009; Houghton *et al.*, 2010). In the national survey of 5 to 14 year olds, only small differences were found between the north and south island in mean 25(OH) concentrations (50 vs 48nmol/L), prevalence of deficiency (25[OH]D <17.5nmol/L) was 2 versus 3%, and insufficiency (25[OH]D <37.5nmol/L) was 27 versus 29% respectively (Rockell *et al.*, 2005).

2.10.3 Age

There is an age-related decline in dermal 7-dehydrocholesterol concentrations, so when exposed to comparable sunlight, a 70 year old individual will produce a quarter of the vitamin D compared with a 20 year old (MacLaughlin and Holick, 1985; Riggs, 2003). Epidemiological studies indicate that average 25(OH)D concentrations in younger children are greater than those in older children and adolescents (Oliveri *et al.*, 1993; Gregory *et al.*, 2000; Dawson-Hughes *et al.*, 2005; Rockell *et al.*, 2005; Mansbach *et al.*, 2009; Papandreou *et al.*, 2009; Looker, 2011), potentially due to skin colour, modern lifestyle or body size. Mansbach *et al* suggest this higher levels in young children are due to an immaturity of skin pigmentation in preschool children, with skin colour developing

once greater exposure to the sun is achieved (Mansbach *et al.*, 2009) whilst Stein *et al* propose adolescents spend more time inside (Kimm *et al.*, 2002; Stein *et al.*, 2006).

In a worldwide systematic review, vitamin D status was lower in the child and adolescent category than in adults, however this age category was wide (from 1 to 18 years of age). It is therefore feasible that this is due to the lower levels of adolescents (Hilger *et al.*, 2014). It is uncertain when the age-related decline in 7-dehydrocholesterol concentration begins and may only become significant in adult life (Reginster, 2005). In addition, less sunshine exposure for vitamin D production may be required for small children compared to adolescents and adults due to their greater surface area for body size (Riggs, 2003; Munns *et al.*, 2006).

There are very few studies specifically looking at the narrower age band of 2 to <5 years. The 25(OH)D concentrations measured in Inuit preschool aged children did not differ between the ages of 3 to 5 years (El Hayek *et al.*, 2010). In Montreal, preschool children in the age group 1.8 to 3.2 years were found to have an 12nmol/L higher plasma 25(OH)D than those aged 4.0 to 5.8 years, however this difference disappeared once weight was included in the analysis (El Hayek *et al.*, 2013). No significant difference was seen in 25(OH)D concentrations for Irish children between 1 and 3 years of age (Carroll *et al.*, 2014) nor for preschool children aged 1 to 5 years living in Adelaide (Zhou *et al.*, 2014).

2.10.4 Gender

Worldwide reviews of vitamin D status report lower 25(OH)D concentrations in females compared to males (McKenna, 1992; van Schoor and Lips, 2011). Gender differences in 25(OH)D status in children and adolescents were not seen in the Asia-Pacific region in a review by Hilger *et al* (Hilger et al, 2014).

In studies which included preschool age children, gender differences vary. Higher levels of vitamin D insufficiency were seen in girls compared with boys in the US 2001-6 NHANES study (Mansbach *et al.*, 2009), in Canadian (El Hayek *et al.*, 2013) and Jordanian preschool children (Nichols *et al.*, 2015), whilst no gender differences were reported in Irish children attending clinics for minor surgery (Carroll *et al.*, 2014) or Canadian children of non-Western immigrants (Omand *et al.*, 2013). In New Zealand, for infants under two years of age, males had lower 25(OH)D concentrations compared to females (Houghton *et al.*, 2010) whilst, in children 5 to 6 years old

enrolled in the Child Nutrition Survey, 25(OH)D concentrations were lower in girls than boys (Rockell *et al.*, 2005).

2.10.5 Ethnicity

Many countries now have a diverse ethnic mix within their population due to migration. Studies of children from various countries have found those with an immigrant background are more likely to have lower serum 25(OH)D concentrations and an increased risk of vitamin D deficiency. Countries and ethnic groups in which this has been described include children of African ethnicity in Ireland (Carroll *et al.*, 2014); of Asian ethnicity in Britain (Bangladeshi, Indian, Pakistani) (Lawson and Thomas, 1999); immigrant children (Turkish, Arab-Islamic, Asian, African) in Germany (Hintzpeter *et al.*, 2008); children of non-Western immigrants in Canada (Omand *et al.*, 2013); and in children in the United States of African-American (Weng *et al.*, 2007), Hispanic and non-Hispanic black compared with non-Hispanic white (Mansbach *et al.*, 2009), and of non-Hispanic black or Mexican -American compared with non-Hispanic white (Kumar *et al.*, 2009). Immigrant preschool children in Greece were found to have lower serum 25(OH)D concentrations than non-immigrants, with the authors noting these children also had lower vitamin D intake and socioeconomic class (Nicolaidou *et al.*, 2006). In the Northern Territory of Australia, no difference was seen in the mean 25(OH)D concentrations between indigenous and non-indigenous children attending hospital, most likely due to the high number of sunshine hours experienced at this latitude (Dyson *et al.*, 2014).

The New Zealand population includes 200 ethnic groups, the predominant groups are European (71%), Maori (14%), Pacific (8%) and Asian (11%) (Statistics New Zealand, 2013). People of Pacific Island descent have been reported as being at the highest risk of vitamin D deficiency in studies of newborns, infants and adults (Grant *et al.*, 2009; Camargo *et al.*, 2010; Ministry of Health, 2012). In a study of newborns in Wellington, those in the 'other' ethnic group of prioritised ethnicity, made up largely of Asians, were also at high risk of vitamin D deficiency (i.e. not-Maori, not-Pacific, not-European) (Camargo *et al.*, 2010) while low vitamin D levels have also been reported in South-East Asian women living in Auckland (von Hurst *et al.*, 2010). In an Auckland study of breast-fed infants 2 to 3 months of age, European children had a significantly higher mean 25(OH)D concentrations than children of other ethnic groups (Wall *et al.*, 2013). Whilst Maori were found to be at higher risk than European children in the studies above and in adult surveys, their risk of vitamin D deficiency is lower than Pacific and 'other' ethnic groups, pointing to the

potential relation of factors such as skin colour and socioeconomic status, which are discussed below (Ministry of Health, 2012).

2.10.6 Skin colour

The pigment melanin is the primary determinant of skin colour. It has been proposed to act as a natural sunscreen by absorbing UVB rays, preventing penetration to the lower stratum basale and stratum spinosum where the highest concentrations of 7-dehydrocholesterol are located, thus adversely affecting vitamin D production (Matsuoka *et al.*, 1991). It has been postulated there is an evolutionary element to skin colour throughout the world (Jablonski and Chaplin, 2000). Peoples originating from close to the equator have a higher melanin concentration and darker skin developed as an adaptive mechanism in response to high sunlight exposure. Migration to higher latitudes caused skin colour evolution to lighter colours to increase cutaneous synthesis from weaker UVB and shorter sunshine hours (Loomis, 1967; Caron-Schreinemachers *et al.*, 2005).

The threshold dose of sun exposure required to cause colouring (pinking of the skin) is the minimal erythemal dose (MED). The contribution of vitamin D_3 to the circulation has been shown to be 10,000 to 20,000IU with 24 hours of a whole-body MED (Hollis, 2005). The exposure time to achieve this MED is dependent on skin pigmentation, being higher for those with darker skin colouring (Lo *et al.*, 1986). To achieve a MED equivalent to a person with white skin, a person of medium skin colour (e.g. Asian or Indian) requires 3 times longer sun exposure, while a black skin colour will require 6 to 10 times more sun exposure (Hollis, 2005). Higher rates of vitamin D deficiency and rickets have been reported in dark skinned children in the United Kingdom, Texas and Canada (Binet and Kooh, 1995; Shah *et al.*, 2000; Callaghan *et al.*, 2006).

The extent to which vitamin D production is affected by melanin is not fully defined. Laboratory studies of sun exposure on differing skin colourings report melanin as inhibiting vitamin D production with the findings of higher 25(OH)D concentrations in those with whiter skin colour, but this is has yet to be confirmed in free-living situations and may be due to the degree of sun exposure (Bogh *et al.*, 2010). In New Zealand, significant differences were seen in the mean 25(OH)D concentrations based on constitutive skin colour and forearm skin colour of both Pacific and European adults, the authors concluding it was tanning, rather than skin colour, being the determinant of 25(OH)D (Rockell *et al.*, 2008).

The association of skin colouring and vitamin D status has been investigated in different countries. In South-Eastern USA, lower serum 25(OH)D concentrations were described in young black compared to white young girls aged 4 to 8 years (Stein *et al.*, 2006). The National Diet and Nutrition Survey in Great Britain of children aged 4 to 18 years reported a 37-fold increase in risk of vitamin D deficiency for non-white children compared to white children (Absoud *et al.*, 2011). In Italian preschool children, those with non-white skin were more likely to be vitamin D deficient than those with white skin (Mazzoleni *et al.*, 2014).

Contradictory findings on the association of skin colour with vitamin D status have also been reported. Skin colour was not a determinant of vitamin D status in a study of French adults (Malvy *et al.*, 2000). Nicolodiau *et al* report 25(OH)D concentrations were not associated with skin phototype in Greek preschool aged children, with the authors acknowledging the participant group largely consisted of children with similar skin phototypes and few children with dark skin so was not a representative sample (Nicolaidou *et al.*, 2006). A Canadian study of infants and toddlers found skin colour was not associated with 25(OH)D concentration (Omand *et al.*, 2013). This finding was unexpected by the authors, who reported the majority of the participants had light skin colour (86%) and the possibility of reduced sun exposure due to parents protecting these young children from the cold weather with enveloping blankets and clothing (Omand *et al.*, 2013).

When the association of skin colour and ethnicity was tested in a study of adult New Zealanders, higher 25(OH)D concentrations were found in those with lighter skin colour, but skin-colour associated differences disappeared when ethnicity was also considered, with the authors concluding the variation in 25(OH)D was determined more by ethnicity than skin colour (Nessvi *et al.*, 2011). In a national survey of 5 to 14 year olds, a large variation in the skin colour of Maori and Pacific children was described, and it was concluded that overall variations in average skin colour most likely accounted for the ethnic difference seen in the vitamin D levels in these children (Rockell *et al.*, 2005). There was no association found between skin colour and 25(OH)D concentrations in Dunedin infants aged 12 to 22 months old infants. This may be due to sun exposure necessary to produce vitamin D being limited for very young children in a cold climate where covering clothing is worn for warmth (Houghton *et al.*, 2010).

2.10.7 Socioeconomic status

Lower socioeconomic status is associated with a range of poorer health outcomes in children and remains one of the principal determinants of child health (Chen *et al.*, 2002). Socioeconomic status is measured in several ways, including income, household crowding, income support and health insurance, with lower socioeconomic status, as described by these various measures, associated with poorer vitamin D status (Xie *et al.*, 2003; Nicolaidou *et al.*, 2006; Weng *et al.*, 2007; Gordon *et al.*, 2008; Absoud *et al.*, 2011; El Hayek *et al.*, 2013; Carroll *et al.*, 2014). This relationship does vary from country to country. For example, 2 to 16 year olds from poorer households in Jordan have higher 25(OH)D concentrations when compared to children of this age living in more affluent households. The researchers who completed this study suggested this is due to children escaping poor surroundings and playing outside in the sunshine for longer periods (Gharaibeh and Stoecker, 2009). In contrast, poorer households in New Zealand are often in built-up urban suburbs with little or no areas for children to play safely, limiting physical activity and sunlight exposure (Hamlin and Ross, 2005).

Whilst dietary vitamin D contributes only a small amount to body vitamin D status, it becomes of relatively greater importance during winter (Davies *et al.*, 1999; Pasco *et al.*, 2001). The intake of vitamin D may be affected by disparities in diet quality associated with socio-economic status (Dubois *et al.*, 2011). In the New Zealand 2008/9 National Nutrition Survey of adults, those living in most deprived households had three times the risk of vitamin D deficiency than those living in least deprived households (Ministry of Health, 2012). A study of infants in Auckland found a two-fold increase in the risk of vitamin D deficiency for those children living in more crowded households (Grant *et al.*, 2009).

Household crowding is associated with poorer health status (Banerji *et al.*, 2009). Crowding is a characteristic of households with lower socio-economic status and a risk factor rickets in small children (Edidin *et al.*, 1980). Inuit preschool children (El Hayek *et al.*, 2013) and New Zealand infants (Grant *et al.*, 2009) who lived in households with crowding have lower 25(OH)D concentrations than those who do not. With over 40% of Pacific people living in crowded houses, Grant *et al* suggests this as an important factor in the high prevalence of vitamin D deficiency in this ethnic group in New Zealand (Grant *et al.*, 2009).

2.10.8 Maternal education level

The education level of the mother potentially influences the dietary intake and sun protection practices of her child. Mothers with higher educational attainment provide their children with a diet more closely aligned to dietary guidelines (North and Emmett, 2000; Xie *et al.*, 2003; Dubois *et al.*, 2011), which may mean higher amounts of dietary vitamin D, increasing the contribution of dietary sources to vitamin D status in winter (Davies *et al.*, 1999; Pasco *et al.*, 2001). There is little literature available of the effect of mother or caregiver education qualifications on child vitamin D status. An inverse association of vitamin D and caregiver education level was found in Dunedin infants less than 2 years of age, with researchers suggesting highly educated caregivers may be more likely to shield children from the sun using clothing and sunscreen (Houghton *et al.*, 2010). In a New Zealand study of sun protection practices in preschool and primary children, the mothers without secondary school qualifications were more likely to allow their child to acquire a tan during summer (Morris *et al.*, 1998). Mothers with higher educational attainment are also more likely to live in affluent, or less deprived, households and participate in research studies (Rogers and Emmett, 2003; Galea and Tracy, 2007).

2.10.9 Dietary: Supplements

Supplementation with vitamin D is not routinely recommended for young children in New Zealand. The use of supplements containing vitamin D has been found to be positively associated with 25(OH)D concentrations in studies of children (Davies *et al.*, 1999; Lawson and Thomas, 1999; Grant *et al.*, 2009; Kumar *et al.*, 2009; Mansbach *et al.*, 2009; Absoud *et al.*, 2011; El Hayek *et al.*, 2013; Omand *et al.*, 2013; Carroll *et al.*, 2014; Madsen *et al.*, 2014), while the use of supplements reduces the variation in vitamin D status seen between winter and summer months (Davies *et al.*, 1999). Supplement usage is likely to be linked to socioeconomic status, with children in the United Kingdom from non-manual households reported as taking more supplements than those with parents with manual jobs (Gregory *et al.*, 1995; Davies *et al.*, 1999).

2.10.10 Dietary: Milk

Milk is a major contributor to vitamin D intake in infants and children, particularly in those countries which routinely fortify milk with vitamin D (Gordon *et al.*, 2008; El Hayek *et al.*, 2010).

For children less than 2 years of age, infant formula consumption has been shown to be associated with a reduced risk of vitamin D deficiency (Grant *et al.*, 2009).

Studies including preschool children in Jordan and Finland found the consumption of fortified versus non-fortified milk to be associated with higher 25(OH)D (Piirainen *et al.*, 2006; Gharaibeh and Stoecker, 2009). Preschool aged children drinking larger volumes of fortified milk in Canada were found to have significantly improved vitamin D status compared with those who consumed smaller amounts (El Hayek *et al.*, 2010; Maguire *et al.*, 2011). Children in the US NHANES survey who drank less than 1 serve of milk per week had a higher risk of vitamin D deficiency than those who drank larger volumes (Kumar *et al.*, 2009). Milk in New Zealand is not routinely fortified with vitamin D (Anchor, 2014) and to date no studies have explored milk consumption patterns and vitamin D status in preschool children in this country.

2.10.11 Body mass index

Body mass index (BMI) is used alongside weight-for-height measurements in the traditional assessment of nutrition status in young children, but has been shown to be inadequate for accurate prediction of body fat levels in this age group (Wells, 2001). Higher body fat levels in older children and adults have been found to be associated with lower 25(OH)D concentrations (Kamycheva *et al.*, 2004; Alemzadeh *et al.*, 2008; Moore and Liu, 2015) as vitamin D is sequestered in the increased volume of adipose tissue (Wortsman *et al.*, 2000) or through modulation of adipogenesis by vitamin D receptor pathways (Moore and Liu, 2015). Negative effects on vitamin D status in children and adolescents with an overweight and obese BMI may also be related to lower physical activity levels and a sedentary indoor lifestyle with decreased sun exposure (Rajakumar *et al.*, 2005).

Studies including preschool children report conflicting findings in the relationship of vitamin D and BMI. An increased risk of poorer vitamin D status in association with increasing BMI was reported in a study of Canadian 2 year olds (Maguire *et al.*, 2011). There was no significant association of overweight or obesity with mean 25(OH)D concentration found in studies of Irish children aged 1 to 3 years (Carroll *et al.*, 2014), Australian children aged 1 to 5 years (Zhou *et al.*, 2014) and over 780 preschool children from New Haven, US (Carpenter *et al.*, 2012). No association was found between vitamin D deficiency and BMI status in Canadian preschool children, however the authors noted a limited range of BMI of the participant group (El Hayek *et al.*, 2013). In studies

including both children and adolescents, overweight or obese participants had a higher risk of vitamin D deficiency in Great Britain (Absoud *et al.*, 2011), the United States (Kumar *et al.*, 2009) and Italy (Vierucci *et al.*, 2013). In Great Britain, a higher risk of vitamin D insufficiency was reported in the children with lower physical activity levels who were overweight and had higher screen time usage (Absoud *et al.*, 2011). The New Zealand National Children Survey found obesity was associated with lower 25(OH)D concentrations in children aged 5 to 14 years (Rockell *et al.*, 2005). It may be BMI as a risk factors is more applicable to the adolescent rather than the preschool age group.

2.10.12 Physical activity

Physical activity is a potential indicator of sun exposure and vitamin D status in children as most of this activity occurs outdoors. However, few studies investigating vitamin D status have measured the physical activity of children. Measurement of physical activity levels in young children is time consuming, labour intensive and requires costly technology (Tucker, 2008). For investigations of the determinant of vitamin D, assessment of time spent outdoors and in sunshine are also required. For this reason, previous studies have used broad questions such as the average time spent in outdoor play per week or day (Hintzpeter *et al.*, 2008; Roth *et al.*, 2011; Maguire *et al.*, 2013; Omand *et al.*, 2013). In Great Britain, Absoud *et al* reported a 50% increase in risk of vitamin D insufficiency in those children and adolescents who spent less than ½ an hour each day outdoors (Absoud *et al.*, 2011). There was no association reported between physical activity levels and risk of vitamin D deficiency in children and adolescents in Germany and Canada (Hintzpeter *et al.*, 2008; Roth *et al.*, 2011; Maguire *et al.*, 2013; Omand *et al.*, 2013).

2.10.13 Daycare attendance

Research around daycare centre attendance and vitamin D status is limited. In response to strong messages from the Cancer Society, early childhood centres have strict sun protection policies (Cancer Society of New Zealand, 2012; St Mary's Early Childhood Education Centre, 2013). Studies in Canada and Italy measuring 25(OH)D concentrations of children attending preschool did not investigate any potential association with the number of hours children attended, and did not include a control group of children not attending formal childcare for comparison (Nicolaidou *et al.*, 2006; El Hayek *et al.*, 2013). The New Zealand Ministry of Education funds children aged 3 and 4 years of age for 20 hours of teacher-led early childhood education per week (Ministry of

Education, 2014). At present, there are more preschool children in formal childcare options such as daycare and kindergarten facilities than family based care (Statistics New Zealand, 2010). The number of hours children attend varies, from one morning to 30+ hours a week. In the New Zealand sun avoidance policy context, it is speculated that increased hours of attendance at formal early childhood centres may negatively affect vitamin D status through reduced sun exposure.

2.10.14 Sunscreen and sun protection practices

Sunscreens are designed to absorb or reflect UV rays on the surface of the skin. Sunscreen has been shown to effectively block cutaneous vitamin D production in laboratory settings (Matsuoka *et al.*, 1987), but in randomised controlled trials of adult usage, no association with vitamin D status and sunscreen usage has been found, suggesting inadequate amounts are routinely applied for a total blockage of UVB rays (Marks *et al.*, 1995).

The wearing of clothing provides an effective barrier for UV rays reaching the skin of humans. The degree of sun penetration is determined by the type and weave of fabric (Matsuoka *et al.*, 1992; Salih, 2004). Clothing with high UV protection rating is included in New Zealand anti-cancer messages for summer, while in colder climates, clothes including hats are worn for warmth (Cancer Society of New Zealand, 2014). The area of skin exposed to the sun's rays is a strong determinant of 25(OH)D concentrations (Kimlin *et al.*, 2007; Kimlin *et al.*, 2014). Certain cultural or religious beliefs require covering clothing to be worn, particularly by women, and has been associated with lower 25(OH)D concentrations (Mukamel *et al.*, 2001). The age when children begin using such covering clothing depends on society norms and family beliefs (Moore, 2007). In New Zealand it is the minority of preschool children who wear covered clothing for cultural reasons, however, as individuals these children have the potential to be at higher risk of vitamin D deficiency (Statistics New Zealand, 2013).

UV exposure is primarily determined by activities undertaken outside and may be affected by outdoor UV protection. The annual dose of UV received by young children has decreased compared to earlier generations and is now comparable to adults who have indoor occupations (Godar, 2005). Structures such as umbrellas, awnings, shade sails and trees decrease an individual's exposure to sunlight, particularly in the summer months (Gies *et al.*, 1998).

Young children are largely reliant on adults for sun protection in application of sunscreen and provision of covering clothing or supervision in sun avoidance (i.e. sitting in shade). The sun protection practices of parents have been found to be a strong influence on those of their young children (Zinman *et al.*, 1995; McGee *et al.*, 1997). Conversely, Morris *et al* found no association in a New Zealand study of parent's sun protection factors. In this study over half of the parents reported they used sunscreen only intermittently and had been sunburnt themselves recently, so may not have had the knowledge to apply adequate amounts of sunscreen to protect their child's skin, or enforce sun avoidance and covering clothing (Morris *et al.*, 1998).

The few studies which have investigated vitamin D and the use of sunscreen in young children report conflicting findings (Paxton *et al.*, 2013). The regular use of sunscreen had a seven-fold increase in the risk of vitamin D deficiency in Italian children and adolescents tested in summer, spring and autumn (Vierucci *et al.*, 2013). In Ireland, children under 16 years of age using sunscreen were found to have higher 25(OH)D concentrations than those who did not (Carroll *et al.*, 2014). The authors suggest this was due to incorrect application of sunscreen, with this usage potentially being a marker of increased sun exposure and time outside, resulting in higher vitamin D production (Carroll *et al.*, 2014).

2.11 Determination of vitamin D by prediction questionnaires

Predictive questionnaires offer a non-invasive, inexpensive method of determining vitamin D status.

2.11.1 Venepuncture and young children

Young children find needles scary and painful, much more so than older children and adolescents (Fassler, 1985; Howe *et al.*, 2011) and may experience distress when approaching blood sampling procedures (Broome *et al.*, 1989; Fradet *et al.*, 1990). Of 89 children aged between 3 to 6 years requiring venepuncture a Canadian hospital, 36 to 64% reported suffering high levels of pain during venepuncture (Fradet *et al.*, 1990). Studies in paediatrics differ from those of adults in aspects such as potential pain and distress, with consent for participation having to be obtained from a parent or caregiver (Caldwell *et al.;* Shaddy and Denne, 2010). Blood sampling in children who participate in medical research is an issue of concern to both parents, and researchers

(Howie, 2011) and may affect recruitment and research participation (Grant *et al.*, 2009; Shaddy and Denne, 2010; Nichols *et al.*, 2015). These factors may contribute to the low number of studies described in the literature which investigate vitamin D in children of preschool age. A significant number of these study participants are children presenting at hospital or outpatient clinics, where 25(OH)D is measured on a blood sample taken for other reasons (Dyson *et al.*, 2014).

2.11.2 Increase in volume of vitamin D blood test requests

Requests for measurement of 25(OH)D concentrations increased 2 to 6 fold in hospitals in Glasgow and London between 2008-10 (Sattar *et al.*, 2012), tripled in Ontario from 2004-9 (Mittelstaedt, 2010) and by 94% in the decade 2000-10 in Australia (Bilinski and Boyages, 2012a; Bilinski and Boyages, 2013). New Zealand has followed this trend, with a four-fold increase in tests for 25(OH)D concentration in 2010 than 2000 (8,500 to 32,800 tests respectively) (Bolland *et al.*, 2012). Approximately 22,000 adults had a blood test measuring 25(OH)D concentration at the central Auckland laboratory in an 18 month period in 2002/3 (Bolland *et al.*, 2008).

A consequence of this increasing number of test requests is the increase in costs for health departments (Bilinski and Boyages, 2012b). The situation in neighbouring Australia has become uneconomic, with the cost of measurement of 25(OH)D concentration rising from $1M in 2001 to $96M in 2010 (Bilinski and Boyages, 2012b). In New Zealand the cost of tests for vitamin D in 2011 was $1M (Bolland *et al.*, 2012). Measurement of 25(OH)D concentration is relatively expensive, in Auckland an assay costs $46 for self-referred testing and is much higher than for other tests (e.g. $9 for cholesterol) due to the length and complexity of the analysis methods used to determine 25(OH)D concentrations (Labtests, 2014).

Recently, there has been unease expressed by the health professionals around over-diagnosis, excessive medicating and related blood testing, with vitamin D being only one example of a broader concern (Moynihan *et al.*, 2012; Bilinski and Boyages, 2013). Routine testing of vitamin D has been challenged on the basis of 25(OH)D assay techniques not using LC-MS/MS technology being able to accurately quantify vitamin D deficiency at lower levels, as well as comparison issues between laboratories (Halpern, 2013). In New Zealand, Bolland *et al* questioned the need for routine testing of a large number of people for a minority who were at risk of vitamin D deficiency (Bolland *et al.*, 2012). Other considerations include clinicians recommending sun exposure to raise vitamin D levels given skin cancer concerns, and the safety of supplementation being prescribed

within current recommendations (Vieth, 1999; Ministry of Health and Cancer Society of New Zealand, 2012).

With spiralling testing costs, various approaches have been taken by health authorities. Vitamin D testing in Canada is only available on a private basis whilst Australia advises health professionals against routine screening (Bilinski and Boyages, 2013). In 2011, the New Zealand District Health Boards advised tests for vitamin D could only be ordered by specialists for bone related investigations (Bolland *et al.*, 2012). This decision resulted in a rapid reduction in the number of tests requested in the first three months, from 2300 to 580 per month (Bolland *et al.*, 2012). Currently, health practitioners assess the risk of vitamin D deficiency of patients on an individual basis. This is cost-effective for the health authorities with the cost of supplementation for one year being less than the cost of screening vitamin D status (Bolland *et al.*, 2012) however there may be an associated risk of over-prescribing of this supplement.

2.11.3 Prediction questionnaires

A prediction tool is non-invasive, inexpensive and has the potential to save young children possible trauma and pain associated with venepuncture (Fradet *et al.*, 1990). Questionnaires based on predictor variables of low vitamin D status have been developed in three categories; 1. prediction of 25(OH)D concentration (Millen *et al.*, 2010; Peiris *et al.*, 2011; Bertrand *et al.*, 2012; Jensen *et al.*, 2013); 2. the likelihood of participant being in a 25(OH)D range (Sohl *et al.*, 2014); 3. predicting participants at risk of vitamin D deficiency or insufficiency (Bolek-Berquist *et al.*, 2009; Absoud *et al.*, 2011; Nabak *et al.*, 2013; Tran *et al.*, 2013; Lopes *et al.*, 2014). In this final category, questionnaires have been developed for specific age groups in different countries; children/adolescents (Great Britain) (Absoud *et al.*, 2011), young adults (United States) (Bolek-Berquist *et al.*, 2009), older adults (Brazil, The Netherlands) (Lopes *et al.*, 2014; Sohl *et al.*, 2014) and postmenopausal females (United States) (Nabak *et al.*, 2013). No questionnaires assessing risk of vitamin D deficiency in preschool aged children have been published in the literature to date, nor questionnaires specific to any New Zealand population group.

For a questionnaire to be used in routine clinical situations, the information for the predictor variables needs to be easily obtained, thus biochemical analysis results and information such as ambient ultraviolet radiation (UVR) readings cannot be used (Tran *et al.*, 2013). Some researchers report intentionally only including variables which the patient could easily answer in a

consultation (Bolek-Berquist *et al.*, 2009; Lopes *et al.*, 2014). The questionnaires predicting vitamin D deficiency or insufficiency using easily obtainable information are described in Table 2.5.

Predictors of vitamin D deficiency or insufficiency are determined for the participant group, thus prediction questionnaires cannot be easily extrapolated for use in different populations, season or latitudes and the predictor variables may change over a passage of time (Absoud *et al.*, 2011; Lopes *et al.*, 2014). Performance has been assessed using Receiver Operating Characteristics (ROC) analysis. For an ROC curve, the true positive rate (sensitivity) is plotted at different cut-off points with the false positive rate (1-specificity), and the Area Under the Curve (AUC) is a measure of how two states (deficient/not deficient) are distinguished (Hanley and McNeil, 1982; Greiner *et al.*, 2000; Field, 2013). In respect to vitamin D, Sohl defines specificity as "the proportion of individuals who are not deficient and who are correctly classified as non-deficient" and sensitivity as "the proportion of individuals who are deficient and who are correctly classified as deficient" (Sohl *et al.*, 2014).

External validation is carried out through the AUC calculation and comparison with another subject group. In a model which could not distinguish between a deficient or non-deficient state, the AUC would equal 0.5 while with a perfect separation of two states the AUC would equal 1.0 (Hanley and McNeil, 1982). Two prediction questionnaires reported validation using AUC, with results 0.82 ($P<0.001$) and 0.685 ($P<0.001$) (Absoud *et al.*, 2011; Lopes *et al.*, 2014). Studies by Absoud *et al* and Lopes *et al* split the participant group in order to provide external validation, while Sohl *et al* used a separate group of participants (Absoud *et al.*, 2011; Lopes *et al.*, 2014; Sohl *et al.*, 2014). This second group was younger in age and conducted some 13 years after, potentially limiting the accuracy of this validation (Sohl *et al.*, 2014). The prediction questionnaires of Bolek-Berquist *et al* and Nabak *et al* did not report any external validation measures, limiting their usefulness (Bolek-Berquist *et al.*, 2009; Nabak *et al.*, 2013).

In a diagnostic situation, positive and negative predictive values (PPV and NPV respectively) are the proportions of results which are true positive and true negative and are affected by disease prevalence (Mausner and Kramer, 1985). Varying PPV and NPV have been reported for prediction questionnaires (Table 2.5). Lopes *et al* and Nabak *et al* report higher PPV (74% and 80%) and lower NPV (53% and 41%), while the questionnaire by Sohl *et al* has very high PPV of 96% and poor NPV of 37%.

Table 2.5. Questionnaires predicting vitamin D deficiency or insufficiency using simple variables

Study	Country	Age Group	Number (n)	Vitamin D concentration predicted, & prevalence	Mean 25(OH)D (nmol/L)	Predictor Variables	AUC	ROC Sensitivity	ROC Specificity	PPV	NPV	Population representative
Absoud (2011) (Absoud *et al.*, 2011)	Great Britain 1997/8 National Diet Nutrition Survey	Children & adolescents 4-18 years Male + female	1102	<50nmol/L 35%	62	White skin colour Age 4-8 y Month of testing June-Nov Not on income support > 1/2 hour exercise per day Watches TV <2.5hours per day Taking vitamin D supplement	0.82	75%	79%	ns	ns	Yes
Bolek-Berquist (2009) (Bolek-Berquist *et al.*, 2009)	Wisconsin US	Young adults 18-40 years Male + female	184	<40nmol/L 26%	63	Suntan Tanning booth use >= 2 milk serves daily	ns	79%	78%	ns	ns	No
Lopes (2014) (Lopes *et al.*, 2014)	San Paulo Brazil	Older adults 65+ years Male + female	908	<50nmol/L 58%	49	Gender female Diabetes present Season winter/spring	0.69	56%	72%	74%	53%	No
Nabak (2013) (Nabak *et al.*, 2013)	Wisconsin US	Older adults Mean 61 years Postmenopausal females	609	<50nmol/L 19%	78	Height Weight Ethnic, black Sun tan in the last 12 months Sunlight exposure, lightly dressed How often sunscreen used Multivitamins Vitamin d supplement Do you use sunscreen	ns	86%	35%	80%	41%	No

Study	Country	Age Group	Number (n)	Vitamin D concentration predicted, & prevalence	Mean 25(OH)D (nmol/L)	Predictor Variables	AUC	ROC Sensitivity	ROC Specificity	PPV	NPV	Population representative
Sohl (2014) (Sohl *et al.*, 2014)	Amsterdam The Netherlands Longitudinal Aging Study	Older adults 55-85 Male + female	1106	2 models <30nmol/L 17% <50nmol/L 46%	53	Age Sex BMI >30 Smoking Alcohol <13 drinks/week Season Vitamin use Bicycling Sporting Gardening Medication use Presence of appetite Limitations in transport use Partner status Remembers year	<30nmol/L 0.80 <50nmol/L 0.71	<30nmol/L 61% <50nmol/L 61%	<30nmolL 84% <50nmolL 82%	<30nmolL 37% <50nmolL 78%	<30nmolL 95% <50nmolL 67%	Yes

PPV positive predictive value, NPV negative predictive value, ns not stated

2.12 The relationship between vitamin D and allergic and respiratory diseases in preschool children

Rates of childhood allergic disease have been increasing over the past few decades throughout the world (Asher *et al.*, 2006). In New Zealand, the prevalence of eczema, allergic rhinoconjunctivitis and asthma is 22%, 16% and 11% respectively, with food allergy estimated to affect 10% of children (Asher *et al.*, 2006; Allergy New Zealand, 2014). The rates of eczema and asthma are higher than those of countries in Europe and the Asia Pacific region, and similar to the United Kingdom (Asher *et al.*, 2006). These diseases have a high burden, both for the children and their families, affecting hospital admissions, quality of life, educational progress and health outcomes (Kiebert *et al.*, 2002; Asher *et al.*, 2006; Marklund *et al.*, 2006).

Also increasing in prevalence in New Zealand are hospital admissions for respiratory infections in young children (Craig *et al.*, 2013). New Zealand has higher hospitalisation rates for acute lower respiratory infections, including pneumonia, than other developed countries (Craig *et al.*, 2013). This is of specific relevance to asthma given that >90% of asthma attacks in children are initiated by a viral respiratory infection (Johnston *et al.*, 1995).

Although the aetiology and pathophysiology of these allergic diseases is complex, research has indicated that vitamin D may have a role in these diseases (Huh and Gordon, 2008). Vitamin D has an established role is in calcium and phosphate homeostasis, and is necessary for optimal skeletal mineralisation (Bischoff-Ferrari *et al.*, 2004; Lips, 2010). In calcium homeostasis, vitamin D facilitates calcium absorption in the duodenum, influences calcium mobilisation by osteoclasts and regulates calcium reabsorption by renal cells (Matsui *et al.*, 1983; Shinki *et al.*, 1999). The skeletal consequences of vitamin D deficiency have long been recognised. They manifest as rickets in children and as osteomalacia in adults (Holick, 2004). Vitamin D deficiency has been also been associated with retardation of skeletal growth and failure to meet maximum potential bone mass (Holick, 2004). Evidence for the increased risk of osteoporosis that occurs with vitamin D deficiency due to calcium malabsorption and secondary hyperparathyroidism has been provided by both epidemiological studies and randomised controlled trials (Bischoff-Ferrari *et al.*, 2004; Lips *et al.*, 2006; Tang *et al.*, 2007).

The proposed links between vitamin D and the immune system came from several routes. One of the earliest pieces of evidence was the observation of a relationship between rickets and pneumonia in young children. For example, in a study of children living in Tehran, those with

nutritional rickets were more likely to also have gastroenteritis and bronchopneumonia (Salimpour, 1975). In Ethiopia, children less than 5 years of age hospitalised with pneumonia had a 13-fold increased risk of rickets compared with control children who did not have pneumonia (Muhe *et al.*, 1997). Among hospitalised Jordanian infants, those with rickets were more likely to have been hospitalised with a respiratory tract infection (Najada *et al.*, 2004). Rickets was associated with an increased risk of death from severe pneumonia in Yemeni children less than 5 years old (Banajeh *et al.*, 1997). More recently, subclinical vitamin D deficiency was shown to be associated with an increased risk of acute lower respiratory infections in young Indian children (Wayse *et al.*, 2004) and Turkish newborns (Karatekin *et al.*, 2007). Demonstration of this association of subclinical vitamin D deficiency with an increased risk of lower respiratory infections implied that the mechanism was likely to be due to factors other than those directly related to skeletal and muscle function.

Observational studies and then randomised clinical trials in patients with tuberculosis is a second area of research that has provided important insights into the relationship between vitamin D and respiratory infections. The treatment of tuberculosis with high-intensity light was first discovered in 1895 by Finsen, and by the 1920's sun exposure had become a widely accepted treatment method (Zasloff, 2006). In 1849, improvements in tuberculosis patients were observed following administration of fish oil (Williams, 1849). In a meta-analysis of studies comparing tuberculosis patients and healthy controls, low serum 25(OH)D concentrations were associated with an increased risk for active tuberculosis (Nnoaham and Clarke, 2008). In Pakistan, patients with tuberculosis who were vitamin D deficient had a five-fold increased risk of progression of their tuberculosis compared with patients with tuberculosis who were not vitamin D deficient (Talat *et al.*, 2010).

More recent research which has focused on the relationship between vitamin D and allergic disease has investigated both of the alternative hypotheses that either low or high vitamin D status increases the risk of allergic disease. In 1999, Wjst *et al* observed that the increasing rates of vitamin D supplementation of pregnant women and infants that occurred in an attempt to prevent infantile rickets, coincided with the rise in prevalence of allergic conditions (Wjst and Dold, 1999). He hypothesised that an adverse effect of this vitamin supplementation was delayed immunological maturity due to the suppressive action of calcitriol on dendritic cell maturation, causing lower IL-12 signalling and less Th_1 cell development (Holt *et al.*, 2005). Conversely a decade later, several researchers theorised that the increase in rates of allergic disease was rather due to increasing rates of vitamin D deficiency reported worldwide in association with an

increasingly sedentary, indoor lifestyles in urban settings, with the reduced sun exposure associated with these modern lifestyles negatively affecting cutaneous synthesis of vitamin D (Camargo *et al.*, 2007; Litonjua, 2008; Vassallo and Camargo Jr, 2010). This theory was based on the known latitudinal variation in UVB exposure and observations of latitudinal gradients, with higher rates of allergic disease in populations living further from the equator. This relationship of higher latitude with an increased prevalence of allergic disease was evident across several different markers of allergic disease including prescription rates for epinephrine auto injector (EpiPen) for anaphylaxis treatment (EpiPen) in Australia (Mullins *et al.*, 2009) and the United States (Camargo Jr *et al.*, 2007), anaphylaxis admission rates in Chile (Hoyos-Bachiloglu *et al.*, 2014), infant hypoallergenic formula prescription rates in Australia (Mullins *et al.*, 2010) and parental report of eczema, egg and peanut allergy in Australian children (Osborne *et al.*, 2012).

Other observational studies which have provided evidence for a relationship of low vitamin D status with an increased risk of allergic disease include those that show childhood eczema prevalence is inversely correlated with UV exposure (Silverberg *et al.*, 2013); that food allergy is more common in children born in months of low UVB intensity (Vassallo *et al.*, 2010) and in children of darker-skinned (African American) ethnicity groups (Bird *et al.*, 2014); and that obese and overweight children are at increased risk of allergic sensitisation to food compared to normal weight children (Visness *et al.*, 2009). Winter season, non-European ethnicity and obesity are each associated with an increased risk of lower vitamin D status (Webb *et al.*, 1988; Rajakumar *et al.*, 2005; Weng *et al.*, 2007).

Understanding of the mechanism underlying the relationship between vitamin D status and allergic disease has advanced by the demonstration, in cells in body tissues other than the kidney, of the existence of vitamin D receptors (VDR) and of enzymes systems to convert 25(OH)D to the active metabolite $1,25(OH)_2D$ (calcitriol) (Hewison *et al.*, 2000). In the 1980s, VDRs were identified within activated inflammatory cells with T-cell proliferation shown to be inhibited by calcitriol (Provvedini *et al.*, 1983; Rigby *et al.*, 1984), and by the expression of 1-α-hydroxylase by activated macrophages (Adams *et al.*, 1983). More recently, receptors have been identified on nearly all the cells of the immune system, including T and B lymphocytes, monocytes, neutrophils and antigen presenting cells (macrophages and dendritic cells) (Holick, 2007; Baeke *et al.*, 2010).

The expression of 1-α-hydroxylase in immune cells is controlled by inflammatory cytokines, such as IFN-γ, interleukin 1(IL-1) and TNR-α, independent of PTH or $1,25(OH)_2D$ (Van Etten *et al.*, 2008). This suggests a potential role for vitamin D in immune mediated conditions such as allergic,

infectious and autoimmune diseases (Székely and Pataki, 2012). These findings may be particularly relevant to the paediatric population, as young children have the highest rates of allergic disease and respiratory infections of any age group, and may offer options in terms of prevention and treatment of these conditions (Litonjua and Weiss, 2007; Bozzetto *et al.*, 2012).

2.13 Vitamin D and the immune system

2.13.1 Allergy and atopy

Allergy is an IgE mediated hypersensitive response to normal harmless allergens (environmental bodies) resulting in an allergic disease such as eczema, food allergy, allergic rhinoconjunctivitis and asthma. Atopy is a predisposition to develop certain allergic diseases, measured as the presence of specific immunoglobulins E mediated antibody responses in serum or via skin prick testing (Ober and Yao, 2011). Allergy and atopy do not always co-exist in individuals, those who test positive for the presence of atopy do not always develop allergic diseases. Conversely, those with allergic diseases do not always have positive atopy test results (Jarvis and Burney, 1998).

2.13.2 Allergic response of the immune system

A 'normal' antigen-specific response needs a balance between the two types of T helper cells, Th_1 and Th_2. Cell-mediated responses to neoplastic cells and intracellular pathogens (viruses) require Th_1 activation, while the role of Th_2 is in the immune response against antigens and extracellular pathogens (bacteria and parasites) (Cantorna *et al.*, 2004). Th_1 cells secrete interleukin-2 (IL-2), interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) and Th_2 cells secrete IL-4, IL-5 and IL-13. When the 'normal' Th_1/Th_2 balance is biased towards Th_2, a hyper-allergenic response to environmental antigens occurs (Cantorna *et al.*, 2004). Allergic diseases are symptoms of Th_2-mediated diseases.

Vitamin D appears to have an immune-modulatory role, affecting both the innate and adaptive immune systems (Benson *et al.*, 2012) (Figure 2.3). Recent research suggests $1,25(OH)_2D$ contributes to the immune systems through mechanisms that modulate immunologic tolerance (Dimeloe *et al.*, 2010), suppress pro-allergic immune responses (Hewison, 2010), maintain the integrity of the epithelial barrier (Kong *et al.*, 2008) and reduce susceptibility to infection (Liu *et al.*, 2007).

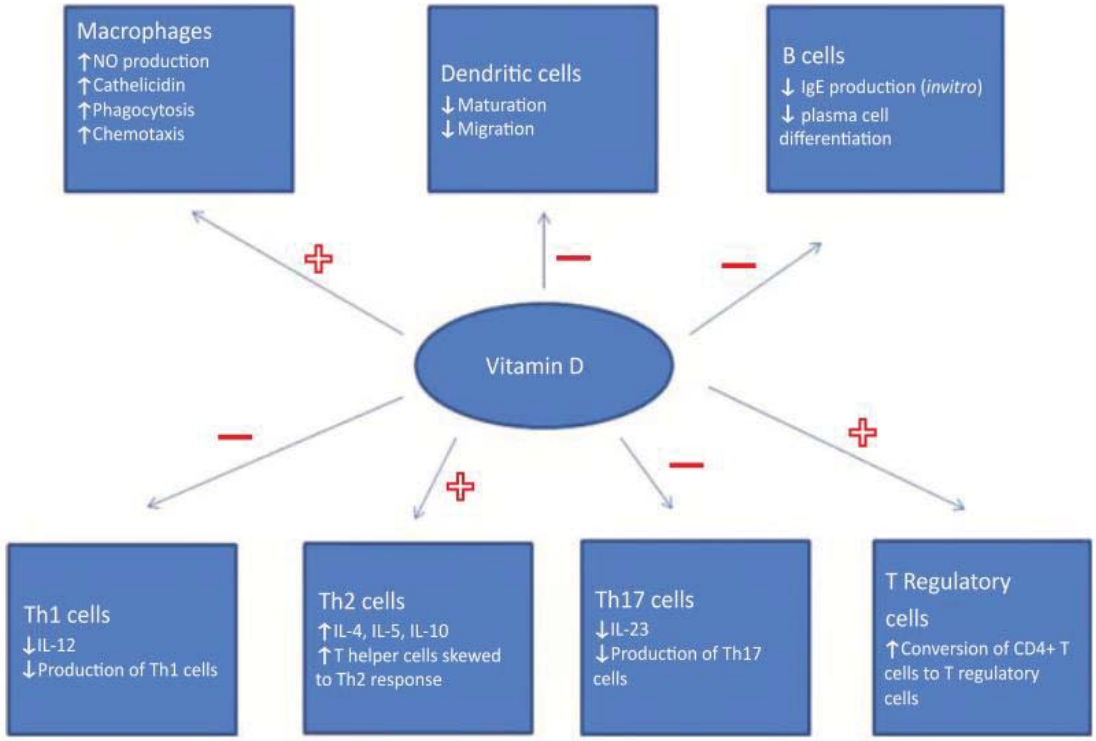


Figure 2.3. The impact of vitamin D on the immune system. Reproduced with permission (Benson *et al.*, 2012)

The immune-modulatory role of vitamin D on the innate system acts to increase antimicrobial peptide synthesis and inhibit pro-inflammatory cytokine production (Adams and Hewison, 2008). In the innate system, expression of genes encoding the toll-like receptors (TLR) have been shown to be induced by vitamin D. Antigen presenting monocytes, macrophages, and also keratinocytes, all express TLR (Baeke *et al.*, 2010). The chemotactic and phagocytic capability of monocytes and macrophages is enhanced by TLR, positively affecting defence against infection (Baeke *et al.*, 2010). Antimicrobial defence is also enhanced through $1,25(OH)_2D$ stimulating production of anti-microbial proteins (AMP), such as cathelicidin and beta-defensin (Liu *et al.*, 2006) whilst reducing levels of pro-inflammatory cytokines IL-1, IL-6, IL-8 and TNF-α (Almerighi *et al.*, 2009). Baeke *et al* describe this as a negative feedback mechanism preventing extreme TLR activation and inflammation at advanced stages of infection (Baeke *et al.*, 2010).

Common to both the innate and adaptive immune system are the antigen presenting cells (APC), the dendritic cells (DC), which capture, process and present antigens to T helper cells (Baeke *et al.*, 2010). These cells express the VDR. The *in vitro* addition of $1,25(OH)_2D$ affects the differentiation and maturation of dendritic cells (Penna and Adorini, 2000), leading to a reduction

in production of the cytokines IL-12 and IL-23 (Baeke *et al.*, 2010). Subsequently, this inhibits differentiation of T helper Th_1 and Th_{17} cells (Baeke *et al.*, 2010). Thus, vitamin D affects the ability of dendritic cells to present antigens, and indirectly contributes to modulation of T helper cell ratios.

Based on their cytokine secretion patterns, naïve CD4+ T helper cells differentiate into Th_1 and Th_2 cells, mediating immune response (Staeva-Vieira and Freedman, 2002). The action of $1,25(OH)_2D$ on dendritic cells increases production of CD4 cells into regulatory T cells (Treg), which secrete anti-inflammatory cytokines IL-10 and TGF-β (Griffin *et al.*, 2003) and are capable of supressing sustained inflammation by effector T cells (Baeke *et al.*, 2010). Vitamin D has been shown to modulate the T-cell antigen receptor (von Essen *et al.*, 2010). In the adaptive immune system, the inhibition of Th_1 cells by $1,25(OH)_2D$ arrests the cell cycle, triggers apoptosis, dampens the Th_1 and Th_{17} inflammatory response resulting in reduced production of IL-2, IL-17, IL-21 and the key Th_1 cytokine IFN-γ in a dose-dependent fashion (Baeke *et al.*, 2010).

Conflicting Th_2 cell responses have been reported. In vitro studies show opposing effects of $1,25(OH)_2D$ concentrations affecting the secretion of Th_2 cytokines, with secretion inhibited by lower concentrations, and induced by higher $1,25(OH)_2D$ concentrations (Dimeloe *et al.*, 2010). In vitro studies by Boonstra *et al* report Th_2 cells were augmented through action of $1,25(OH)_2D$ and increased production of anti-inflammatory cytokines IL-4, IL-5 and IL-10 (Boonstra *et al.*, 2001) while Staeva-Vieira *et al* found an inhibition of expression of Th_2 cells and suggest this is due to attenuation of IL-4 transcription by the VDR (Staeva-Vieira and Freedman, 2002). These varying results may be due to a dose-dependent response of Th_2 lymphocytes to vitamin D.

Dendritic cells play a major role in the commencement of the allergic response through differentiation and maturation (Grabbe *et al.*, 1995). Immature DCs mature into potent APC during movement from non-lymphoid tissue to lymphogenic organs, with Langerhans cells an example of DC found in skin, promoting Treg cell populations (Cella *et al.*, 1997). T cell response requires both T cell and dendritic cell activation by engagement of CD28 and CD154 by CD80/CD86 on T cells, and CD 40 on DC (Piemonti *et al.*, 2000). In addition, activated T cells express CD152 which has higher affinity for CD80/CD60, promoting immunological tolerance through inhibited cytokine IL-2 production, IL-2 receptor expression and cell survival (Lenschow *et al.*, 1996).

$1,25(OH)_2D$ has been shown to inhibit DC differentiation and maturation into potent APC through inhibition of IFN-γ secretion and up regulation of CD152 and down regulation of CD154 molecules (Penna and Adorini, 2000). Human dendritic cells treated with $1,25(OH)_2D$ convert CD4+ T cells into IL-10 responses, supressing proliferation of T cells (Unger *et al.*, 2009). An example of the clinical significance of this is provided by the observation that children who outgrow clinical milk allergy have higher levels of T regulatory cells than those whom milk allergy persists (Shreffler *et al.*, 2009). These findings suggest a potential for a role for vitamin D in development of tolerance to allergic diseases.

Antimicrobial peptides (AMP) are protective against bacteria, viruses and fungi. They are produced within circulating white cells and on epithelial surfaces (Zasloff, 2002). The most common AMPs produced in response to tissue injury (cytokines including IL-1) or microbes are β-defensins 2 & 3 and cathelicidin (LL-37) (Zanetti, 2004). These AMP (particularly cathelicidin) have dual roles, anti-infection and, by combining with the epidermal growth factor receptor, in epithelial tissue repair and growth (Zasloff, 2002). VDR receptors have been located on cathelicidin, *in vitro* studies showed the concentration of cathelicidin in human neutrophils and expression of cathelicidin in keratinocytes was increased by vitamin D (Wang *et al.*, 2004). Vitamin D stimulates toll like receptors (TLR) on monocytes, resulting in expression of cathelicidins and greater microbiocidal capability (Liu *et al.*, 2006).

Immunoglobulin (IgE) is a class of antibodies which play a major role in immune response in allergic disease by binding to allergens, resulting in the release of inflammatory substances by mast cells (Geha *et al.*, 2003). Concentrations of serum IgE have been shown to be affected by vitamin D, with laboratory studies reporting IgE production was reduced, and IL-10 production stimulated, with the introduction of $1,25(OH)_2D$, firstly to human beta cells (Heine *et al.*, 2002) and secondly, in murine allergy mouse models (Hartmann *et al.*, 2012).

However, conflicting effects of vitamin D on AMP production and inflammatory regulators (Treg) have also been reported. In vitro testing of AMP production with $1,25(OH)_2D$ has found both increasing and decreasing IL-17 response (Palmer *et al.*, 2011; Peelen *et al.*, 2011). Murine studies have reported both reduction, and no effect, in Treg levels with $1,25(OH)_2D$ (Chang *et al.*, 2010). In human in vitro studies, IL-10 production was enhanced with vitamin D, stimulating Treg, and regulation of Th_2, response (Xystrakis *et al.*, 2006). This influence has also been seen with corticosteroid treatment for asthma (Dimeloe *et al.*, 2010).

2.14 Vitamin D and allergic and respiratory diseases

2.14.1 Vitamin D and eczema

Eczema is commonly the first step in the 'atopic march', with other atopic diseases such as food allergy, allergic rhinoconjunctivitis and asthma following (Zheng *et al.*, 2011). In comparison with mild eczema, severe eczema is characterised by higher IgE levels, greater Th_2 polarisation, larger areas of skin affected and more viral and bacterial skin infections (Beck *et al.*, 2009). Individuals affected with eczema are also more likely to also have food allergy, asthma and be sensitised to common allergens (Beck *et al.*, 2009).

The pathogenesis of acute lesions of eczema is illustrated in Figure 2.4. Eczema is due to a combination of defective epidermal barrier (with epidermal proteins affected by gene mutations causing trans-epidermal water loss, leading to dryness, with increased entry of allergens and microbes into the skin) (Cork, 1997). This leads to irritation and mechanical injury through scratching, and dermal and epidermal allergic inflammatory response (Borzutzky and Camargo Jr, 2013). This skin injury and immune response drives keratinocytes and dendritic cells to secrete cytokines IL-4 and IL-13, recruiting CD4+ T cells to the skin whilst naïve T cells are polarised to a Th_2 response, stimulating β-cells to a hyper allergenic response (Soumelis *et al.*, 2002). A decreased level of inflammation control in eczema patients may be caused by differences in immunopathology of dendritic cells and absence of FOXP3+ Treg cells in lesional skin compared to healthy patients (Verhagen *et al.*, 2006).

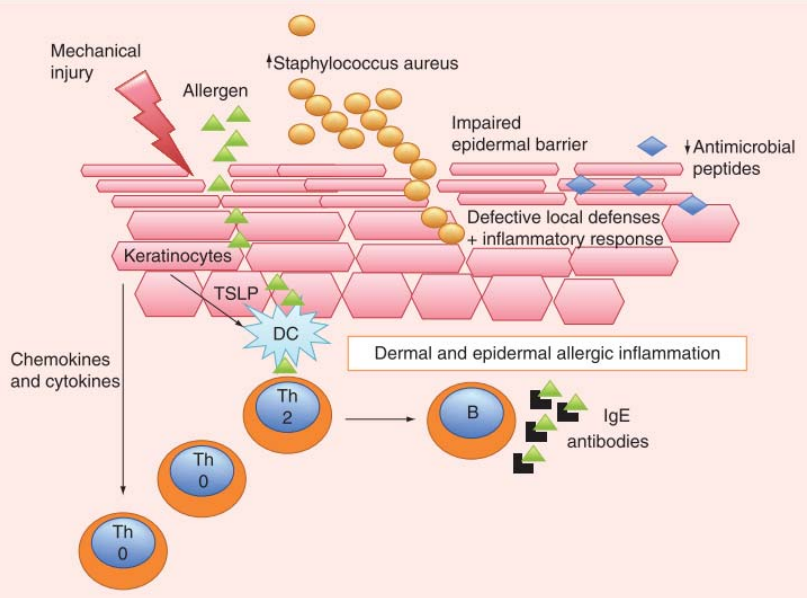


Figure 2.4. Overview of the pathogenesis of acute lesions of eczema. Reproduced with permission (Borzutzky and Camargo Jr, 2013)
Th = T helper cell; DC = dendritic cell; B = β-cell; IgE = Immunoglobulin E

In the skin, activation of vitamin D can occur independently through keratinocytes in the stratum basale, due to their 1-α-hydroxylase (CYP27A1 and CYP27B1) content (Sawada *et al.*, 2000; Bikle, 2012). These genes encode a cytochrome P450 oxidase, regulating the expression of around 900 genes (Bikle, 2012). A key component of epidermal barrier integrity is the gene filaggrin, responsible for aggregation of the keratin bundles in the two lowest layers of the stratum corneum (O'Regan *et al.*, 2009). In patients with eczema abnormalities in the filaggrin encoding gene leads to fragile corneocytes and decreased Natural Moisturising Factor (NMF) (Cork, 1997; Palmer *et al.*, 2011), and also to differences in other proteins that result in tight junction defects (Cork, 1997).

Eczema affected skin is commonly colonised by *S. aureus* due to decreased control of bacterial and viral infections (Ong *et al.*, 2002), and this colonisation is believed to contribute to disease pathogenesis. This is most likely due to down regulation in AMPs (and corresponding decrease in cathelicidin and β-defensins reducing anti-pathogen action), alterations in TLR2 induced inflammation and reduced antiviral activity through Th_1 (Ong *et al.*, 2002). A feedback loop causes chronic eczema, with Th_{17} cells preferentially penetrating the dermis and the reduction in AMP intensified by IL-4, IL-13 and IL-10 Th_2 cytokine action permits bacterial overgrowth which then drives further inflammation (Howell, 2007). In chronic eczema, inflammation is also heightened by involvement of CD8+ T cells secreting both IFN-γ and IL-13 cytokines (Hijnen *et al.*, 2012).

Potential areas where sufficient vitamin D status may make a positive contribution to eczema are through increased epidermal differentiation and skin barrier function (Bikle, 2012). Possible mechanisms include tolerogenic action of dendritic cells, increased cathelicidin production, filaggrin expression in combination with decreased β-cell proliferation, IgE and Th_2 cytokine production and pathogen colonisation (Borzutzky and Camargo Jr, 2013).

Vitamin D has an important role in skin barrier permeability, demonstrated in a laboratory study of mice deficient in 1-α-hydroxylase. The resultant lack of VDR brought about decreased expression of epidermal barrier proteins and barrier recovery following injury (Meindl *et al.*, 2005). Administration of VDR in another mouse model of eczema showed increased induction of skin barrier genes, including filaggrin and AMP expression (Hartmann *et al.*, 2012). Improved maintenance of mucosal barrier function (regulation of tight junction proteins including ZO-1, claudin 1, claudin 2 and E-cadherin) and mucosal response to damage was been seen in murine cell cultures with $1,25(OH)_2D$ (Kong *et al.*, 2008). These *in vitro models* suggest a protective effect of vitamin D on skin barrier function.

Vitamin D has been shown to affect the pathogen *S. aureus,* which stimulates and spreads skin inflammation in eczema. Vitamin D status is a determinant of *S. aureus* colonisation and carriage in healthy people (Olsen *et al.*, 2012). In eczema patients, 25(OH)D levels are inversely associated with S. aureus enterotoxins (Peroni *et al.*, 2011) and also associated with serum cathelicidin levels (Kanda *et al.*, 2012), while supplementation resulted in improved cathelicidin production in affected skin (Hata *et al.*, 2008).

2.14.2 Vitamin D and food allergy

Food allergy is an exaggerated IgE mediated immune response to common and otherwise harmless food antigens. Children have higher rates of food allergy than adults, with most children outgrowing their food allergy by school age, particularly to egg and milk. In contrast, allergies to peanut, tree-nut, fish and shellfish are usually lifelong (Sicherer and Sampson, 2010).

The proposed link between rising rates of food allergy and vitamin D status have been discussed previously. It has been hypothesised that the combined effect of vitamin D deficiency and infections is to decrease intestinal barrier integrity and allow greater antigen access to the immune system (Vassallo *et al.*, 2010). In 2010, Vassallo and Camargo proposed a model where

vitamin D deficiency in a critical time interval early in life (in utero or early infancy) increases vulnerability to gastrointestinal infections and colonisation of the gastrointestinal tract with abnormal microbial flora. This leads to abnormal intestinal barrier function, with increased permeability, allowing excessive dietary allergens to be exposed to the immune system. In conjunction, there may be a synergistic effect of vitamin D deficiency promoting a Th_2 immune balance compromising tolerance (Vassallo *et al.*, 2010).

Lack *et al* suggests cutaneous sensitisation may impact the development of food allergy (Lack, 2008). Cutaneous sensitisation occurs through impaired skin barrier function (increased skin permeability and decreased filaggrin linked with transepidermal water loss, found prior to eczema development (Palmer *et al.*, 2006). On exposure to low doses of food proteins, via table tops, hands and dust, these proteins infiltrate the impaired skin barrier and are taken up by antigen presenting Langerhans cells, leading to increased IgE production by β-cells and allergenic Th_2 immune responses (Perry *et al.*, 2004). A prospective birth cohort study found low-dose exposure of peanut (arachis oil) to infantile inflamed skin was associated with an increased risk of childhood food allergy at age 5 (Lack *et al.*, 2003).

The gastrointestinal tract is the mucosal site of greatest antigen exposure, with gastrointestinal infections damaging the epithelial barrier, allowing dietary and microbial antigens to penetrate into the intestinal lumen (Vassallo *et al.*, 2010). Food allergy is potentially a clinical manifestation of this process, particularly in patients with a family history of allergic disease (Lack, 2008).

Potential mechanisms where vitamin D may be a determinant of food allergy are the effect of vitamin D on healthy microbial ecology and integrity of the gastrointestinal tract and via is effect on the immune response to allergens (Vassallo *et al.*, 2010). The role of vitamin D in immune function and infection defence has been discussed previously in this thesis, with these same mechanisms also potentially applicable to the pathogenesis of food allergy.

2.14.3 Vitamin D and allergic rhinoconjunctivitis and asthma

Children with allergic rhinoconjunctivitis commonly present with other allergic diseases, with asthma and allergic rhinitis both being expressions of an atopic phenotype (Chiang *et al.*, 2012; Hong *et al.*, 2012). The concept of 'one airway, one disease' suggests a common airway and mucosal susceptibility between these two conditions (Durham, 2002). Data from epidemiological

studies suggests rhinitis is an independent risk factor for asthma (Leynaert *et al.*, 2000) in addition to being a manifestation of atopy (Guerra *et al.*, 2002; Peroni *et al.*, 2003). Adult patients with respiratory airway diseases commonly report rhinitis prior to the development of asthma (Guerra *et al.*, 2002). With respect to clinical severity, these conditions run in parallel. Poorly controlled allergic rhinoconjunctivitis can exacerbate symptoms of allergic asthma (Leynaert *et al.*, 2000), resulting in increased healthcare costs and decreased personal wellbeing (Sazonov Kocevar *et al.*, 2005).

The most frequent form of asthma is atopic asthma. In atopic individuals allergen exposure results in a Th_2 immune system response involving eosinophilic inflammation (Arbes Jr *et al.*, 2007; Kim *et al.*, 2010), with IgE production increasing the risk of asthma continuance and severity (Martinez, 2002; Rasmussen *et al.*, 2002; Holloway and Holgate, 2004).

Potential mechanisms for the role of vitamin D on asthma include prevention of airway obstruction or improvement in pulmonary physiology, reversal of steroid resistance, improvement of respiratory infection control (Taylor and Camargo Jr, 2011).

The lung utilises vitamin D as a component of its defence mechanism to induce Th_2 cell responses and inflammation with VDR expression needed for induction of IL-4, IL-5 and IL-13 (Lloyd and Hessel, 2010). These cytokines have individual roles; IL-4 promotes IgE production (responsible for asthma exacerbations and airway inflammation), IL-5 assists eosinophil survival and IL-13 alters immune and structural cells (Mann *et al.*, 2014). Eosinophils express VDR and secrete pro-inflammatory cytokines, cytokines, leukotrienes and matrix metalloproteinases, all implicated in asthma (Lloyd and Hessel, 2010).

2.14.3.1 Vitamin D and airway obstruction and pulmonary physiology

Evidence for a role of vitamin D in asthma comes from bench, animal model and human subject research. *In vitro* treatment of peripheral blood with $1,25(OH)_2D$ increases CXCR4 expression in eosinophils through IL-5 action (Hiraguchi *et al.*, 2011). Mice born to mothers with diet-induced vitamin D deficiency had altered pulmonary tissue structure with reduced lung volume and function, (Zosky *et al.*, 2011). Asthmatic children with vitamin D deficiency have increased airway responsiveness, higher eosinophil counts and IgE levels (Brehm *et al.*, 2009) and lower lung function as measured by forced expiratory volume (FEV) (Brehm *et al.*, 2010). A strong

relationship has been reported between serum 25(OH)D concentration and measures of lung function of US adults participating in the NHANES III study (Black and Scragg, 2005).

Chronic asthma involves poorly reversible airflow obstruction and thickening of the airway smooth muscle (Panettieri Jr *et al.*, 1998), governed by airway remodelling. VDR is expressed in airway smooth muscle cells, with stimulation by vitamin D inducing microarray gene expression signature affecting remodelling, cell maturation and survival (Bossé *et al.*, 2009), affecting pathophysiology and treatment. Human airway smooth muscle cell proliferation and expression of matrix metalloproteinases is suppressed by 1,25(OH2D_3 *in vitro* (Song *et al.*, 2007). This modulation of airway smooth muscle gene expression results in decreased airway inflammation and alveolar smooth muscle proliferation (Taylor and Camargo Jr, 2011). An inverse relationship between vitamin D levels and worsening airflow limitation was seen in asthmatic children (Searing *et al.*, 2010b).

2.14.3.2 Vitamin D and pulmonary immune function

Higher concentrations of 1,25$(OH)_2$D may cause a shift to Th_2 immune response whilst in the lung simultaneously cause contrasting anti-inflammatory effects (Rothers *et al.*, 2011). These dual effects of vitamin D were seen in mice treated with ovalbumin, where vitamin D supplementation increased T-cell proliferation, Th_2 cytokine IL-4 and IL-13 and IgE production. Lung function was improved through impaired eosinophil recruitment and lower levels of IL-5 (Matheu *et al.*, 2003). Similarly, despite high IgE concentrations and elevated Th_2 cytokines, allergic asthma-induced mice with DVR knockout exposed to 1,25$(OH)_2$D failed to develop airway inflammation, eosinophilia or airway hyper-responsiveness (Wittke *et al.*, 2004).

Treg cells have been proposed to suppress Th_2 response and prevent allergic diseases. The number of CD4+CD25+ regulatory T are decreased in asthmatic children compared to healthy controls, suppressing activity (Hartl *et al.*, 2007). Supplementation of mice or cell cultures with 1,25$(OH)_2$D or UV irradiation enhanced the suppressive activity of CD4+CD25+ cells (Gorman *et al.*, 2007). Serum 25(OH)D concentrations are positively correlated with FoxP3 expression (Chambers *et al.*, 2012), with *in vivo* studies showing 1,25$(OH)_2$D treatment of CD4+T cells increased FoxP3 expression (Penna *et al.*, 2005). In asthmatic Costa Rican children aged between 6 and 14 years, serum 25(OH)D concentrations were found to be inversely correlated with sputum eosinophil counts (Brehm *et al.*, 2009).

2.14.3.3 Vitamin D and reversal of steroid resistance

Currently, the most common treatment prescribed for asthma involves anti-inflammatory glucorticosteroids (Prevention Program, 2007). Insensitivity or resistance to these steroids increases asthma severity, requiring higher dosage regimes to regain asthma control (Prevention Program, 2007).

Supplementation with vitamin D *in vitro* increases production of the anti-inflammatory IL-10 from Treg cells in glucocorticoid resistant cells from asthmatic patients (Xystrakis *et al.*, 2006) and enhances the immunosuppressive response to dexamethasone in peripheral blood mononuclear cells (PBMC) of asthmatic patients (Searing *et al.*, 2010b). PBMC cultures of patients with moderate to severe asthma stimulated with $1,25(OH)_2D$ had inhibited production of the Th_{17} cytokines IL-17 and IL-22 (Nanzer *et al.*, 2013).

In children with asthma, lower serum 259OH)D concentrations are associated with increased use of inhaled and oral corticosteroids (Searing *et al.*, 2010b). Improved lung function in asthmatic children treated with inhaled corticosteroid was greater in those with vitamin D sufficiency (Wu *et al.*, 2012).

2.14.3.4 Vitamin D and respiratory infection control in patients with asthma

The final aspect of asthma potentially affected by vitamin D is through bacterial and viral infections contributing to asthma exacerbation (Johnston *et al.*, 1995). A weaker antiviral response in asthmatics has been proposed as the mechanism underlying the increased morbidity infections cause in asthmatic children compared to healthy controls (Jackson *et al.*, 2013). Asthma exacerbations caused by respiratory tract infections may be contributed to by vitamin D deficiency weakening pulmonary defences. In wheezing children who required hospitalisation, serum 25(OH)D concentrations are inversely associated with detection of respiratory syncytial virus (RSV) (the major pathogen causing infection) and of rhinovirus (the main trigger of wheezing) infection (Jartti *et al.*, 2010).

2.14.3.5 Vitamin D and respiratory infections

When exposed to bacterial infection, expression of 1-α-hydroxylase is upregulated in macrophages and converts 25(OH)D to the active $1,25(OH)_2D$, inducing gene expression encoding

for AMP (White, 2010). Antimicrobial peptides (AMP) are protective against viruses, fungi and bacteria and are produced within circulating white cells and on the epithelial surface (Zasloff, 2002). The most common AMPs produced in response to tissue injury (cytokines including IL-1) or microbes are β-defensins 2 & 3 and cathelicidin (LL-37) (Zanetti, 2004).

VDR receptors have been located on cathelicidin, with the concentration of cathelicidin in human neutrophils and expression of cathelicidin in keratinocytes increased following exposure to $1,25(OH)_2D$ (Wang *et al.*, 2004). Vitamin D stimulates toll like receptors (TLR) on monocytes, resulting in expression of cathelicidins and greater microbiocidal capability through clearance of bacteria from barrier sites and immune cells (Liu *et al.*, 2006). In a study of tuberculosis, Liu *et al* highlight this connection of vitamin D to human infection with *Mycobacterium tuberculosis* (Liu *et al.*, 2006). Higher rates of tuberculosis occur among African-American populations, who also have high rates of vitamin D deficiency (Liu *et al.*, 2006). Extracted serum monocyte samples showed double the cathelicidin levels in subjects with light compared to dark pigmented skin. When supplemented with vitamin D_3, cathelicidin concentrations on activated macrophage levels from participants with dark pigmented skin increased to those observed in participants with light pigmented skin, suggesting monocytes in individuals with more pigmented skin are less efficient at killing the *M. tuberculosis* bacterium, and that cathelicidin is the mechanism which enhances the vitamin D mediated immune response to this pathogen (Liu *et al.*, 2006).

Defence against infection with respiratory pathogens is also enhanced through the action of vitamin D in strengthening the epithelial barrier through stimulation of genes expressing junction proteins (Schwalfenberg, 2011). Vitamin D has been shown to decrease respiratory syncytial virus induction of pro-inflammatory cytokines and chemokines without conceding an anti-viral state, and may be responsible for reduction in asthma exacerbations without an associated decrease in infection rate (Hansdottir *et al.*, 2008).

2.15 Points in the lifecourse when vitamin D status may be more critical

There may be critical times in the life course where vitamin D has the potential to influence immune system development and the subsequent deveopment of clincial disease (Figure 2.5). Findings from birth cohort studies suggest that the developing foetal immune system may sensitive to vitamin D status, both deficiency and excess.

Murine studies suggest that intestinal bacteria play an important role in early immune system development, with there only being a short timeframe for development of tolerance (Sado *et al.*, 1988). In mice maintained in a germ-free environment, oral tolerance was only established in mice colonised with intestinal flora before the first four weeks of life (Sado *et al.*, 1988). Colonisation by healthy microbial bacteria may be interrupted by infection and vitamin D deficiency (Vassallo and Camargo Jr, 2010). That many children outgrow childhood food allergies may be due to maturation of the immune system and microbial bacteria environment (Vassallo and Camargo Jr, 2010).

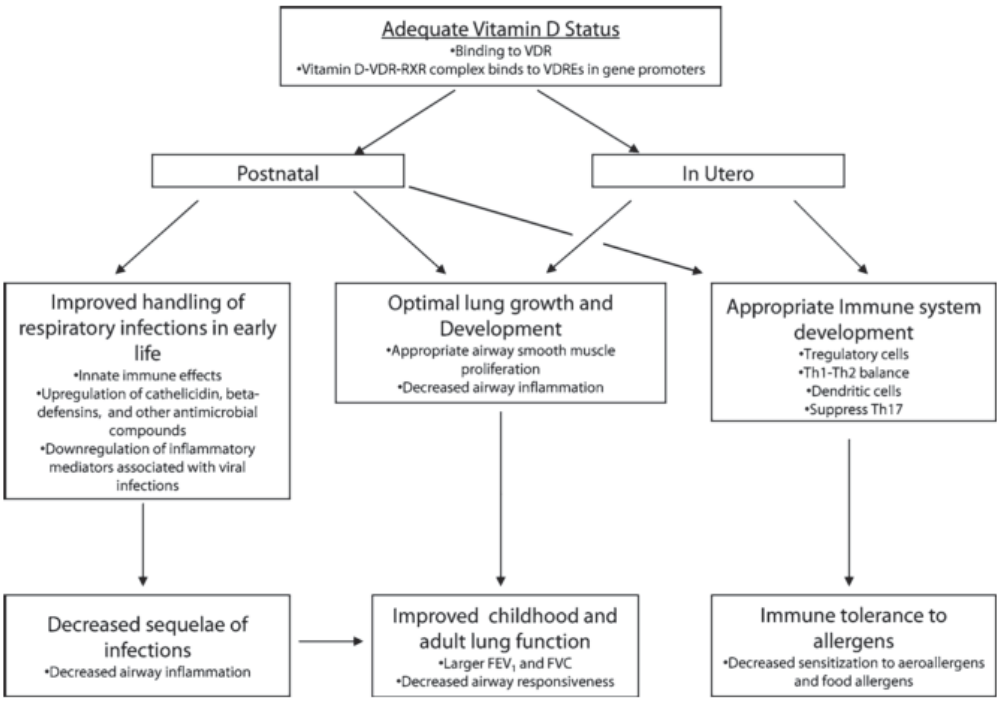


Figure 2.5. Proposed concept for adequate vitamin D status and the prevention of allergies and asthma. Reproduced with permission (Litonjua, 2012b).

2.15.1 Vitamin D and allergic diseases: birth cohort studies

Birth cohort studies suggest an early life role for vitamin D in the development of the immune system. Earlier studies of the role of vitamin D during pregnancy used maternal vitamin D intake from specific vitamin D-rich food sources or from the whole diet as measured by food frequency questionnaires as a proxy measure of vitamin D status. Later studies have measured maternal serum 25(OH)D concentrations during pregnancy or cord-blood 25(OH)D concentration to determine newborn vitamin D status. Findings from these studies have been mixed, with both

lower and higher early life vitamin D status of mothers during pregnancy and of infants at birth associated with higher risk of allergy in these children at various subsequent ages.

These birth cohort studies provide an insight into relationships between vitamin D status and allergic diseases. Conflicting results for the relationship of vitamin D and eczema, allergic rhinoconjunctivitis, asthma and respiratory diseases have been reported from birth cohort studies. These studies are described below, and listed in Table 2.6. Food allergy has not been specifically measured to date, therefore allergic sensitisation (as measured by IgE) is also discussed below.

2.15.1.1 Vitamin D and eczema

Lower maternal intake of vitamin D during pregnancy has been associated with an increased risk of eczema in Japanese children at age 2 (Miyake *et al.*, 2010) and Finnish children at age 5 (Erkkola *et al.*, 2009). Lower 25(OH)D concentrations in newborn cord blood were associated with increased risk of eczema in Australian one year olds (Jones *et al.*, 2012) and French five year olds (Baïz *et al.*, 2014). In contrast, in the United States no association was found between maternal vitamin D intake and eczema at 3 years, although the authors subsequently suggested this was due to the classification of eczema (Camargo *et al.*, 2007). Increased maternal 25(OH)D concentrations during pregnancy were associated with increased risk of eczema at age 9 months (but not at 9 years) and 15 years in UK children (Gale *et al.*, 2007; Tolppanen *et al.*, 2013) while higher vitamin D intake during infancy was associated with increased risk of eczema at age 6 in Swedish children (Back *et al.*, 2009). Higher maternal 25(OH)D concentrations were associated with a lower risk of eczema in Taiwanese children at age 4 (Chiu *et al.*, 2015).

2.15.1.2 Vitamin D and allergic rhinoconjunctivitis

In a population based birth cohort study of 1669 women from Finland, a protective association was observed between increased maternal vitamin D intake during pregnancy and the risk of childhood allergic rhinitis at five years of age (Erkkola *et al.*, 2009). In contrast, in child cohort studies from France (Baïz *et al.*, 2014) and Arizona, USA, newborn cord blood 25(OH)D concentration were not associated with risk of allergic rhinitis at age 5 years (Rothers *et al.*, 2011).

2.15.1.3 Vitamin D and asthma

An increased risk of childhood wheezing has been associated with low maternal vitamin D intake during pregnancy and with low cord blood 25(OH)D concentrations (Camargo *et al.*, 2007; Devereux *et al.*, 2007; Erkkola *et al.*, 2009; Camargo *et al.*, 2010). An increased risk of transient early childhood wheezing associated with lower cord blood 25(OH)D concentration was seen in French children at age 5 but no association was evident between cord blood 25(OH)D concentration and asthma (Baïz *et al.*, 2014). An inverse association between 25(OH)D concentration and risk of wheeze was reported in Polish children at age 2 (Stelmach *et al.*, 2015).Lower serum 25(OH)D concentrations at age 6 years were shown to be a predictor of asthma and atopy at age 14 years in Australian children (Hollams *et al.*, 2011). Lower 25(OH)D concentration at age 4 years was associated with an increased risk of asthma at ages 4 and 8 years in Dutch children (Belderbos *et al.*, 2011), whilst a lower risk of asthma was associated with higher maternal 25(OH)D concentrations at age 4 in a Taiwanese cohort (Chiu *et al.*, 2015).

In contrast with these studies showing that poorer vitamin D status is associated with an increased risk of asthma, in Japan higher maternal vitamin D intake during pregnancy was associated with increased risk of childhood asthma (Miyake *et al.*, 2010) and in a study from Finland, vitamin D supplementation during infancy was associated with increased risk of asthma later in life (Hypponen *et al.*, 2004). Higher maternal 25(OH)D concentrations were associated with increased risk of hospitalisations due to asthma in Polish offspring at age 25 years (Hansen *et al.*, 2015).No association was evident between maternal 25(OH)D concentration during pregnancy and asthma or wheeze in childhood from Italy, nor for cord blood 25(OH)D concentrations and asthma in children at age 5 years living in Tucson, Arizona (Rothers *et al.*, 2011; Morales *et al.*, 2012).

2.15.1.4 Vitamin D and respiratory infection

The most important pathogen causing lower respiratory infection in young children is the respiratory syncytial virus (Simoes, 1999). A prospective birth cohort in the Netherlands found children who were vitamin D deficient (cord blood 25(OH)D <50nmol/L) had a six-fold increased risk of RSV-induced bronchiolitis in the first year compared to those with 25(OH)D concentrations ≥75nmol/L (Belderbos *et al.*, 2011). No association between cord blood 25(OH)D concentration and frequency of respiratory infection was found in Polish children at age 2 (Stelmach *et al.*, 2015).

2.15.1.5 Vitamin D and sensitisation (IgE)

In a birth cohort in Arizona, US, both high (≥75nmol/L) and low (<50nmol/L) infant cord blood 25(OH)D concentrations were associated with an increased risk of skin prick test (SPT) sensitivity and increased total IgE levels at age 5 years (Rothers *et al.*, 2011). Similarly, a cross-sectional study of over 7000 British adults aged 45 years found both low and high 25(OH)D concentrations (25[OH]D <25nmol/L and >135nmol/L respectively) were associated with elevated IgE concentrations (Hyppönen *et al.*, 2009). Deficient maternal 25(OH)D concentrations (≥125nmol/L) appeared to be associated with an increased prevalence of allergen sensitisation in Taiwanese children at age 2 (Chiu *et al.*, 2015). In other prospective studies, a high intake of vitamin D in infancy was associated with an increased risk of allergic sensitisation in 123 Swedish children at age 6 years (Back *et al.*, 2009). Atopy defined by skin prick test positivity was found to be more prevalent at age 31 in those participants of the Northern Finland Birth Cohort who received regular vitamin D supplementation during the first year of life (Hypponen *et al.*, 2004). In a Swedish birth cohort study, supplementation of infants with vitamins A & D during the first year of life was associated with an almost 2-fold increase risk of food hypersensitivity and allergic sensitisation at age 4 years (Kull *et al.*, 2006).

Vitamin D deficiency (25[OH]D <27.5nmol/L) was not associated with food sensitivity (measured as plasma specific IgE) in a prospective Boston birth cohort study. However, there was an increased risk of food sensitivity for children of a certain genotype with vitamin D deficiency (Liu *et al.*, 2011).

The birth cohort studies described in Table 2.6 have some limitations including modes of determination of vitamin D status, limited numbers of participants with higher vitamin D status, differing latitude and variable impact and measurement of confounding factors. Serum 25(OH)D concentration reflects status at only one point in time while food frequency provides a measure, at best semi-quantitative, of dietary vitamin D intake over several weeks. It obviously does not take into account the major source of vitamin D, that which is generated from UV light exposure (Litonjua, 2008). The apparently conflicting results of the birth cohort studies to date are likely to be contributed to by these factors, and these studies do not provide sufficient evidence to determine if there is, or is not, a casual relationship between vitamin D at an early point in the lifecycle and subsequent risk of allergic and respiratory diseases.

Table 2.6. Birth cohort studies investigating vitamin D and allergy

Author and country	Study cohort	25(OH)D measurement	Reported result
Baiz (2014) France (Baïz *et al.*, 2014)	Etude des Determinants pre et post natals de l'Enfant (EDEN)	Infant cord blood 25(OH)D concentration	Inverse association between cord blood 25(OH)D concentration and risk of transient early wheeze and early and late onset atopic dermatitis at ages 1, 2, 3 and 5 years. No association between 25(OH)D concentration and asthma or allergic rhinitis at age 5 years.
Belderbos (2011) The Netherlands (Belderbos *et al.*, 2011)	156 neonates	Infant cord blood 25(OH)D concentration	Mean 25(OH)D concentrations were lower in neonates who subsequently developed RSV-induced bronchiolitis. A six-fold increased risk of RSV lower respiratory tract infections in the first year was seen for neonates born with cord blood 25(OH)D <50nmol/L compared to those with 25(OH)D ≥75nmol/L.
Camargo (2007) Boston, US (Camargo *et al.*, 2007)	Project Viva 1,194 children	Maternal vitamin D intake FFQ, mean intake vitamin D	Inverse association of maternal vitamin D intake and recurrent wheeze at age 3. No association with eczema at age 3
Camargo (2011) Wellington, New Zealand (Camargo *et al.*, 2011)	New Zealand Asthma and Allergy Cohort Study 922 children	Infant cord blood 25(OH)D concentration	Inverse association of cord blood 25(OH)D concentration risk of wheeze at ages 15 months, 3 years and 5 years. No association with asthma at age 5 years
Chiu (2015) Taiwan (Chiu *et al.*, 2015)	Prediction of Allergies in Taiwanese Children Study (PATCH) 164 children	Maternal serum 25(OH) concentration	Deficient maternal 25(OH)D concentration (<50nmol/L) associated with higher prevalence of allergen sensitisation before age 2 years. A lower risk of eczema and asthma at age 4 with higher maternal 25(OH)D concentration
Devereux (2007) Scotland, UK (Devereux *et al.*, 2007)	SEATON 1,253 children	Maternal vitamin D intake FFQ	Inverse association of maternal vitamin D intake with ever wheeze, wheeze in previous year and persistent wheeze at age 5 years

Author and country	Study cohort	25(OH)D measurement	Reported result
Erkkola (2009) Helsinki, Finland (Erkkola *et al.*, 2009)	Type 1 Diabetes Prediction and Prevention (DIPP) 1,669 children	Maternal vitamin D intake FFQ	Inverse association of asthma and allergic rhinitis at age 5 years. No association with eczema.
Gale (2007) Southampton, UK (Gale *et al.*, 2007)	466 children	Maternal serum 25(OH) concentration	Positive association of maternal 25(OH)D concentration and eczema at age 6 months and asthma at age 9 years, but not eczema at age 9 years
Hansen (2015) (Hansen *et al.*, 2015) Denmark	Danish Fetal Origins Cohort 965 children	Maternal serum 25(OH) concentration	Higher maternal 25(OH)D concentration ≥125nmol/L associated with increased risk of asthma hospitalisations, during first 25 years. Lower 25(OH)D <50nmol/L associated with lower risk of asthma hospitalisation, and asthma medicine use. No association of maternal 25(OH)D concentration and allergen-specific IgE, total IgE, asthma or hayfever or lung function at age 20
Hyponnen (2004) Finland (Hypponen *et al.*, 2004)	Northern Finland Birth Cohort 5007 adults	Vitamin D supplementation in first year of life	Supplementation with vitamin D in first year of life associated with increased risk of atopy, asthma and allergic rhinitis at age 31 years
Jones (2012) Perth, Australia (Jones *et al.*, 2012)	231 high risk children from prospective birth cohort study	Infant cord blood 25(OH)D concentration Maternal vitamin D intake	Lower cord blood 25(OH)D concentration more common in infants with eczema at age 1 year. Higher risk of eczema in infants with cord blood 25(OH)D <50nmol/L than those ≥75nmol/L. No significant association between 25(OH)D concentration and eczema severity.
Liu (2011) Boston, US (Liu *et al.*, 2011)	Boston Birth Cohort 649 mother and infant pairs	Infant cord blood (25(OH)D concentration IgE to common allergens Genotyping data of 11 candidate genes	Vitamin D deficiency (25[OH]D <27.5nmol/L) not associated with food sensitivity. SNPs examination, significant interaction of IL4 polymorphism and vitamin D deficiency for certain genotypes
Miyake (2010) Osaka, Japan (Miyake *et al.*, 2010)	Osaka Maternal and Child Health Study (OMCHS) 763 children	Maternal vitamin D intake FFQ	Higher maternal intake of dairy products inversely associated with infant wheeze and eczema at age 16-24 months

Author and country	Study cohort	25(OH)D measurement	Reported result
Morales (2012) Spain (Morales *et al.*, 2012)	Infancia y Medio Ambiente Project (INMA) 1,724 children	Maternal serum 25(OH) concentration	No association between maternal 25(OH)D concentration and wheeze at age 1 year or 4 years, nor asthma at age 4-6 years
Rothers (2011) Tucson, US (Rothers *et al.*, 2011)	Tucson Infant Immune Study 219 children	Infant cord blood 25(OH)D concentration	Association of low and high cord blood 25(OH)D concentration with higher total and allergen-specific IgE. No association between cord blood 25(OH)D concentration and allergic rhinitis or asthma
Stelmach (2015) (Stelmach *et al.*, 2015) Poland	Polish Mother and Child Cohort Study 190 children	Infant cord blood 25(OH)D concentration	Cord blood 25(OH)D inversely associated with risk of multi-triggered wheeze, especially viral, by age 2 years. No association of 25(OH)D concentration and food allergy, atopic dermatitis and frequency of infection
Tolpannen (2013) United Kingdom (Tolppanen *et al.*, 2013)	Avon Longitudinal Study of Parents and Children	Prospective association mean serum 25(OH)D concentration	At mean age 9.8 years, higher 25(OH)D_3 concentration associated with increased risk of wheezing, flexural dermatitis

FFQ food frequency questionnaire, IGE immunoglobulin E, RSV respiratory syncytial virus, SNP single nucleotide polymorphism

2.16 Studies examining the relationship of vitamin D status and allergic and respiratory diseases in children of preschool age

Very few studies have directly measured vitamin D status and examined the prevalence of allergic diseases or respiratory infection specifically in the 2 to <5 years age group. Epidemiological and RCT studies which have studied various aspects of the relationship of vitamin D with eczema, food allergy, allergic rhinoconjunctivitis, asthma and respiratory infection involving the preschool age group 2 to <5 years are discussed below, and listed in Table 2.7.

2.16.1 Vitamin D and eczema

Observational studies involving preschool age children that have investigated associations between vitamin D status and eczema report contradictory findings, with both lower and higher 25(OH)D concentrations associated with increased eczema prevalence and severity. Those reporting an inverse association include an Italian study of 37 children aged 8 months to 12 years attending outpatient clinics diagnosed with eczema. Eczema severity was classified, with the mild disease group having significantly higher 25(OH)D concentrations than those with moderate or severe eczema (93 vs 69 & 51nmol/L, P<0.05) (Peroni *et al.*, 2011). Results were similar when stratified by age, however no other adjustment for confounding was possible die to the small sample size. In a case control study of 826 Hong Kong Chinese children under 18 years of age recruited from paediatric clinics, mean serum 25(OH)D concentrations were lower in those diagnosed with eczema compared with the control group who did not have eczema (29 vs 34nmol/L, P<0.001) (Wang *et al.*, 2014). A larger proportion of the children with eczema had vitamin D deficiency (25[OH]D <25nmol/L). An inverse relationship between serum 25(OH)D concentration and eczema severity as measured SCORing Atopic Dermatitis (SCORAD) and the Nottingham Eczema Severity Score (NESS) (Wang *et al.*, 2014). In this study of a single ethnic group of children, the associations between 25(OH)D and eczema were adjusted for age, sex, month of testing and immunoassay batch. A cross-sectional study of 251 Korean hospital patients aged 3 months to 18 years found an inverse relationship between serum 25(OH)D concentration and eczema severity as determined by the SCORAD index (R= -0.24, P<0.000) (Shin *et al.*, 2014). The recruitment from clinics rather than the community increases the potential for existing health conditions in the children to potentially affect 25(OH)D concentrations.

An inverse association between serum 25(OH)D concentrations and presence of eczema based on physician diagnosis of eczema was reported in a small cross-sectional study of Korean children

and adolescents (Han *et al.*, 2015). This study was conducted over winter with a small sample size (n=33) (Han *et al.*, 2015).

Contrasting associations between vitamin D status and eczema severity have also been reported. No difference in mean 25(OH)D concentrations was found in 89 Italian children aged 0.5 to 16 years with moderate or severe eczema compared to mild eczema (Galli *et al.*, 2015). Supplementations with 2000IU vitamin D_3 daily did not influence eczema severity compared to controls who did not receive supplementation (Galli *et al.*, 2015). Eczema severity was not associated with serum 25(OH)D concentrations in a small Korean study (n=33) of children aged 1 to 15 years (Han *et al.*, 2015). Potential confounders were not examined in this study.

In an earlier Korean study, the severity of eczema in 157 children and adults (age 4 months to 56 years) attending an atopy clinic was not significantly correlated with serum 25(OH)D concentrations, however for the patients with food sensitisation, mean serum 25(OH)D concentrations were higher in patients with mild eczema than those with moderate or severe eczema ($P<0.05$) (Lee *et al.*, 2013). In a subset of 36 patients with food sensitisation, mean serum 25(OH)D concentrations were significantly higher in patients with mild compared to moderate or severe eczema, with the authors suggesting a role for vitamin D may exist in a sub-group of eczema patients. Benson *et al* hypothesise these conflicting results are due to a bimodal relationship between vitamin D status and allergic skin diseases (Benson *et al.*, 2012), and Hypponen *et al* suggest that there may be a threshold effect for both low and high 25(OH)D concentrations associated with elevated IgE concentrations (Hyppönen *et al.*, 2009). The age ranges included in this study by Han *et al* from Korea were broad incorporating infants to adolescents, who have different prevalence rates of eczema (Han *et al.*, 2015). Almost half of the eczema experienced by children in infancy disappears by 3 years of age, dependent on severity and presence of atopic sensitisation, which may impact vitamin D status of children around this age (Illi *et al.*, 2004).

Results from cross-sectional and case control studies in other age groups are also contradictory. Lower serum 25(OH)D concentrations in patients with eczema compared to those without eczema have been reported in obese adults in the United States (Oren *et al.*, 2008), dietary vitamin D intake was found to be lower in patients with eczema than controls in Norwegian adults (Solvoll *et al.*, 2000). In contrast, higher mean 25(OH)D concentrations were observed in German children with eczema compared to those without (Heimbeck *et al.*, 2013) and no significant association

between eczema and low serum 25(OH)D concentration was found in Australian infants at age 1 year (Allen *et al.*, 2013).

The theory that early infancy is of particular importance in the subsequent development of allergic diseases has been supported by observational studies. In two cross-sectional studies of Japanese infants and school children, the highest incidence of eczema was found in those children born in autumn, and lowest in those born in spring (Kusunoki *et al.*, 1999; Kuzume and Kusu, 2007). Sunshine hours over the 3 months before and after birth of Japanese infants were inversely correlated with the incidence of eczema that was related to each month of birth (Kuzume and Kusu, 2007). In contrast, in a small study of 210 Danish patients who were diagnosed with eczema, a higher likelihood of birth in summer and autumn months was found (Beck and Hagdrup, 1986). Denmark is a country of higher latitude and has routine infant vitamin D supplementation. At present, the relationship between month of birth and subsquent eczema is unclear.

2.16.2 Vitamin D and food allergy

To date, only one study has investigated direct measurement of serum 25(OH)D concentration and prevalence of food allergy. In 12 month old infants of Australian born parents aged 12 months, those with vitamin D insufficiency (25[OH]D ≤50nmol/L) were three times as likely to have a challenge proven food allergy to peanut and/or egg (Allen *et al.*, 2013). This association was not seen for children of parents born overseas or for children of all parents.

Research that has used proxy indicators of vitamin D status - latitude and season of birth – report an indirect association of these proxy measures of vitamin D status with food allergy, with rates of food allergy. This may be hypothesised to be related to the decrease in UV exposure dose that occurs with increasing distance from the equator (Godar, 2005). Studies using surrogate markers of food allergy report higher rates at increasing latitude. An increase in prescription rates of epinephrine auto-injectors (EpiPen) for treatment of anaphylaxis and in hospital admission rates for anaphylaxis with increasing distance from the equator has been reported from studies performed in the southern states of Australia (Mullins *et al.*, 2009), and northern states of the United States, where the highest rates of EpiPen prescriptions were reported from New England (Camargo Jr *et al.*, 2007). A strong north-south gradient was also seen for hospital anaphylaxis admission rates in Chile (Hoyos-Bachiloglu *et al.*, 2014). In Chile, the rate of food-induced

anaphylaxis was associated with latitude in children but not adults (β 0.01, P=0.006 and β 0.003, P=0.16) (Hoyos-Bachiloglu *et al.*, 2014). More southern latitudes in Australia (further from the equator) were also associated with higher prescription rates of infant hypoallergenic formula (β 89.69, 95%CI 2.90-176.49, P=0.04) (Mullins *et al.*, 2010), and higher parental reports of egg and peanut allergy in cohorts of Australian children aged 4-5 years and 8-9 years (Osborne *et al.*, 2012).

Season of birth may contribute to the development of food allergy through maternal and in-utero status, and lifestyle factors related to the amount of time spent outdoors in early infancy. Season of birth potentially affects exposure to infections (e.g. increased in winter), outside allergens and vitamin D status of both the pregnant mother and infant through exposure to UVB radiation (Keet *et al.*, 2012). Season of birth has been associated with food allergy prevalence in epidemiological studies. In a cohort of primarily African-American inner-city children aged 5-8 years, a winter season of birth was associated with a trend to sensitisation to egg, peanut and soy (Bird *et al.*, 2014). The majority of these children had a dark skin colour and were found to be vitamin D deficient or insufficient. In a study of clinic records of 835 children born in the Australian Capital Territory between 1995 and 2009, an autumn/winter season of birth was more common for children diagnosed with food allergy between 0 to 4 years (autumn/winter vs spring/summer, 57% vs 43%, P<0.001) (Mullins *et al.*, 2011). In the same study, analysis of national prescription data found autumn/winter seasons of birth were more likely for children prescribed an EpiPen (54% vs 46%, P<0.001) and infant hypoallergenic formula (54% vs 46%, P<0.001) compared to other seasons (Mullins *et al.*, 2011). Children under 5 years of age living in Boston who were born in autumn or winter and visiting a hospital emergency department were 53% more likely to be diagnosed with food allergy than three control groups; children a non-food allergy emergency department visit, all births in Boston and all births in Massachusetts (Vassallo *et al.*, 2010). Autumn season of birth was more common in food allergic children based on both parent or self-reported food allergy (5862 children from the National Health and Nutrition Examination Survey (NHANES III)) and physician diagnosis food allergy (1514 child patients from the John Hopkins Paediatric Clinic) (Keet *et al.*, 2011). Those born in autumn and at highest risk of food allergy were of Caucasian ethnicity, who are most likely to experience seasonal variation in 25(OH)D concentrations in infancy. Also at high risk were children with a history of eczema, with the authors suggesting skin barrier function may be implicated in the seasonal associations with food allergy (Keet *et al.*, 2012).

2.16.3 Vitamin D and allergic rhinoconjunctivitis

To date, no study has examined the relationship between vitamin D status and allergic rhinoconjunctivitis in preschool aged children. A case-control study in Qatar compared asthmatic Qatari children less than 15 years of age diagnosed with allergic rhinitis to healthy control of children of similar age. A higher prevalence of vitamin D deficiency was seen among the children with allergic rhinitis than among the controls (Ehlayel *et al.*, 2011). This observation is consistent with data from other age groups. A study reporting data from the NHANES 1988-1994 survey in the United States, showed that serum concentrations of 25(OH)D were positively associated with prevalence of allergic rhinitis and number of allergic episodes in non-Hispanic white and African-American adults (Wjst and Hyppönen, 2007). Stronger associations were noted in adults less than 20 years of age, with the authors suggesting vitamin D status may be more important at an earlier disease state during childhood (Wjst and Hyppönen, 2007). An Iranian study of 50 adults diagnosed with allergic rhinitis reported 30% had severe vitamin D deficiency (25(OH)D <12.5nmol/L) compared to 5% of adults in a national population survey of Iranian adults (Arshi *et al.*, 2012). No control group was used in this study. Nor was there adjustment for potential confounders. No association between serum 25(OH)D concentrations and risk of self-reported allergic rhinitis was evident in a national survey 15,212 Korean adults (Cheng *et al.*, 2014). Lower plasma 25(OH)D concentrations were reported in Turkish adults with allergic rhinoconjunctivitis compared to controls (18 vs 33nmol/L, P=0.01) (Yenigun *et al.*, 2015). This was a small study of 42 adults diagnosed with allergic rhinoconjunctivitis with 35 controls matched for age and sex.

In contrast, a recent case-control study of Turkish children aged 8 to 14 years has reported higher mean 25(OH)D concentrations in children with allergic rhinoconjunctivitis than healthy controls (85 vs 55nmol/L, P=0.001) (Goksugur *et al.*, 2015). This was a small study, 22 participants, ranging from school age to adolescents, who were diagnosed with allergic rhinoconjunctivitis by paediatric and ophthalmological examination.

2.16.4 Vitamin D and asthma

Conflicting data on the relationship between vitamin D status and asthma has been reported in studies which included preschool children aged from 2 to <5 years. Studies have reported poorer vitamin D status is associated with an increased risk of asthma. In a case control study of 170 Turkish children aged 2-14 years attending paediatric outpatient clinics, mean serum 25(OH)D concentrations were lower in asthmatic than non-asthmatic children (43 vs 70nmol/L, P<0.001)

(Uysalol *et al.*, 2014). This study had a small sample size, with the authors suggesting the lower 25(OH)D concentration may be secondary to these children spending less time spent outdoors and participating in less physical activity as attempts by their parents to lessen the risk of an asthma attack (Uysalol *et al.*, 2014). Lower mean 25(OH)D concentrations were found in Egyptian children and adolescents diagnosed with bronchial asthma compared to controls (19 vs 58nmol/L, P=0.008) (Ismail *et al.*, 2015). This was also a small study, with 36 asthmatic children and 36 healthy controls over a wide age range of 3 to 14 years. Confounders such as skin colour or sun exposure were not explored (Ismail *et al.*, 2015).

In a study of children under 16 years recruited from paediatric hospital clinics in Qatar, mean serum 25(OH)D concentrations were lower, and the proportion of children with vitamin D deficiency (25[OH]D <50nmol/L) was greater in asthmatic compared with non-asthmatic children (26% vs 11%, P<0.001) (Bener *et al.*, 2011). Almost half (42%) of children were under 5 years of age but the vitamin D status of this younger age group and relationship with asthma prevalence was not reported. In comparison with non-asthmatic children, the proportion of asthmatic children with white or darker skin colouring was smaller, and with wheatish skin tone was larger, with this difference in skin pigmentation also potentially contributing to differences in 25(OH)D concentrations. Sun exposure and physical activity were measured in this study, with lower levels of both found in asthmatic children (Bener *et al.*, 2011).

Asthma prevalence was significantly correlated with lower serum 25(OH)D concentrations in a study of 99 children and adolescents aged 0 to 18 years hospitalised in Denver with asthma, atopic dermatitis and/or food allergy (P=0.047) (Searing *et al.*, 2010a). This association was adjusted for age and latitude. Of the asthmatic patients in this study, 47% had 25(OH)D concentrations <75nmol/L and 17% <50nmol/L (Searing *et al.*, 2010b). A higher prevalence of 25(OH)D concentrations <50nmol/L was reported in Iranian children aged from 1 to 15 years with asthma compared to healthy controls (55% vs 10%) (Ahmadabadi *et al.*, 2015). Although there were 100 children in this hospital study diagnosed with asthma, only 20 healthy controls were used to compare 25(OH)D concentrations.

Swedish children aged 6 months to 4 years attending hospital for treatment of acute wheeze had significantly lower serum 25(OH)D concentrations than healthy controls awaiting surgery, with 25(OH)D concentrations <75nmol/L associated with an increased risk of acute wheeze (P<0.05) (Stenberg Hammar *et al.*, 2014). Serum 25(OH)D concentration were not tested at time of admission, rather blood sampling occurred 12 weeks later.

In contrast, no differences were seen in mean 25(OH)D concentrations between 547 asthmatic and non-asthmatic children and adolescents aged 2 to 19 years in a retrospective case-control study performed in a Massachusetts hospital (Menon *et al.*, 2012). A small study of 36 Egyptian children aged from 2 to 9 years attending hospital with bronchial asthma reported a higher mean 25(OH)D concentration than the 18 control children without asthma, however the small participant numbers limit extrapolation of these findings (El-Sayed and Amer).

Serum 25(OH)D concentration has been inversely associated with corticosteroid usage in studies of asthmatic US and Egyptian children. In a small cross-sectional study of the hospital records of 100 asthmatic children aged 0 to 18 years in Colorado, the use of inhaled and oral corticosteroids was inversely associated with serum 25(OH)D D concentrations (P=0.047 and P=0.02 respectively) (Searing *et al.*, 2010b). In a small study of 60 Egyptian children aged 2 to 18 years hospitalised with asthma, lower serum 25(OH)D concentrations were associated with increased inhaled and nasal corticosteroid usage (P=0.001) (Abd and El Banna). The types and amounts of corticosteroid usage were not fully described by the authors in this study.

There is considerable variation in results from the studies investigating the relationship of vitamin D status detailed in Table 2.7. These apparently conflicting results for allergic diseases are likely to be contributed to by variable study design, sample sizes, country and measurement of 25(OH)D concentration. To date, no studies of allergic rhinoconjunctivitis (rather allergic rhinitis) have been reported in the preschool age group. Because of these issues, the studies published to date do not provide sufficient quality evidence to determine if there is, or is not, a causal relationship between vitamin D status and eczema, food allergy, allergic rhinoconjunctivitis and asthma in preschool children.

2.16.5 Vitamin D and respiratory infection

Although respiratory disease is more common in the paediatric population than any other age group, only two studies including preschool children aged 2 to <5 years have investigated the association of vitamin D status and prevalence of lower respiratory disease. In Ecuadorian children aged 3-36 months, no association between vitamin D deficiency and acute lower respiratory infection (ALRI) was found, with a non-significant trend of a higher risk of pneumonia in deficient compared to children with sufficient vitamin D status (Mokhtar *et al.*, 2014). In a Canadian case-control study of children less than 5 years old, no difference in 25(OH)D

concentration was found for children with ALRI compared to the control group (McNally *et al.*, 2009).

Studies which investigated respiratory infection severity have reported associations of this with vitamin D status. Inamo *et al* reported a correlation of vitamin D deficiency with increased ALRI severity in Japanese children aged 1 to 4 years hospitalised with ALRI (Inamo *et al.*, 2011). In the study of Canadian children above, mean serum 25(OH)D concentrations were lower in those children with ALRI admitted to the intensive care unit than children with ALRI admitted to the general ward, or age-matched children without a respiratory infection (McNally *et al.*, 2009). In Indian children aged 2 to 60 months, serum 25(OH)D concentrations >56nmol/L were associated with a decreased risk of severe ALRI (Wayse *et al.*, 2004).

Of the randomised controlled trials that have investigated whether vitamin D supplementation can prevent acute respiratory infections, only one trial has enrolled preschool children. In this study 453 children aged 1 to 36 months from Kabul, Afghanistan who had been diagnosed with pneumonia were randomised to receive a single bolus dose of 100,000 IU vitamin D_3 or placebo. These children were recruited from a community previously identified as high risk of vitamin D deficiency. At 90 days, there was a reduced risk of repeat ALRI occurrence in the intervention group (Manaseki-Holland *et al.*, 2010).

In a recent RCT of vitamin D supplementation during pregnancy (27 weeks until birth) and infancy (birth to 6 months) in New Zealand where woman/infant pairs were randomised to: placebo/placebo, 1000IU/400IU, or 2000IU/800IU D_3 daily, a post-hoc analysis was performed to determine if vitamin D supplementation prevented health care visits for acute respiratory infections. A smaller portion of children randomised to the higher dose vitamin D supplementation made any primary care visits for acute respiratory infections from age 6 to 18 months old (Grant *et al.*, 2014). Similarly, supplementation of vitamin D through fortified milk (300IU) daily in Mongolian schoolchildren in winter reduced the number of parental reported acute respiratory infections (Camargo *et al.*, 2012). Latino inner-city children from New York aged 6 months to 5 years were supplemented with 1 teaspoon of cod liver oil for up to six months. Supplementation was associated with a decrease in paediatric visits due to reduced upper respiratory tract (Linday *et al.*, 2004).

However, not all clinical trials have reported a protective effect, with Urashima *et al* reporting vitamin D supplementation in Japanese schoolchildren lowered the risk of influenza A but did not

affect influenza B (Urashima *et al.*, 2010). In this RCT, children received placebo or 1200 IU D_3 daily over the winter and spring months. Baseline and follow-up 25(OH)D concentrations were not measured so the relation of vitamin D status to these effects is unclear. Daily supplementation of 2000 IU D_3 for 12 weeks during winter of New York adults found no difference in self-reported upper respiratory tract infection duration or frequency (Li-Ng *et al.*, 2009). The researchers note this lack of effect may be due to the small differences in serum 25(OH)D concentrations of the supplementation (88nmol/L) compared with the placebo (63nmol/L) group (Li-Ng *et al.*, 2009).

Most upper respiratory tract infections are viral (Wald *et al.*, 1991). Although literature abounds on the mechanistic role of vitamin D in respiratory infection, there is a paucity of data investigating vitamin D status and recent respiratory infections in preschool children. In a cohort US study, lower newborn cord blood 25(OH)D concentrations were associated with an increased risk of acute respiratory infection in the first 3 months of life (Camargo *et al.*, 2011). In a randomised controlled trial that recruited newly-diagnosed asthmatic children and adolescents from Poland, supplementation with vitamin D was associated with a reduced risk of asthma exacerbations triggered by acute respiratory infection (Majak *et al.*, 2011). A secondary outcome of the randomised controlled trial by Urashima *et al* of vitamin D supplementation in Japanese schoolchildren was a statistically significant decrease in asthma exacerbations in supplemented children. The authors suggest vitamin D may be mediating the reduction in asthma exacerbations through fewer upper respiratory tract infections in children receiving vitamin D supplementation (Urashima *et al.*, 2010).

The disparity in results of studies investigating the relationship between vitamin D and respiratory disease are likely to also be contributed to by variable study design, sample sizes, country and method of measurement of 25(OH)D concentration. Thus, the studies outlined in Table 2.7 do not provide sufficient quality evidence to determine if there is or is not a causal relationship between vitamin D status and respiratory disease in preschool children.

Table 2.7. Studies investigating vitamin D and allergic and respiratory diseases involving preschool children aged from 2 to <5 years.

Study author, Country	Study type	Participants	Age (years)	Disease Definitions	Outcome
ECZEMA					
Baek (2014) Korea (Baek *et al.*, 2014)	Cross sectional	251 children with atopic dermatitis	1 – 18	Physician diagnosed AD SCORAD	Inverse correlation between serum 25(OH)D concentration and values of SCORAD (R-0.24, P<0.000) and total IgE (R= -0.29, P<0.000) and age (R= -0.49, P<0.000)
Chiu (2013) Wisconson, US (Chiu *et al.*, 2013)	Cross sectional	94 patients with atopic dermatitis	1 - 18	Physician diagnosed AD SCORAD	No significant correlation between 25(OH)D concentration and eczema severity (r= -0.001, P=0.99)
Galli (2015) Italy (Galli *et al.*, 2015)	Cross sectional	89 patients with atopic dermatitis	0.5 - 16	Hanifin & Rajka criteria for AD SCORAD	No difference in 25(OH)D concentrations in moderate and severe AD compared to mild AD. No correlation between vitamin D concentrations, total IgE and SCORAD index
Han (2015) Korea (Han *et al.*, 2015)	Case control	33 patients with atopic dermatitis, 70 healthy controls	1 16	Hanifin & Rajka criteria for AD SCORAD	Mean 25(OH)D concentration significantly reduced in children with AD than control (37.5 vs 41nmol/L, P=0.04). Serum 25(OH)D not associated with AD severity
Heimbeck (2013) Germany (Heimbeck *et al.*, 2013)	Cross sectional	9838 population representative	1 - 17	Doctor diagnosed AD	Mean 25(OH)D concentration significantly higher in children with eczema than those without (P<0.001). Inverse association between low 25(OH)D and eczema, quartile 1 (<29nmol/L) versus quartile 2 (29-42nmol.L) (OR=0.76, 95%CI 0.61-0.94)
Lee (2013) Korea (Lee *et al.*, 2013)	Cross sectional	157 with atopic dermatitis	0.3 – 57	Physician diagnosed AD SCORAD	No significant correlation between serum 25(OH)D concentration and atopic dermatitis severity. In 36 patients with food sensitisation mean serum concentrations were significantly higher in patients with mild (21ng/ml), moderate (18ng/ml) or severe atopic dermatitis (13ng/ml), P<0.05
Peroni (2011) Italy (Peroni *et al.*, 2011)	Cross sectional	37 children with atopic dermatitis	0.5 – 12	Physician diagnosed AD SCORAD	Vitamin D deficiency may be related to severity of atopic dermatitis Mean (25OH)D concentration was higher in mild (36.9ng/ml) compared to moderate (27.5ng/ml) and severe atopic dermatitis (20.5ng/ml). Serum IgE levels increased in relation to vitamin D deficiency and atopic dermatitis severity
Shin (2014) Korea (Shin *et al.*, 2014)	Cross sectional	251 children with atopic dermatitis	1 – 18	Physician diagnosed AD SCORAD	Significant inverse correlation between serum 25(OH)D concentration and values of SCORAD index (R= -0.24, P<0.000) and total IgE (R= -0.29, P<0.000)

Study author, Country	Study type	Participants	Age (years)	Disease Definitions	Outcome
Sidbury (2008) Boston, US (Sidbury *et al.*, 2008)	Double blind, randomised controlled trial	11 children with winter dermatitis	2 - 13	Physician diagnosed AD	1,000IU ergocalciferol or placebo daily for 1 month. Significant improvement in IgA, but not EASI, scores 4/5 treated with vitamin D had improved IgA scores compared with 1/6 in placebo group (P=0.04)
Wang (2014) Hong Kong (Wang *et al.*, 2010)	Case control	498 patients with atopic dermatitis 328 non-allergic controls	<18	Physician diagnosed atopic dermatitis SCORAD Ness	Mean 25(OH)D concentration in atopic dermatitis patients lower than controls (29 vs 24, P<0.001). Larger proportion of atopic dermatitis patients with 25(OH)D deficiency (<25nmol/L) compared to controls Inverse association between serum 25(OH)D and atopic dermatitis severity Larger proportion of atopic dermatitis patients with vitamin D deficiency had elevated IgE (43%) than those without vitamin D sufficiency (20%)
ALLERGIC RHINOCONJUNCTIVITIS					
Elhayel (2011) Qatar (Ehlayel *et al.*, 2011)	Case control	483 asthmatic children 483 controls	<15	Physician diagnosis	Higher prevalence of allergic rhinitis in asthmatic children compared with controls (37% vs 20%, P=0.001). No significant difference found between 25(OH)D category and allergic rhinitis
ASTHMA					
Abd (2013) Egypt (Abd and El Banna)	Case control	600 asthmatic 40 controls	2-18	GINA guidelines	Significant correlation of vitamin D deficiency with severity of asthma Lower 25(OH)D concentration associated with intensity of corticosteroid use
Ahmadabadi (2015) Iran (Ahmadabadi *et al.*, 2015)	Cross sectional	100 asthmatic 20 control	1 - 15	Physician diagnosis	25(OH)D deficiency (<50nmol/L) more common in asthmatic children than controls (55% vs 10%, P=0.02))
Awasthi (2014) Lucknow, India (Awasthi and Vikram, 2014)	Case-control	50 asthmatic 25 control	1 - 15	Physician diagnosed asthma, classified by GINA control guidelines	25(OH)D insufficiency (<75nmo/L) associated with increased risk of bronchial asthma and level of control. 25(OH)D concentration associated with asthma occurrence (OR 13.5, 95%CI 4.25-42.85, P=0.000)

Study author, Country	Study type	Participants	Age (years)	Disease Definitions	Outcome
Bener (2011) Qatar (Bener *et al.*, 2011)	Case-control	483 asthmatic 483 control	<16	Diagnosis by paediatric immunologist	Mean 25(OH)D concentrations significantly less for asthmatic than non-asthmatic subjects (P<0.001) 68% of asthmatic subjects had vitamin D deficiency, 25(OH)D<50nmol/L Prevalence of vitamin D deficiency higher in asthmatic than non-asthmatic for both moderate ((25(OH)D<50nmol/L) 42% vs 25% and severe 25(OH)D<25nmol/L) 26% vs 11%) Vitamin D was the strongest predictor of asthma (OR 4.82 95%CI 2.41-8.63, P<0.001)
El Sayed (2014) Egypt (El-Sayed and Amer)	Case control	36 asthmatic 16 control	>2	Doctor diagnosed bronchial asthma	Higher mean 25(OH)D concentration in asthmatic children versus control (178 vs 89nmol/L, P=0.000)
Ismail (2015) Egypt (Ismail *et al.*, 2015)	Cross sectional	36 bronchial asthma 36 control	3 - 14	Physician diagnosed asthma, classified by GINA control guidelines	Mean 25(OH)D concentrations lower in asthmatic compared to control (7.7 vs 23.3ng/dL, P<0.001). Serum 25(OH)D concentrations lower in children with severe compared to moderate or mild eczema (5.9, 8.5 & 8.9ng/dL, P=0.008)
Menon (2012) Massachusetts, United States (Menon *et al.*, 2012)	Case-control retrospective	265 asthma 284 non-asthma control	2 - 19	Asthma symptom & severity incidence by NHLBI guidelines	No significant difference in mean 25(OH)D concentrations between asthmatic and control subjects (28.6 vs 28.4ng/ml, P=1.0). No association of 25(OH)D concentration and asthma severity Significant difference in 25(OH)D mean concentration between obese asthma and non-obese asthma subjects (23 vs 30ng/ml, P<0.0001), and obese control and non-obese control (25 vs 32ng/ml, P=0.01)
Elhayel (2011) Qatar (Ehlayel *et al.*, 2011)	Case-control	483 asthmatic 483 control	<16	Diagnosis by paediatric immunologist	Mean 25(OH)D concentration significantly lower in asthmatic children than non-asthmatic control, 17.5 vs 20.8 ng/ml, P<0.001. Lower vitamin D level associated with more allergic disease and elevated serum IgE Vitamin D deficiency prevalence higher in children with asthma, allergic rhinitis, atopic dermatitis, acute urticaria, food allergy Greater vitamin D deficiency association with IGE atopy markers in asthmatic children than controls

Study author, Country	Study type	Participants	Age (years)	Disease Definitions	Outcome
Searing (2010) Denver, US (Searing *et al.*, 2010b)	Retrospective cross-sectional	99 asthmatic, atopic dermatitis and/or food allergy	0 - 18	Physician diagnosis	Asthma and taking an inhaled corticosteroid significantly correlated with lower vitamin D concentrations Presence of asthma significantly correlated with lower vitamin D concentration, P<0.047 In asthmatic children, vitamin D was inversely associated with use of inhaled corticosteroid, P=0.036
Searing (2010) Denver, US (Searing *et al.*, 2010a)	Cross-sectional	100 asthmatic	0 - 18	Physician diagnosis	Lower serum 25(OH)D concentrations significantly associated with usage of inhaled (P=0.0475) and oral steroids (P=0.02) and total steroid dose (P<0.01)
Uysalol (2013) Tekirdag, Turkey (Uysalol *et al.*, 2013)	Case control	85 asthmatic 85 control	2 - 14	GINA guidelines for asthma and severity	Mean 25(OH)D concentration significantly lower for asthmatic children than non-asthmatic control 17 vs 28ng/ml, P<0.001) Lower vitamin D level significantly associated with increased asthma severity (P<0.001) and decreased frequency of controlled asthma (P=0.01)
RESPIRATORY INFECTION					
Banerji (2009) Yemen (Banerji *et al.*, 2009)	Cross sectional	529 severe pneumonia hospitalisation	< 5	Physician diagnosis	Prevalence of clinical rickets was 50% in children hospitalised with pneumonia. Increased risk of dying from severe pneumonia in children with rickets
Binks (2014) Northern Territory, Australia (Binks *et al.*, 2014)	Cross sectional	44 ALRI classified hospitalisations 30 non-ALRI classified hospitalisations	< 3	Physician diagnosis	Vitamin D insufficiency (25(OH)D <75nmol/L) less common in children hospitalised with ALRI (23%) than hospitalised for other reasons (47%) (OR0.34, 95%CI 0.11-1.03, P=0.043)
Inamo (2011) Japan (Inamo *et al.*, 2011)	Cross sectional	28 ALRI hospitalisation	1 - 4	Physician diagnosis	Significant correlation of vitamin D deficiency (25[OH]D <37.5nmol/L) and ALRI severity (need for supplementary oxygen and ventilator management)
Leis (2012) Saskatchewan, Canada (Leis *et al.*, 2012)	Case control	197 Bronchiolitis or pneumonia hospitalisation	< 5	Physician diagnosis	Vitamin D intake <80 IU/kg/day associated with 4 fold increased risk of ALRI (OR 4.9, 95%CI 1.5-16.4)

Study author, Country	Study type	Participants	Age (years)	Disease Definitions	Outcome
Manaseki-Holland (2010) Kabul, Afghanistan (Manaseki-Holland *et al.*, 2010)	Randomised placebo-controlled trial	453 children diagnosed with non-severe or severe pneumonia	1 - 3	Physician diagnosis	Supplementation of 100,000 IU of vitamin D_3. Follow-up to 90 days. No significant difference in mean days to recovery between intervention and placebo (4.74 vs 4.98 days, P=0.17). Risk of repeat pneumonia in 90 days lower in vitamin D_3 group than placebo (RR 0.78, 95%CI 0.64-0.94, P=0.01). Time to repeat episode was longer in supplementation group than placebo (72 vs 59 days, OR 0.71, 95%CI 0.53-0.95, P=0.02)
McNally (2009) Saskatchewan, Canada (McNally *et al.*, 2009)	Case control	55 bronchiolitis 50 pneumonia 92 control	<5	Doctor diagnosed on hospital admission	No significant difference in mean 25(OH)D concentration of ALRI group than control (81 vs 83nmol/L, P=0.71) Disease severity - mean 25(OH)D concentration of ALRI patients in paediatric intensive care was lower than control (49 vs 83nmol/L, P=0.001) and ALRI patients on general ward versus control (49 vs 87nmol/L, P=0.001)
Mokhtar (2014) Ecuadorian Andes (Mokhtar *et al.*, 2014)	RCT – Vitamin A and Zinc Prevention of Pneumonia Study	526 community based children	0.6 - 3	Pneumonia incidence	No association between 25(OH)D deficiency and ALRI Non-significant trend for children with vitamin D deficiency (25(OH)D <50) in developing pneumonia compared with vitamin D sufficiency (RR 1.67, 95%CI0.93-2.98, P=0.086)
Wayse (2004) India (Wayse *et al.*, 2004)	Case control	80 severe ALRI 70 controls	<5	WHO definition severe ALRI	Mean 25(OH)D concentration lower in ALRI than controls (23 vs 38nmol/L, P<0.01) Lower risk of severe ALRI with 25(OH)D concentrations >22.5nmol/L (OR 0.09, 95%CI 0.03-0.24)

AD atopic dermatitis, SCORAD SCORing Atopic Dermatitis, NESS Nottingham Eczema Severity Score, EASI Eczema Area and Severity Index, GINA Global Initiative for Asthma , ALRI acute lower respiratory infection

Chapter 3: Method

This method chapter describes the recruitment of children and collection of data. The statistical analysis methods used in development of the predictive questionnaire and investigation of relationship between vitamin D status and allergic and respiratory diseases are outlined. Definitions of vitamin D status and allergic and respiratory diseases are included in this chapter.

3.1 Study Design

A cross-sectional study design was used to recruit a national sample of children over a ten week period – August to October 2012 (winter to early spring) – and to measure their 25(OH)D concentrations in dried blood spot samples. We used innovative methods including an internet based method of data collections and a community pharmacy based strategy for the collection of dried blood spots from children throughout New Zealand. The study was named Te Ra Whakaora, which in Maori means sunshine and health.

Ethical approval was granted by the Health and Disability Ethics Committee, Northern Region, reference number NTX/12/04/036, and a parent or caregiver of each participant signed informed consent forms for participation of their child in the study.

3.1.1 Participants

Participants were preschool children aged 2 to <5 years of age. Children in New Zealand commence primary school on their 5th birthday. The vitamin D status of newborns, infants below 24 months and school children has been determined previously but there are no data that describe the vitamin D status of children from 2 to <5 years old (Rockell *et al.*, 2005; Grant *et al.*, 2009; Camargo *et al.*, 2010; Houghton *et al.*, 2010; Wall *et al.*, 2013). There were no exclusion criteria.

3.1.2 Setting and recruitment

The collection of data took place at 54 testing centres in 17 New Zealand cities and towns. We achieved good geographical coverage. The majority of testing took place at 49 community pharmacies (Figure 3.1). The study was promoted nationwide in visits to pharmacies by the marketing manager of Pharmacy Brands (healthcare services provider incorporating six pharmacy chains). Pharmacies were then recruited to act in a voluntary capacity in this study through

advertising in three pharmacy publications and by direct approach. Appendix 1 contains the information sheet sent to pharmacies. Testing centres also included the Human Nutrition Units at Massey University, Auckland, and the University of Otago, Dunedin, two Auckland health services who carry out before-school checks for 4 year old children, and a Wellington general practice clinic.

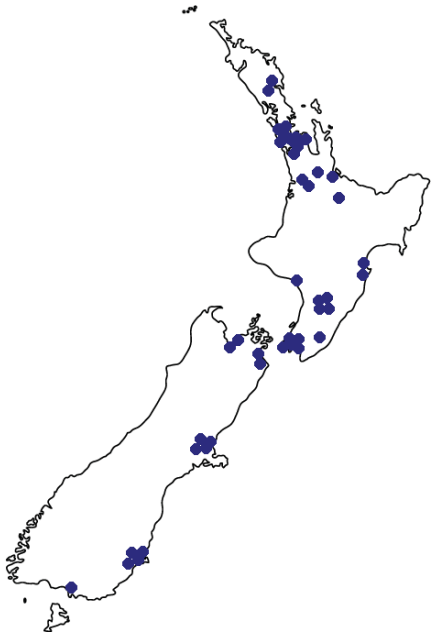


Figure 3.1: The location of the 49 pharmacies used as testing centres throughout New Zealand.

Participants were recruited using a variety of techniques during the ten-week period from August to October 2012. The primary recruitment method was via the purchased access to an email database of the online services company ReachME (www.reachme.co.nz) (ReachMe, 2014). The company provides products and information for parents of newborn and young children and estimate their database contains 160,000 families with children aged from birth up to seven years old. Emails advertising the study were sent out to families via the company on a rolling basis throughout the recruitment period.

A study website was created containing information about the study where parents could register their interest to participate in the study. Posters and flyers with colourful illustrations were designed to appeal to the parents of young children and printed to advertise the study. Early childhood education facilities and kindergartens in close proximity to testing centres were contacted and ethical approval obtained for the display of posters and distribution of flyers to families. A national daycare organisation included information about the study in their e-newsletter, displayed posters and distributed flyers to families. Ethical approval was obtained to

display posters in Plunket rooms (provider of publically funded well-child services). Participating pharmacies displayed posters and distributed brochures. Various businesses, organisations and parents advertised the study on their Facebook page. Press releases were distributed to media outlets, with several articles appearing in local newspapers throughout New Zealand. Awareness was generated through interviews on two radio stations and in a Sunday evening TV news program. All advertising material is contained in Appendix 1.

3.1.3 Procedures

Parents registered interest in participating in the study through the website, email, phone or text/SMS. They were then contacted by a member of the study office who explained the study in more detail. Once enrolled, an information sheet and consent form were sent by mail (Appendix 2). Parents completed a questionnaire about their child's medical history and vitamin D deficiency risk factors in a paper or online version (www.surveymonkey.com) then visited a local testing centre/participating pharmacy with their child with the completed consent form. Anthropometric measurements were obtained, as was a blood spot from fingerprick test for subsequent measurement of 25(OH)D concentration. This measurement visit took no longer than 15 minutes. The study flow is described in Figure 3.2.

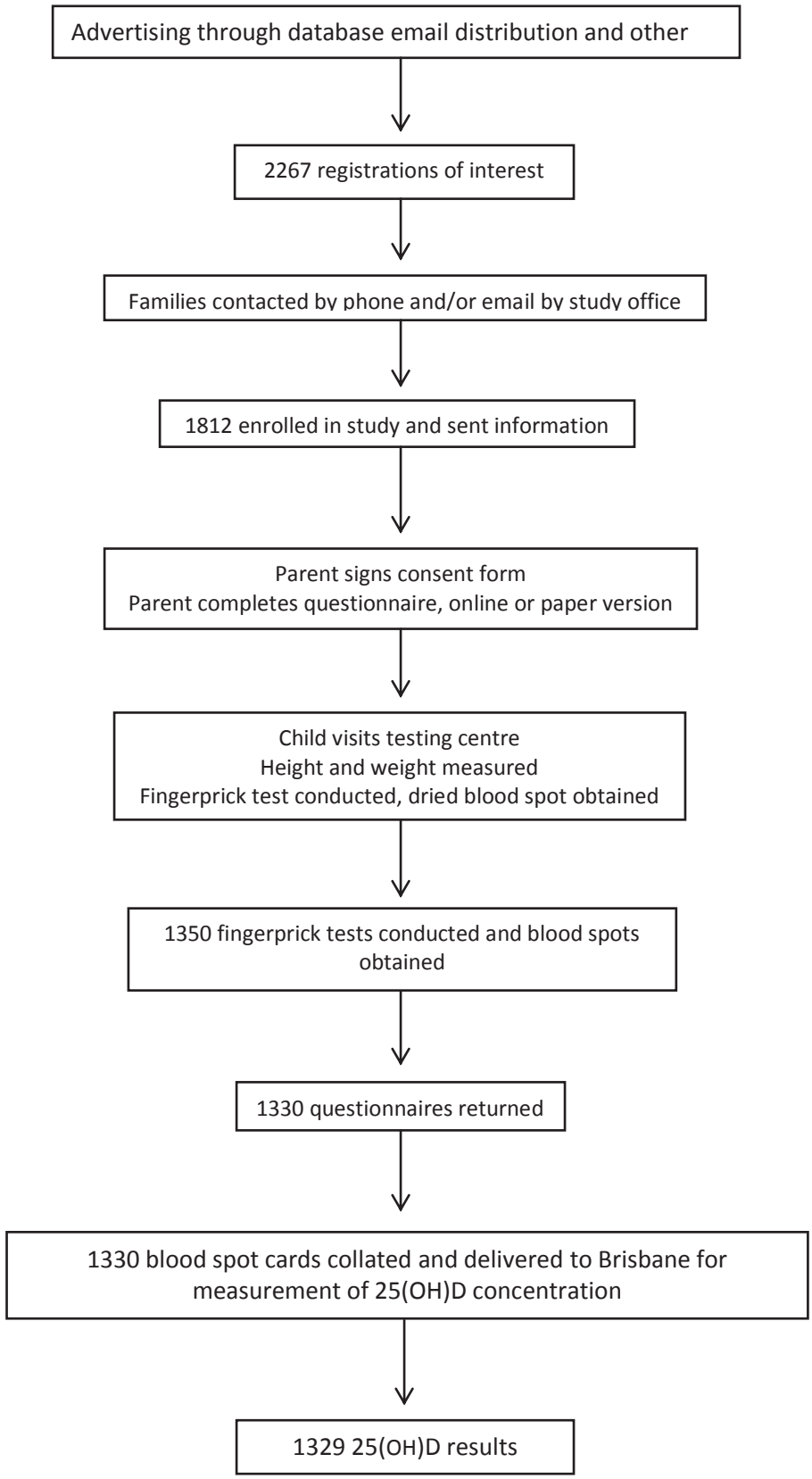


Figure 3.2: Study Flow

3.1.4 Blood sampling

A fingerprick method was used to obtain a capillary blood spot sample from each child, with this sample collected by trained testers. A standardised operating procedure was developed in consultation with Associate Professor Darryl Eyles, University of Queensland, who approved the final version (Appendix 3). All testers were visited and trained in the fingerprick test and blood collection methodology by study team personnel. Each testing centre was supplied with a comprehensive kit containing all procedures and equipment (Appendix 4). Blood was dropped onto a pre-stamped circle on Whatman 305 filter paper until 50µl was obtained and allowed to air dry for at least one hour before the flap on the blood spot card was closed. Cards had dual identifiers, study number and date of birth. Samples were stored in protective envelopes at room temperature before being couriered back to the Auckland study office. At the conclusion of the ten week testing period, samples were collated, photographed and flown to the Queensland Brain Institute, University of Queensland, Australia, for measurement of 25OHD concentration (Appendix 5).

3.1.5 Biochemical analysis

The liquid chromatography, tandem mass spectrometry analytical method for measurement of dried blood spot 25(OH)D concentration has been described elsewhere (Eyles *et al.*, 2009). This assay measures both 25(OH)D_2 and 25(OH)D_3, and results are reported as total 25(OH)D.

Precise 3.2 mm whole blood punches were obtained from each dried blood spot. This is equivalent to 3µl of whole blood. The 25(OH)D concentration measured on the dried blood spot was corrected for the haematocrit fraction in serum of children this age (0.41 for girls and 0.45 for boys) (Bain, 2011) using the following equation:
Plasma [25(OH)D] (nM) = dried blood spot (25[OH]D) (nM) /(1 - haematocrit fraction)

3.1.6 Anthropometric measurements

Height and weight were measured without shoes by the trained testers prior to the fingerprick test, using household non-calibrated scales and wall-mounted measuring devices. Body mass index (BMI) adjusted-for-age was calculated (Cole *et al.*, 2000).

3.1.7 Questionnaire

The questionnaire was offered in online (www.surveymonkey.com) and paper formats and completed by a parent or caregiver (Appendix 6). The 59-item questionnaire collected information that would enable a description of demographics, presence of known risk factors of vitamin D deficiency and relevant recent medical history. The questionnaire was piloted in a focus group of mothers with preschool age children, and face validity achieved through review and revision by a pediatrician and experts in vitamin D research.

Caregivers were asked to list all ethnicities they identified for their child, with a free-text option available. For analytical purposes, ethnicity was then assigned using the following prioritisation; Maori > Pacific > non-European other > NZ European. Mothers were asked to describe their highest educational qualification obtained, and maternal education was then classified into three groups; no secondary qualifications, secondary qualifications or post-secondary qualifications.

A geographical small area measure of socioeconomic status was used. Specifically, each household was allocated to a national household deprivation index of 1 to 10, with 1 being the least deprived (affluent) of households, and 10 being the most deprived (poorest) of households, as defined by the NZ Index of Social Deprivation, a census-based measure (Salmond *et al.*, 2007). Geo-coding of residential address was made using ArcGIS geographic information system software (Esri), along with the subsequent allocation of deprivation index, in conjunction with the Epidemiology and Biostatistics section, Faculty of Medical and Health Sciences, University of Auckland. These 10 deprivation index scores were then collapsed into 5 quintile categories, with quintile 1 incorporating deprivation index 1 and 2, quintile 2 (3 & 4), quintile 3 (5 & 6), quintile 4 (7 & 8) and quintile 5 (9 & 10). Residential address was also used to establish latitude, with the country then separated into 3 regions; northern (34-38^{o}S), central (39-42^{o}S) and southern (43-46^{o}S) New Zealand.

Parents were asked for information on known risk factors for vitamin D deficiency. For determination of skin colour, the Fitzpatrick scale of skin reaction to sun exposure was used (Fitzpatrick, 1988). With the strong sun protection messages in New Zealand, some young children have had insufficient sun exposure to allow an assessment of skin reaction to be made. Therefore we also used a six-point parental report of skin colour (very fair, fair, medium, olive, dark and very dark) (Houghton *et al.*, 2010). Parental reported skin colour was then collapsed into 3 categories - very fair/fair, medium, olive/very dark.

New Zealand does not have mandatory fortification of foods with vitamin D. Thus, with few dietary sources of vitamin D commonly consumed by young children, measurement of dietary intake being relatively complex and the incompleteness of the data available in the New Zealand Food Composition Database for vitamin D content of food, we did not measure total dietary vitamin D intake. Questions regarding dietary sources were restricted to the type of milk the child consumes to identify any fortified with vitamin D (with a free-text option available for the recording of brand name), and any current use of vitamin D containing supplements or cod liver oil. Each milk type was then classified as being fortified or not fortified with vitamin D. A child taking a supplement was defined as those who reported usage of a supplement containing vitamin D and/or cod liver oil.

Parents were asked to specify who cared for their child during the day, and if attending formal preschool education facilities such as daycare, preschool or kindergarten, for how many hours per week. The relationship between 25(OH)D concentrations and daycare attendance has not been previously studied.

Questions relating to household living conditions which may impact on respiratory or allergic diseases included household crowding, exposure to cigarette smoke and pets living in the house (Burr *et al.*, 1999). The number of older and younger children living in the same house was queried. Exposure to cigarette smoke was defined by the number of days per week the child was in the same room as someone smoking. This was then collapsed to 2 categories, exposed to cigarette smoking on one or more days of the week or never. Parents were asked to report pets (dog and/or cat) living in the child's home in the last year.

Information on sun protection practices was sought for both summer and winter seasons. Parents were asked to indicate on a 4 point range (always, usually, sometimes, never) their child's use of sunscreen, sunhat, covering clothing and midday sun avoidance for each season. This was then collapsed down to 2 categories for each sun protection variable; ever used in the season, or never. The amount of time children played outside, on average, per day in summer and winter was asked, for <½ hour per day, ½-1 hour per day or >1 hour per day.

Items regarding recent medical history focused on allergic and respiratory diseases. Prevalence of eczema, allergic rhinoconjunctivitis and asthma were sought using the core questionnaire modules for wheezing, rhinitis and eczema for 6-7 year olds of the International Studies of Asthma and Allergies in Childhood (ISAAC) (Asher *et al.*, 1995). The ISAAC study is a large,

international study of asthma epidemiology and the questionnaire is commonly used to establish prevalence of asthma, eczema and related diseases (Asher *et al.*, 2006). This questionnaire is designed for parents to complete on behalf of their child. Further details were provided by parents of medications which had been prescribed for their child in treatment of asthma and eczema, which acted as an additional confirmation of diagnosis and treatment option. With infection an important component of respiratory disease, parents were asked to recount how many respiratory infections their child had experienced in the last year, as common colds and cough or chest infections, in 4 categories (none, 1-2, 3-4, >5 times). Finally, parents were asked if their child had been diagnosed with bronchitis, bronchiolitis, pneumonia or tuberculosis in the last year. Definitions of asthma, allergic rhinoconjunctivitis, eczema, food allergy and respiratory infection are detailed below.

The questionnaire sought information on food allergy based on parental report. Those parents who indicated their child had ever had a food allergy were asked if the food allergy had been diagnosed by a doctor, and if it had resulted in their child having to visit hospital. A short list of foods commonly associated with allergic response in preschool children was provided, and parents asked to list which foods their child had reacted to; egg, milk, peanut and seafood, with an open-text option also being available. Children who have experienced acute allergic reaction as anaphylaxis are prescribed epinephrine (adrenaline) in the form of EpiPen for the emergency treatment of future acute reactions. Parents were asked whether their child had an EpiPen.

3.2 Definitions

3.2.1 Vitamin D deficiency and insufficiency

Vitamin D deficiency was defined as a dried blood spot 25(OH)D concentration <25nmol/L in line with the New Zealand guidelines (Consensus Statement on Vitamin D and Sun Exposure in New Zealand), and below this level the risk of rickets is increased (Ministry of Health and Cancer Society of New Zealand, 2012). Vitamin D insufficiency was defined as a dried blood spot 25(OH)D concentration <50nmol/L in line with the New Zealand guidelines of below recommended amount (Ministry of Health and Cancer Society of New Zealand, 2012).

3.2.2 Allergic diseases and respiratory infection

Eczema

Parents were asked an initial question “has your child ever had an itchy rash which was coming and going for at least six months?”. A diagnosis of eczema was made from a positive response to both the subsequent questions; "has your child had this itchy rash at any time in the last 12 months?" and "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?" (Asher *et al.*, 2006).

Severe eczema was defined by two measures; 1. Children whose eczema symptoms resulted in sleep disturbance for one or more nights per week (Williams *et al.*, 1999), and 2. Children who reported use of topical steroid treatment.

Food allergy

In this section of the questionnaire, parents were initially asked “has your child ever had food allergy?” with a positive response defined as parental report food allergy. The subsequent question “was your child’s allergy confirmed or diagnosed by a doctor?” defined parental report of doctor diagnosed food allergy. Possession of an EpiPen was described by parental report of “does your child have an EpiPen?” and a food related hospital visit through “has your child ever been to hospital as a result of a food allergy?”.

Allergic rhinoconjunctivitis

Allergic rhinoconjunctivitis was defined as a positive response to two questions; "has your child had a problem with sneezing, or a runny nose, when he/she did not have a cold or the flu in the last 12 months?" and "has this nose problem been accompanied by itchy-watery eyes?" (Asher *et al.*, 2006).

Parents were asked to detail the months of the year these nose problems had occurred in the last 12 months. These month responses were then collapsed into seasons: summer (December, January, February), autumn (March, April, May), winter (June, July, August) and spring (September, October, November).

Asthma

Asthma/wheeze was defined as a positive parental response to the question 'has your child had wheezing or whistle in the chest in the last 12 months?' [13].

A subgroup of the children with asthma who were more likely to have atopic asthma was identified by a positive response to the above question combined with a positive response to the question about the presence of eczema and/or food allergy (definitions above).

Medication use is one way of measuring asthma management and control. Parents were asked “which asthma medications has your child used”. Parental responses were coded into 5 categories: inhaled and oral beta antagonist, inhaled and oral steroid, and other asthma medication.

Respiratory infection

Three types of respiratory infections were investigated.

Any respiratory infection (ARI) was defined as a positive response to the question "has your child been diagnosed with any of the following illnesses in the last 12 months - bronchitis, bronchiolitis, pneumonia?" or any parental report of cough, chest infection and common cold in the last 12 months.

Report of lower respiratory infections (LRI) was obtained from the question "has your child been diagnosed with any of the following illnesses in the last 12 months - bronchitis, bronchiolitis, pneumonia?", with diagnosis of LRI made with a positive response for any of these illnesses.

The frequency of upper respiratory tract infections (URI) was determined from the question; "how many times has your child had a common cold in the last 12 months". Responses were collapsed into two categories; lower frequency (0-2 times) and higher frequency (≥3 times).

3.3 Statistical analysis

3.3.1 Sample size

Sample size estimates were based upon the power needed to identify associations with vitamin D deficiency (25[OH]D <25nmol/L) in a cross-sectional study. Sample size calculations were performed using G*Power 3 (Heinrich Heine University, Dusseldorf). A sample of 1100 was more than sufficient to provide 80% power to demonstrate statistical significance for a risk factor acting across the whole population for which there was a relative risk of 1.8 for the association of this factor with the presence of vitamin D deficiency (alpha=0.05 two-tailed, ratio unexposed:exposed=1:4, frequency of deficiency in unexposed=8%, odds ratio=1.9: minimum sample needed = 1018).

For the development of a predictive questionnaire to assess the risk of vitamin D deficiency, a further 300 participants were required to allow random selection from the sample to be used for estimating the probability of correct classification (deficient/ non-deficient). Thus, a total of 1300 participants was the desired sample size.

3.3.2 Data analysis

All analyses were performed with the Statistical Package for the Social Sciences (SPSS 21.0). All tests with statistical significance were determined by $P<0.05$.

The normal distribution of dried blood spot 25(OH)D concentrations was confirmed by the Kolmogorov-Smirnov and Shapiro-Wilk tests of normality. Descriptive statistics were used to describe the distribution of 25(OH)D concentrations and the prevalence of vitamin D deficiency. As the 25(OH)D concentration was normally distributed, mean values were reported. Differences between groups in mean 25(OH)D concentration were determined using independent t-tests or ANOVA. Post-hoc analysis was by linear regression and Dunnett test for comparison with a single control. Associations between categorical variables were determined using the chi-squared test.

Independent predictors of vitamin D deficiency were identified using multivariable logistic regression. All assumptions for multicollinearity were met. Associations were described using odds ratios (OR) and 95% confidence intervals (CI).

3.3.3 Development of a predictive questionnaire for vitamin D deficiency

For the predictive questionnaire, two models were developed; 1. vitamin D deficiency (25[OH]D <25nmol/L) and 2. vitamin D insufficiency (25[OH]D <50nmol/L). The 1329 participants were randomly selected into two datasets, 929 (70%) in the 'development' and 400 (30%) in the 'validation' groups. A 70/30 split was chosen to optimise sensitivity in identification of predictors of deficiency in the predictive tool development. The 'development' group was used to develop the prediction tool, with the 'validation' group acting as an internal validation and indication of how useful the tool might be for new cases.

An alternate prioritisation of ethnicity was used in the development of the predictive questionnaire to increase accuracy by inclusion of specific ethnicities which have reported higher rates of vitamin D deficiency in prior studies. Children were coded into six categories from the parental report of ethnicity. From analysis of the mean 25(OH)D concentrations (Appendix 8) and P values, the following prioritisation order was followed: children of Indian/African > Pacific > Any European> Maori/Asian ethnicities. Although household deprivation was a significant predictor of vitamin D deficiency, this variable was not used in the prediction equation. Deprivation index is calculated based on residential address and used primarily as a research instrument in epidemiological studies, thus this information is not readily available to individuals or in clinical situations. However, as deprivation index was a significant predictor of vitamin D deficiency, in order to explore all options in obtain the best performing predictive tool, additional analysis was carried out and a model developed which included the deprivation index variable.

There were no children who drank toddler milk with 25(OH)D concentrations <25nmol/L. These children have a zero risk of vitamin D deficiency and thus were removed from the 'development' dataset for the development for model predicting vitamin D deficiency. This resulted in 870 children in the dataset. All 400 children in the 'validation' dataset were included in the validation process. For the model predicting vitamin D insufficiency, all children in the 'development' dataset were included.

Independent predictors of vitamin D deficiency or insufficiency were identified using logistic regression. The significant variables were then fitted in a multivariable logistic regression, and any redundant variables eliminated, until the best-fitting model was obtained which had all variables significant. Non-significant variables were then examined, and those of borderline significance

were tested in the model and retained if they added to the model's performance. Significance was determined by odds ratios (OR) with $P<0.05$.

A mathematical scoring system was developed to predict vitamin D deficiency or insufficiency. The risk score associated with each predictor was weighted based on the regression coefficient values and an equation developed. Receiver Operating Characteristic (ROC) analysis was used to determine the cut-off criterion for vitamin D deficiency and insufficiency by choosing the best balance of sensitivity and specificity. For internal validation in the vitamin D deficiency model, a negative score of 8 (higher than the total score) was added to the equation for children who drank toddler milk. This modified equation was applied in ROC analysis of the 'validation' group (n=400). The equation developed for the model of vitamin D insufficiency was applied in ROC analysis of the 'validation' group (n=400) for internal validation.

Performance of the predictive questionnaire was assessed by ROC analysis, using area under the ROC curve (AUC), sensitivity and specificity. In clinical applications, clinicians wish to know the chance of a child having a positive result from the questionnaire as being truly deficient (Mausner and Kramer, 1985). Predictive values will differ depending on the prevalence of deficiency in the sample group. For this reason, positive predictive value (PPV) and negative predictive value (NPV) were also calculated. The calculation used is outlined in Table 3.1, and below.

Table 3.1. Sensitivity, specificity, positive and negative predictive values

	Deficient (number)	**Non-deficient (number)**	**Total (number)**
Positive (number)	a (true positive)	b (false positive)	$T_{\text{test positive}}$
Negative (number)	c (false negative)	d (true negative)	$T_{\text{test negative}}$
Total	$T_{\text{deficient}}$	$T_{\text{non deficient}}$	Total

Adapted from Mausner 1985 (Mausner and Kramer, 1985)

In Table 3.1, the columns show the actual status of the children (deficient or non-deficient) and the rows show the results of the predictive questionnaire (positive or negative).

Using sensitivity and specificity as determined by ROC analysis, PPV and NPV are calculated as:

Sensitivity = true positive rate = a / (a + c)

Specificity = true negative rate = d / (b + d)

PPV = number of true positives / number of true positives + number of false positives = a / (a + b)

NPV = number of true negatives / number of true negatives + number of false negatives = d (d + c) (Mausner and Kramer, 1985)

3.3.4 Vitamin D and allergic and respiratory diseases

The relationship between dried blood spot 25(OH)D concentration and prevalence of eczema, food allergy, allergic rhinoconjunctivitis, asthma and respiratory infections were investigated initially using bivariate analyses. Differences between groups in mean 25(OH)D concentration were determined using Independent t-tests or ANOVA. Dried blood spot 25(OH)D concentration was treated as a continuous variable. For the purposes of analysis 25(OH)D was also classified into 4 categories; <25, 25 to <50, 50 to <75 and ≥75nmol/L. Associations of each of the disease outcomes with these categorised 25(OH)D values was determined using the chi-square test.

The association between the presence of each disease outcome and the categorised 25(OH)D concentration was investigated using unadjusted and multivariable logistic regression analysis, and reported using OR and 95% CI. The reference 25(OH)D category used in logistic regression was 50 to <75nmol/L (Ross *et al.*, 2011; Ministry of Health, 2012).

A total of 15 variables were considered as potential confounders for each disease outcome. These variables described gender, age, BMI, household deprivation, region of New Zealand, total number of children in household, skin colour, maternal education, daycare attendance, ethnicity, vitamin D supplement usage, toddler milk consumption, exposure to cigarette smoke, cat or dog living in the house and season of birth.

A logistic regression model was created for each disease outcome. Those confounding variables with a P value of <0.20 in one predictor model analysis were entered into the logistic regression model. Those variables of non-significance were removed singly using a backwards stepwise method. The resultant confounders varied according to disease outcome as detailed in Table 3.2.

If a variable was a significant confounder for one disease outcome it was included in the multivariable logistic regression analysis for all disease outcomes to avoid unintentional bias in estimation of the relationship between disease outcome and dried blood spot 25(OH)D concentration. Thus, the 7 confounder variables included in all of the disease outcome models were gender, skin colour, maternal education, ethnicity, daycare attendance and usage of vitamin

D supplements and toddler milk. Each disease outcome model was also subsequently adjusted for confounding variables specific to each condition (Table 3.2).

Interaction effects were tested for each disease outcome. No significant interactions were seen. The presence of interaction was assessed using two methods, firstly through logistic regression models where the variable was multiplied by 25(OH)D and significance calculated, and secondly, through generation of scatterplot of response versus 25(OH)D with smoother lines.

Table 3.2. Confounders for disease outcomes as determined through logistic regression analysis

	Gender	**Skin colour**	**Maternal education**	**Ethnicity prioritised**	**Daycare attendance**	**Supple-ment usage**	**Toddler milk usage**
Asthma	✓	✓	✓	✓			✓
Atopic asthma	✓	✓	✓	✓			✓
Eczema		✓	✓				✓
Doctor diagnosed food allergy		✓				✓	✓
Parental report food allergy		✓					✓
Allergic rhinoconjunctivitis					✓		✓
Lower respiratory infection		✓		✓			
Upper respiratory infection			✓				

3.4 Provision of results to participants

On receipt of the analysis results, participants were informed of their vitamin D status by letter or email. This communication included general information about vitamin D which could be taken to a medical professional for further advice on their result (Appendix 7). All participants will be sent a brief report summarising the main findings of the project.

Chapter 4: Results

This chapter presents the data which, firstly, describes the vitamin D status and predictors of vitamin D deficiency in the enrolled children; secondly, describes the development, validation and performance of the vitamin D deficiency predictive questionnaire; and thirdly, are the analysis of the data which investigates the relationship of vitamin D status with allergic and respiratory diseases.

4.1 Vitamin D status and predictors of vitamin D deficiency in preschool children

4.1.1 Participant sample recruitment and characteristics

The 1329 children were recruited and tested in 17 cities and towns throughout New Zealand. The majority (49%) lived in the northern region with 28% and 23% in the central and southern regions respectively.

Travelling from north to south New Zealand, the number of children recruited from each town was: Whangarei 47(4%), Auckland 500(38%), Morrinsville 4(0.5%), Tauranga 27(2%), Hamilton 48(4%), Rotorua 16(1%), Napier 17(1%), Hastings 16(1%), Wanganui 15(1%), Palmerston North 78(6%), Masterton 20(2%), Wellington 183(14%), Nelson 28(2%), Blenheim 16(1%), Christchurch 118(9%), Dunedin 150(11%) and Invercargill 38(3%) (Figure 4.1).

The mean age of the 1329 children was 41 months (range 24-60 months). The majority (70%) of the children were of NZ European ethnicity. Most (81%) of the mothers had post-secondary school qualifications and 51% of children lived in affluent areas (the two least deprived quintiles of New Zealand households). Most (83%) of the children had a BMI-adjusted-for-age that was within the normal range, and most (86%) attended day care. A supplement containing vitamin D or cod liver oil was being taken by 19% of the children at the time the questionnaire was completed (Tables 4.1 & 4.2).

4.1.2 Dried blood spot 25(OH)D concentrations

The mean (S.D.) dried blood spot 25(OH)D concentration was 52 (19)nmol/L. Dried blood spot 25(OH)D concentrations ranged from 5 to 173nmol/L (Table 4.1, Figure 4.2).

Unadjusted analyses of 25(OH)D concentrations by child characteristics are presented in Tables 4.1 and 4.2.
Mean 25(OH)D concentration was lower in girls than boys (49 vs 51nmol/L, P<0.001).
In comparison with NZ European children, mean 25(OH)D concentrations were lower in Maori (50nmol/L, P<0.001) and Pacific (40nmol/L, P<0.001) children, and those of other non-European ethnic groups (46nmol/L, P<0.001).

In comparison with women with post-secondary education (mean 25(OH)D concentrations 53nmol/L), mean serum 25(OH)D concentration was lower in children of women with no secondary (40nmol/L, P<0.001) but not in children of women with secondary education (51nmol/L, P=0.21).

Children with olive-very dark skin colour (41nmol/L) had a mean 25(OH) concentration that was lower than either children with very fair or fair skin (53nmol/L, P<0.001) or with skin of medium colour (53nmol/L, P<0.001).

Children not attending formal daycare had a lower mean 25(OH)D concentration than those attending daycare (49 vs 53nmol/L, P=0.01). Although there was a significant difference in mean 25(OH)D concentration found between the four categories of hours spent by children in daycare (P=0.04), the mean 25(OH)D concentrations for each category did not exhibit a linear trend.
Almost all (98%) of children reported using sunscreen while playing outdoors in summer, and those who did, had a higher mean 25(OH)D concentrations than those who did not (52 vs 41nmol/L, P<0.001).

Mean 25(OH)D concentrations were higher in children who took vitamin D containing supplements (58 vs 51nmol/L, P<0.001) and for children whose parents reported they drank toddler milk (61 vs 54nmol/L, P<0.001). For children who drank milk, those who consumed non-fortified milk had lower 25(OH)D concentrations than those consuming fortified milk (51 vs 57nmol/L, P<0.001).

Whilst significant differences were seen in mean 25(OH)D concentration by residential latitude these difference did not show a clear latitudinal gradient. In comparison with children living in the northern third of the country (mean 25(OH)D concentration 53nmol/L), mean 25(OH)D concentration was lower in children living in the middle (50nmol/L, P<0.001) but not the more southern (52nmol/L, P=0.25) third of the country.

4.1.3 Vitamin D categories

The prevalence of 25(OH)D concentrations <25nmol/L was 6.5% (86/1329). Almost half (41.8%) of the children (556/1329) had a 25(OH)D concentration from 25 to <50nmol/L, with 40.7% (541/1329) from 50 to <75nmol/L and 11.0% (146/1329) had 25 (OH)D concentrations ≥75nmol/L. The highest prevalence of vitamin D deficiency (25[OH]D <25nmol/L) was found in children of Pacific ethnicity (23%), or who had darker skin colouring (26%), or whose mothers did not have high school qualifications (35%). There were no children who drank toddler milk who had 25(OH)D concentrations <25nmol/L (Tables 4.1 and 4.2).

4.1.4 Predictors of vitamin D deficiency (25[OH]D <25nmol/L)

There were 80 children who drank toddler milk in the study group, and none had 25(OH)D concentrations <25nmol/L. The sample of probability of deficiency is 0/80, thus the odds ratio for children who drink toddler milk is 0. In a multivariable analysis which only included children who did not drink toddler milk, factors independently associated with the risk of vitamin D deficiency were female gender (OR=1.92, 95%CI 1.17-3.14), children of other non-European ethnicities (not including Maori or Pacific) (3.51, 1.89-6.50) compared to NZ European, children whose mothers had less than secondary school qualifications (5.00, 2.44-10.21), who had olive-dark skin colour (4.52, 2.22-9.16) and who did not take vitamin D supplements (2.56, 1.06-6.18). Analysed on a covariate basis, each quintile increase in deprivation index was associated with an increase in risk of vitamin D deficiency (1.27, 1.06-1.53). Factors not associated with an increased risk of vitamin D deficiency were age, BMI, attendance at daycare, consumption of milk fortified with vitamin D (not toddler milk), latitude and number of children in the household (Table 4.3).

4.1.5 Predictors of vitamin D insufficiency (25[OH]D <50nmol/L)

Independent risk factors associated with 25(OH)D concentrations below 50nmol/L differed to those for vitamin D deficiency. From multivariable analysis, independent predictors were female gender (OR=1.55, 95%CI 1.24-1.95), children of Pacific (2.46, 1.25-4.84) and 'other' non-NZ European ethnicities (2.21 (1.57, 3.10), children whose mothers had less than secondary school qualifications (2.45, 1.34, 4.51), who were not attending a formal daycare centre (1.50, 1.08, 2.07), who were not taking a vitamin D supplement or cod liver oil (2.05, 1.52, 2.76) and not drinking toddler milk (3.19, 1.85-5.48) (Table 4.4).

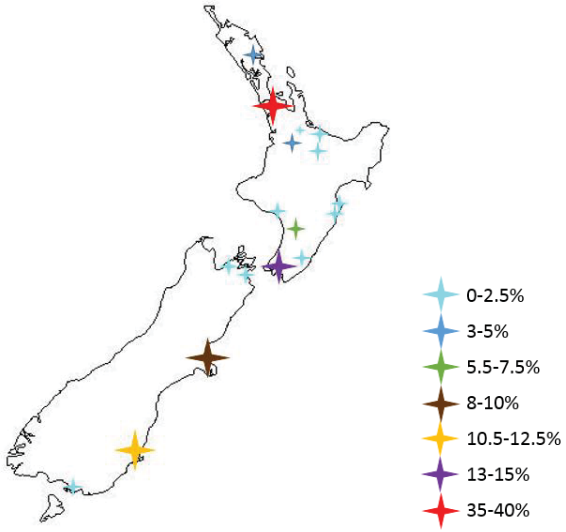


Figure 4.1. Distribution of children recruited and tested in each of the 17 towns throughout New Zealand

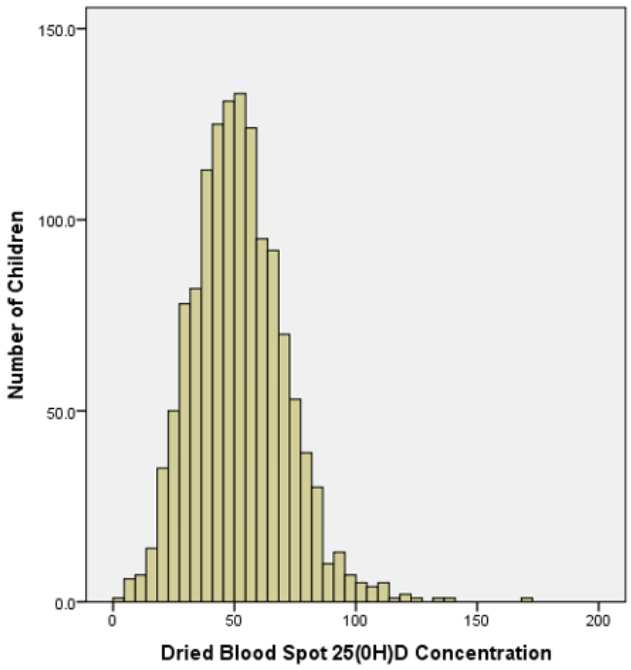


Figure 4.2. Histogram of dried blood spot 25(OH)D concentrations of participants (n=1329)

Table 4.1: Vitamin D status by child demographics, sunlight exposure and main dietary sources of vitamin D

Variable	n (%)	Mean (S.D.*) 25(OH)D (nmol/L)	25(OH)D categories (nmol/L) n (%)				P value
							Comparison of means†
			<25	25 to <50	50 to <75	>75	
Total	1329	52 (19)	86 (7)	556 (42)	541 (41)	146 (11)	--
Child demographics							
Gender							<0.001
Female	648 (49)	49 (18)	51 (8)	296 (46)	248 (38)	53 (8)	
Male	681 (51)	51 (20)	35 (5)	260 (38)	293 (43)	93 (14)	
Age (years)							0.47
2 years old	496 (37)	52 (10)	30 (6)	223 (45)	183 (37)	60 (12)	
3 years old	448 (34)	53 (20)	25 (6)	180 (40)	194 (43)	49 (11)	
4 years old	385 (29)	51 (19)	31 (8)	153 (40)	164 (43)	37 (10)	
BMI, kg/m^2 (n=1315)‡							0.08
Normal	1094 (83)	52 (19)	70 (6)	449 (41)	452 (41)	123 (11)	
Overweight	173 (13)	52 (19)	9 (5)	79 (46)	65 (38)	21 (12)	
Obese	48 (14)	46 (19)	7 (15)	22 (46)	16 (33)	3 (6)	
Ethnicity							<0.001
Maori	174 (13)	50 (20)[a]	16 (9)	74 (43)	66 (38)	18 (10)	
Pacific	44 (3)	40 (18)[b]	10 (23)	21 (48)	12 (27)	1 (2)	
Other non-European	185 (14)	46 (19)[b]	28 (15)	83 (45)	62 (34)	12 (7)	
NZ European	926 (70)	54 (19)[a]	32 (3)	378 (41)	401 (43)	115 (12)	
Sunlight exposure related variables							
Skin colour - parental report							<0.001
Very fair – fair	767 (58)	53 (18)[a]	27 (4)	339 (44)	313 (41)	88 (11)	
Medium	445 (33)	53 (20)[a]	29 (7)	172 (39)	193 (43)	51 (11)	
Olive – very dark	117 (9)	41 (20)[b]	30 (26)	45 (38)	35 (30)	7 (6)	
Attend formal daycare							0.01
Yes	1137 (86)	53 (19)	71 (6)	464 (41)	473 (42)	129 (11)	
No	192 (14)	49 (18)	15 (8)	92 (48)	68 (35)	17 (9)	
Hours attended formal daycare							0.27
1-10	257 (19)	54 (19)	12 (5)	110 (43)	100 (39)	35 (14)	
11-20	460 (35)	52 (21)	39 (9)	177 (38)	194 (42)	50 (11)	
21-30	234 (18)	54 (19)	9 (4)	96 (41)	102 (44)	27 (12)	
30+	186 (14)	51 (18)	11 (6)	81 (44)	77 (41)	17 (9)	

Variable	n (%)	Mean (S.D.*) 25(OH)D (nmol/L)	25(OH)D categories (nmol/L) n (%)				P value
							Comparison of means†
			<25	25 to <50	50 to <75	>75	
Sunscreen use in summer							<0.001
Ever	1298 (98)	52 (19)	75 (6)	548 (42)	530 (41)	145 (11)	
Never	31 (2)	41 (23)	15 (36)	8 (26)	11 (36)	1 (3)	
Dietary sources of vitamin D							
Vitamin D Supplement use∫							<0.001
Yes	253 (19)	58 (20)	6 (2)	78 (31)	131 (52)	38 (15)	
No	1076 (81)	51 (19)	80 (7)	478 (44)	410 (38)	108 (10)	
Drinks milk (n=1250)							0.01
Non-fortified	1159 (93)	51 (19)	81 (7)	500 (43)	458 (40)	120 (10)	
Fortified	91 (7)	57 (18)	3 (3)	27 (30)	49 (54)	12 (13)	
Drinks toddler milk							<0.001
Yes	80 (6)	61 (16)	0 (0)	20 (25)	44 (55)	16 (20)	
No	1249 (94)	54 (19)	86 (7)	536 (43)	497 (40)	130 (10)	

* Standard deviation

† Comparison using Independent t-test or ANOVA

‡ Cole *et al*, 2000. Normal BMI: <25kg/m^2; Overweight BMI: 25-<30kg/m^2; Obese BMI: >30kg/m^2

∫ Includes cod liver oil

[a,b] Values with different superscript letters indicate significant differences (Dunnetts test, P<0.05)

Table 4.2: Vitamin D status by maternal and household characteristics

Variables	n (%)	Mean (S.D.*) 25(OH)D (nmol/L)	25(OH)D categories (nmol/L) n (%)				P value
							Comparison of means†
			<25	25 to <50	50 to <75	>75	
Mother education qualifications							<0.001
Less than secondary	59 (4)	40 (24)[b]	20 (34)	23 (39)	13 (22)	3 (5)	
Secondary	198 (15)	51 (19)[a]	10 (5)	93 (47)	77 (39)	18 (9)	
Post secondary	1072 (81)	53 (19)[a]	56 (5)	440 (41)	451 (42)	125 (12)	
Household deprivation							0.21
Quintile 1	343 (26)	54 (19)	17 (5)	142 (42)	138 (40)	45 (13)	
Quintile 2	331 (25)	52 (19)	13 (4)	144 (44)	140 (42)	34 (10)	
Quintile 3	287 (22)	52 (19)	18 (6)	114 (40)	126 (44)	29 (10)	
Quintile 4	227 (17)	51 (20)	19 (8)	103 (45)	78 (34)	27 (12)	
Quintile 5	141 (11)	49 (19)	19 (13)	52 (37)	59 (42)	11 (8)	
Residential latitude							0.01
Northern: 35 to39°S	651 (49)	53 (19)[a]	29 (4)	265 (41)	281 (41)	76 (12)	
Central: 40 to 42°S	378 (28)	50 (20)[b]	42 (11)	155 (41)	155 (41)	35 (9)	
Southern: 43 to 46°S	300 (23)	52 (19)[a]	15 (5)	136 (45)	136 (45)	35 (12)	
Number of children in house							<0.001‡
1	247 (19)	54 (22)	13 (5)	102 (41)	101 (41)	31 (13)	
2	695 (52)	52 (18)	36 (5)	298 (43)	284 (41)	77 (11)	
3	281 (21)	51 (18)	21 (7)	122 (43)	111 (40)	27 (10)	
4	68 (5)	58 (24)	5 (7)	19 (28)	34 (50)	10 (15)	
5+	38 (3)	41 (20)	11 (29)	15 (39)	11 (29)	1 (3)	

*Standard deviation

†Comparison using Independent t-test or ANOVA

‡ No significant difference in mean 25(OH)D concentration by linear regression analysis

[a,b] Values with different superscript letters indicate significant differences (Dunnetts test, P<0.05)

Table 4.3. Factors independently associated with vitamin D deficiency as defined by dried blood spot 25-hydroxyvitamin D (25[OH]D) concentration <25nmol/L for children who do not drink toddler milk (n=1249)

Variable	**n**	**Association with vitamin D deficiency**	
		Multivariable Odds Ratio (95% confidence intervals)	**P value**
Gender			
Male	35	1.00	
Female	51	1.92 (1.17, 3.14)	0.01
Ethnicity			
NZ European	32	1.00	
Maori	16	1.56 (0.77, 3.17)	0.22
Pacific	10	2.53 (0.96, 6.68)	0.06
Other	28	3.51 (1.89,6.50)	<0.001
Maternal education level			
Post secondary qualification	56	1.00	
Secondary qualification	10	0.73 (0.34, 1.52)	0.40
No secondary qualification	20	5.00 (2.44, 10.21)	<0.001
Skin colour			
Fair – very fair	27	1.00	
Medium	29	1.37 (0.77, 2.44)	0.29
Olive –very dark	30	4.52 (2.22, 9.16)	<0.001
Vitamin D supplements			
Yes	6	1.00	
No	80	2.56 (1.06, 6.18)	0.04
Household deprivation*			
Change of one quintile from less to more deprived		1.27 (1.06, 1.53)	0.01

Children who drink toddler milk, sample of probability of vitamin D deficiency is 0/80, odds of deficiency = 0
Vitamin D deficiency defined as 25(OH)D <25nmol/L
*Deprivation index covariate

Table 4.4. Factors independently associated with vitamin D insufficiency as defined by dried blood spot 25-hydroxyvitamin D (25[OH]D) concentration <50nmol/L

Variable	n	Association with vitamin D insufficiency	
		Multivariable Odds Ratio (95% confidence intervals)	P value
Gender			
Male	295	1.00	
Female	347	1.55 (1.24, 1.95)	<0.001
Ethnicity			
European	410	1.00	
Maori	90	1.22 (0.87, 1.71)	0.24
Pacific	31	2.46 (1.25, 4.84)	0.01
Other	111	2.21 (1.57, 3.10)	<0.001
Maternal education level			
Post secondary qualification	496	1.00	
Secondary qualification	103	1.19 (0.87, 1.63)	0.28
No secondary qualification	43	2.45 (1.34, 4.51)	0.01
Daycare attendance			
Yes	535	1.00	
No	107	1.50 (1.08, 2.07)	0.02
Vitamin D supplements			
Yes	84	1.00	
No	558	2.05 (1.52, 2.76)	<0.001
Toddler milk			
Yes	20	1.00	
No	622	3.19 (1.85, 5.48)	<0.001

Vitamin D insufficiency defined as 25(OH)D <50nmol/L

4.2 Development of a predictive questionnaire to assess risk of vitamin D deficiency

The participant characteristics of the 'total', 'development' and 'validation' groups are described in Appendix 8. The groups were similar for all characteristics. The mean 25(OH)D concentrations for the alternate ethnicity prioritisation used in the tool development are also presented in Appendix 8.

4.2.1 Model for prediction of vitamin D deficiency (25[OH]D <25nmol/L)

The regression coefficients from analysis of the 'development group' minus children who drank toddler milk (n=870) were used to create the following simple mathematical equation to calculate the risk of vitamin D deficiency (Table 4.5):
Total score = 0.59 * female + 1.66 * mother with no secondary education qualification + 1.28 * olive-dark skin colour + 1.47 * Indian or African ethnicity + 1.07 * Pacific ethnicity + 1.26 * Asian ethnicity.
When added, total scores range from 0 to 6.0.

The best balance of sensitivity and specificity were determined at a cut-off of ≥1.86 in the 'development group', as there is a drop-off in both sensitivity and specificity below this point (Table 4.6). As there were no children who drank toddler milk in the deficient category, a coefficient of -8 was chosen and included in the equation for children who drank toddler milk. This coefficient would make it impossible to exceed the cut-off for vitamin D deficiency for any child drinking toddler milk.

When this modified equation and cut-off were applied in ROC analysis of the 'validation' group (n=400), the predictive tool was able to identify children at risk for vitamin D deficiency with a sensitivity of 42%, a specificity of 97% and an ROC area under the curve (AUC) of 0.76 (95%CI 0.67-0.86, P<0.001) (Table 4.7, Figure 4.4). Positive and negative predictive values were calculated to be PPV 52% (95%CI 31-72%) and NPV 95% (95%CI 93-97%). A sample layout of the predictive model is provided in Table 4.8.

The performance of a model for vitamin D deficiency which included deprivation index (significant in the logistic regression analysis) was inferior to the model above. This predictive tool was able to identify children at risk for vitamin D deficiency with a sensitivity of 36%, a specificity of 97% and

an ROC area under the curve (AUC) of 0.76 (95%CI 0.66-0.86, P<0.001). Positive and negative predictive values were calculated to be PPV 46% (95%CI 26-67%) and NPV 95% (95%CI 92-97%) (Appendix 8).

4.2.2 Model for prediction of vitamin D insufficiency (25[OH]D <50nmol/L)

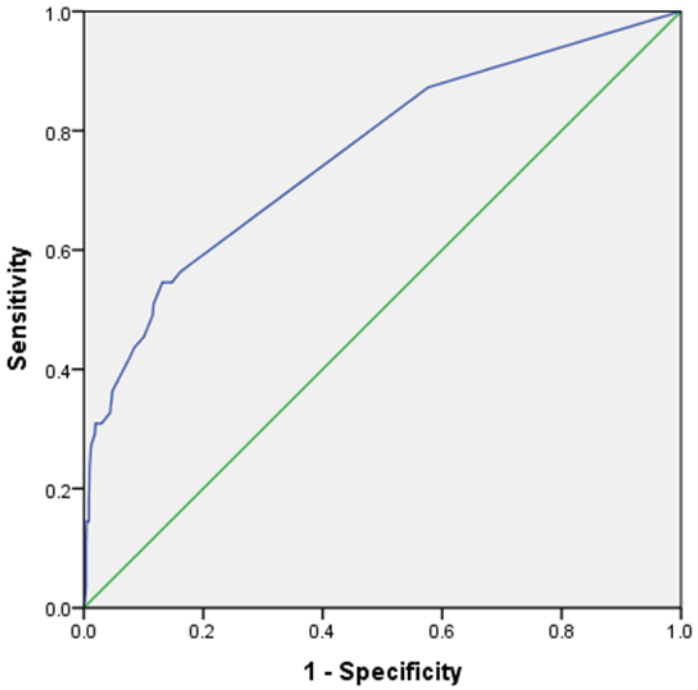
Using regression coefficients of significant variables in the logistic regression analysis of the 'development group' (n=929) (Table 4.9), the equation to calculate the risk of vitamin D insufficiency was:
Total score = 0.51 * female + 0.83 * mother with no secondary education qualification + 0.79 * not taking vitamin D supplement + 0.92 * not drinking toddler milk + 1.58 * Indian or African ethnicity + 0.76 * Pacific ethnicity + 1.03 * Asian ethnicity.

A cut-off score of ≥2.21 was determined by examining the best sensitivity and specificity from the range of total scores from 0 to 5.63, as below this point there is a sharp drop-off in both sensitivity and specificity (Table 4.10). When this equation and cut-off were applied to the 'validation' group (n=400), the ROC AUC was 0.63 (95%CI 0.57-0.68, P<0.001) with a sensitivity of 52% and a specificity of 66%. Positive and negative predictive values were calculated to be PPV 58% (95%CI 50-65%) and NPV 60% (95%CI 53-66%) (Table 4.11, Figure 6). A sample layout of the predictive model is provided in Table 4.12.

Table 4.5. Logistic regression analysis for vitamin D deficiency (25[OH]D <25nmol/L) for 'development' dataset minus children who drink toddler milk (n=870)

	B	S.E.	P value	Odds Ratio (OR)	95% C.I.	
Female	0.59	0.31	0.06	1.80	0.98	3.33
Mother with no secondary education qualifications	1.66	0.43	<0.001	5.25	2.28	12.06
Olive -dark skin colour	1.28	0.39	0.001	3.61	1.69	7.72
Indian or African ethnicity	1.47	0.58	0.01	4.36	1.41	13.49
Pacific ethnicity	1.07	0.48	0.03	2.91	1.14	7.43
Asian ethnicity	1.26	0.66	0.06	3.54	0.97	12.93

B regression coefficient; S.E. standard error



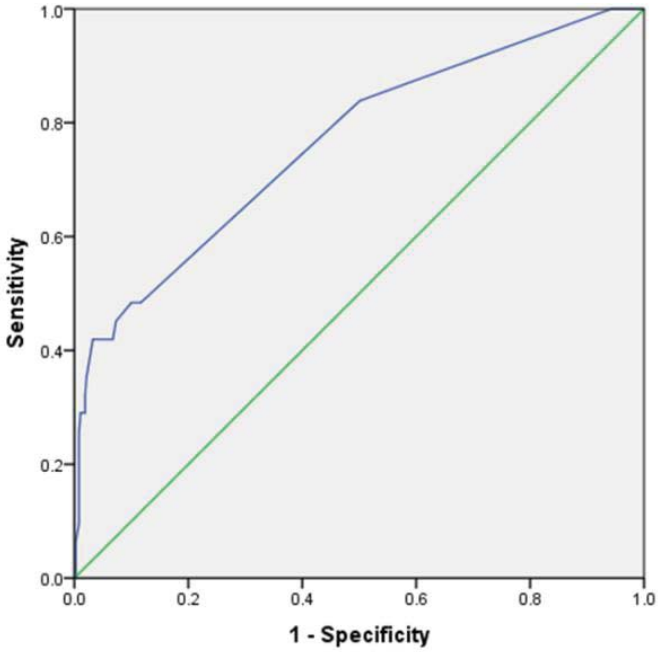
Area Under Curve (AUC): 0.76 (95% CI 0.69-0.83, P<0.01)

Figure 4.3. Receiver Operating Characteristic (ROC) for identifying preschool children with vitamin D deficiency (25[OH]D <25nmol/L) -'development' dataset minus children who drink toddler milk (n=870)

Table 4.6: Performance of model equation in identifying preschool children with vitamin D deficiency (<25nmol/L) - 'development' dataset minus children who drink toddler milk (n=870)

Total score	Sensitivity	1 - Specificity	PPV (%)	NPV (%)
-1.0000	1.000	1.000	-	-
.2950	.873	.577	9.3	98.0
.8300	.564	.162	18.9	96.6
1.1650	.545	.147	20.0	96.5
1.2700	.545	.131	21.9	96.6
1.3750	.509	.117	22.8	96.4
1.5650	.491	.115	22.3	96.3
1.6600	.455	.101	23.4	96.1
1.7550	.436	.085	27.9	96.1
1.8600	**.418**	**.076**	**27.1**	**95.9**
1.9650	.364	.048	33.9	95.7
2.1550	.327	.044	33.3	95.5
2.3000	.309	.029	41.5	95.5
2.4450	.309	.020	51.2	95.5
2.6450	.291	.018	51.6	95.4
2.8450	.273	.012	60.0	95.3
2.9400	.236	.010	61.9	95.1
3.0350	.182	.009	58.8	94.7
3.2250	.164	.009	56.3	94.6
3.3300	.145	.009	53.3	94.5
3.6750	.145	.005	66.7	94.5
4.2100	.109	.004	66.7	94.3
4.5050	.036	.004	40.0	93.9
4.8000	.018	.002	50.0	93.8
6.0000	.000	.000	0	0

Score ≥1.86 = risk of vitamin D deficiency
Score <1.86 = not at risk of vitamin D deficiency
Vitamin D deficiency 25(OH)D <25nmol/L



Area Under Curve (AUC): 0.76 (95% CI 0.67-0.86, P<0.01)

Figure 4.4. Internal validation: Receiver Operating Characteristic (ROC) for identifying preschool children with vitamin D deficiency (25[OH]D <25nmol/L) -'validation' subset (n=400)

Table 4.7. Internal validation: performance of model equation in identifying preschool children with vitamin D concentration (25[OH]D <25nmol/L) – 'validation' dataset (n=400)

Total score	Sensitivity	1 – Specificity	PPV (%)	NPV (%)
-9.0000	1.000	1.000	-	-
-7.7050	1.000	.976	-	-
-7.0750	1.000	.957	-	-
-6.4450	1.000	.951	-	-
-5.9500	1.000	.949	-	-
-5.1100	1.000	.946	-	-
-2.2350	1.000	.943	-	-
.2950	.839	.501	8.4	97.4
.8300	.484	.117	25.9	95.3
1.1650	.484	.108	33.3	95.4
1.2700	.484	.100	35.7	95.4
1.3750	.452	.073	34.1	95.3
1.5650	.419	.068	39.4	95.1
1.6600	.419	.054	32.5	95.0
1.7550	.419	.041	46.3	95.2
1.8600	**.419**	**.033**	**52.4 (95% CI 31-72)**	**95.2 (95%CI 93-97)**
1.9650	.355	.022	57.9	94.8
2.1550	.323	.019	55.6	94.5
2.3950	.290	.019	56.3	94.3
2.6450	.290	.011	69.2	94.3
2.8450	.258	.008	72.7	94.1
3.0350	.194	.008	66.7	93.6
3.2250	.129	.008	33.2	93.0
3.3300	.097	.008	27.3	92.8
3.8750	.065	.003	4.9	91.9
4.5050	.000	.003	0	0
5.6000	.000	.000	0	0

Score ≥1.86 = risk of vitamin D deficiency
Score <1.86 = not at risk of vitamin D deficiency
Vitamin D deficiency 25(OH)D <25nmol/L
CI confidence interval, PPV positive predictive value; NPV negative predictive value
(Total score = 0.59 * female + 1.66 * mother with no secondary education qualification + 1.28 * olive-dark skin colour + 1.47 * Indian or African ethnicity + 1.07 * Pacific ethnicity + 1.26 * Asian ethnicity – 8 * drink toddler milk)

Table 4.8. Sample layout of predictive questionnaire for assessment of risk of vitamin D deficiency (25[OH]D <25nmol/L) in winter in preschool children

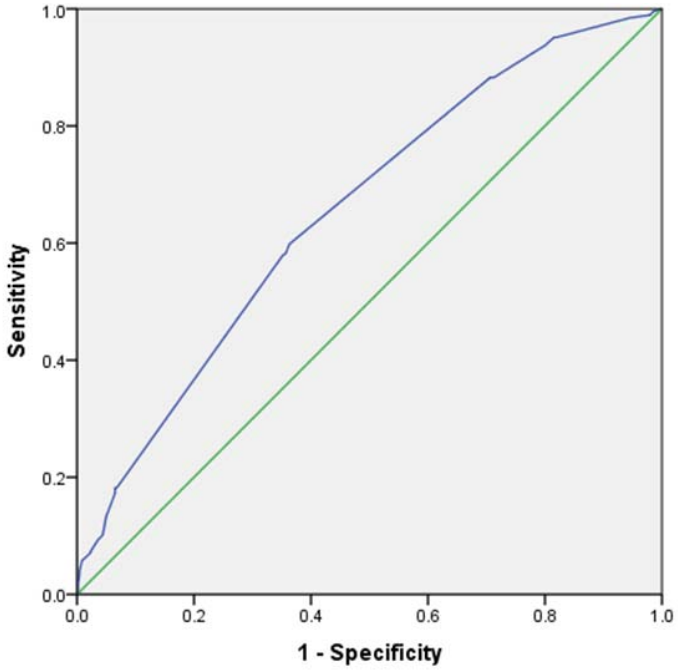
Question	Answer	Answer Score	Child Score
Is your child male or female?	Male Female	0 0.59	
Which ethnic group does your child identify with?	Maori Pacific Island NZ European Indian or African Asian	0 1.07 0 1.47 1.26	
Has the child's mother completed any secondary school qualifications?	Yes No	0 1.66	
Which category best fits the child's skin colouring	Very fair to fair Medium Olive to dark	0 0 1.28	
Does the child drink infant formula or toddler milk?	Yes No	-8 0	
Total Score			

Total Score for child:
≥1.86: child is at risk of vitamin D deficiency in winter, further investigation is recommended
<1.86: child is not at risk of vitamin D deficiency in winter

Table 4.9. Logistic regression analysis for vitamin D insufficiency (25[OH]D <50nmol/L) for 'development' dataset (n=929)

	B	S.E.	P value	Odds Ratio (OR)	95% C.I.	
Female	0.51	0.14	<0.001	1.66	1.27	2.17
Mother with no secondary education qualifications	0.83	0.36	0.02	2.29	1.13	4.62
Not taking a vitamin D supplement	0.79	0.18	<0.001	2.21	1.54	3.16
Not drinking toddler milk	0.92	0.31	0.003	2.50	1.35	4.62
Indian or African ethnicity	1.58	0.58	0.01	4.83	1.57	14.91
Pacific ethnicity	0.76	0.32	0.02	2.15	1.15	3.99
Asian ethnicity	1.03	0.39	0.01	2.79	1.29	6.00

B regression coefficient; S.E. standard error, OR odds ratio, CI confidence interval



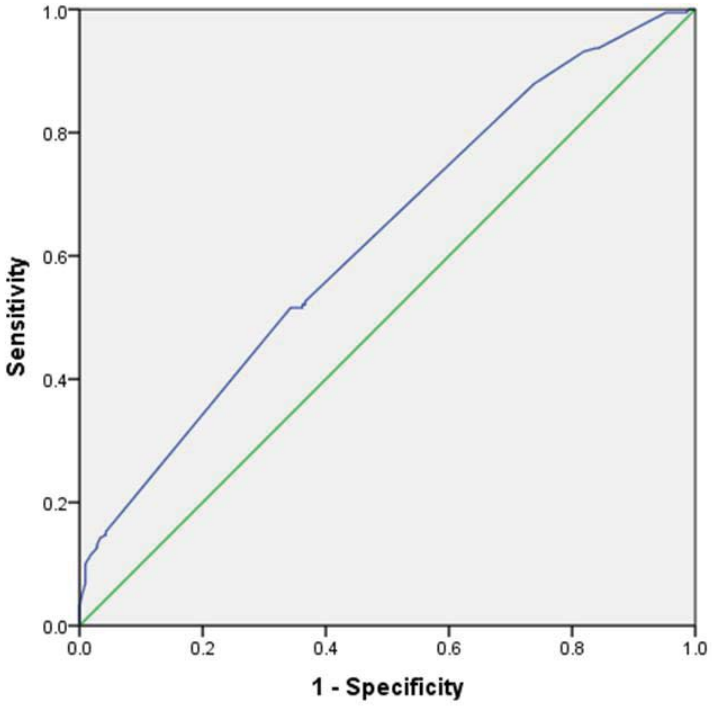
Area under the curve = 0.66 (95%CI 0.62-0.69, P<0.001)

Figure 4.5. Receiver Operating Characteristic (ROC) for identifying preschool children with vitamin D insufficiency (25[OH]D <50nmol/L) -'development' subset (n=929)

Table 4.10. Performance of model equation in identifying preschool children with vitamin D insufficiency (25[OH]D <50nmol/L) - 'development' dataset (n=929)

Total Score	Sensitivity	1 - Specificity	PPV (%)	NPV (%)
-1.0000	1.000	1.000		
.2550	.996	.985	48.9	77.8
.6350	.989	.979	48.9	66.7
.7750	.989	.977	49.0	68.8
.8550	.985	.945	50.0	78.8
.9750	.951	.820	52.4	79.6
1.1650	.951	.816	52.5	80.0
1.3650	.938	.801	52.6	77.2
1.4850	.883	.713	54.0	72.1
1.5450	.883	.711	54.0	72.6
1.5850	.883	.709	54.1	72.4
1.6650	.883	.706	54.2	72.5
1.7650	.600	.365	62.3	62.6
1.8850	.597	.363	62.4	62.6
2.0050	.586	.358	61.6	62.1
2.1250	.582	.356	61.2	61.9
2.2050	**.580**	**.352**	**60.9**	**61.9**
2.2750	.184	.069	71.6	54.6
2.3950	.181	.065	72.6	54.7
2.4650	.173	.065	71.6	54.4
2.4850	.139	.052	71.6	53.8
2.5200	.135	.050	71.8	53.7
2.6400	.102	.044	68.7	52.9
2.8600	.093	.036	71.2	52.9
2.9950	.073	.023	66.0	52.3
3.0300	.071	.023	65.3	52.3
3.1500	.058	.008	86.7	52.6
3.2700	.049	.006	88.0	52.4
3.2950	.040	.004	90.0	52.3
3.5500	.033	.004	88.2	52.1
3.8050	.020	.002	90.0	51.8
3.9650	.013	.002	85.7	51.6
4.3750	.004	.002	66.7	51.4
5.6300	.000	.000	0	0

Score ≥2.21 = risk of vitamin D insufficiency
Score <2.21 = not at risk of vitamin D insufficiency
Vitamin D insufficiency 25(OH)D <50nmol/L
PPV positive predictive value; NPV negative predictive value



Area under the curve = 0.63 (95%CI 0.57-0.68, P<0.001)

Figure 4.6. Internal validation: Receiver Operating Characteristic (ROC) for identifying preschool children with vitamin D insufficiency (25[OH]D <50nmol/L) - 'validation' dataset (n=400)

Table 4.11. Internal validation: performance of model equation in identifying preschool children with vitamin D insufficiency (25[OH]D <50nmol/L) – 'validation' subset (n=400)

Total score	Sensitivity	1 - Specificity	PPV (%)	NPV (%)
-1.0000	1.000	1.000	-	-
.2550	1.000	.990	-	-
.6500	.995	.986	47.7	75.0
.8550	.995	.952	48.6	90.9
.9750	.937	.843	50.1	73.3
1.1650	.937	.838	50.3	73.8
1.3650	.932	.819	50.7	73.9
1.5700	.879	.738	51.9	56.1
1.7300	.526	.367	56.5	59.6
1.7850	.521	.367	56.3	59.4
1.8850	.521	.362	56.6	59.6
2.0400	.516	.362	56.3	59.3
2.1600	.516	.352	57.0	59.7
2.2050	**.516**	**.343**	**58 (95%CI 50-65)**	**60% (95%CI 53-66)**
2.2750	.153	.043	78.4	55.7
2.3950	.147	.043	77.8	55.5
2.4650	.142	.033	79.4	55.5
2.4850	.132	.029	80.7	55.3
2.5200	.126	.029	80.0	55.1
2.6400	.116	.019	78.6	54.8
2.8600	.100	.010	86.4	54.8
3.0150	.074	.010	82.4	54.2
3.1700	.068	.010	81.3	54.1
3.5450	.053	.005	90.9	53.7
3.8050	.032	.000	0	0
3.9650	.021	.000	0	0
5.1200	.000	.000	0	0

Score ≥2.21 = risk of vitamin D insufficiency
Score <2.21 = not at risk of vitamin D insufficiency
Vitamin D insufficiency 25(OH)D <50nmol/L
CI confidence interval, PPV positive predictive value; NPV negative predictive value

Table 4.12. Sample layout of predictive questionnaire for assessment of risk of vitamin D insufficiency (25[OH]D <50nmol/L) in winter in preschool children

Question	Answer	Answer Score	Child Score
Is your child male or female?	Male Female	0 0.51	
Which ethnic groups does your child identify with?	Maori Pacific Island NZ European Indian or African Asian	0 0.76 0 1.58 1.03	
Has the child's mother completed any secondary school qualifications?	Yes No	0 0.83	
Is the child taking a supplement containing vitamin D, or cod liver oil?	Yes No	0 0.79	
Does the child drink infant formula or toddler milk?	Yes No	0 0.92	
Total Score			

Total Score for child:
≥2.21: child is at risk of vitamin D insufficiency in winter
<2.21: child is not at risk of vitamin D insufficiency in winter

4.3 Relationship of vitamin D status with prevalence of allergic and respiratory diseases

Dried blood spot 25(OH)D concentrations and parental reports of allergic diseases and respiratory infections were available for 1329 participants.
The odds rations (OR) in the tables which show the association between vitamin D status and disease condition are presented unadjusted for any confounders, and then after adjustment for a common set of confounders (Table 3.2). The association of vitamin D status with each disease was also examined using confounders specific for that condition. These alternative sets of confounders did not result in any significant differences to the findings. For consistency, only the results adjusted for common confounders are shown.

4.3.1 Vitamin D and eczema

Participant characteristics and mean dried blood spot 25(OH)D concentrations stratified for the prevalence of eczema are presented in Appendix 8.

The prevalence of parent reported eczema in this study cohort was 23% (299). With respect to eczema severity, there were 4% (57) of children reporting sleep disturbance and 20% of children who used topical steroid treatments (Table 4.13).

The mean 25(OH)D concentrations were not significantly different for children with eczema compared to those without (P=0.50) nor for children in whom markers of more severe eczema were present; for children who reported using topical steroid treatment (P=0.94) or for children who reported sleep disturbance (P=0.12) compared to those who did not (Table 4.13).

There was no trend observed across the four 25(OH)D categories in prevalence of eczema (P= 0.41) or in eczema severity (P= 0.48 and P=0.08) (Table 4.14).

No association between 25(OH)D concentrations and eczema was found in both unadjusted and multivariable logistic regression analysis. This lack of association was also seen for eczema severity (Tables 4.15, 4.16, 4.17).

The prevalence of eczema was not associated with the child's season of birth (P=0.68) (Table 4.18).

Table 4.13. Mean dried blood spot 25-hydroxyvitamin D concentration for children with and without eczema and severe eczema

Parental report of eczema and severe eczema	**n (%)**	**Mean 25(OH)D (S.D.) (nmol/L)**	**P value***
Eczema			
			0.50
Present	299 (23)	53 (20)	
Absent	1030 (77)	52 (19)	
Severe Eczema – sleep disturbed			
			0.12
Sleep disturbed	57 (4)	56 (21)	
No sleep disturbance	1272 (96)	52 (19)	
Severe eczema - topical steroid used			
			0.97
Steroid used	267 (20)	53 (19)	
No steroid used	1062 (80)	53 (20)	

S.D. standard deviation
*P values from Independent t-tests

Table 4.14. Prevalence of eczema by dried blood spot 25-hydroxyvitamin D concentration

Eczema and severe eczema category	**25(OH)D concentration in nmol/L n (%)**				**P value***
	<25	25 to <50	50 to <75	≥75	
Eczema					
					0.41
Eczema present	15 (17)	132 (24)	115 (21)	37 (25)	
Eczema absent	71 (83)	424 (76)	426 (79)	109 (75)	
Severe Eczema – sleep disturbed					
					0.48
Sleep disturbed	4 (5)	19 (3)	25 (5)	9 (6)	
No sleep disturbed	82 (95)	537 (97)	516 (95)	137 (11)	
Severe Eczema – topical steroid used					
					0.88
Steroid used	13 (15)	120 (22)	101 (19)	33 (23)	
No steroid used	73 (85)	436 (78)	440 (81)	113 (79)	

*P values from chi squared test

Table 4.15. Odds ratio of eczema by dried blood spot 25-hydroxyvitamin D category

25(OH)D concentration (nmol/L)	n	Unadjusted OR (95% CI)	P value	Adjusted* OR (95% CI)	P value
<25	15	0.78 (0.43, 1.42)	0.42	0.66 (0.34, 1.29)	0.22
25 to <50	132	1.15 (0.87, 1.53)	0.33	1.20 (0.92, 1.55)	0.26
50 to <75	115	1.00		1.00	
≥75	37	1.26 (0.82, 1.93)	0.29	1.23 (0.79, 1.89)	0.36

*Adjusted for gender, ethnicity, maternal education level, skin colour, vitamin D supplement and toddler milk usage, daycare attendance
OR odds ratio, CI confidence interval

Table 4.16: Odds ratio of severe eczema for children with sleep disturbance by dried blood spot 25-hydroxyvitamin D category

Dried blood spot 25(OH)D concentration (nmol/L)	n	Unadjusted OR (95% CI)	P value	Adjusted* OR (95% CI)	P value
<25	4	1.01 (0.34, 2.97)	0.99	0.86 (0.26, 2.83)	0.80
25 to <50	19	0.73 (0.40, 1.34)	0.31	0.79 (0.42, 1.45)	0.43
50 to <75	25	1.00		1.00	
≥75	9	1.36 (0.62, 2.97)	0.45	1.31 (0.59, 2.90)	0.51

*Adjusted for gender, ethnicity, maternal education level, skin colour, vitamin D supplement and toddler milk usage, daycare attendance
OR odds ratio, CI confidence interval

Table 4.17: Odds ratio of severe eczema for children who use topical steroid treatment by dried blood spot 25-hydroxyvitamin D category

Dried blood spot 25(OH)D concentration (nmol/L)	n	Unadjusted OR (95% CI)	P value	Adjusted* OR (95% CI)	P value
<25	13	0.83 (0.46, 1.50)	0.53	0.69 (0.35, 1.35)	0.27
25 to <50	120	1.31 (0.98, 1.74)	0.07	1.33 (0.99, 1.79)	0.06
50 to <75	101	1.00			
≥75	33	1.33 (0.87, 2.04)	0.45	1.30 (0.84, 2.01)	0.25

*Adjusted for gender, ethnicity, maternal education level, skin colour, vitamin D supplement and toddler milk usage, daycare attendance
OR odds ratio, CI confidence interval

Table 4.18. Season of birth and prevalence of eczema

Parental report of eczema	Season of birth n(%)				P value*
	Summer	Autumn	Winter	Spring	
					0.41
Present	61 (20)	74 (25)	72 (24)	92 (29)	
Absent	238 (80)	227 (75)	247 (67)	410 (71)	

*P values from chi squared test

4.3.2 Vitamin D and food allergy

Participant characteristics and mean dried blood spot 25(OH)D concentrations stratified for prevalence of doctor diagnosed food allergy are presented in Appendix 8.

The prevalence of doctor diagnosed food allergy was lower than that of parent reported food allergy, 12% and 19% respectively. The mean 25(OH)D concentration of children with doctor diagnosed food allergy was significantly higher than those without (56 vs 52nmol/L, P<0.007), and higher for those with parent reported food allergy than those without (55 vs 51nmol/L, P=0.02) (Table 4.19).

The prevalence of doctor diagnosed and parent reported food allergy in children differed across 25(OH)D categories (P=0.001 and P=0.04), being highest in those children with a 25(OH)D concentration ≥ 75nmol/L and lowest in those with a 25(OH)D concentration <25nmol/L (Table 4.21). In a multivariable analysis that adjusted for confounding factors, the odds of doctor diagnosed food allergy were increased for children with a 25(OH)D concentration ≥75nmol/L (OR 2.21, 95%CI 1.33-3.68, P=0.002), while odds of parent reported food allergy were decreased for children with a 25(OH)D concentration <25nmol/L (OR0.41, 95%CI 0.18-0.93, P=0.04) (Tables 4.21 & 4.22).

Neither the children's season of birth nor latitude were associated with doctor diagnosed or parent reported food allergy, nor with the child having an EpiPen or food related hospital visits. There was no association between either doctor diagnosed or parent reported food allergy and season of birth (Table 4.23).

Table 4.19. Mean dried blood spot 25-hydroxyvitamin D concentration for children with and without doctor diagnosed food allergy, parental report food allergy, child having an EpiPen and food related hospital visits

Food allergy category	**n (%)**	**Mean 25(OH)D (S.D.) (nmol/L)**	**P value***
Doctor diagnosed food allergy			
Present	153 (12)	56 (21)	0.007
Absent	1176 (88)	52 (19)	
Parent reported food allergy			
Present	249 (19)	55 (19)	0.02
Absent	1080 (81)	51 (19)	
Child has EpiPen			
EpiPen	23 (2)	54 (16)	0.57
No EpiPen	1306 (98)	52 (19)	
Food related visit to hospital			
Visited hospital	39 (3)	52 (19)	0.97
Not visited hospital	1290 (97)	52 (19)	

S.D. standard deviation
*P values from Independent t-test

Table 4.20. Prevalence of food allergy by dried blood spot 25-hydroxyvitamin D concentration

Food allergy category	**25(OH) D concentration in nmol/L n(%)**				**P value***
	<25	25 to <50	50 to <75	≥75	
Doctor diagnosed food allergy					
					0.001
Present	4 (5)	67 (12)	53 (10)	29 (20)	
Absent	82 (95)	489 (88)	488 (90)	117 (80)	
Parent reported food allergy					
					0.04
Present	8 (10)	103 (18)	102 (19)	36 (25)	
Absent	78 (90)	453 (82)	439 (81)	110 (75)	

*P values from chi squared test

Table 4.21: Odds ratio of doctor diagnosed food allergy by dried blood spot 25-hydroxyvitamin D category

25(OH)D concentration (nmol/L)	n	Unadjusted OR (95% CI)	P value	Adjusted* OR (95% CI)	P value
<25	4	0.45 (0.16, 1.27)	0.13	0.45 (0.15, 1.36)	0.16
25 to <50	67	1.26 (0.86, 1.85)	0.23	1.39 (0.93, 2.06)	0.21
50 to <75	53	1.00		1.00	
≥75	29	2.82 (1.39, 3.75)	<0.001	2.21 (1.33, 3.68)	0.002

*Adjusted for gender, ethnicity, maternal education level, skin colour, vitamin D supplement and toddler milk usage, daycare attendance
OR odds ratio, CI confidence interval

Table 4.22: Odds ratio of parental report of food allergy by dried blood spot 25-hydroxyvitamin D concentration

25(OH)D concentration (nmol/L)	n	Unadjusted OR (95% CI)	P value	Adjusted* OR (95% CI)	P value
<25	8	0.44 (0.21, 0.94)	0.04	0.41 (0.18, 0.93)	0.04
25 to <50	103	0.98 (0.72, 1.33)	0.89	1.03 (0.75, 1.41)	0.89
50 to <75	102	1.00		1.00	
≥75	36	1.41 (0.91, 2.17)	0.12	1.35 (0.87, 2.10)	0.18

*Adjusted for gender, ethnicity, maternal education level, skin colour, vitamin D supplement and toddler milk usage, daycare attendance
OR odds ratio, CI confidence interval

Table 4.23. Prevalence of parent reported food allergy, doctor diagnosed food allergy, EpiPen ownership and food related visits to hospital with season of birth and region

	Parent reported food allergy n(%)	No parent reported food allergy n(%)	P value*	Doctor diagnosed food allergy n(%)	No doctor diagnosed food allergy n(%)	P value*
Season of birth			0.10			0.19
Summer	51 (17)	248 (83)		30 (10)	269 (90)	
Autumn	68 (23)	233 (77)		40 (13)	261 (87)	
Winter	65 (20)	254 (80)		44 (14)	275 (86)	
Spring	65 (16)	345 (84)		39 (10)	271 (90)	
Region			0.34			0.35
North	112 (17)	539 (83)		71 (11)	581 (89)	
Central	74 (20)	304 (80)		51 (13)	327 (87)	
South	63 (21)	237 (79)		31 (10)	269 (90)	

*P values from chi squared test

4.3.3 Vitamin D and allergic rhinoconjunctivitis

Participant characteristics and mean dried blood spot 25(OH)D concentrations stratified for prevalence of allergic rhinoconjunctivitis are presented in Appendix 8.

Ten percent of the children had allergic rhinoconjunctivitis. There were seasonal differences in the frequency of nose problems associated with allergic rhinoconjunctivitis (P<0.001), with higher prevalence reported in winter (11%) and spring (10%) compared to summer (6%) and autumn (8%) (Table 4.24).

No difference in mean 25(OH)D concentration was found between children with and without allergic rhinoconjunctivitis (52 vs 52nmol/L, P=0.98) or for comparisons of those with and without allergic rhinoconjunctivitis within each season; summer (P=0.52), autumn (P=0.96), winter (P=0.21) and spring (P=0.68) (Table 4.24).

Although there was no linear trend across 25(OH)D categories, there was an increased prevalence of allergic rhinoconjunctivitis found in the lowest and highest 25(OH)D categories (Table 4.25).

In unadjusted analysis, children with low concentration of 25(OH)D (<25nmol/L) were found to have increased risk of allergic rhinoconjunctivitis approaching significance (OR 1.91, 95%CI 1.01, 3.63, P=0.05). However, this association was not present in the multivariable logistic regression analysis (Table 4.26). There was no association found between the risk of allergic rhinoconjunctivitis and dried blood spot 25(OH)D concentration in either unadjusted or multivariable analysis (Table 4.26).

Table 4.24. Mean dried blood spot 25-hydroxyvitamin D concentration for children with and without allergic rhinoconjunctivitis

Allergic rhinoconjunctivitis category	**n (%)**	**Mean 25(OH)D (S.D.) (nmol/L)**	**P value***
Allergic rhinoconjunctivitis			
Present	139 (10)	52 (23)	0.98
Absent	1190 (90)	52 (19)	
Problem with sneezing, runny nose without cold/flu last 12 months?			
Present	278 (21)	52 (20)	0.86
Absent	1051 (79)	52 (19)-	
Nose problem with itchy watery eyes in last 12 months			
Present	142 (11)	52 (22)	0.96
Absent	1187 (89)	52 (19)	
Season of nose problems			
			0.001**
Summer			
Present	81 (6)	53 (24)	0.52
Absent	1248 (94)	52 (19)	
Autumn			
Present	109 (8)	52 (19)	0.96
Absent	1120 (92)	52 (19)	
Winter			
Present	150 (11)	50 (20)	0.21
Absent	1179 (89)	52 (19)	
Spring			
Present	139 (10)	53 (19)	0.68
Absent	1190 (90)	52 (19)	

S.D. standard deviation
*P values from Independent t-test
**P value from chi squared test

Table 4.25. Prevalence of allergic rhinoconjunctivitis by dried blood spot 25-hydroxyvitamin D concentration

Parental report of allergic rhinoconjunctivitis	**25(OH) D concentration in nmol/L n(%)**				**P value***
	<25	25 to <50	50 to <75	≥75	
					0.17
Present	14 (16)	56 (10)	50 (9)	19 (13)	
Absent	72 (84)	500 (90)	491 (91)	127 (87)	

*P value from chi squared test

Table 4.26. Odds ratio of allergic rhinoconjunctivitis by dried blood spot 25-hydroxyvitamin D category

25(OH)D concentration (nmol/L)	n	Unadjusted OR (95% CI)	P value	Adjusted* OR (95% CI)	P value
<25	14	1.91 (1.01, 3.63)	0.05	1.82 (0.89, 3.72)	0.10
25 to <50	56	1.10 (0.74, 1.64)	0.64	1.14 (0.75, 1.72)	0.54
50 to <75	50	1.00	--	1.00	
≥75	19	1.47, (0.84, 2.58)	0.18	1.45 (0.82, 2.58)	0.20

*Adjusted for gender, ethnicity, maternal education level, skin colour, vitamin D supplement and toddler milk usage, daycare attendance
OR odds ratio, CI confidence interval

4.3.4 Vitamin D and asthma

Participant characteristics and mean dried blood spot 25(OH)D concentrations for children stratified by asthma are presented in Appendix 8.

The prevalence of reported asthma in the children the last 12 months was 32% (420/1329). When limited to those children in whom eczema or food allergy were also reported, atopic asthma prevalence was 12% (162/1329) (Table 4.27).

There were no differences found in mean dried blood spot 25(OH)D concentrations for children with asthma and atopic asthma compared to those without (any asthma: 52 vs 52nmol/L, P=0.94) and (atopic asthma: 54 vs 52nmol/L, P=0.25) (Table 4.27). There was no relationship seen between both the prevalence of asthma or atopic asthma and dried blood spot 25(OH)D concentration category (P=0.98 and P=0.22 respectively) (Table 4.28). In both unadjusted and multivariable logistic regression analysis, there was no association between dried blood spot 25(OH)D concentration and presence of asthma, or atopic asthma (Tables 4.29 & 4.30).

In the subset of children with atopic asthma, there was no significant differences in the mean dried blood spot 25(OH)D concentrations and the reported use of either inhaled or oral asthma medications (Table 4.31).

Table 4.27. Mean dried blood spot 25-hydroxyvitamin D concentration for children with and without asthma and atopic asthma

Parental report of asthma and atopic asthma	**n (%)**	**Mean 25(OH)D (S.D.) (nmol/L)**	**P value ***
Asthma			
Present	420 (32)	52 (19)	0.94
Absent	909 (68)	52 (20)	
Atopic asthma			
Present	162 (12)	54 (19)	0.25
Absent	1167 (88)	52 (19)	

S.D. standard deviation
*P values from Independent t-tests

Table 4.28. Prevalence of asthma and atopic asthma by dried blood spot 25-hydroxyvitamin D concentration

Parental report of asthma and atopic asthma	**25(OH)D concentration in nmol/L n (%)**				**P value***
	<25	25 to <50	50 to <75	≥75	
Asthma					
					0.17
Present	25 (29)	184 (33)	162 (30)	49 (34)	
Absent	61 (71)	372 (67)	379 (70)	97 (66)	
Atopic asthma					
					0.22
Present	7 (8)	73 (13)	59 (11)	23 (16)	
Absent	79 (92)	483 (87)	483 (89)	123 (84)	

*P values from chi squared test

Table 4.29. Odds ratio of asthma by dried blood spot 25-hydroxyvitamin D category

25(OH)D concentration (nmol/L)	**n**	**Unadjusted OR (95% CI)**	**P value**	**Adjusted OR (95% CI)**	**P value**
<25	25	0.96 (0.58, 1.58)	0.87	0.81 (0.46, 1.41)	0.45
25 to <50	184	1.16 (0.90, 1.49)	0.26	1.19 (0.91, 1.55)	0.21
50 to <75	162	1.00		1.00	
≥75	49	1.18 (0.80, 1.75)	0.40	1.13 (0.76, 1.69)	0.55

*Adjusted for gender, ethnicity, maternal education level, skin colour, vitamin D supplement and toddler milk usage, daycare attendance
OR odds ratio, CI confidence interval

Table 4.30. Odds ratio of atopic asthma by dried blood spot 25-hydroxyvitamin D category

25(OH)D concentration (nmol/L)	n	Unadjusted OR (95% CI)	P value	Adjusted OR (95% CI)	P value
<25	7	0.72 (0.32, 1.64)	0.44	0.56 (0.22, 1.42)	0.22
25 to <50	73	1.24 (0.86, 1.78)	0.26	1.31 (0.89, 1.92)	0.17
50 to <75	59	1.00		1.00	
≥75	23	1.53 (0.91, 2.57)	0.11	1.43 (0.83, 2.44)	0.20

*Adjusted for gender, ethnicity, maternal education level, skin colour, vitamin D supplement and toddler milk usage, daycare attendance
OR odds ratio, CI confidence interval

Table 4.31. Mean dried blood spot 25-hydroxyvitamin D concentration of children with atopic asthma (n=162) and their use of asthma medication

Medication used by children with atopic asthma (n=162)	n (%)	Mean 25(OH)D (S.D.) (nmol/L)	P value*
Beta antagonist inhaled			
Used	100 (62)	53 (20)	0.86
Not used	62 (38)	54 (19)	
Beta antagonist oral			
Used	8 (5)	67 (15)	0.05
Not used	154 (95)	53 (19)	
Inhaled steroid			
Used	54 (33)	57 (19)	0.16
Not used	108 (67)	52 (19)	
Oral steroid			
Used	16(10)	50 (18)	0.48
Not used	146 (90)	54 (20)	
Other asthma medication			
Used	3 (2)	38 (7)	0.16
Not used	159 (98)	54 (19)	

S.D. standard deviation
*P values from Independent t-test

4.3.5 Vitamin D and respiratory infections

4.3.5.1 Any respiratory infection respiratory infection (ARI)

Ninety-nine percent of the children (1318/1329) were reported to have had any respiratory infection in the last 12 months. Mean dried blood spot 25(OH)D concentrations did not differ in comparisons between children with and without reported ARI (52 vs 46nmol/L, P=0.29) (Table 4.32).

Table 4.32. Mean dried blood spot 25-hydroxyvitamin D concentration for children with any respiratory infection

Parental report of any respiratory infection in last 12 months	**n (%)**	**Mean 25(OH)D (S.D.) (nmol/L)**	**P value ***
Any respiratory infection	1318 (99)	52 (19)	0.29
No respiratory infection	11 (1)	46 (16)	

S.D. standard deviation
*P value from Independent t-test

4.3.5.2 Lower respiratory infection (LRI)

Thirteen percent of the children (169/1329) were reported to have had a LRI in the last 12 months. No child was reported to have had tuberculosis in the last 12 months. (Table 4.33).

Mean dried blood spot 25(OH)D concentrations did not differ in comparisons between children with and without reported LRI (52 vs 52nmol/L, P=0.72), pneumonia (53 vs 52nmol/L, P=0.86), bronchitis (52 vs 52nmol/L, P=0.95) or bronchiolitis (51 vs 52nmol/L, P=0.50) (Table 4.33). There was no association between the proportion of children with reported LRI and dried blood spot 25(OH)D concentration category (P=0.98) (Table 4.34).

In both unadjusted and multivariable logistic regression analysis, there was no association between 25(OH)D concentration and prevalence of LRI (Table 4.35).

Table 4.33. Mean dried blood spot 25-hydroxyvitamin D concentration for children with and without lower respiratory infection

Parental report of lower respiratory infection in last 12 months	**n (%)**	**Mean 25(OH)D (S.D.) (nmol/L)**	**P value ***
Any lower respiratory infection			
Present	169 (13)	52 (19)	0.72
Absent	1160 (87)	52 (18)	
Pneumonia			
Present	32 (2)	53 (20)	0.86
Absent	1297 (98)	52 (19)	
Bronchitis			
Present	60 (5)	52 (17)	0.95
Absent	1269 (95)	52 (19)	
Bronchiolitis			
Present	98 (7)	51 (17)	0.50
Absent	1231 (83)	52 (19)	

S.D. standard deviation
*P values from Independent t-test

Table 4.34. Prevalence of lower respiratory infection in last 12 months by dried blood spot 25-hydroxyvitamin D concentration

Parental report of lower respiratory infection in last 12 months	**25(OH)D concentration in nmol/L n(%)**				**P value***
	<25	25 to <50	50 to <75	≥75	
					0.98
Present	12 (14)	71 (13)	67 (13)	19 (13)	
Absent	74 (86)	485 (87)	474 (87)	127 (87)	

*P value from chi squared test

Table 4.35. Odds ratio of lower respiratory infection in last 12 months by dried blood spot 25-hydroxyvitamin D concentration

25(OH)D concentration (nmol/L)	n	Unadjusted OR (95% CI)	P value	Adjusted* OR (95% CI)	P value
<25	12	1.15 (0.60, 2.22)	0.68	1.15 (0.54, 2.32)	0.77
25 to <50	71	1.04 (0.73, 1.48)	0.85	1.05 (0.73, 1.52)	0.80
50 to <75	67	1.00		1.00	
≥75	19	1.06 (0.61, 1.83)	0.84	1.03 (0.59, 1.80)	0.91

*Adjusted for gender, ethnicity, maternal education level, skin colour, vitamin D supplement and toddler milk usage, daycare attendance
OR odds ratio, CI confidence interval

4.3.5.3 Upper respiratory infection (URI)

Ninety-eight percent of the children (1302/1329) were reported to have had a URI in the last 12 months. Participant characteristics and mean dried blood spot 25(OH)D concentrations stratified by upper respiratory infection are presented in Appendix 8.

Mean dried blood spot 25(OH)D concentrations did not differ in comparisons between children with parental report of lower and higher frequency of URI (common cold) over the last year (51 vs 52nmol/L, P=0.28) (Table 4.36). There was no association between the proportion of children with reported URI (common cold) and dried blood spot 25(OH)D concentration category (P=0.11) (Table 4.37).

In unadjusted analysis, in comparison with children with a 25(OH)D concentration of 50 to <75nmol/L, there was an increased odds of more frequent URI (common cold) in children with 25(OH)D concentrations ≥75nmol/L (OR1.51, 95%CI 1.03-2.21). In the multivariable analysis this association became non-significant (Table 4.38).

Table 4.36. Mean dried blood spot 25-hydroxyvitamin D concentration for children with upper respiratory infection

Parental report of number of common colds in last 12 months	**n (%)**	**Mean 25(OH)D (S.D.) (nmol/L)**	**P value ***
Number of URI (common cold) in last 12 months			
			0.38
0	27 (2)	52 (15)	
1-2 times	535 (40)	51 (19)	
3-4 times	519 (39)	53 (19)	
>5 times	248 (19)	51 (19)	
Combined frequency of URI (common cold) in last 12 months			
0 to 2	562 (42)	51 (19)	0.28
3 or more	767 (58)	52 (19)	

S.D. standard deviation
*P values from Independent t-test

Table 4.37. Prevalence of parental report of higher frequency of URI (common cold) over last 12 months by dried blood spot 25-hydroxyvitamin D concentration

Parental report of upper respiratory infection (common cold)	**25(OH)D concentration in nmol/L n(%)**				**P value***
	<25	25 to <50	50 to <75	≥75	
					0.11
0 to 2	41 (43)	228 (41)	242 (45)	51 (35)	
3 or more	45 (57)	328 (59)	299 (55)	95 (65)	

*P value from chi squared test

Table 4.38. Odds ratio of a higher frequency of URI (common cold) over last 12 months by dried blood spot 25-hydroxyvitamin D concentration

Dried blood spot 25(OH)D concentration in nmol/L	**n**	**Unadjusted OR (95% CI)**	**P value**	**Adjusted OR (95% CI)**	**P value**
<25	41	0.89 (0.56, 1.40)	0.61	1.01 (0.62, 1.65)	0.98
25-49.9	228	1.16 (0.92, 0.48)	0.21	1.24 (0.97, 1.59)	0.09
50-74.9	242	1.00		1.00	
≥75	51	1.51 (1.03, 2.21)	0.03	1.46 (0.99, 2.14)	0.06

*Adjusted for gender, ethnicity, maternal education level, skin colour, vitamin D supplement and toddler milk usage, daycare attendance
OR odds ratio, CI confidence interval

Chapter 5: Discussion

This thesis described the vitamin D status, investigated predictors of vitamin D deficiency in New Zealand preschool children, sought to develop a predictive questionnaire that could be used to assess the risk of vitamin D deficiency being present, and also investigated the relationship between dried blood spot 25(OH)D concentration and allergic and respiratory diseases in this age group. This discussion chapter will provide a summary of the main findings, followed by a detailed discussion of vitamin D status and predictors of vitamin D deficiency, the development of a predictive questionnaire for vitamin D deficiency and the relationship of vitamin D with allergic diseases – eczema, food allergy, allergic rhinoconjunctivitis and asthma - and with respiratory infections. Strengths and limitations of the study will be discussed in two parts, firstly general considerations and secondly, considerations of the relationship between vitamin D status and allergic and respiratory diseases.

5.1 Statement of the main study findings

To date, the vitamin D status of preschool children in New Zealand has not been determined. The use of capillary blood sampling methods facilitated the measurement of 25(OH)D concentrations of a large number of young children from throughout New Zealand.

In this study, the mean dried blood spot 25(OH)D concentration of children aged 2 to <5 years was 52nmol/L as measured in late winter. The prevalence of vitamin D deficiency (25[OH]D <25nmol/L) was 7% and for vitamin D insufficiency (25[OH]D <50nmol/L) was 48%.

Predictors of vitamin D deficiency in New Zealand preschool children have not been previously investigated. None of the children who drank toddler milk were vitamin D deficient. The predictors of vitamin D deficiency in children who did not drink toddler milk were female gender, being of non-European (not Maori or Pacific) ethnicity, having a mother with less than secondary school education level, darker skin colouring, living in a more deprived household and not taking a vitamin D supplement. Predictors of vitamin D insufficiency were female gender, being of Pacific and other non-European ethnicity, not attending a formal daycare centre, not taking a vitamin D supplement and not drinking toddler milk.

There is little literature on the effect of daycare centre attendance on vitamin D status. These centres have formal sun protection policies. In this study, the mean 25(OH)D concentrations of children who attended daycare was higher than those who did not. In addition, there was no

linear gradient seen between the number of hours spent in daycare and vitamin D status, while not attending daycare was an independent predictor of vitamin D insufficiency.

A limited number of predictive questionnaires have been published for assessment of vitamin D deficiency in other countries and population groups. The identified predictors of vitamin D deficiency above were used to develop and validate a predictive questionnaire to assess the risk of vitamin D deficiency (25[OH]D <25nmol/L) in preschool aged children in winter. Overall predictability of this questionnaire was favourable (AUC 0.76) and specificity was high (95%), however sensitivity was poor (42%). As a result of this low sensitivity, the first of the study hypotheses was rejected. A second questionnaire was developed to assess risk of vitamin D insufficiency (25[OH]D <50nmol/L), which had a lower overall predictability (AUC 0.63), with a sensitivity of 54% and specificity of 66%.

The literature review highlighted a lack of research in this age group about the relationship between vitamin D status and allergic and respiratory diseases in preschool children aged between 2 to <5 years, both worldwide and in New Zealand, especially of studies which have directly measured the vitamin D status of participants in relation to the presence of each of these diseases. The results of this current study found a lack of association of vitamin D status with presence of eczema, allergic rhinoconjunctivitis, asthma and respiratory infection in the preschool children, while there was a two-fold increase in risk of parental report, doctor diagnosed food allergy in children with 25(OH)D concentrations ≥75nmol/L. Hence, the second of the study hypotheses was rejected.

5.2 Vitamin D status and predictors of vitamin D deficiency in preschool children

This is the first large scale study in New Zealand to measure the vitamin D status of preschool aged children. Vitamin D status has been described in various population groups in New Zealand including newborns (Camargo *et al.*, 2010), breastfed infants aged 2 to 3 months (Wall *et al.*, 2013), infants aged 6 to 23 months in Auckland and Dunedin (Grant *et al.*, 2009; Houghton *et al.*, 2010), school children aged 5 to 14 years (Rockell *et al.*, 2005) and from adults (NZ National Nutrition Surveys of 1997/8 and 2008/9) (Rockell *et al.*, 2006; Ministry of Health, 2012). The mean serum 25(OH)D concentrations for each age group were 44nmol/L (newborn) (Camargo *et al.*, 2010), 53nmol/L (breastfed infants) (Wall *et al.*, 2013), 55nmol/L (infants, Auckland) (Grant *et al.*, 2009), 52nmol/L (infants, Dunedin) (Houghton *et al.*, 2010), 50nmol/L (school children) (Rockell *et*

al., 2005) and in adults 50nmol/L (1997/8) (Rockell *et al.*, 2006) and 53nmol/L (2008/9) (Ministry of Health, 2012).

The dried blood spot mean 25(OH)D concentration (52nmol/L) found in the current study is similar to those of other age groups of children in New Zealand. The winter mean serum 25(OH)D concentrations of infants and schoolchildren ranged from 39 to 47nmol/L (Rockell *et al.*, 2005; Grant *et al.*, 2009; Houghton *et al.*, 2010). However, the prevalence of vitamin D deficiency in the current study (7%) is lower than that seen in these previous studies of 10-16% (25[OH]D <27.5nmol/L) in infants (Grant *et al.*, 2009; Houghton *et al.*, 2010) and higher than that reported for schoolchildren (4%), however the 25(OH)D concentration cut-off used to define deficiency in this study (25[OH]D <17.5nmol/L) was lower (Rockell *et al.*, 2005).

The estimates of vitamin D deficiency prevalence reported in these previous studies are from year-round sampling whereas the prevalence of deficiency estimated in the current study was from blood samples obtained only in the late winter months. At a similar latitude to Dunedin (43^{o}S), 15% of Italian preschool children were found to have vitamin D deficiency (25[OH]D <25nmol/L) in winter compared to 5% in summer months (Vierucci *et al.*, 2013). A study of preschool children in Adelaide, Australia, reported 4% of the children had 25(OH)D concentrations below 30nmol/L in winter, and 0% in the summer season (Zhou *et al.*, 2014). At 35^{o}S, Adelaide has an equivalent latitude to Northland, which is at a more equatorial latitude than the towns where children were recruited from in this study. Studies from New Zealand have shown the seasonal variation in 25(OH)D concentrations in adults is between 10-20nmol/L (Bolland *et al.*, 2012), with differences between winter and summer means varying from 24-35nmol/L in infants (Grant *et al.*, 2009; Houghton *et al.*, 2010) and 15nmol/L in school aged children (Rockell *et al.*, 2005). Overseas studies involving preschool children report differences between summer and winter 25(OH)D means of 10-15nmol/L in Canada (Newhook *et al.*, 2009; El Hayek *et al.*, 2010; El Hayek *et al.*, 2013), 20-33nmol/L in Great Britain (Absoud *et al.*, 2011), Ireland and the United States (Stein *et al.*, 2006), 28nmol/L in Australia (Zhou *et al.*, 2014) and 35-40nmol/L in Italy (Vierucci *et al.*, 2013; Mazzoleni *et al.*, 2014). The mean 25(OH)D concentrations of the current study cohort would be expected to be higher, and the prevalence of deficiency lower, in the summer and autumn months and on a year-round average compared to the late winter/early spring measurements of this study. The lower prevalence rates in winter in the current study may also be due to the children in this cohort not being representative of the preschool population due to the sampling biases that occurred as a result of the sampling methodology (discussed in section 5.5.1).

The risk factors for vitamin D deficiency in this age group are consistent with those found in previous studies of New Zealand children; female gender (Rockell *et al.*, 2005), non-European ethnicity (Rockell *et al.*, 2005; Grant *et al.*, 2009; Houghton *et al.*, 2010; Ministry of Health, 2012), low maternal education level (Houghton *et al.*, 2010), lower socioeconomic status (Grant *et al.*, 2009), not taking supplements containing vitamin D (Grant *et al.*, 2009) and not drinking infant formula or toddler milk (Grant *et al.*, 2009). Skin colour was not a risk factor for vitamin D deficiency in New Zealand infants (vitamin D deficiency defined as 25[OH]D <27.5nmol/L) or in school children (vitamin D deficiency defined as 25[OH]D <17.5nmol/L) (Rockell *et al.*, 2005; Grant *et al.*, 2009; Houghton *et al.*, 2010). This is in contrast to the finding of this current study where those children with darker skin colouring were at increased risk of vitamin D deficiency. Wide variations in skin colour of Maori and Pacific children were noted in a national survey of schoolchildren, with the authors concluding that these differences most likely accounted for the ethnic differences in 25(OH)D concentrations (Rockell *et al.*, 2005). Being of Maori and Pacific heritage was not an independent risk factor for vitamin D deficiency in the multivariable model.

Consistent with the findings of the current study, studies of preschool aged children from Australia (Zhou *et al.*, 2014), Canada (El Hayek *et al.*, 2013), Ireland (Carroll *et al.*, 2014) and New Haven, US (Carpenter *et al.*, 2012), and also New Zealand infants (Grant *et al.*, 2009; Houghton *et al.*, 2010), found no association of BMI with vitamin D status. In contrast, obesity was associated with lower 25(OH)D concentrations in the Children's Nutrition Survey of 5 to 14 year old New Zealand children (Rockell *et al.*, 2005). It is likely the inclusion of adolescents in this national survey, and the low rate of obesity in the preschool group studied compared with that present in the population-representative national survey, contributed to the lack of association with BMI in the current study.

No latitudinal gradient was evident in the comparison of dried blood spot 25(OH)D concentrations from samples obtained in the northern versus southern regions of New Zealand, a finding that was consistent with that observed in the national surveys for children and adults (Ministry of Health, 2003; Ministry of Health, 2012). This lack of gradient may be contributed by the late winter - early spring collection period where nadir levels may be more uniform and more likely to be equal throughout the country. This lack of association of vitamin D status with latitude in New Zealand has positive implications for policymakers, as it helps to simplify any future public health recommendations being made on a nationwide basis.

Children drinking non-vitamin D fortified milk at home had lower mean 25(OH)D concentrations than those who consumed fortified milk (51 vs 57nmol/L, P<0.001), however, this was not found to be a predictor of vitamin D deficiency in the multivariable model. This is an interesting finding given that vitamin D is only permitted to be added to reduced fat milks in New Zealand, bringing the vitamin D level up to match that of full fat milk (Food Standards Australia New Zealand, 2014). This may suggest that overage in the fortified milks is contributing to the higher 25(OH)D concentration of children consuming fortified milks in this sample group and should be considered in future research investigating strategies to increase vitamin D status. Milk intake has been shown to be the major source of vitamin D for preschool children in countries with a fortified milk supply, with consumption positively associated with 25(OH)D concentrations (El Hayek *et al.*, 2010; Whiting *et al.*, 2011). With the small stature and body size of preschool children the contribution of dietary vitamin D may be more significant than in other age groups, particularly during winter months. Previous research in children and adults suggests lower dietary vitamin D intakes were associated with poorer vitamin D status during winter (Davies *et al.*, 1999; Pasco *et al.*, 2001; Roth *et al.*, 2005). The higher 25(OH)D concentrations of children consuming fortified milk in the current study suggests a potentially simple vehicle for improving vitamin D status throughout the preschool years via fortification of this staple food. However, consumption patterns of milk have changed over the past decade, with parents in this study reporting 6% of children do not drink milk, and 9% drink non-dairy milk options (soy, almond, oat and rice milks). There is little data available on the dietary intake of vitamin D in young children in New Zealand, particularly for this dietary staple and potential source of vitamin D (Nowson *et al.*, 2012). Future research efforts into the contribution of vitamin D from milk and other dietary sources to the vitamin D status of preschool children, along with the potential for fortification of milk to improve vitamin D levels, are warranted.

Discovery of an association between vitamin D status and consumption of toddler milk in these preschool children was an unexpected finding. When the questionnaire was designed, we assumed that children over the age of 2 years would not be still drinking formulated milk products (toddler milk or infant formula), as in New Zealand, toddler milks are designed for children aged 1 to 2 years of age (Watties, 2014). Thus, an individual option for this dietary milk was not provided in the list of milks and the 80 children (6%) who drank toddler milk were identified through a parent providing information in the free-text panel. Not having toddler milk (or infant formula) as an option in the list of milks thus became a limitation in our data collection instrument. It is assumed that parents who continue to give their child a formulated milk after 2 years of age will do so for specific reasons (e.g. parental concern about food allergy or fussy eating patterns or the

child's nutritional status) and may be conscious enough to write this down in a free-form text option. The not-insignificant proportion providing this free text response may also be reflective of a study sample being of higher socio-economic status and hence able to afford the more expensive formula compared with standard milks. Our study was unable to determine if the absence of vitamin D deficiency among those drinking toddler milk was due to the consumption of this milk or due to the other characteristics of toddler milk drinkers that were protective against vitamin D deficiency. Future studies should explore the relationship further between consumption patterns of toddler milk in the preschool population and 25(OH)D concentrations as this may be a potential strategy for preventing vitamin D deficiency in this age group.

A second unexpected finding of this study was that the mean dried blood spot 25(OH)D concentration was higher in those children who attended formal daycare than those who did not (53 vs 49nmol/L, P=0.01). With strict sun protection policies in formal preschool establishments, it was hypothesised that hours of attendance of formal daycare would be inversely associated with 25(OH)D concentrations. It may be that those children who do not attend childcare have more indoor lifestyles with caregivers who are more vigilant in sun protection, with both of these factors having a negative impact on 25(OH)D concentrations. No association was found between the number of hours of daycare attendance by the children and mean 25(OH)D concentrations. This lack of association may be due to variation in adherence to the sun protection policies between centres and between different centre staff. Future public health policies in the area of vitamin D should include consideration of young children attending daycare centres, and perhaps daycare specific policies around sun protection.

With New Zealand having the highest rates of melanoma in the world there is a perception that parents of preschool children can be strident in ensuring their children are protected from the sun (Ferlay *et al.*, 2010b). The very small proportion (2%) of children in this study whose parents reported their child never used sunscreen in summer is consistent with this. These children had a lower mean 25(OH)D concentration compared to those who used sunscreen (41 vs 52nmol/L, P<0.001). It may be that parents of these children are aware, due to their child's skin pigmentation, that they do not burn with prolonged sun exposure and therefore choose not to use sunscreen. In less affluent households, the purchase and use of sunscreen may be limited by cost of these products. The finding of higher 25(OH)D concentrations associated with sunscreen usage is consistent with previous studies in both adults (Marks *et al.*, 1995) and children (Nicolaidou *et al.*, 2006; Carroll *et al.*, 2014). This may be due to inadequate amounts of sunscreen applied, the children who use sun protection having less skin pigmentation and/or being outdoors

more and exposed to the greater amounts of sun, or the higher surface area of young children facilitating higher cutaneous synthesis of vitamin D (Riggs, 2003; Munns *et al.*, 2006).

No New Zealand studies of infants or school children have reported the prevalence of vitamin D insufficiency (25[OH]D <50nmol/L) to date. Almost half of the children in this study had a 25(OH)D concentration <50nmol/L. The Institute of Medicine and New Zealand guidelines recommend an annual average 25(OH)D concentration above 50nmol/L for health (Diamond *et al.*, 2005; Institute of Medicine (US), 2010; Ministry of Health and Cancer Society of New Zealand, 2012). The implication of low 25(OH)D concentrations during winter is unknown (Holick, 2007) and the definition of optimal levels, in terms of human health as well as disease prevention, continues to be debated (Holick, 2009; Heaney and Holick, 2011). There is currently no vitamin D preparation suitable for young children in New Zealand and supplementation is only recommended only for those at risk of deficiency, which raises questions as to how best to increase 25(OH)D concentrations in children who are vitamin D insufficient or deficient. These are important issues for further research and policy development.

5.3 Prediction of vitamin D deficiency in preschool children

There are potential benefits of prediction questionnaires to assess risk of vitamin D deficiency. These include: firstly, providing a non-invasive method for determining the risk of vitamin D deficiency and hence eliminating the need for blood sampling in preschool aged children who find this procedure particularly unpleasant; secondly, the avoidance of unnecessary supplementation through both identification of those children not at risk and; thirdly, healthcare cost savings through reduced use of diagnostic blood tests and improved public health outcomes by the timely identification and treatment of those who are not vitamin D sufficient.

5.3.1 Prediction of 25(OH)D concentrations <25nmol/L

To our knowledge, no prediction tool has been developed for assessment of preschool children at risk of vitamin D deficiency. Comparison with previously published predictive questionnaires is complicated by the differing definitions of vitamin D deficiency or insufficiency used. No other study has developed a prediction tool for 25(OH)D <25nmol/L, with the closest being the study by Sohl *et al*, predicting a range of 25(OH)D concentrations, the lowest being 25(OH)D <30nmol/L, in older adults from the Netherlands Longitudinal Ageing Study (Sohl *et al.*, 2014). The performance

of the questionnaire developed in the current study, as measured by area under the curve (AUC) (AUC 0.76, P<0.001) is similar to that reported by Sohl *et al* (AUC 0.80, P<0.001) (Sohl *et al.*, 2014). The sensitivity of the questionnaire developed by Sohl *et al* is higher than that of the current study (61% vs 42%), but specificity lower (84% vs 97%). The positive predictive value (PPV) of the questionnaire developed in the current study is higher than that reported by Sohl *et al*, 52% vs 37%, while the negative predictive values (NPV) are similar, 95% vs 94% (Sohl *et al.*, 2014).

The lower sensitivity, and higher specificity, of the questionnaire developed in this study may be due to the population studied, the number of variables used in the model, and the different cutoff point chosen for vitamin D deficiency in preschool children compared to that of the older adults in the study by Sohl *et al*. The current study sample consisted of children of a diverse range of ethnicities with significant differences in mean 25(OH)D concentrations, whereas Sohl *et al* was a homogeneous group of Dutch adults. The predictive tool developed from the current study contained 5 variables compared to the 10 included in the tool developed by Sohl *et al* (Sohl *et al.*, 2014). Several of these 10 variables would not be pertinent to a preschool population (alcohol consumption, smoking, medication use and indicators of age status including loss of appetite and memory). Inclusion of season and measurement of gardening, bicycling and sporting variables are indicators of sun exposure which may contribute to a better identification by the predictive questionnaire of those with deficient 25(OH)D concentrations. The only sun exposure data collected in the current study was a very general question, asking how often children played outdoors in summer and winter seasons (0, 0.5-1 and >1 hours/day). This was not a predictor of vitamin D deficiency in this sample of children, the hours exposed to direct sunlight has been previously shown to be a predictor of vitamin D status in New Zealand adults (Scragg *et al.*, 1990).

The statistical power of the developed predictive questionnaire may have been affected by the low prevalence of vitamin D deficiency in the preschool population (7%) compared to that of Sohl *et al* (17%). The low sensitivity of the predictive questionnaire may also be due to the majority of the cases of vitamin D deficiency occurring below an estimated probability of deficiency of 0.1 (Figure 5.1). This could be described as the questionnaire attempting to predict a specific outcome (vitamin D deficiency) by using a range of very general variables (e.g. skin colour). The distribution of cases according to the cut-off score of the developed predictive questionnaire is illustrated in Figure 5.2, where the score of ≥1.86 indicates a child is at risk of vitamin D deficiency. At this cut-off, 61% of children who are deficient have already been classified by the questionnaire as non-deficient, reducing the predictive performance. In comparison, at this cut-off 91% of non-deficient children have been correctly classified as non-deficient. Future research

in groups with a higher prevalence of vitamin D deficiency may assist in development of predictive questionnaires with higher predictive power.

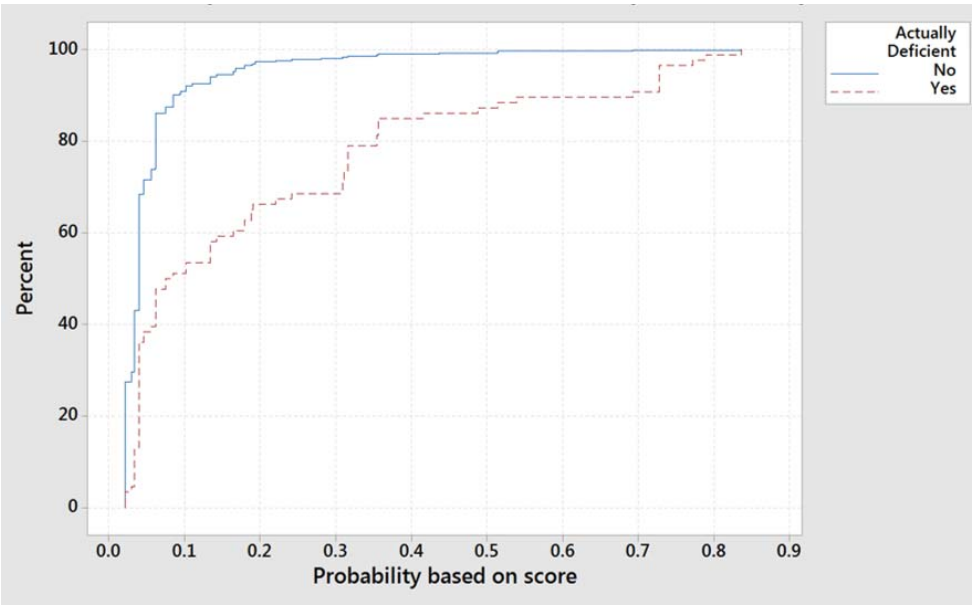


Figure 5.1. Empirical distribution function of estimated probability of vitamin D deficiency (25[OH]D <25nmol/L) in 1329 children.

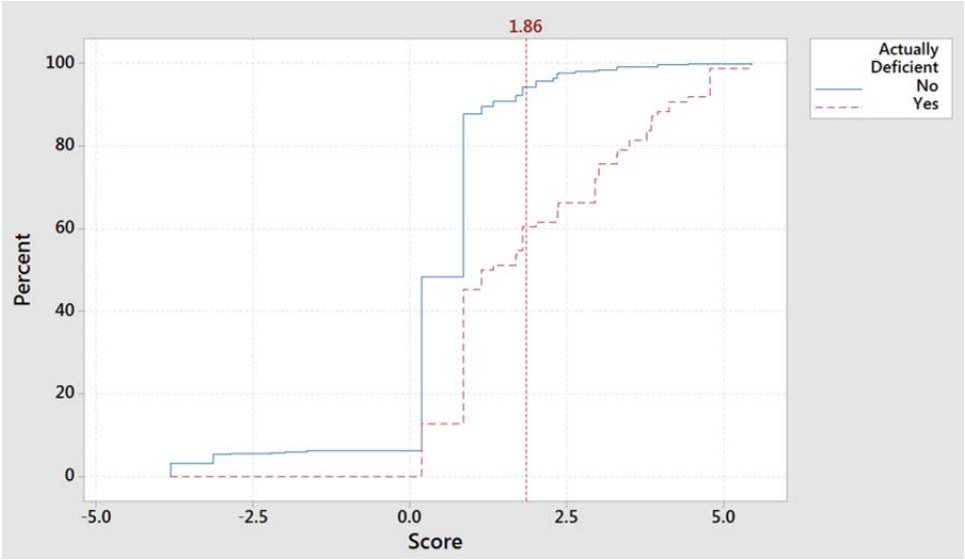


Figure 5.2. Empirical distribution function of predictive score for vitamin D deficiency (25[OH]D <25nmol/L) at cut-off of 1.86. (Logistic regression score adjusted by -4 if child drinks toddler milk).

The best balance of sensitivity and specificity was required when assigning a cut-off during the development of the predictive questionnaire. In the 'development' dataset, the cut-off of ≥1.86 was chosen (Table 4.6). It can be seen in Table 4.6 that sensitivity drops below the score of 1.86. Although PPV increases at higher scores, sensitivity (being the probability of the test result being

positive if a child is deficient) will detect more positives at a lower score and is more important than PPV in a screening tool, in order to decide whether further investigation (e.g. a blood test) is necessary.

In this study, household deprivation was a predictor of vitamin D deficiency in the multivariable logistic regression analysis. Inclusion of this variable in the logistic regression model that generated the predictive tool showed that only children living in the most deprived 10% of households were at increased risk of vitamin D deficiency. This variable was excluded from the predictive model as deprivation index information is not readily known by individuals, is complex and costly to calculate and may create issues for parents being labelled as living in an area of highest deprivation in a clinical situation where anonymity cannot be maintained. Variables reported in previous studies which are not readily available for inclusion in the predictive model of this study were disease biomarkers (e.g. for diabetes) and ambient UV exposure (Tran *et al.*, 2013). In order for predictive tools to be applicable to the clinical setting they should only include variables which can be created from readily available. It made little difference to the overall performance of the predictive questionnaire whether household deprivation was included or not.

The strength of this questionnaire is the high negative predictive value (95% prediction of non-deficient children). Although contrary to our hypothesis (that the developed questionnaire should be able to accurately predict vitamin D deficiency), the developed questionnaire may provide an initial tool to identify those children *not* at risk of vitamin D deficiency, and therefore in whom measurement of serum 25(OH)D concentrations and prescribing of vitamin D supplements is not necessary. This identification of a child being at low risk of vitamin D deficiency could potentially reduce prescription of unnecessary supplementation.

5.3.2 Prediction of 25(OH)D concentrations <50nmol/L

There is currently a lack of consensus on the optimal level of vitamin D required for optimal health. Currently in New Zealand, a 25(OH)D concentration below 25nmol/L signifies to clinicians that intervention is required (Ministry of Health and Cancer Society of New Zealand, 2012). A predictive model was also developed to investigate the risk of children with insufficient vitamin D concentrations (25[OH]D <50nmol/L), as recommended by the New Zealand guidelines and the Institute of Medicine (Ross *et al.*, 2011; Ministry of Health and Cancer Society of New Zealand, 2012). Other published questionnaires have predicted 25(OH)D concentrations <50nmol/L in

children and adolescents in Great Britain (Absoud *et al*), older adults in Brazil (Lopes *et al*) and Wisconsin, US (Nabak *et al*) and the Netherlands (Sohl *et al*) (Absoud *et al.*, 2011; Nabak *et al.*, 2013; Lopes *et al.*, 2014; Sohl *et al.*, 2014).

The performance of the questionnaire predicting vitamin D insufficiency (25[OH]D <50nmol/L) in the current study as measured by AUC (AUC 0.63) was lower than these other studies; Absoud *et al* (0.82), Lopes *et al* (0.69), Sohl *et al* (0.71), with Nabak *et al* not reporting an AUC result (Absoud *et al.*, 2011; Nabak *et al.*, 2013; Lopes *et al.*, 2014; Sohl *et al.*, 2014). The sensitivity and specificity of the current predictive questionnaire (52% and 66% respectively) were both lower compared with those achieved in the studies by Absoud *et al* (75% and 79%), Lopes *et al* (56% and 72%) and Sohl *et al* (61% and 82%), with the study by Nabak *et al* (86% and 35%) having a higher sensitivity but lower specificity. The PPV of the current study (58%) was lower than that reported by Lopes *et al* (74%) and Nabak *et al* (80%) but not Sohl *et al* (37%). The NPV (60%) was higher than Lopes *et al* (53%), Nabak *et al* (41%) but not Sohl *et al* (67%).The study by Absoud *et al* did not report PPV and NPV. Despite vitamin D insufficiency being prevalent in the current study sample, the diagnostic performance of the developed predictive questionnaire is relatively poor, which would preclude use in clinical situations.

The prevalence of vitamin D insufficiency of the current study was 48%, which lies between the prevalence reported in the other studies; Absoud *et al* (35%), Lopes *et al* (58%), Nabak *et al* (19%), Sohl *et al* (46%) (Absoud *et al.*, 2011; Nabak *et al.*, 2013; Lopes *et al.*, 2014; Sohl *et al.*, 2014).

The number of variables used in these various predictive questionnaires varies, but all have one or more indicators of sun exposure, or take into account season of testing. Neither of these indicators of vitamin D status were included in the questionnaire developed in this current study. Conducting year-round testing and measurement of sun exposure in preschool children (e.g. physical activity, screen time, UV readings) has the potential to add to the performance of this questionnaire predicting vitamin D insufficiency. Given the large proportion of children in this study with 25(OH)D concentrations that were in the insufficient range in winter, development of tools able to more accurately identify this subclinical deficiency would be appropriate.

5.4 The relationship between vitamin D and allergic diseases and respiratory infection

5.4.1 Vitamin D and eczema

In the current study, no association between vitamin D status and prevalence of eczema, and severe eczema, was found. To date, there are no other studies to have investigated 25(OH)D concentrations in relation to eczema prevalence in the age group 2 to <5 years included in this study. This lack of association is in contrast to studies in Korea and Germany. Lower mean serum 25(OH)D concentrations were reported in Hong Kong children ≤18 years old with eczema compared to children of the same age without eczema. A larger proportion of the children with eczema had vitamin D deficiency (25[OH]D <25nmol/L) (Wang *et al.*, 2014). Reduced odds of eczema was seen in German children and adolescents who had lower vitamin D concentrations (Heimbeck *et al.*, 2013).

These two studies included children of wider age ranges which limits comparability to the current study, as 25(OH)D concentrations in adolescents are known to be lower than that of young children (Gregory *et al.*, 2000; Rockell *et al.*, 2005; Mansbach *et al.*, 2009) and eczema prevalence rates are highest in young children (Illi *et al.*, 2004). The study by Wang *et al* was a case control study of children recruited from paediatric clinics with eczema diagnosed by physicians, while the study by Heimbeck *et al* was a nationally representative survey with eczema diagnosis based on report of physician diagnosed eczema ever. These diagnosis criteria are different to the parental report in the current study, further limiting direct comparison of results.

The finding of no association of 25(OH)D concentration and severe eczema is consistent with results from Lee *et al*, in Korean children and adults aged 4 months to 56 years, and Galli *et al* in Italian children aged 0.5 to 16 years, where eczema severity was not correlated with serum 25(OH)D concentrations (Lee *et al.*, 2013; Galli *et al.*, 2015). However, the finding is in contrast to other studies which have reported lower 25(OH)D concentrations in children with severe eczema compared to mild or moderate eczema (Peroni *et al.*, 2011; Wang *et al.*, 2014) and an inverse correlation between serum 25(OH)D and eczema severity (Shin *et al.*, 2014; Wang *et al.*, 2014). In all of these previous studies, the participants were hospitalised or recruited from paediatric clinics and the existing health conditions of the children may have the potential to affect vitamin D status. The physician diagnosis and clinical scoring criteria for eczema severity and wide age range

of participants in these studies limits comparability with the parental report of eczema in the current study.

5.4.2 Vitamin D and food allergy

To date, only one other study has investigated the relationship between 25(OH)D concentration and the prevalence of food allergy. In 12 month old infants of Australian born parents, those with vitamin D insufficiency (25[OH]D ≤50nmol/L) were three times as likely to have a challenge proven food allergy to peanut and/or egg (Allen *et al.*, 2013). This association was not seen for infants of parents born overseas or for children of all parents. Comparison between this and the current study is limited by factors including; different countries with cultural and ethnic variations, diagnosis of food allergy (food challenge versus parental report), specific food allergy to peanut/egg at age 12 months versus cumulative incidence for all food allergy, difference in age of infants versus children aged 2 to <5 years, and data on parental place of birth not collected for the current study.

In the current study, there was an increased risk of doctor diagnosed food allergy in children with higher dried blood spot 25(OH)D concentrations. Results from observational studies and supplementation trials suggest both higher and lower 25(OH)D concentrations may increase the risk of atopy and allergic conditions. In a birth cohort in Arizona, US, both high (25[OH]D ≥75nmol/L) and low (25[OH]D <50nmol/L) infant cord blood 25(OH)D concentrations were associated with an increased risk of skin prick test (SPT) sensitivity and increased total IgE levels at age 5 years (Rothers *et al.*, 2011). Similarly, a cross-sectional study of over 7000 British adults aged 45 years found both low and high 25(OH)D concentrations were associated with elevated IgE concentrations (25[OH]D <25nmol/L and >135nmol/L respectively) (Hyppönen *et al.*, 2009). In other prospective studies, a high intake of vitamin D in infancy was associated with increased risk of eczema and allergic sensitisation in 123 Swedish children at age 6 years (Baek *et al.*, 2013) and a higher maternal intake of vitamin D during pregnancy was associated with increased risk of eczema in offspring of 596 UK mothers when the children were aged 9 months (Gale *et al.*, 2007). Asthma, allergic rhinitis and atopy defined by skin prick test were found to be more prevalent at age 31 in those participants of the Northern Finland Birth Cohort who received regular vitamin D supplementation during the first year of life (Hypponen *et al.*, 2004). In a Swedish birth cohort study, supplementation of infants with vitamins A & D during the first year of life was associated

with almost 2-fold increase risk of asthma, food hypersensitivity and allergic sensitisation at age 4 (Kull *et al.*, 2006).

A "U" shaped relationship has been suggested to exist between vitamin D status and allergic diseases with both higher and lower 25(OH)D concentrations associated with an increased risk (Rothers *et al.*, 2011). Rothers *et al* suggest these findings may be due to two conflicting vitamin D dependent mechanisms operating concurrently in the immune system; increased levels of 25(OH)D may cause a proliferation of Th_2 lymphocytes while simultaneously generating anti-inflammatory effects in lung tissues (Rothers *et al.*, 2011). *In vitro* studies by Boonstra *et al* report Th_2 cells were augmented through action of $1,25(OH)_2D$ and increased production of anti-inflammatory cytokines IL-4, IL-5 and IL-10 (Boonstra *et al.*, 2001).

The current study had limited capacity to determine whether such "U" shaped relationship was evident due to the small number of children (n=29) with food allergy and 25(OH)D concentrations ≥75nmol/L. However, the association we observed would be consistent with those reported form the birth cohort studies described above in which vitamin D may increase the risk of allergic diseases.

There was a decreased odds of parental report food allergy for children with dried blood spot 25(OH)D concentrations <25nmol/L (P=0.02) and a non-significant trend for the odds of doctor diagnosed food allergy to also be decreased in those with 25(OH)D <25nmol/L. The small numbers of children in these categories (n=8 and n=4 respectively) limit the potential to interpret this data. We are not aware of any previous studies which have reported a reduced risk of food allergy in association with lower serum 25(OH)D concentrations. Because of both the small numbers and the relative lack of robustness in diagnosis of food allergy, this interesting observation should be investigated further in a larger, more robust study.

Previous studies have shown parental or self-report of adverse reactions to food overestimate the rate of food allergy as determined by food challenge (Bock, 1987; Venter *et al.*, 2006). Our findings are consistent with this, with 19% of parents reporting their child had ever had food allergy, compared with 12% who reported a doctor had diagnosed their child's food allergy. The increased odds of doctor diagnosed food allergy in children with 25(OH)D concentrations ≥75nmol/L was not seen in children with parental report food allergy. This may be due to the parental report of food allergy including non-IgE mediated reactions (food intolerances) while the doctor diagnosed food allergy report more likely to be IgE mediated.

Season of birth has been used as a proxy indicator of vitamin D status in previous studies. A winter and autumn season of birth was found to be more common for children diagnosed with food allergy in Australia (Mullins *et al.*, 2011) and the United States (Vassallo *et al.*, 2010; Keet *et al.*, 2012; Bird *et al.*, 2014) and for children prescribed EpiPen autoinjectors (Mullins *et al.*, 2011). Allergic sensitisation usually occurs in early life. Although there was no significant association of season of birth with food allergy prevalence found, the higher rates of food allergy in autumn and winter months found in this study are suggestive of a relationship and deserve further investigation.

As we only had a measure of the current 25(OH)D concentrations in the preschool children we studied we need to be cautious in consideration of causality for its relationship with food allergy which has already developed. In addition, data on other factors which may influence sensitisation in early life, such as parental history of atopy and timing of infant complementary feeding, was not collected.

5.4.3 Vitamin D and allergic rhinoconjunctivitis

In contrast with the research effort that has been focussed on the relationship between vitamin D status and asthma or eczema, few studies have investigated the relationship between vitamin D status and allergic rhinoconjunctivitis, particularly in preschool children. The findings from the research that has been conducted to date have been mixed. In the only study including preschool age children, a case-control study compared asthmatic Qatari children under 15 years of age diagnosed with allergic rhinitis to healthy control of children of similar age. The prevalence of vitamin D deficiency was greater among the children with allergic rhinitis than controls (Ehlayel *et al.*, 2011). Potential confounders were not considered, and the co-existence of asthma in these children with allergic rhinitis limits comparison to healthy children.

In the current study children with 25(OH)D concentrations <25nmol/L had a non-significant higher risk of allergic rhinoconjunctivitis in unadjusted analysis (P=0.05), however this trend disappeared in the multivariable analysis (P=0.10). A lack of statistical power due to the small numbers in this 25(OH)D category (n=14) limits the interpretation of these data.

5.4.4 Vitamin D and asthma

Our finding of no significant difference in mean dried blood spot 25(OH)D concentration between children of preschool age with and without asthma is consistent with the case-control study in Massachusetts, US (Menon *et al.*, 2012), but not with other hospital based studies in Egypt (Ismail *et al.*, 2015), Qatar (Bener *et al.*, 2011) and Turkey (Uysalol *et al.*, 2014). In Massachusetts, no differences were seen in mean 25(OH)D concentrations between 547 asthmatic and non-asthmatic children and adolescent hospital patients (Menon *et al.*, 2012). Mean 25(OH)D concentrations were lower in asthmatic compared to non-asthmatic children and adolescents attending hospital and outpatient clinics in Egypt, Qatar and Turkey (Bener *et al.*, 2011; Uysalol *et al.*, 2014; Ismail *et al.*, 2015).

The lack of association of 25(OH)D concentration and asthma in the current study is in contrast to an inverse correlation of 25(OH)D concentrations with asthma reported in studies involving preschool children in Denver and Sweden (Searing *et al.*, 2010a; Stenberg Hammar *et al.*, 2014). Swedish children aged 6 months to 4 years attending hospital for treatment of acute wheeze had significantly lower 25(OH)D concentrations than healthy controls awaiting surgery, with 25(OH)D concentrations below 75nmol/L associated with an increased risk of acute wheeze ($P<0.05$) (Stenberg Hammar *et al.*, 2014). However, vitamin D status was not tested at time of admission, rather blood sampling occurred 12 weeks later. In Denver, asthma prevalence was significantly correlated with lower 25(OH)D concentrations in patients under 18 years of age (Searing *et al.*, 2010a).

Comparison of the findings of the current study to those of previous research is limited by a number of factors which have the potential to affect vitamin D status and asthma prevalence rates. These include differences in the number and age range of participants, recruitment from medical clinics or hospitalised children versus the community, diagnostic criteria used (physician versus parental report), ethnicity and country of study location.

Serum 25(OH)D concentration has been inversely associated with corticosteroid usage in studies of US and Egyptian children with asthma. In children and adolescents in Colorado, the use of inhaled and oral steroids was inversely associated with serum 25(OH)D concentrations (Searing *et al.*, 2010b). In hospitalised, asthmatic Egyptian children and adolescents, lower serum 25(OH)D concentrations were associated with increased inhaled and nasal corticosteroid usage (Abd and El Banna).

Such an inverse relationship between 25(OH)D concentrations and steroid usage was not seen in our study, with mean dried blood spot 25(OH)D concentrations of those children with asthma whose parents reported inhaled or oral steroid medication usage not significantly different to those not taking asthma medications. This may be due to the small number of participants in the previous studies (100 and 60 respectively), differences between physician diagnosis of asthma and parental report, and current steroid usage of the hospital patients recorded compared to the 'ever' report of asthma medication usage from the questionnaire used in the current study.

5.4.5 Vitamin D and respiratory infection

Although respiratory disease is more common in the paediatric population than any other age group, few studies investigating the association of vitamin D and respiratory disease have included preschool children aged between 2 to <5 years of age. The lack of association in the current study between 25(OH)D concentrations and lower respiratory infection was consistent with the findings of two studies involving preschool aged children. In Ecuadorian children aged 3 to 36 months, no association was evident between vitamin D deficiency and acute lower respiratory infection (ALRI) (Mokhtar *et al.*, 2014). In a Canadian case-control study of children aged less than 5 years, no difference in vitamin D concentrations was found for children with ALRI compared to the control group (McNally *et al.*, 2009).

Although results of studies have reported both inverse and positive relationships of vitamin D status and ALRI severity (Wayse *et al.*, 2004; McNally *et al.*, 2009; Inamo *et al.*, 2011), only data on the prevalence of lower respiratory infection were collected in the current study, meaning a potential relationship of vitamin D status and lower respiratory infection severity could not be investigated.

Studies that have demonstrated associations of vitamin D deficiency with an increased risk of lower respiratory infection, wheezing and viral infections (Wayse *et al.*, 2004; Camargo *et al.*, 2007; Camargo *et al.*, 2010; Jartti *et al.*, 2010), and supplementation with vitamin D decreasing upper respiratory infection rates (Linday *et al.*, 2004; Urashima *et al.*, 2010), suggest vitamin D may play a role in antiviral defence. It is speculated that the lack of association between vitamin D status and lower respiratory infection in this study suggests this role may not be present for preschool aged children, or at least, there may not be a concurrent relationship between vitamin D status and lower respiratory infection risk. Recent research suggests adequate vitamin D levels

are required for optimal lung development, with vitamin D deficiency in utero or in early infancy predisposing to diminished lung function and poorer respiratory outcomes in offspring of vitamin D deficient rodent mothers at 2 and 7 weeks of age (Gaultier *et al.*, 1984; Zosky *et al.*, 2014). It may be speculated that respiratory and immune function in preschool years has matured to a state that is no longer responsive to vitamin D status, but vitamin D status in utero or infancy may be a determinant of lower respiratory risk in the preschool years.

To our knowledge, there are no studies of vitamin D status and prevalence of upper respiratory tract infections in preschool children aged 2 to <5 years. In a US birth cohort study, lower newborn cord blood 25(OH)D concentrations were associated with an increased risk of acute respiratory infection in the first 3 months of life (Camargo *et al.*, 2011). Two supplementation RCT's from Poland and Japan suggest vitamin D may be mediating reductions in asthma exacerbations through fewer upper respiratory tract infections in children receiving vitamin D supplementation (Urashima *et al.*, 2010; Majak *et al.*, 2011). The non-significant trend found in this study of an increased risk for more frequent upper respiratory infections in children with 25(OH)D concentrations ≥75nmol/L (P=0.03 unadjusted, P=0.06 adjusted) implies the possibility of a "U" shaped relationship as has been described for vitamin D with food allergy.

Specific limitations on our study pertaining to respiratory disease include the questionnaire based, symptom based report by parents rather than diagnosis by physician or laboratory testing. There is a limited capacity within a study of this design to confirm the presence or absence or severity of each of these respiratory diagnoses, e.g. lack of detailed medical history including general practice or hospital admission records.

A recent study of respiratory infections in children in Auckland from age 0 to 18 months found that parents underestimated the number of primary care visits due to acute respiratory infection when compared to the number determined from an audit of general practice records (Grant *et al.*, 2014). It is possible the number of infections reported in this current study may be an underestimate of the true prevalence. Early life information which may have an effect on respiratory health, such as infection history and maternal or newborn vitamin D status, was not collected in this study.

Several features of this study may bias any measure of association between vitamin D status and respiratory infection prevalence. This study group was more affluent, with a higher maternal education level and lower BMI than the national population (section 5.5.1). The small number of

Pacific children recruited into the study (n = 40) limits our ability to generalise our findings to this population group which is known to have both poorer vitamin D status and a larger burden of disease from respiratory infections (Grant *et al.*, 1998; Grant *et al.*, 2009; Houghton *et al.*, 2010).

5.5 Methodological strengths and limitations

A unique feature of the design of this study was the use of pharmacies as testing centres which facilitated recruiting participants from throughout New Zealand. The use of capillary blood sampling was acceptable for parents, contributing to this research being the first large-scale study in New Zealand to use dried blood spot methods to measure 25(OH)D concentrations. Although this study has a number of strengths, the limitations also need to be addressed. These will be discussed in two parts; general strengths and limitations followed by those pertaining to the investigation of a relationship between vitamin D status and allergic and respiratory diseases in this study group.

5.5.1 General strengths and limitations of the study

5.5.1.1 Study population

An advantage of this study was the participants were non-hospitalised healthy children. Many other studies measuring 25(OH)D concentrations in young children use blood samples from hospital or outpatient clinics taken for other health reasons, which may affect vitamin D status (Mallet *et al.*, 2005; Roth *et al.*, 2005; Carroll *et al.*, 2014; Dyson *et al.*, 2014).

The primary recruitment mode of email sent through the online company database was effective. It allowed the recruitment of a large number of participants within a short timeframe which was necessary in order to recruit all participants during the late winter/early spring period. The complementary recruitment strategies used were successful in raising awareness, with many parents reporting these acted as a prompt to express interest after hearing about the study from different sources (e.g. kindergarten newsletter, newspaper article). The online company ReachMe (www.reachme.co.nz) offers free gifts for newborn infants in exchange for including their details on their database for future communications, and claims to have 160,000 families included on this database. This database has been successfully used in previous Massey University research, where the knowledge, attitudes and behaviours of 7000 mothers of young children about vitamin D and sun exposure was collected (Conlon and von Hurst, 2011). Compared to national statistics, the study group had higher overall maternal education levels (mothers without secondary qualifications 4% vs 21%) (Statistics New Zealand, 2013), children live in more affluent households (26% vs 14% from quintile 1 and 10% vs 30% from quintile 5) (Salmond *et al.*, 2007) (Figure 5.2) and have lower BMI levels (4% vs 11% obese BMI and 13% vs 24% overweight BMI) (Ministry of

Health, 2013b). Children with more highly educated mothers and living in less deprived households are more likely to have greater access to better nutrition and a healthier living standard (Rogers and Emmett, 2003), which may positively affect overall health (such as BMI) and result in higher 25(OH)D concentrations. Socioeconomic status has been inversely linked with 25(OH)D concentrations in national surveys, with adults living in households of high deprivation having a higher prevalence of vitamin D deficiency (Houghton *et al.*, 2010; Ministry of Health, 2012). This association was also found in the current study. Thus, the feasibility of extrapolating the findings of this study to the entire preschool-age population is limited, and as socio-economic factors have been linked with vitamin D levels previously, it is possible the results reported for this cohort could overestimate 25(OH)D concentration and under-estimate the prevalence of vitamin D deficiency in this age group in New Zealand.

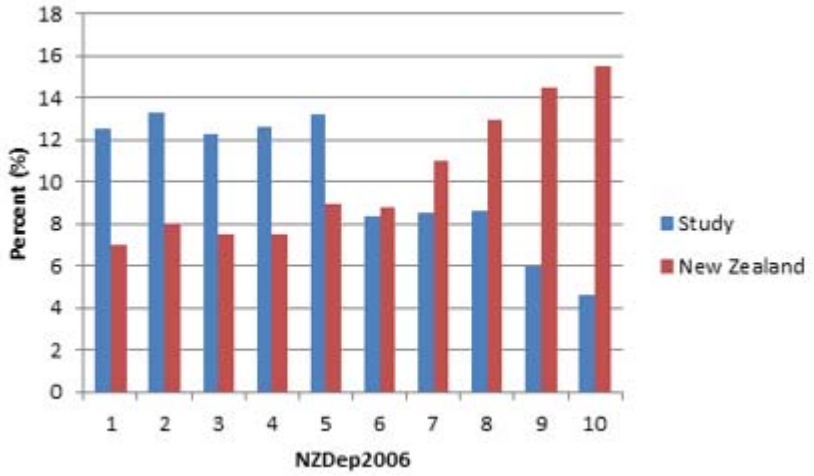


Figure 5.3. Comparison of family household deprivation index of study participants and New Zealand families (NZDep 2006) (Salmond *et al.*, 2007)

The use of supplements containing vitamin D or cod liver oil is infrequently recorded in epidemiological studies of vitamin D. We asked participants in this study about their child's use of supplements or cod liver oil. We considered this potentially important in a country which does not have a food supply routinely fortified with vitamin D. Around one fifth (19%) of parents reported their child took a supplement containing vitamin D or cod liver oil. Data on supplement usage in preschool children in New Zealand is limited. In a 2006 Auckland study of 355 European preschool children aged 3.5 years, 24% reported a taking a supplement ≥1 per day and 39% ≥1 per week while the 2002 Child Nutrition Survey reported approximately 5% of children were taking supplements (Ministry of Health, 2003; Theodore *et al.*, 2006). In both studies, none were reported as being specific vitamin D supplements. The higher supplement usage of the 3.5 year

old group in this study is likely due to the higher socioeconomic status of this group in comparison with the 2002 Child Nutrition Survey which recruited a sample that was representative of the national population. Although the 19% of participants reporting taking supplements containing vitamin D or cod liver oil may limit extrapolation of results to the general preschool population, our findings do provide the only evidence currently available on vitamin D supplement use within this population group in New Zealand.

5.5.1.2 Questionnaire

The self-administered questionnaire contained 59 questions. Face validity of the questionnaire was achieved through review and approval by a paediatrician and experienced vitamin D researchers. A focus group of mothers with preschool children was held, testing comprehension and length, with minor wording amendments made.

The two options offered for completion of the questionnaire - paper and online - assisted in data collection, with over two-thirds of parents utilising the online format. The online questionnaire was designed with safeguards to ensure all questions were answered. Researchers were available by email or phone to answer parental queries while completing the questionnaire.

Use of the pharmacies promoted community awareness of the study and engaged pharmacy staff, not only being trained in the procedures for undertaking capillary blood spots but also in supporting parents to complete the questionnaire. The trained personnel conducting the fingerprick tests often assisted parents who spoke English as a second language or those who struggled with the complexity of the questionnaire, and the support of the pharmacy staff in this role was appreciated. Regular newsletters and contact with pharmacies by the researcher encouraged pharmacy staff to contact the research team if they encountered problems with blood sampling or supporting parents to complete the questionnaire. However, the self-administered nature may have caused bias in the participant group due to the comprehension level required to complete the questionnaire. The pharmacy staff reported the questionnaire length and medical history components as hurdles for those with English as a second language. Ideally, the questionnaire would be conducted by trained interviewer in order to capture all demographics in future studies, however this would add considerably to the expense of conducting such a study.

Questions regarding dietary sources of vitamin D were limited to the type of milk consumed and supplement or cod liver oil usage. The consumption patterns of milk in this preschool cohort were not recorded in this study. Previous research has found lower vitamin D intakes were associated with lower vitamin D levels in the winter season (Davies *et al.*, 1999; Pasco *et al.*, 2001; Roth *et al.*, 2005). At present, the NZ Food Composition Database for vitamin D is incomplete and has precluded intake for this nutrient being measured in national nutrition surveys. Milk intake has been shown to be the major source of vitamin D for preschool children in countries with a fortified milk supply, with consumption positively associated with 25(OH)D concentrations (El Hayek *et al.*, 2010; Whiting *et al.*, 2011). Around 15% of parents in this study reported their child does not drink any milk or drinks non-dairy beverages. As consumption of non-dairy milk by young children has been linked to decreased 25(OH)D concentrations (Lee *et al.*, 2014), together with the finding from this study of increased 25(OH)D concentrations in children who drink toddler milk, development of a measurement tool to assess vitamin D intake in young children should be considered for future research.

5.5.1.3 Capillary blood sample analysis of 25(OH)D

Parents were willing for their young children to undergo a fingerprick blood sampling method, as confirmed by 1350 participants recruited and tested in a ten week period. Overall, researchers found parents were accepting of their child undergoing a fingerprick test to obtain blood, with the vast majority of children experiencing minimal trauma during testing. The positive experience of using this technique has been shown in another study with primary school participants in London (Griffiths *et al.*, 2014). Specialised paediatric lancets were successfully used in the current study to minimise discomfort for the children, with very few reports of child pain.

Blood collection by venepuncture requires trained phlebotomists. An advantage of this study was that people could be trained in the fingerprick procedure in a short period of time. All pharmacy and research staff who undertook this procedure received a standardised training and initial supervision to undertake the procedure. Preprinted circles on the filter paper gave testers an indication of the volume of blood required. Variability existed in the ability of testers in the volume of blood collected from children for the dried blood spot, also contributed to by the disparity in capillary blood flow from the puncture site. Nevertheless, all blood spots collected were of sufficient size to be successfully analysed for 25(OH)D. All testers reported increased confidence and blood volume was achieved with repetition. For future research it would be

beneficial to incorporate into a training schedule a minimum number of trials of fingerpricks to be conducted on children prior to the study commencing.

Blood sampling by venepuncture requires large blood volume, specialised facilities, storage and processing equipment when compared with blood obtained by a fingerprick method. Only a small volume (~3µL) of blood is required, which proved advantageous for sampling in this population of young children (Craft *et al.*, 2000; Keevil, 2011). Blood spots stored at room temperature have been shown to be stable over a 4 month period for measurement of 25(OH)D (Craft *et al.*, 2000; Newman *et al.*, 2009). Extreme temperatures in postal transportation in summer months may negatively affect the blood spots, thus the samples were flown to Brisbane for measurement of 25(OH)D (Eyles *et al.*, 2010; Fraser and Milan, 2013).

There may be limitations in comparing the 25(OH)D results obtained here from dried blood spots and analysis by LC-MS/MS with previous studies of serum samples using a variety of assay techniques in other laboratories. However, good correlation has been reported between 25(OH)D concentrations from dried blood spot and serum samples in previous studies (Newman *et al.*, 2009; Larkin *et al.*, 2011; Heath *et al.*, 2014). In this study, the 25(OH)D in dried blood spot was extracted in the laboratory which had developed the validated methodology for a paediatric population. In validation, 25(OH)D concentrations of neonatal cord serum blood were highly correlated with that obtained from dried blood spot (r=0.85) (Eyles *et al.*, 2010). This laboratory was well experienced in the primary factors of blood spot volume and hole punch position, taking into account the gradient of 25(OH)D concentration within the blood spot (Eyles *et al.*, 2009). The other primary factor for accurate 25(OH)D analysis is variability in type of filter paper used (Kvaskoff *et al.*, 2012). The use of Whatman 903 paper for all samples removed this variable.

Comparison of 25(OH)D results of blood obtained from capillary and serum sampling was not considered feasible in this study. Future studies could consider validation of dried blood spot versus serum 25(OH)D concentrations within the study cohort to enable accurate conclusions to be drawn as to differences between results from the two methods. Future research may consider validation of a method for analysis of dried blood spots for 25(OH)D in New Zealand laboratories to prevent the need for samples to be sent overseas for analysis and associated ethical approvals.

Our measurement of 25(OH)D was a single measurement during the late winter and early spring months, when vitamin D status would be expected to be lower than during summer and autumn due to seasonal variation. The seasonal variation of vitamin D status has been well described. The

lowest 25(OH)D concentrations have been shown to occur in late winter to early spring months in Australia and New Zealand (Pasco *et al.*, 2001; Bolland *et al.*, 2008; Ministry of Health, 2012; Zhou *et al.*, 2014). The prevalence of vitamin D deficiency is also highest at this time of year, as found in the NZ Adult Nutrition Survey (Ministry of Health, 2012).

Data collection over a short timeframe of 10 weeks in the nadir period enabled the lowest 25(OH)D concentrations to be investigated in preschool children, and predictors of vitamin D status identified in the absence of seasonal confounding. However, due to the short sampling timeframe, the effect of seasonality was not explored. No literature exists on the seasonal variation of 25(OH)D concentrations on New Zealand preschool-aged children. In New Zealand, seasonal variations in 25(OH)D concentration of 10 to 20nmol/L were reported for adults tested in Auckland (Bolland *et al.*, 2008). In contrast, there was a larger seasonal variation of 50nmol/L found in studies of newborns in Wellington and breastfed infants in Auckland (Camargo *et al.*, 2010; Wall *et al.*, 2013). Seasonal differences in 25(OH)D concentrations were found to be smaller in infants aged 2 to 23 months, of 25nmol/L in Dunedin (Houghton *et al.*, 2010) and 35nmol/L in Auckland infants aged 6 to 23 months (Grant *et al.*, 2009). In the 2002 Children's National Nutrition Survey a seasonal variation of 25(OH)D of 15nmol/L for children aged 5-14 years was reported (Ministry of Health, 2003). Overseas studies involving preschool children report differences between summer and winter 25(OH)D concentration means of 10 to 15nmol/L in Canada (Newhook *et al.*, 2009; El Hayek *et al.*, 2010; El Hayek *et al.*, 2013), 20 to 33nmol/L in Great Britain (Absoud *et al.*, 2011), in Australia (Zhou *et al.*, 2014), Ireland (Carroll et al, 2014) and the United States (Stein *et al.*, 2006), and 35 to 40nmol/L in Italy (Vierucci *et al.*, 2013; Mazzoleni *et al.*, 2014). Given this wide range of seasonal variation in the age groups of children both younger and older than preschool age, it is difficult to accurately predict the summer levels of this study cohort. Further testing of 25(OH)D concentrations on a year-round basis is warranted to assess seasonal variation of this age group in New Zealand.

5.5.1.4 Pharmacy as testing centre

A novel aspect of this study was the use of pharmacies as the location for blood sampling. This enabled widespread coverage throughout New Zealand. Pharmacies volunteered to be involved with this study for a variety of reasons, including an interest and desire to support interesting research, wanting to improve the skills of their staff and intern pharmacists, increase in foot traffic through their store, potential for new customers and an attitude of community service. Our experience was that pharmacies were a viable avenue for capillary testing and dried blood spot

collection, and this may be a feasible method for a nationwide collection of capillary blood for future research.

At the commencement of the study 25 pharmacies had volunteered as testing centres. As parents expressed interest in the study through the email recruitment, pharmacies in areas of high interest were identified and approached, so at the end of the data collection period 49 pharmacies were involved as testing centres. The two major reasons pharmacies gave for declining to be part of the study were unavailability of time or staff to conduct fingerpricks and lack of remuneration for their services. The researcher minimised the burden for pharmacies as much as possible in view of their voluntary capacity, limiting their activities to conducting the fingerprick tests, storing dried blood spot cards and couriering these back to the study office at the completion of data collection.

There were several limitations associated with the pharmacies in this study. Pharmacy involvement was on a volunteer basis, so a uniform coverage throughout the country was not achieved. This restricted enrolment numbers, as there were several areas with many interested parents but no local pharmacy willing to take part. It would be desirable in future research to have pharmacies close to populations of interested parents, which could be achieved by providing remuneration for services. Pharmacy is a demanding retail operation, and the majority of pharmacists requested parents make appointments for the fingerprick test, as staff were unable to stop their prescription dispensing duties in busy times. With only certain staff trained in the fingerprick test, appointment times were limited, often to weekdays. This meant some parents were not able to have their child take part in the study, particularly working parents who were unable to obtain weekend appointments. To overcome this in future studies, more testers would need to be trained in the pharmacies to enable a wider range of appointments to be available.

5.5.1.5 Attrition during the study

There was attrition experienced between the expression of interest in the study by parents and enrolment. The email recruitment method proved successful in capturing parent's attention, with 2267 registrations of interest in a ten week period. All parents were contacted by phone or email within 2 to 3 days by the Auckland study office and had the study explained to them, resulting in 1812 children enrolled. This attrition was due to several main factors; the email database was nationwide and emails were sent in error to some towns or cities which did not have testing centres, the testing centre was not close enough to the participant’s house, the parent had

reconsidered since their expression of interest or another parent was uncomfortable with their child being involved in the study. For future studies involving this recruitment method, tighter control of the email distribution to locations where testing centres were located would facilitate a lower attrition at this stage.

Attrition was also experienced between enrolment and the dried blood spot sample being obtained. There were very few reports of children refusing to undergo the procedure in the pharmacy, approximately ten children. Feedback from parents indicated the causes of this attrition included; they could not make appointments with the pharmacy at a convenient time, those who enrolled early often agreed to go to a pharmacy some distance from their home but logistics prevented them making the trip, the information sheets got lost at home, school holiday absence and sick children prevented attending the pharmacy. Reminders were sent by email and text by the study office throughout the data collection period. Improvements which could benefit future studies would include having all pharmacies available at the commencement of the study and devolving responsibility for making appointments and reminders to pharmacy staff. This would require remuneration for the increased amount of staff time involved.

There were 20 children who had a fingerprick test but their parents or caregivers failed to complete the questionnaire. This may have been due to intermittent technical issues with the Survey Monkey program experienced by some computer users or that parents may felt incapable of completing the questionnaire on their own. The researcher contacted all these families on numerous occasions, offering to visit or complete the questionnaire over the phone, unfortunately without success.

5.5.2 Questionnaire predicting vitamin D deficiency

Another unique feature of this study was the development of predictive questionnaires to assess vitamin D deficiency and insufficiency in winter in preschool children. Although the performance of the questionnaires by ROC Area Under the Curve were comparable to that achieved by questionnaires which have been developed overseas (Absoud *et al.*, 2011; Lopes *et al.*, 2014), the low sensitivity and specificity means the predictive performance is not robust enough for these questionnaires to be used in clinical situations. The applicability of predictive questionnaires is limited to the population it was developed for, and thus any additional validation with another group would need to be preschool children from New Zealand. It would also be an interesting

research exercise to validate this questionnaire with another age group, however this would require additional funding. The small number of children who were vitamin D deficient limited the ability of the predictive questionnaire to assess vitamin D deficiency in this sample, future work in this area would require collection of data on vitamin D status in a population representative sample with larger numbers of deficient children of preschool age.

5.5.3 Relationship of vitamin D and allergic and respiratory diseases

A strength of this study is the large number (n=1329) of participants who were free living, healthy children from throughout New Zealand. The use of the eczema, allergic rhinoconjunctivitis and asthma modules from the internationally recognised ISAAC survey enabled prevalence of these conditions to be assessed in a manner consistent with that frequently used in epidemiological studies worldwide (Asher *et al.*, 1995; Mallol *et al.*, 2013).

This study has several potential limitations, the first being the cross-sectional nature. These types of studies are useful in planning future research, reporting associations; however a major limitation is that they cannot determine causality. For example, it is not known whether high 25(OH)D concentrations cause a higher prevalence of food allergy or whether presence of food allergy and higher 25(OH)D concentration are due to a separate unrelated factor. A longitudinal study design, where children are followed for an extended period of time, would be necessary to further understand the relationship between vitamin D status and allergic diseases. This would ideally be from pregnancy onwards, which would include the phase of life when the immune system is developing and the early life initiators of allergic disease.

The prevalence estimate of allergic and respiratory diseases were based on parental report, collected on a self-completed questionnaire based on their child's symptoms. The diseases were not confirmed by the diagnosis of a doctor nor the presence of intermittent or perennial symptoms. There is a limited capacity within a study of this design to confirm the presence or absence or severity of each of these diagnoses, e.g. intermittent or perennial symptoms for allergic rhinoconjunctivitis, lung function tests to demonstrate reversible airways obstruction for asthma, serum IgE for atopy, scoring atopic dermatitis (SCORAD) for eczema, skin prick test, radioallergosorbent test (RAST) and double blind food challenge for food allergy. The estimate of prevalence of each allergic or of respiratory infection frequency will be imprecise due to the

nature of parental recall and report. This imprecise measurement may affect associations between vitamin D status and risk of disease.

The International Study of Asthma and Allergies in Childhood (ISAAC) is worldwide epidemiological research program with 233 centres in 98 countries providing information on prevalence and severity of asthma, allergic rhinoconjunctivitis and eczema (Asher *et al.*, 2006). Medical history for these three conditions in this study was based on the respective modules of the ISAAC questionnaire for children aged 6-7 years (Asher *et al.*, 1995). The ISAAC questionnaire is a well-respected, validated international tool frequently used to determine prevalence and severity of asthma and allergic conditions. Whilst parents or caregivers completed the questionnaire on behalf of their child, these modules were not validated for use in a New Zealand preschool age group in this study. The prevalence of food allergy was determined through parental report, with the question of doctor diagnosed food allergy used in an attempt to obtain a more rigorous definition. The questions on food allergy were not specifically validated for use in a preschool population.

Considerable variation exists in the published literature on the definitions of allergic and respiratory diseases. This limits comparability, and differences in prevalence rates between worldwide studies may be due to the definition chosen. In this study, definitions of asthma, allergic rhinoconjunctivitis, eczema and respiratory prevalence were symptoms in the last year, while food allergy was a lifetime prevalence. With some children outgrowing food allergy, current allergy status was not defined in this study.

Early life information which may have an effect on the development of allergic and respiratory diseases, such as infection history and maternal or newborn vitamin D status, was not collected in this study. These could be considered for inclusion in future studies of allergic diseases in this age group.

This study cohort was not representative of this age group in the New Zealand population due to the manner of enrolment and recruitment. Parents participating in the study were self-selected through a primary recruitment tool of email, contributing to a cohort which is more affluent, has a higher maternal education level and lower BMI than the general population. The advertising brochures and posters for this study described the study as “investigating the relationship of vitamin D and asthma, eczema and food allergy”, with this wording seeming to resonate with parents whose children bear the burden of these diseases. The prevalence of eczema, allergic

rhinoconjunctivitis and asthma in this study cohort was (23%, 10% and 32%) compared with those 6-7 year old children in New Zealand using the same ISAAC questions as this study (22%, 11% and 22% respectively) (Williams *et al.*, 2008). Lower socioeconomic status has been linked to increased prevalence of allergic diseases, such as asthma (Mansbach *et al.*, 2009). This high percentage of enrolled children with eczema and allergic rhinoconjunctivitis, coupled with a study group with higher socioeconomic status, limit the extrapolation of results from this study to the general preschool population. In addition, people who take part in research studies have a higher likelihood of following health guidelines (e.g. sun protection, dietary intake) (Trauth *et al.*, 2000), which may affect vitamin D status and also the size of the association of vitamin D and prevalence of allergic and respiratory diseases.

It may be possible children and their parents with allergic diseases adopt behavioural patterns which impact on vitamin D status. This behaviour may be to improve symptoms and avoid exacerbations, for example the avoidance of the outdoors and associated allergens or physical exertion in those with asthma or allergic rhinoconjunctivitis, or to cover eczema lesions considered unsightly with concealing clothing. Each of these behaviours may limit sun exposure. Measurement of sun exposure and outdoor physical activity levels in future studies would explore this factor. Children with food allergies may not consume seafood, eggs and milk to avoid food allergens. These foods are potentially rich sources of vitamin D, avoidance may decrease dietary vitamin D intake and affect vitamin D status, particularly in winter months when sun exposure is reduced.

Allergic conditions are more common in urban areas and developed nations compared with rural locations and developing countries (Williams *et al.*, 1999; Downs *et al.*, 2001; Kabesch and Lauener, 2004; Naleway, 2004). Increased exposure to allergens and sun through a rural outdoor lifestyle may be a factor in these different rates. Data on this potential confounder of urban versus rural location was not collected in this study, and should be investigated in future research.

Currently there is debate about the appropriate methods for analysing prevalence studies, as there may be a marked difference between prevalence ratios and prevalence odds if the disease is common in the study population (Pearce, 2004). A limitation of this study may be the use of prevalence odds ratios rather than prevalence ratios in analysis of the data.

When designed, this study was intended to have sufficient power to be able to investigate relationships between vitamin D status and prevalence of allergic conditions. However, this ability

may have been compromised with the small number of children with low and high 25(OH)D concentrations (7% with 25[OH]D <25nmol/L and 11% with 25[OH]D ≥75nmol/L). It may be argued the smaller numbers of children with 25(OH)D concentrations <25nmol/L and ≥75nmol/L may constrain the ability to detect modest associations within the children. The clinical significance of any significant associations will be limited by the sample size and cross-sectional nature of the study. It would be desirable to extend the number of participants in future studies to include more children with these lower and higher 25(OH)D concentrations.

Chapter 6: Conclusion

6.1 Implications of the study findings

Measuring the vitamin D status of a large number of young children in multiple centres throughout the country was facilitated by the use of a capillary blood sampling method. Two factors in particular were important, firstly parents being receptive to their young child undergoing a fingerprick test to obtain a blood sample, and secondly, the lower level of training required for testers which increased the number of testers and testing centres available to collect blood samples. The measurement of 25(OH)D concentration on dried blood spots was carried out using a validated assay methodology by the laboratory which developed this analysis technique. Currently, blood spots must be transported overseas for analysis. Development of assay capability in New Zealand would increase the opportunity for vitamin D research in this country. The experience of the current study suggests a fingerprick test as a capillary blood collection methods is suitable for large scale studies in this age group.

The mean dried blood spot 25(OH)D of this self-selected sample of preschool children in winter was 52nmol/L, which is consistent with results reported for infant and schoolchildren age groups in New Zealand (Rockell *et al.*, 2005; Grant *et al.*, 2009; Houghton *et al.*, 2010). Vitamin D concentrations <25nmol/L were present in 7% of this cohort of preschool children, while 48% had 25(OH)D concentrations below 50nmol/L. An important focus for further research includes the potential avenues to increase 25(OH)D concentrations of young children to an optimum level.

The predictors of vitamin D deficiency (25[OH]D <25nmol/L) were consistent with those previously reported in infant and schoolchildren age groups in New Zealand - female gender, being of other non-European ethnicity (not Pacific or Maori), having a mother with less than secondary school education level, living in a household of higher deprivation, not drinking infant formula (toddler milk) and not taking a vitamin D supplement. A unique finding of this study was not attending a daycare centre was a predictor for vitamin D insufficiency (25[OH]D <50nmol/L), which needs to be confirmed in a population representative sample of preschool children. These results and the identification of predictors of vitamin D deficiency add to the vitamin D literature in a previously unstudied population group, helping to inform future public health policy and vitamin D trials in this age group.

To the best of our knowledge, the predictive questionnaire developed in this study was the first to assess risk of vitamin D deficiency in any age group in New Zealand. The predictive questionnaire was developed on data from 929 children, and validated in a group of 400. Future studies of

vitamin D status in this age group in a population-representative sample with higher rates of vitamin D deficiency are needed in order to try and develop a questionnaire that provides clinicians with an inexpensive, convenient and non-invasive method of identification of preschool children at risk of vitamin D deficiency. However, based upon the experience of this study, and of studies that have attempted to develop such predictive questionnaires for other age groups, it may not be possible to develop a questionnaire that is sufficiently predictive of vitamin D status to be clinically useful. This study measured children only in winter, but future research to measure 25(OH)D concentrations of New Zealand preschool children throughout the year is indicated to extend the applicability of this predictive questionnaire to status across all seasons.

In this large-scale study investigating the relationship of vitamin D status with allergic and respiratory diseases for preschool children aged 2 to <5 years in New Zealand we found no apparent association between dried blood spot 25(OH)D concentration and parental-reported prevalence of eczema, allergic rhinoconjunctivitis, asthma and respiratory infection. The lack of association of vitamin D status with prevalence of these diseases in the preschool children does not support the increase in serum 25(OH)D concentrations as a potential option for preventing these diseases in this age group. Future research is needed with a population representative study of preschool aged children testing for atopic sensitisation (e.g. antigen-specific serum IgE) and using clinical tools (e.g. SCORAD, lung function testing) for assessment of disease severity (e.g. eczema and asthma). Further research into the optimal age and 25(OH)D concentration required to prevent atopic sensitisation is of high priority in the quest to reduce prevalence of these conditions.

Previous observations that season of birth and latitude are related to prevalence of allergy were not supported in this study for eczema and food allergy. The non-significant finding of higher rates of these diseases in children born in autumn and winter seasons are suggestive of a relationship and deserve investigation in the search to determine if conditions *in utero* or early infancy are of particular importance in the subsequent development of allergies.

The two-fold increase in risk of parentally reported, doctor diagnosed food allergy for children with 25(OH)D concentrations ≥75nmol/L provides food for thought in the debate of what is the optimal 25(OH)D concentration for children. The implications of potential adverse outcomes of food allergy with higher 25(OH)D concentrations cautions the supplementation of preschool children with vitamin D with the goal of prevention or treatment of respiratory or allergic diseases. Detailed studies directly measuring 25(OH)D and the relationship with food allergy in

the child lifecycle stage are warranted, with high priority given to research into determination of optimal 25(OH)D concentrations for preschool children.

The optimal level of 25(OH)D for overall health in young children continues to be a topic of intense interest, and debate, amongst researchers. This study adds to the literature relevant to the preschool population. The increased risk for food allergy and non-significant trend of increased risk for more frequent upper respiratory infections in children with 25(OH)D ≥75nmol/L indicates the need for defining an optimal range of 25(OH)D for non-bone health outcomes to remain a priority research issue. Given the potential for vitamin D status in earlier life being a determinant of subsequent respiratory health, defining this range is likely to require both clinical trials and longitudinal studies. In addition, investigation of the timing and involvement of vitamin D in immune system and lung function development during pregnancy and early infancy, and association with allergic and respiratory diseases throughout the lifespan, is warranted.

6.2 Recommendations for future research

Determine vitamin D status and seasonal variation of vitamin D concentrations in a representative population survey of the preschool age group in New Zealand.

Conduct further investigations into the relationship of vitamin D status and attendance of preschool children at formal daycare centres.

Develop a dietary assessment method for vitamin D intake, in order to determine the proportion of vitamin D obtained from dietary sources (e.g. milk) in the preschool age group, which may be significant in winter months. This can also inform future public health strategies that aim to improve vitamin D status in this age group.

Develop an assay for 25(OH)D in New Zealand laboratories, and validate measurement of 25(OH)D concentration in blood collected by capillary sampling versus venepuncture methods. This would increase accessibility for future research studies and remove the necessity of sending samples overseas, which requires ethical and parental approval.

Explore how the vitamin D status of preschool children affects the prevalence of allergic and respiratory diseases in later life. Consider the use of a longitudinal study to assess these factors.

Future research should consider the extent to which vitamin D status and early life experiences affect allergic status of preschool children. For example, this could be the focus of a longitudinal study of maternal and newborn vitamin D status and history of infections from birth.

Conduct year round testing of 25(OH)D concentrations on preschool age children to ascertain seasonal variation, and incorporate the findings in a predictive questionnaire to assess vitamin D deficiency throughout the year. Subsequently, investigate the use of such a predictive questionnaire in clinical settings, and acceptance by health professionals.

Determine the optimal level of vitamin D for child health through clinical trials and longitudinal studies. Explore new preparations to increase those children with deficient levels (i.e. supplements) to an optimal level. For children with insufficient levels, explore strategies to achieve optimal levels using foods commonly consumed by preschool children (e.g. fortification of milk).

6.3 Conclusion

Capillary blood sampling methods facilitated the measurement of 25(OH)D concentrations in a large sample of preschool children from throughout New Zealand. Using this approach, it was found the mean dried blood spot 25(OH)D concentration in winter was 52nmol/L, with 7% of children having vitamin D deficiency (25[OH]D<25nmol/L) and 48% vitamin D insufficiency (25[OH]D <50nmol/L). The predictors of vitamin D deficiency were female gender, being of other non-European ethnicity, having a mother with less than secondary school education qualifications, darker skin colouring, living in a household of higher deprivation, not taking a vitamin D supplement and not drinking toddler milk. Predictors of vitamin D insufficiency were female gender, being of Pacific or other non-European ethnicity, having a mother with less than secondary school education qualifications, not attending a formal daycare centre, not taking a vitamin D supplement and not drinking toddler milk. These factors were then used in the development and validation of a predictive questionnaire to assess children at risk of vitamin D deficiency. This questionnaire was only modestly effective at predicting vitamin D deficiency with a sensitivity of 42% and specificity of 95%. A second questionnaire was developed to assess risk of vitamin D insufficiency, which also had relatively poor diagnostic performance (sensitivity of 54% and specificity of 66%). These performances impair the ability of the questionnaires to be used in clinical situations. There was a two-fold increase in odds of parental report, doctor diagnosed food allergy for children with 25(OH)D concentrations ≥75nmol/L. No association between vitamin D status and prevalence of eczema, allergic rhinoconjunctivitis, asthma and respiratory infection was found in the preschool children. The relationship between vitamin D status and health in preschool children is complex, with future research into the optimal vitamin D concentration for preschool children in New Zealand warranted.

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A 4 year old girl had her finger-pricked at the pharmacy as part of the study.

She was asked why she had a plaster on her finger, this was her answer:

“There was a special needle and they got blood out to see how much sunshine is in it”

Appendix 1

Recruitment and advertising information

- Recruitment information for pharmacies
- Advertising poster
- Advertising brochure
- Study website material
- Media advertising

MASSEY UNIVERSITY
COLLEGE OF SCIENCES
TE WĀHANGA PŪTAIAO

Te Ra Whakaora

An investigation of the vitamin D status of New Zealand 2-4 year-olds; the factors affecting vitamin D status, and the relationship between vitamin D status, respiratory infection and allergy

Information Sheet for Pharmacists

This study will be measuring the vitamin D levels of 1600 New Zealand children aged 2-4 years through a finger-prick blood test, and parents will also complete a questionnaire. The main aim is to develop a validated, quick, non-invasive screening tool for health professionals to estimate vitamin D deficiency in this age group.

Why test for Vitamin D?
Research has found many New Zealander adults and infants have low levels of vitamin D levels. There is very little data on the preschool population of this country so it is not known if their levels are also low.

Vitamin D is essential in calcium absorption and bone health. The current low levels in New Zealand are being seen in a re-emergence of infantile rickets (soft bones causing bowed legs) and are a risk factor for osteoporosis later in life. Sunlight is the major source of vitamin D for our bodies, as UVB rays are converted in the body to active vitamin D. UVB levels decrease the further south you go in New Zealand, meaning that there may be insufficient to maintain adequate levels for health, especially in winter. The body's ability to absorb vitamin D is also affected by darker skin colour, using sunscreen and clothing as protection from the sun.

What are the study outcomes?
This study will not only test the levels of vitamin D in cities throughout the country with different sunlight levels, it will see if there is any link between low vitamin D levels and respiratory disease, asthma, wheezing and allergies, which are common in New Zealand.

The data will be used to create a screening tool, where doctors can ask a quick series of questions and work out a child's risk of being deficient in vitamin D, without the need and expense of a blood test. This provides many benefits - the child avoids the pain of an invasive test, their health is improved with appropriate and timely supplementation/treatment, and the health system has monetary savings in the blood test and future health treatments (e.g. by the reduction in osteoporosis rates).

How will the study work?

- Parents contact the study organiser and are sent a questionnaire, which names their closest participating pharmacy.
- Parents fill out the questionnaire at home, and then bring their child into the pharmacy for the finger-prick blood test.
- The pharmacist records the weight and height of the child, and carries out the finger-prick test.
- The child chooses from a bright range of plasters for their finger after the blood test, and then receives a small reward gift.
- When the blood spot has dried, the pharmacist places the card into an envelope. When all the samples have been collected, the cards and questionnaires are couriered back to Massey University.

The study will provide all equipment needed (gloves, biohazard bags, paediatric lancets, plasters, reward packs, courier bags, etc). Training in the collection of bloodspots would be provided in the pharmacy by study personnel in late July.

The blood fingerpicks will be collected in August and September this year.

The number tested by a pharmacy is expected to be 30-50 children, and for each visit would take 10-15 minutes of staff time. Over the two month period, this equates to less than 15 minutes per day. Parents can be asked to call the pharmacy to arrange an appointment, which could be scheduled into a quieter periods during the week. The total number of children can be agreed with each pharmacy as the study co-ordinator has control of numbers through the enrolment process.

This study may particularly appeal to pharmacies which currently offer blood glucose testing, as they would already be setup for this type of work, and staff would be familiar with the blood testing scenario.

Benefits for pharmacies

- The opportunity for new customers to visit and experience the pharmacy, to establish a rapport between tester and parent, potentially leading to business.

- Each pharmacy would be provided with an A4 brochure on vitamin D. This could be used as an information resource and would be useful in staff training on providing customer information on vitamin D supplements. There is currently confusion regarding the amount of sun required for adequate vitamin D levels and the important sun safe messages with skin cancer. There is a Government consensus statement on sun exposure and vitamin D supplementation for certain populations due to be released in late 2012 – this would be included in the brochure and could be used as a basis for advice by pharmacy staff to customers both in sunscreen and supplement sales.

- Opportunity to participate in research in this vulnerable group of the population, leading to increased health outcomes.

If you would like further information or to discuss this research project, please contact Dr Pamela von Hurst, (09) 414 0800 x 41205, p.r.vonhurst@massey.ac.nz or Carolyn Cairncross at c.t.cairncross@massey.ac.nz , 021 292 6646.

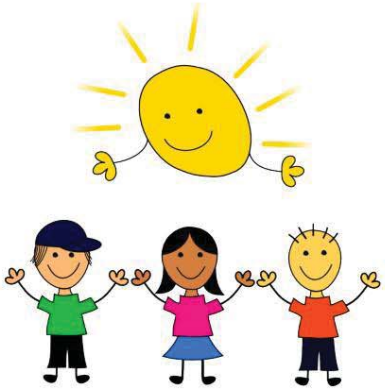
Te Ra Whaka-ra

Study Advertising Poster

MASSEY UNIVERSITY

ARE NEW ZEALAND CHILDREN AT RISK FROM VITAMIN D DEFICIENCY?

We need your help to find out !



BE PART OF A NATIONWIDE SURVEY BEING CARRIED OUT BY MASSEY UNIVERSITY THIS WINTER AND HELP US FIND OUT:

- The current vitamin D status of our children
- If protecting our preschoolers from the sun is resulting in low vitamin D levels
- The possible link between vitamin D and coughs, colds, allergies and eczema
- If we can create a tool for health professionals which can be used to find out if a child is at risk from vitamin D deficiency

For the study we need to recruit 1600 preschoolers from around the country, aged between 2 and 4 years. As a survey participant your child will find out their vitamin D status.

INTERESTED ?

LIKE TO FIND OUT MORE ?

Call **0800MASSEY**, ask for Te Ra Whakaora (9am-5pm)
Or call / text 021 422531 (anytime)
Or visit **TeRaWhakaora.massey.ac.nz**

Te Ra Whakaora

This study is funded by the Health Research Council, & has been reviewed & approved by the Health & Disability Ethics Committee, NTX/12/04/036

Study advertising brochure

LAND CHILDREN AT
AMIN D DEFICIENCY?

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to
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WIDE SURVEY BEING CARRIED
IVERSITY THIS WINTER AND

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Te Rā Whakaora

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ARE NEW ZEALAND CHILDREN AT
RISK FROM VITAMIN D DEFICIENCY?

We need
your help to
find out !

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OUT BY MASSEY UNIVERSITY THIS WINTER AND
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MASSEY UNIVERSITY Te Rā Whakaora

This study is funded by the Health Research Council & has been reviewed
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ARE NEW ZEALAND
RISK FROM VITAMIN

We need
your help to
find out !

BE PART OF A NATIONWIDE S
OUT BY MASSEY UNIVERSITY
HELP US FIND OUT:

- The current vitamin D children
- If protecting our pres the sun is resulting in levels
- The possible link betw and coughs, colds, all eczema
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Or visit **http://TeRaWhakaora.m**

MASSEY UNIVERSITY Te

This study is funded by the Health Research
& approved by the Health & Disability Ethi

Appendix 2

Study information sheet
Study consent form

MASSEY UNIVERSITY

Institute of Food, Nutrition and Human Health
Massey University
Private Bag 102-904
North Shore Mail Centre
Auckland, New Zealand

Te Ra Whakaora Study

An investigation of the vitamin D status of New Zealand 2-4 year-olds; the factors affecting vitamin D status, and the relationship between vitamin D status, respiratory infection and allergy

Information for Parents / Guardians / Caregivers

You are invited to take part in a university research project investigating the vitamin D status of New Zealand pre-school children. The research is being conducted by the Human Nutrition Research team at Massey University, the contact details are below:

Principal Investigator: Dr Pamela von Hurst Institute of Food, Nutrition and Human Health Massey University, Albany Tel: (09) 414 0800 ext 41205 Email: p.r.vonHurst@massey.ac.nz	**Study administrator:** Carolyn Cairncross Institute of Food, Nutrition and Human Health Massey University, Albany Tel: (09) 443 9387 or 021 422531 Email: TeRaWhakaora@massey.ac.nz

Why is this research important?

Vitamin D, which we mostly get from the sun, is very important for building strong bones and maintaining good health. Previous studies conducted in New Zealand and overseas suggest that many people are failing to achieve good vitamin D levels and this potentially affects their long term health.

Although there is some information available about the vitamin D status of New Zealand adults, and a little about newborn babies, we know nothing about the vitamin D status of our pre-school age children. These children are not only very vulnerable to the adverse health effects of vitamin D deficiency; their tender skin is also more likely to suffer damage from too much sun exposure.

As a parent you are probably very aware of the importance of protecting your child from sunburn. This study aims to find out if our concerns about sunburn are having a negative effect on the vitamin D status of our children, and if low vitamin D levels are related to the coughs, colds and allergies that young children suffer from, especially in winter.

The information gathered in this study will also be used to create a simple questionnaire-type tool which can be used by health professionals and parents to determine if a child is at risk of vitamin D deficiency, therefore identifying children at risk before their health is potentially affected.

Who are we looking for?

For this study we need to recruit 1600 2-4 year-olds from all around New Zealand.

What is going to happen?

If you decide, on behalf of your pre-schooler, to participate in the study, you will be sent a consent form and a questionnaire to complete about your child and your family. Your consent is required as

the parent of the child, however it is important that, as much as possible, your child is comfortable about taking part. Therefore you are asked to give consent on your own behalf and on behalf of your child. You will be directed to a participating pharmacy where your child will have their height and weight measured, and have a drop of blood taken from a finger to test their vitamin D levels.

The finger prick is very quick and painless. The person in the pharmacy will be well trained and skilled in the procedure. The finger is cleaned with an alcohol wipe and then a special automatic lancet designed for use in young children is pressed against the finger. Most people don't even realise that their skin has been punctured, and at the most there is minor discomfort. Two drops of blood are put on a special card, your child's finger will be dressed with a colourful sticking plaster, and they will be given a reward in the form of a sticker or a stamp, depending on their age.

The pharmacy staff will take your questionnaire from you, record the height and weight, enclose the blood spot card, and courier it all back to Massey University.

The card will be sent to a laboratory at the University of Queensland, Australia, which specialises in analysis of vitamin D. A hole is punched in the middle of the blood spot and this is analysed, the rest of the card is destroyed, meaning that this sample cannot be used again.

What are the benefits and risks of taking part in this study?

You will receive a letter with the results of your child's vitamin D level in around six months (this is how long the laboratory will take to analyse all 1600 samples). Because vitamin D levels are very variable and change considerably depending on season and amount of sun exposure, the results of the test are indicative of your child's vitamin D level ***at the time of the test only***. However, you will also receive a letter explaining the results, and suggestions for further action if required.

You will also receive a brief report summarising the main findings of the project via mail or email, we anticipate this will be in 2014.

The principal benefit of taking part in this study is that you will contribute to research which will potentially be of considerable benefit to the health of all New Zealand children.

Project Procedures

The data will be used only for the purposes of this project and no individual will be identified. Only the investigators of the study will have access to personal information and this will be kept secure and strictly confidential. Participants will be identified only by a study identification number. Results of this project may be published or presented at conferences or seminars. No individual will be able to be identified.

At the end of this study the list of participants and their study identification number will be disposed of. Any raw data on which the results of the project depend will be retained in secure storage for 10 years, after which it will be destroyed.

Who is funding the research?

This research is funded by the Health Research Council of New Zealand

Participant's Rights

You are under no obligation to accept this invitation. If you decide to participate, you have the right to:

- decline to answer any particular question;
- withdraw from the study at any time
- ask any questions about the study at any time during participation;

- provide information on the understanding that your name will not be used,
- be given access to a summary of the project findings when it is concluded.

If you have any queries or concerns regarding your rights as a participant in this study, you may wish to contact an independent health and disability advocate. Free phone 0800 555 050. Free fax 0500 2SUPPORT (0800 2787 7678) Email: advocacy@hdc.org.nz

Project Contacts

If you have any further questions or concerns about the project, either now or in the future, please contact either Dr Pamela von Hurst or the study administrator (details on page 1).

Committee Approval Statement

This project has been reviewed and approved by the Health and Disability Ethics Committee, Northern Region, application number NTX/12/04/036

Compensation for injury

If physical injury results from your participation in this study, you should visit a treatment provider to make a claim to ACC as soon as possible. ACC cover and entitlements are not automatic and your claim will be assessed by ACC in accordance with the Injury Prevention, Rehabilitation and Compensation Act 2001. If your claim is accepted, ACC must inform you of your entitlements, and must help you access those entitlements. Entitlements may include, but not be limited to, treatment costs, travel costs for rehabilitation, loss of earnings, and/or lump sum for permanent impairment. Compensation for mental trauma may also be included, but only if this is incurred as a result of physical injury.

If your ACC claim is not accepted you should immediately contact the researcher. The researcher will initiate processes to ensure you receive compensation equivalent to that to which you would have been entitled had ACC accepted your claim.

Thank you for considering participating in this study!

The **Te Ra Whakaora** research team

Te Ra Whakaora

MASSEY UNIVERSITY

Institute of Food, Nutrition and Human Health
Massey University
Private Bag 102-904
North Shore Mail Centre
Albany, Auckland
New Zealand

T 09 414 0800
F 09 443 9640

Te Ra Whakaora Study

Consent Form for Parents / Guardians / Caregivers

This consent form will be held for a period of five (5) years

I have read the Information Sheet and have had the details of the study explained to me. My questions have been answered to my satisfaction, and I understand that I may ask further questions at any time.

I agree to participate in this study under the conditions set out in the Information Sheet.

As the parent/main caregiver of the child named below, I give consent for participation on behalf of the child.

Signature: ..... **Date:** .....

Full Name - printed .....
Parent / Guardian / Caregiver

Full Name - printed .....
Child's name

The findings of this study may be the basis of further investigations by our team. Would you be happy for us to contact you in future about research related to this study?

☐ Yes ☐ No

Appendix 3

Standard operating procedures

- Standard operating procedure for blood collection
- Outline for child visit to pharmacy

Te Ra Whakaora

Finger-Prick Test Procedure

Getting Started

- Is the child's hand warm?
- Lay out material
  - Alcohol pad
  - Cotton ball
  - Blood Spot Card
  - Lancet
  - Plaster

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Getting Started

- Open the blood spot card out flat, and place on a flat surface around the height of the child's wrist.

- Put on gloves
- Open the alcohol wipe package

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The finger-prick site

- Choose the 2nd, 3rd or 4th finger of the child's non-dominant hand

- Wipe the **underside** tip of this finger with the alcohol wipe
- Allow to dry

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Piercing the skin

- Twist the cap off the lancet – twist **2-3** times and it will pop, then pull the cap out
- 'Milk' the finger from the middle joint upwards, until the finger tip is crimson in colour
- Place the lancet against the ***under-side*** of the finger, right up near the tip

- Press the lever firmly until it activates - you should hear a 'click'

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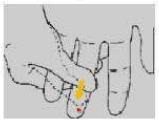
Collecting the blood spots

- Position the finger over the blood spot card



- Gently 'milk' the blood drop from the finger
- Wait until a **good sized** blood spot forms (a raindrop) then gently touch the blood itself to the centre of a circle

- Do not touch the card with the child's finger

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Collecting the blood spots

- Don't worry if the blood isn't in the middle of a circle
- Place a second drop of blood ***DIRECTLY*** on top of the first drop

Te Ra Whakaora

- If the drops of blood are very small, place a third drop ***DIRECTLY*** on top of the other drops on the card

Ve

Valid Sample

Inv

Invalid Sample
More blood needed

When the blood has been collected

- Hold the cotton bud tightly to the finger for one minute

- Place plaster over the wound

- Present child with stickers, lollipop & certificate

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Drying the blood spot

- Form a 'tent' by folding the cover backwards to create a stand
- Allow the blood spot card to dry for **at least one hour.**

- Once dried, fold the flap over the card and place in the bag

Te Ra Whakaora

Problems?

- Problems –

call us on
021 422531
anytime.

Te Ra Whakaora

Thank you for being part of the Te Ra Whakaora Study !!

Te Ra Whakaora

Outline for child visit to pharmacy

Child Visit at Pharmacy

1. Collect questionnaire and consent form.
2. Ensure consent form is signed.
3. Measure weight and height & record on form.
4. Place questionnaire and consent form in the courier bag.
5. Write the study number AND child's date of birth onto the back of the blood spot card. Open out ready for fingerprick test.
6. Prepare child for fingerprick – explain small prick, choose the finger, ensure their hands are warm, child chooses plaster
7. Put on disposable gloves, and wipe child's finger with alcohol wipe.
8. Gently press at the top knuckle of the finger so blood is concentrated at the tip, becoming slightly red.
9. Place lancet onto fingertip and squeeze, should hear 'click'
10. Have finger above the blood spot card.
11. Collect **at least two drops DIRECTLY on top of each other** which fill the equivalent of ONE circle on the blood spot card.
12. Press cotton ball onto finger to stop blood.
13. Place plaster on finger.
14. Praise and thank the child, and present the certificate & small gifts.

15. Create 'tent' with blood spot card open and allow to stand for at least one hour until **completely** dry.
16. Fold cover over card, place in box.

Thank you for being involved in this study, your generous input is very much appreciated.

Te Ra Whakaora www.TeRaWhakaora.massey.ac.nz

Appendix 4

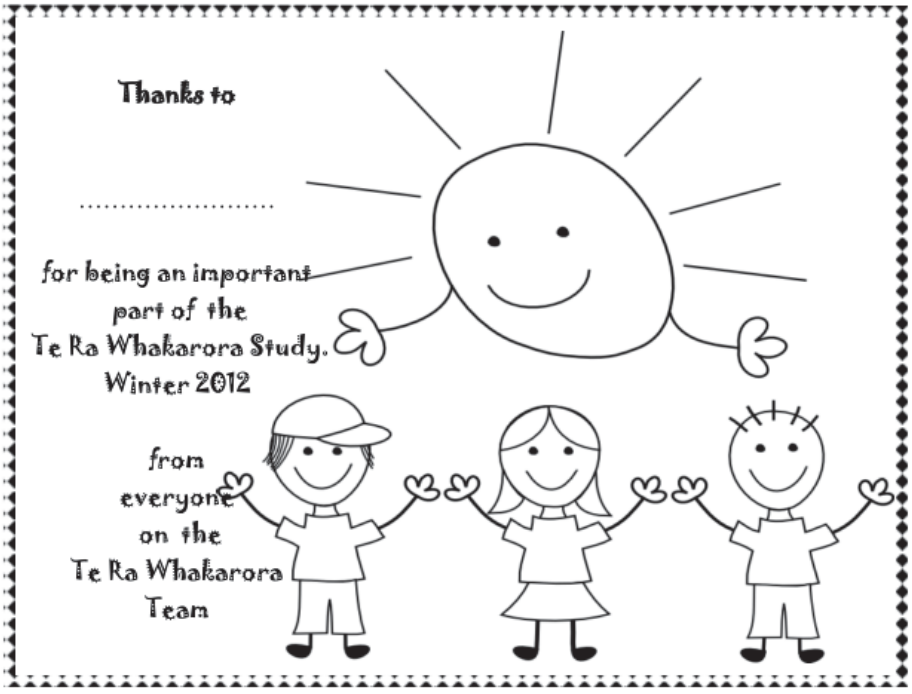
Equipment supplied to testing centres

Equipment supplied to testing centres

Alcohol swabs Blood spot (protein saver) cards (Whatman™903) Cotton Balls Courier bag Drying rack & storage box Gloves, disposable Lancets, single use pediatric (Unistik™ 3) Plaster – child themed Sharps disposal container Reward – certificates, lollipops, stickers	Advertising poster/brochures Copy of consent form, information sheet FAQ - study FAQ - vitamin D Outline of child visit to centre Standard Operating Procedure for fingerprick test & card processing Certificate of recognition

Individual bags provided for Auckland health services conducting testing (before-school checks)

Bravery Certificate



Te Ra Whakaora

Vitamin D deficiency risk and respiratory/allergy diseases in NZ 2-4 year olds.

Frequently Asked Questions about the Te Ra Whakaora Study

Who is being tested in this study?
We are aiming to test 1600 preschool children, aged 2-4 years, from all around New Zealand during winter 2012.

What does the questionnaire ask about?
It asks questions about your child & their home, their recent health (in particular respiratory disease, allergy and eczema) and some risk factors we know affect vitamin D levels (such as sun protection)

How long does the questionnaire take?
The questionnaire takes 10-15 minutes to complete.

Will the fingerprick test hurt?
Special thin lancets are used to make minimize any pain. The test is very quick, the staff have been trained and are practiced. Most children don't even realize their skin has been punctured, and at most there is minor discomfort. The staff will have colourful children's plasters to put on afterwards, and stickers to distract them during the actual fingerprick.

How much blood is needed?
Only 2-3 drops – this is put onto a blood spot card.

When will I be told the result of the fingerprick test?
It will take six months for all the samples to be collected, sent to Australia and analysed. Parents will then be sent a letter with the result, around April next year. This letter will include information about the level of vitamin D which was found, which you can then use to talk to a health professional if you wish. The study researchers will be happy to answer any questions once you receive your letter.

What will the blood test result tell us?
It will tell you the level of vitamin D which is in the child's blood on the day of the test, which will be an indication of their level in winter. Usually vitamin D levels are lowest in winter as the UVB rays from the sun aren't as strong, and we are covered over with clothing to stay warm.

How do they analyse the blood spot?
The spot will analysed at the University of Queensland. Laboratory staff will punch a hole in the middle of the spot, remove the blood off the card using a range of chemicals then put this liquid through a mass spectrometer (like they use on TV shows such as Bones or NCIS). The rest of the blood spot card is then destroyed so the blood cannot be used for any further purposes.

Why is this study being done?
With some of the highest rates of skin cancer in the world, New Zealanders are careful of getting too much sun, especially on our young children's tender skin. Our bodies get most of their vitamin D from sunshine, so this may be impacting on the amount of vitamin D preschoolers have in their bodies. Low vitamin D is known to affect bone strength, recent research thinks there may also be a link with other diseases, such as asthma, eczema and food allergy. We know New Zealand has high rates of these diseases, but we don't have any information about the levels of vitamin D in preschoolers in this country. The results from this study will tell us about any links. We know some of the risk factors for being low in vitamin D – darker skin colour, where you live in the country – but we hope to find out more specifically relevant to preschoolers.

A key part of the study will be developing a simple quiz-type tool - a series of quick questions - which a health professional can quickly fill out to decide if a child is at risk of having low levels of vitamin D. This avoids a blood test, and means any treatment is timely for improving the health of a child.

Who is doing this study?
This research is by the Institute of Food, Nutrition & Human Health at Massey University, in Albany, Auckland.

Has it had ethics approval?
Yes. This study is funded by the Health Research Council, & has been reviewed & approved by the Health & Disability Ethics Committee, NTX/12/04/036.

Te Ra Whakaora?
This study title was chosen as it combines the major source of vitamin D and the necessity of this vitamin for health. It has Maori origin, Te Ra being the sun, and whakaora meaning wellness or health.

I would like more information…..
Please contact the study staff:
call 0800MASSEY and ask for Te Ra Whakaora Study (9am-5pm)
call/text 021 422531
email TeRaWhakaora@massey.ac.nz
visit TeRaWhakaora.massey.ac.nz

This study is funded by the Health Research Council, & has been reviewed & approved by the Health & Disability Ethics Committee, NTX/12/04/036

Te Ra Whakaora

Vitamin D deficiency risk and respiratory/allergy diseases in NZ 2-4 year olds.

Frequently Asked Questions about Vitamin D

What does vitamin D deficiency mean?
Deficiency means a severe lack of this vitamin in the body. Scientists have know for a long time that Vitamin D is essential for healthy bones, where it helps the calcium from our food to be absorbed into bones. Deficiency in vitamin D causes rickets, where soft bones cause bowed legs in children.

A low blood level of vitamin D may not be severe enough to cause rickets, but it may increase the risk of bone fractures. Vitamin D is being studied all around the world at the moment as researchers look at the links between low vitamin D levels and many other medical conditions, such as heart disease, type 1 diabetes, Multiple Sclerosis, immune function and some cancers. This study will be looking to see if low levels are linked with respiratory disease, eczema and allergy in young NZ children.

Where do we get vitamin D from?
Vitamin D is unique among the vitamins because it can be made by the body from sunshine absorbed by our skin. Vitamin D is often called 'the sunshine vitamin' as we only get a small amount from our food. All other vitamins come directly from our food.
It is the UVB rays in sunshine which generate vitamin D in our body, it is the UVA which causes the sunburn.

Which foods contain vitamin D?
There are small amounts of vitamin D in oily fish (tuna and salmon), eggs, meat and dairy products. You can now buy margarines, milks and yoghurts fortified with vitamin D available in New Zealand. Most of our vitamin D comes from sunshine exposure - you cannot get enough vitamin D from food alone, which is why it so important everyone has a certain amount of exposure to the sun.

Why might we not be getting enough vitamin D?
<u>Sun protection</u>
New Zealand has particularly strong sun, and some of the highest rates of skin cancer in the world. We have become very good at protecting ourselves with sunscreen, clothing and shaded areas, particularly the tender skin of our young children. This may be affecting our levels of vitamin D.

<u>Skin type</u>
People with darker coloured skin originally came from close to the equator, where the sun is very strong, and their skin has it's own sunscreen (melanin). This is very effective in stopping sunburn, but unfortunately also blocks the UVB, meaning they have to stay out in the sun for longer to get the same amount of vitamin D as someone with whiter European skin.

<u>Elderly</u>
People who live inside most of the time don't get a lot of sun hitting their skin, so can't make vitamin D. This is common in elderly people. As we age, our body becomes less efficient at making vitamin D too.

Winter

In winter, we wear more clothing to stay warm, which doesn't let the sun's rays through. Those people who wear modest clothing, perhaps for religious reasons, will also not be exposed to many of the suns rays.

Latitude

The UVB rays are stronger in the north of the country than the south. In the south island the sun rays have further to travel, and are at a lower angle, meaning there isn't as much UVB. This means people need to spend longer in the sun to make vitamin D, especially in the winter months.

Do I need to tan or burn to get enough vitamin D?

In a quarter (25%) of the time it takes your skin to start to turn pink you will have your daily dose of vitamin D. Your body won't create anymore vitamin D after this time, so more is not better. There isn't any reason to turn pink or be sunburnt to get a good dose of vitamin D.

How long do I need to be out in the sun each day?

This varies with where you live in New Zealand and the colour of your skin.
In the summer months, 8-15 minutes outside the peak sun hours of 11am-3pm should be sufficient, depending on the amount of sun.
In winter, 30min – 1 hour between 12-2pm while exposing your hands, face & arms. This length of time increases the further south you live, or have darker skin.

Can I make vitamin D with sun coming through a window?

Unfortunately, none. The UVB rays cannot go through glass, but you can still get burnt by the UVA rays. So if you are sitting in your car or by a window in the sun, you won't be increasing your vitamin D levels.

Should I be taking vitamin D supplements?

Talk to your doctor or pharmacist. Those particularly at risk are older people who are mainly indoors, if you have a darker skin colour, fair-skinned people who avoid the sun, people who wear modest clothing and South Island residents (during the winter months).

Te Ra Whakaora

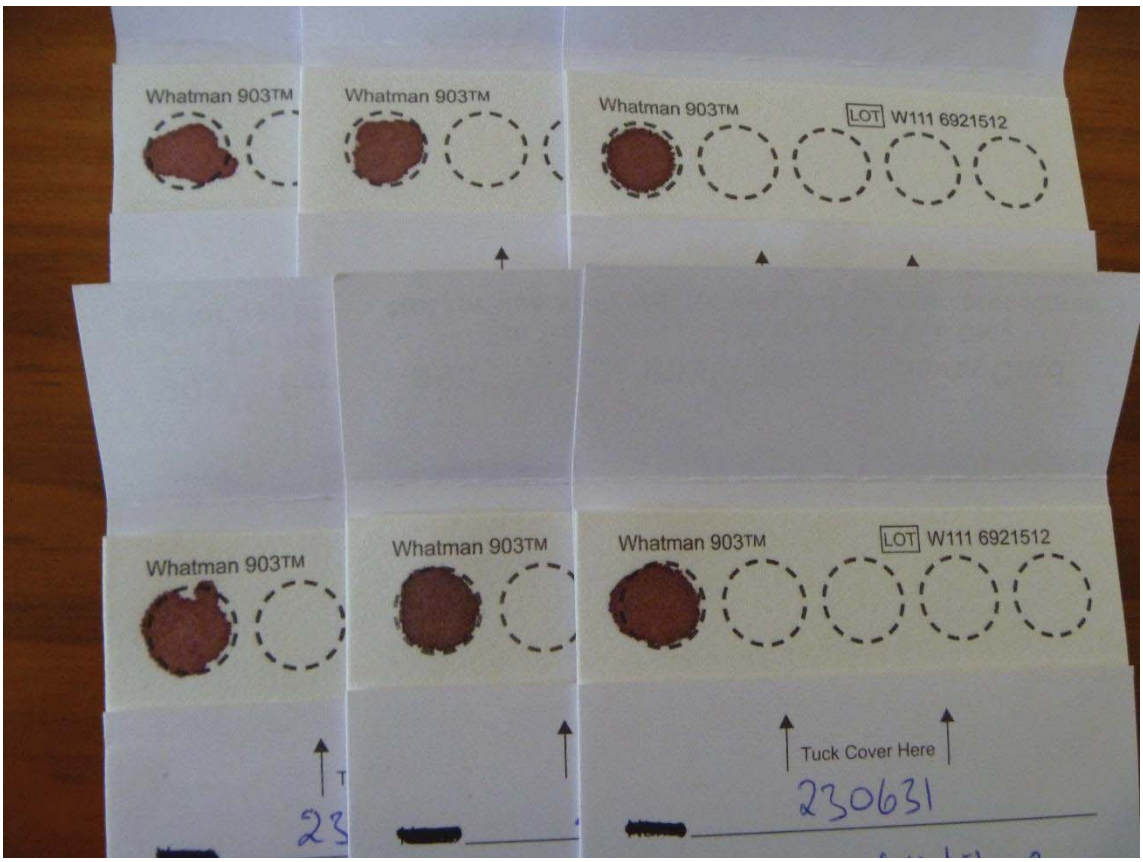
www.TeRaWhakaora.massey.ac.nz

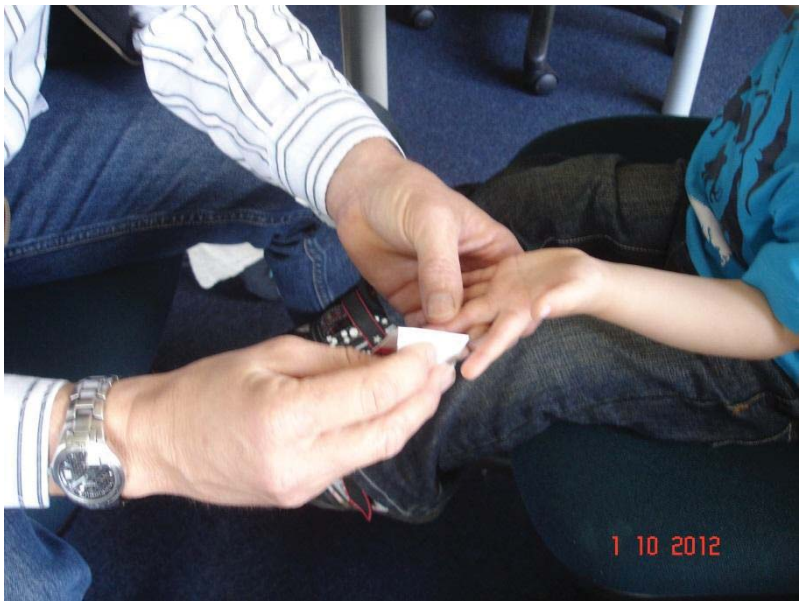
Certificate of appreciation given to each pharmacy

Appendix 5

Photograph of blood spot cards

Photograph of blood spot cards and sampling





Appendix 6

Questionnaire

MASSEY UNIVERSITY

Study Number

Te Ra Whakaora

Vitamin D deficiency risk and respiratory/allergy diseases in NZ 2-4 year olds

QUESTIONNAIRE

Thank you for allowing your child to be part of our study!

The next step is for a parent or guardian to fill out this questionnaire.

Once it is completed, please place it in the pre-paid (Freepost) envelope and mail back to us.

Prefer online?

An online version is available at www.surveymonkey.com/s/TeRaWhakaora

It will ask you for your study number – this is the number at the top of this page.

Have a question??

We would be more than happy to help you fill out the questionnaire –

just call us on

0800MASSEY, ask for the Te Ra Whakaora study (9am – 5pm) or 021 422531

email us at TeRaWhakaora@massey.ac.nz

Instructions for the questionnaire: Place a *tick* in the circle to indicate your answer.

About your child

These questions are about your child

1. How old is your child?

- ◯ 2 years
- ◯ 3 years
- ◯ 4 years

2. Is your child...

- ◯ Female
- ◯ Male

3. Which ethnic group does your child belong to? Please tick all which apply.

- ☐ NZ European
- ☐ Other European
- ☐ Maori
- ☐ Pacific Island
- ☐ Chinese
- ☐ Indian
- ☐ Other Asian

Other (please specify)

4. In your child's home, how many children who are OLDER than your child live there?

- ◯ 0
- ◯ 1
- ◯ 2
- ◯ 3
- ◯ 4
- ◯ 5+

5. In your child's home, how many children who are YOUNGER than your child live there?

- ◯ 0
- ◯ 1
- ◯ 2
- ◯ 3
- ◯ 4
- ◯ 5+

6. How would you describe the colour of your child's skin?

- ◯ Very Fair
- ◯ Fair
- ◯ Medium
- ◯ Olive Dark
- ◯ Very Dark
- ◯ Black

7. What reaction does your child's skin have to sun exposure?

- ◯ Always burns, never tans
- ◯ Usually burn, tan less than average (with difficulty)
- ◯ Sometimes mild burn, tan about average
- ◯ Rarely burn, tan more than average (with ease)
- ◯ Don't know

8. Who usually looks after your child during the day? Please check all which apply.

- ☐ A Parent or Guardian
- ☐ A daycare or preschool centre
- ☐ Kindergarten
- ☐ Other (please specify)
- ☐ A nanny, babysitter or in-home care provider
- ☐ A family member

9. How many hours PER WEEK does your child spend at daycare, preschool or kindergarten?

- ◯ 0
- ◯ 1 - 10
- ◯ 11 - 20
- ◯ 21 - 30
- ◯ 30+

About the child's mother

10. Which is the highest educational qualification the child's mother has completed?

- ◯ Attended intermediate or secondary school but no qualification
- ◯ School certificate / NCEA level 1, or similar overseas qualification
- ◯ Completed high school or NCEA level 2 or 3, or similar overseas qualification
- ◯ Trade certificate, technical institute qualification or other full-time study since leaving school
- ◯ University degree or diploma

Other (please specify)

Your child and the sun

11. How long, on average, would your child play outside in SUMMER?

- ◯ Less than 1/2 hour per day
- ◯ 1/2 - 1 hour per day
- ◯ 1+ hours per day
- ◯ Don't know

12. How long, on average, would your child play outside in WINTER?

- ◯ Less than 1/2 hour per day
- ◯ 1/2 - 1 hour per day
- ◯ 1+ hours per day
- ◯ Don't know

13. When your child is playing outside in SUMMER, do they...

	Always	Usually	Sometimes	Never
Use sunscreen SPF15+	☐	☐	☐	☐
Wear a sun hat	☐	☐	☐	☐
Wear long sleeves and pants	☐	☐	☐	☐
Avoid the midday sun	☐	☐	☐	☐

14. When your child is playing outside in WINTER, do they...

	Always	Usually	Sometimes	Never
Use sunscreen SPF15+	☐	☐	☐	☐
Wear a sun hat	☐	☐	☐	☐
Wear long sleeves and pants	☐	☐	☐	☐
Avoid the midday sun	☐	☐	☐	☐

Eating habits

This section looks at several foods your child may eat.

15. What type of milk does you child usually drink. Tick all which apply.

- ☐ Standard (Blue top)
- ☐ Lite (Light blue top)
- ☐ Trim (Green top)
- ☐ Anchor Calci Plus
- ☐ Anchor Mega
- ☐ Anchor Superblue
- ☐ Meadowfresh Calcitrim or Calcistrong
- ☐ My child does not drink milk

Other (please specify)

16. Does your child take any vitamins or supplements which contain Vitamin D?

- ◯ Yes
- ◯ No

If YES, please tell us the name

17. Do you give your child cod liver oil?

- ◯ Yes
- ◯ No

If YES, how much do they have each day?

Health - Respiratory

In this section we will be asking questions about the recent respiratory health of your child

18. Has your child EVER had wheezing or whistling in the chest at any time in the past?

- ◯ Yes
- ◯ No - please skip to QUESTION 23

19. Has your child had wheezing or whistling in the chest in the last twelve months?

- ◯ Yes
- ◯ No - Please skip to QUESTION 23

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

- ◯ None
- ◯ 1 - 3
- ◯ 4 - 12
- ◯ More than 12

21. In the past 12 months, how often, on average, has your child's sleep been disturbed due to wheezing?

- ◯ Never woken with wheezing
- ◯ Less than one night per week
- ◯ One or more nights per week

22. 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?

- ◯ Yes
- ◯ No

Health - Asthma

23. Has your child EVER had asthma?

- ◯ Yes
- ◯ No - Please skip to QUESTION 25

24. Which asthma medicines has your child used? Please tick all those which have been used.

- ☐ Ventolin or Salapin or Salbutamol syrup
- ☐ Ventolin or Respogen or Salbutamol inhaler
- ☐ Ventolin or Asthalin nebulizer
- ☐ Beclomethasone or Beclozone or Qvar inhaler
- ☐ Budesonide or Entocort or Pulmicort inhaler
- ☐ Budesonide or Entocort or Pulmicort Turbuhaler
- ☐ Flizotide Inhaler
- ☐ Flixotide Diskhaler
- ☐ Not sure
- ☐ Other (please specify)

Health

In this section we will be asking questions about the recent health of your child

25. In the LAST 12 MONTHS, has your child's chest sounded wheezy during or after activity?

- ◯ Yes
- ◯ No

26. In the LAST 12 MONTHS, has your child had a dry cough at night, apart from a cough associated with a cold or chest infection?

- ◯ Yes
- ◯ No

27. In the LAST 12 MONTHS how many times has your child had a cough or chest infection?

- ◯ None
- ◯ 1 - 2 times
- ◯ 3 - 4 times
- ◯ 5+ times

28. In the LAST 12 MONTHS how many times has your child had a common cold?

- ◯ None
- ◯ 1 - 2 times
- ◯ 3 - 4 times
- ◯ 5+ times

29. How many times has your child been prescribed antibiotics in the LAST 12 MONTHS?

- ◯ None
- ◯ Once
- ◯ Two times
- ◯ Three times or more

30. How many ear infections has your child had in the LAST 12 MONTHS?

- ◯ None
- ◯ 1 to 2
- ◯ 3 to 4
- ◯ More than 5

31. How many ear infections has your child EVER had?

- ◯ None
- ◯ 1 to 2
- ◯ 3 to 4
- ◯ More than 5

32. Has your child been diagnosed with any of the following illnesses in the last 12 months?

- ◯ Bronchitis
- ◯ Bronchiolitis
- ◯ Pneumonia
- ◯ Tuberculosis
- ◯ None of these illnesses

33. 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?

- ◯ Yes
- ◯ No - Please skip to QUESTION 38

34. In the PAST 12 MONTHS, has your child had a problem with sneezing, or a runny nose when he/she DID NOT have a cold or the flu?

- ◯ Yes
- ◯ No

35. In the PAST 12 MONTHS, has this nose problem been accompanied by itchy-watery eyes?

- ◯ Yes
- ◯ No

36. In which of the PAST 12 MONTHS did this nose problem occur? Please tick all which apply.

- ☐ January
- ☐ February
- ☐ March
- ☐ April
- ☐ May
- ☐ June
- ☐ July
- ☐ August
- ☐ September
- ☐ October
- ☐ November
- ☐ December
- ☐ Not sure

37. In the PAST 12 MONTHS, how much did this nose problem interfere with your child's daily activities?

- ◯ Not at all
- ◯ A little
- ◯ A moderate amount
- ◯ A lot

Health - Hayfever

38. Has your child EVER had hayfever?

- ◯ Yes
- ◯ No

Health

In this section we will be asking questions about your child's skin

39. Has your child EVER had an itchy rash which was coming and going for at least six months?

- ◯ Yes
- ◯ No - Please skip to QUESTION 50

40. Has your child had this itchy rash at any time in the last 12 months?

- ◯ Yes
- ◯ No - Please skip to QUESTION 50

41. 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

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

- ◯ Under 2 years
- ◯ Age 2-3 years
- ◯ Age 4 years

43. Has this rash cleared completely at any time during the last 12 months?

- ◯ Yes
- ◯ No

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

- ◯ Never in the last 12 months
- ◯ <1 night a week
- ◯ 1+ nights a week

45. Is this rash worse in winter?

- ◯ Yes
- ◯ No

46. Have you used any steroid cream or steroid ointment on your child's rash?

- ◯ Yes
- ◯ No - Please skip to QUESTION 48

47. Which cream or ointment did you use on the rash? (Please tick all the ones you have used.)

- [ ] Dirposone
- [ ] Beta cream
- [ ] Hydrocortisone cream or ointment
- [ ] Locoid cream, ointment or lipocream
- [ ] Triamcinolone
- [ ] Not sure of the name

Other (please specify)

48. Have you used any moisturising cream or ointment on your child's rash?

- ◯ Yes
- ◯ No - please skip to QUESTION 50

49. Which moisturising cream or ointment did you use on the rash? Please tick all the ones you have used.

- [ ] Aqueous Cream
- [ ] DP Lotion
- [ ] healthE Fatty cream
- [ ] Not sure of the name

Other (please specify)

Health - eczema

50. Has your child EVER had eczema?

- ◯ Yes
- ◯ No

Health

These questions are about food allergy. By food allergy we mean a reaction within half an hour of eating the food, including either swelling, hives (red blotches on the skin that move around and are itchy), difficulty breathing, wheezing or vomiting within an hour.

51. Has your child ever had food allergy?

- ◯ Yes
- ◯ No - Please skip to QUESTION 56

52. Which food(s) has your child reacted to? Please tick all which apply.

- ◯ Egg
- ◯ Milk
- ◯ Peanut
- ◯ Seafood

Other (please specify)

53. Was your child's allergy confirmed or diagnosed by a doctor?

- ◯ Yes
- ◯ No

54. Has your child ever been to hospital as a result of a food allergy?

- ◯ Yes
- ◯ No

55. Does your child have an Epipen?

- ◯ Yes
- ◯ No

Health

This section asks about some specific conditions.

56. Does your child suffer from any of the following?

- ☐ Crohn's Disease
- ☐ Celiac Disease
- ☐ Inflammatory Bowel Disorder
- ☐ Cholestatic Liver Disease
- ☐ Cystic Fibrosis
- ☐ None of the above

Home

This section looks at the child's home.

57. How many days, in an average week, is your child in the same room as someone who is smoking?

- ◯ Never
- ◯ 1 day per week
- ◯ 2 days per week
- ◯ 3 days per week
- ◯ 4 days per week
- ◯ 5 days per week
- ◯ 6 days per week
- ◯ 7 days per week

58. In the past 12 months, has there been a dog living in the child's home?

- ◯ Yes
- ◯ No

59. In the past 12 months, has there been a cat living in the child's home?

- ◯ Yes
- ◯ No

Thank You!

Thank you, we appreciate the time you have given to complete this survey.

Please place this completed questionnaire in the pre-paid (Freepost) envelope,

and pop into a mailbox.

Te Ra Whaka  ra

Appendix 7

Letter to parents – child dried blood spot 25(OH)D analysis result

Dear Parent/Guardian of <child name>,

Late last year your family took part in the Te Ra Whakaora - Children's Vitamin D study, with you generously allowing us to take a blood spot from your preschool child. We ended up collecting blood spots from 1350 children throughout New Zealand, which were sent to the University of Brisbane for analysis of vitamin D level. We had hoped to have the result back to you earlier, unfortunately there were some unavoidable delays in the research laboratory. This was outside our control, and we apologise for any inconvenience this delay may have caused you.

The laboratory tested the blood spot for 25-hydroxyvitamin D (or 25(OHD)) which is an indicator of vitamin D status. Blood levels of 25(OHD) vary throughout the year, with highest levels seen in summer and lowest levels in winter. This is due to the sun being the major source of vitamin D, and we have more exposure in the warmer summer months. Your child's vitamin D level reported below will be at its lowest point of the year as the blood spot was taken at the end of winter. It is not yet known if people should aim to have consistent levels throughout the year, or whether the natural changes with the seasons happen for a biological reason.

The blood spot test for your child has reported their vitamin D status level in winter last year to be <25OHD range>.

The Ministry of Health have recently released a Consensus Statement on Vitamin D and Sun Exposure in New Zealand. This is available at http://www.health.govt.nz/publication/consensus-statement-vitamin-d-and-sun-exposure-new-zealand In this statement, they report it is not possible to determine an optimal status level for vitamin D. The results of this study are being reported as 'adequate', 'insufficient' and 'deficient'. You may also be interested in reading the 'question and answer' page on Vitamin D which comes with the above statement and is attached to this letter.

If you have any concerns over your child's vitamin D level, please consult your family doctor. There is a section in the Consensus Statement on page 10 which outlines 'Recommendations for Supplementation', which you may like to take along with you.

By being part of this study, your family has directly contributed to the future health of New Zealand pre-schoolers. The Consensus Statement has information for adults, and there has just been a companion statement released for pregnant women and infants. At this stage, there is a gap around recommendations for young children. This study is in fact listed under the Emerging Research (page 11), and the results will be providing data for the development of recommendations for young children in the future.

The next step for the study team is to analyse the data collected. Like all research this unfortunately takes time but we will be in touch with you each time a set of results is released.

I would like to share with you that this research has been a very personal experience for all involved. We had over 130 people taking fingerpricks throughout the country - many have told me they how they were touched by the children and parents who visited them with everyone having a story to share. I myself have fond memories of the gorgeous wee girl who gave me a hug at the end, even after I pricked her fingers twice, I think the Barbie plasters may have been a big hit!

Once again, I would like to thank your family for being involved in this research. Please don't hesitate to contact the study team if you have any questions regarding this result, our email address is terawhakaora@massey.ac.nz.

Kind regards,
Carolyn Cairncross
Study Manager

On behalf of the
Te Ra Whakaora Study team

Appendix 8

Supplementary results

Supplementary results: List of Tables and Figures

Predictive questionnaire development

Predictive model for vitamin D deficiency including household deprivation

Relationship of vitamin D status with allergic and respiratory diseases

Predictive questionnaire development

Table 8.1. Predictive questionnaire development - mean dried blood spot 25-hydroxyvitamin D (25[OH]D) concentration by reported ethnicity

Ethnicity	**n (%)**	**Mean (S.D.) 25(OH)D (nmol/L)**	**P value***
Total	1329	52 (19)	
NZ European Not NZ European	1144 (86) 185 (14)	54 (18) 40 (20)	<0.000
Other European Not other European	137 (10) 1192 (90)	49 (17) 52 (19)	0.06
Maori Not Maori	174 (13) 1155 (87)	50 (20) 52 (19)	0.15
Pacific Not Pacific	64 (5) 1265 (95)	40 (18) 53 (19)	<0.000
All Asian Not all Asian	48 (4) 1281 (96)	48 (20) 52 (19)	0.20
Indian Not Indian	25 (2) 1304 (98)	29 (15) 52 (19)	<0.000
African Not African	14 (1) 1315 (99)	31 (19) 52 (19)	<0.000

S.D. standard deviation
*P value from Independent t-test

Table 8.2. Comparison of participant characteristics in the 'total', 'development' and 'validation' groups

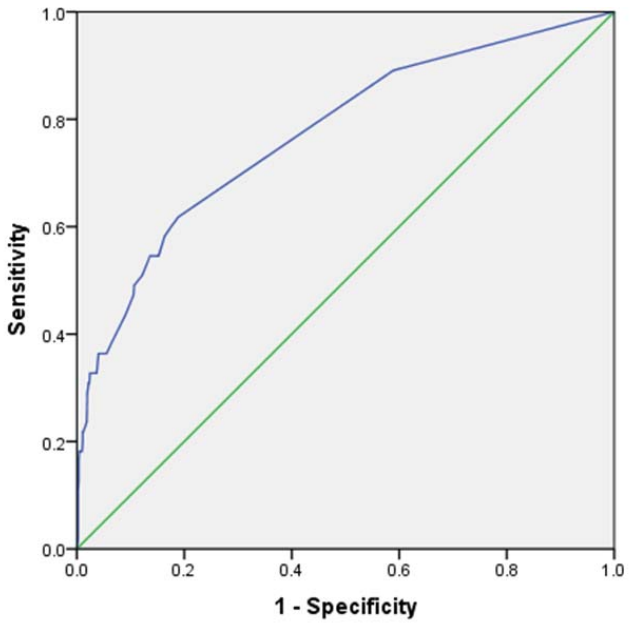
Variable	Total n (%)	'Development' n (%)	'Validation' n (%)
Total	1329 (100)	929 (70)	400 (30)
Gender			
Female	648 (49)	461 (50)	187 (47)
Male	681 (51)	468 (50)	213 (53)
Age (years)			
2 years old	496 (37)	349 (38)	147 (37)
3 years old	448 (34)	310 (33)	138 (35)
4 years old	385 (29)	270 (29)	115 (29)
Ethnicity			
Indian/African	38 (3)	24 (3)	14 (4)
Pacific	62 (5)	50 (5)	12 (3)
Any European	1166 (88)	810 (87)	356 (89)
Maori/Asian	63 (5)	45 (5)	18 (5)
Skin colour			
Very fair – fair	767 (58)	258 (56)	239 (60)
Medium	445 (33)	315 (34)	130 (33)
Olive – very dark	117 (9)	86 (9)	31 (8)
Attend formal daycare			
Yes	1137 (86)	791 (85)	293 (85)
No	192 (14)	138 (15)	58 (15)
Vitamin D supplement use[§*]			
Yes	253 (19)	159 (17)	68 (17)
No	1076 (81)	770 (83)	332 (83)
Toddler milk			
Yes	80 (6)	59 (6)	21 (5)
No	1249 (94)	870 (94)	379 (95)
Mothers education qualifications			
No secondary education	59 (4)	45 (5)	14 (4)
Secondary	198 (15)	147 (16)	51 (13)
Post-secondary	1072 (81)	737 (80)	335 (84)
Household deprivation			
Quintile 1	343 (26)	231 (25)	112 (28)
Quintile 2	331 (25)	224 (24)	107 (27)
Quintile 3	287 (22)	229 (25)	78 (20)
Quintile 4	227 (17)	173 (18)	54 (14)
Quintile 5	141 (11)	92 (10)	49 (12)

Predictive model for vitamin D deficiency including household deprivation.

Table 8.3. Logistic regression analysis for vitamin D deficiency (25[OH]D <25nmol/L) for 'development' dataset minus children who drink toddler milk (n=870). Model includes deprivation index.

	B	S.E.	P value	Odds Ratio (OR)	95% C.I.	
Female	.597	.314	.049	1.817	.982	3.363
Mother with no secondary education qualifications	1.550	.433	.000	4.714	2.019	11.006
Olive -dark skin colour	1.256	.389	.001	3.510	1.637	7.524
Indian or African ethnicity	1.610	.576	.005	5.004	1.617	15.481
Pacific ethnicity	1.000	.485	.039	2.718	1.050	7.033
Asian ethnicity	1.351	.663	.041	3.863	1.054	14.164
Deprivation index 10	1.187	.501	.018	3.277	1.228	8.748

B regression coefficient; S.E. standard error



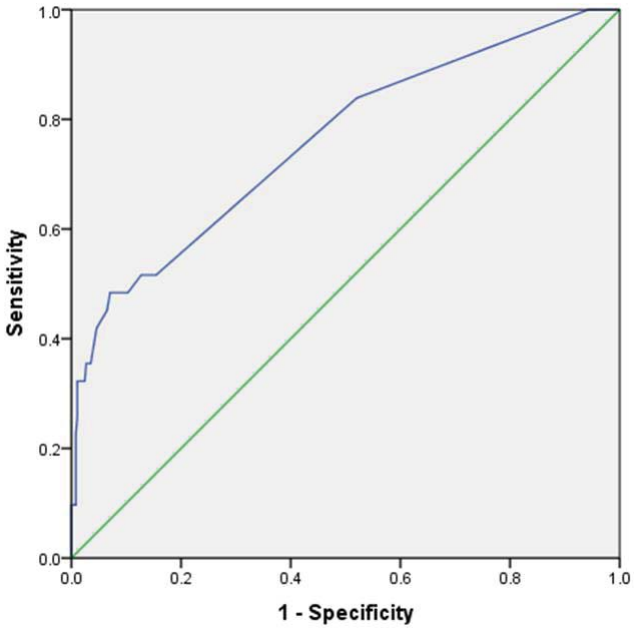
Area Under Curve (AUC): 0.77 (95% CI 0.70-0.84)

Figure 8.1. Receiver Operating Characteristic (ROC) for identifying preschool children with vitamin D deficiency (25[OH]D <25nmol/L) -'development' dataset minus children who drink toddler milk (n=870). Model includes deprivation index.

Table 8.4. Performance of model equation in identifying preschool children with vitamin D deficiency (25[OH]D <25nmol/L) - 'development' dataset minus children who drink toddler milk (n=870). Model includes deprivation index.

Total score	Sensitivity	1 - Specificity
-1.0000	1.000	1.000
.3000	.891	.589
.8000	.618	.189
1.0600	.600	.175
1.1900	.582	.163
1.3050	.545	.152
1.4500	.545	.136
1.5750	.509	.121
1.6050	.491	.107
1.6650	.473	.106
1.7900	.436	.091
1.9050	**.382**	**.064**
2.0350	.364	.055
2.1350	.364	.054
2.1800	.364	.040
2.2350	.327	.037
2.3200	.327	.028
2.4950	.327	.025
2.6650	.309	.023
2.7650	.309	.022
2.8350	.291	.020
2.8650	.236	.018
2.9250	.218	.012
3.0950	.218	.011
3.2400	.200	.011
3.3250	.182	.010
3.4250	.182	.009
3.6400	.182	.005
3.8700	.164	.004
4.1000	.145	.004
4.3400	.127	.004
4.4150	.109	.002
4.6750	.036	.002
4.9750	.018	.002
6.0200	.000	.000

Score ≥1.91 = risk of vitamin D deficiency
Score <1.91 = not at risk of vitamin D deficiency
Vitamin D deficiency 25(OH)D <25nmol/L
PPV positive predictive value; NPV negative predictive value



Area Under Curve (AUC): 0.76 (95% CI 0.66-0.86)

Figure 8.2. Internal validation: Receiver Operating Characteristic (ROC) for identifying preschool children with vitamin D deficiency (25[OH]D <25nmol/L) -'validation' subset (n=400). Model includes deprivation index.

Table 8.5. Internal validation: performance of model equation in identifying preschool children with vitamin D concentration (25[OH]D <25nmol/L)– 'validation' dataset (n=400)

Total score	Sensitivity	1 - Specificity	PPV	NPV
-9.0000	1.000	1.000		
-7.7000	1.000	.976		
-7.0250	1.000	.957		
-6.2500	1.000	.951		
-5.3900	1.000	.949		
-4.7600	1.000	.946		
-2.2950	1.000	.943		
.3000	.839	.520		
.8000	.516	.154		
1.0600	.516	.146		
1.1900	.516	.127		
1.3050	.484	.103		
1.4500	.484	.095		
1.5750	.484	.084		
1.6050	.484	.070		
1.6650	.452	.065		
1.7900	.419	.046		
1.9050	**.355**	**.035**	**46% (26-67)**	**95% (92-97)**
2.0800	.355	.027		
2.2950	.323	.024		
2.4950	.323	.022		
2.6400	.323	.014		
2.7650	.323	.011		
2.8650	.258	.011		
3.0100	.226	.008		
3.1550	.194	.008		
3.2150	.129	.008		
3.3700	.097	.008		
3.9400	.097	.003		
4.4150	.097	.000		
4.5050	.032	.000		
5.5900	.000	.000		

Score ≥1.91 = risk of vitamin D deficiency
Score <1.91 = not at risk of vitamin D deficiency
Vitamin D deficiency 25[OH]D <25nmol/L
PPV positive predictive value; NPV negative predictive value

Table 8.6. Sample layout of predictive questionnaire for assessment of risk of vitamin D deficiency (25[OH]D <25nmol/L) in winter in preschool children

Question	Answer	Answer Score	Child Score
Is your child male or female?	Male Female	0 0.60	
Which ethnic group does your child identify with?	Maori Pacific Island NZ European Indian or African Asian	0 1.00 0 1.61 1.35	
Has the child's mother completed any secondary school qualifications?	Yes No	0 1.55	
Which category best fits the child's skin colouring	Very fair to fair Medium Olive to dark	0 0 1.26	
Does the child live in a household of deprivation index 10?	Yes No	1.12 0	
Does the child drink infant formula or toddler milk?	Yes No	-8 0	
Total Score			

Total Score for child:
≥1.91: child is at risk of vitamin D deficiency in winter, further investigation is recommended
<1.91: child is not at risk of vitamin D deficiency in winter

Relationship of vitamin D status with allergic and respiratory diseases

Table 8.7. Participant characteristics and mean dried blood spot 25-hydroxyvitamin D concentrations for children with and without eczema

	n (%)			Mean 25(OH) (S.D.) in nmol/L		
Variable	**Eczema**	**No Eczema**	**P value***	**Eczema**	**No Eczema**	**P value†**
Total	299 (23)	1030(77)		53 (20)	52 (19)	0.50
Country section			0.17			
North	134 (20)	517 (80)		53 (20	54 (20)	0.91
Central	97 (26)	281 (74)		52 (19)	49 (20)	0.22
South	68 (23)	232 (77)		53 (20)	52 (18)	0.65
Household Deprivation Quintile			0.38			
1	81 (24)	262 (76)		56 (20)	52 (19)	0.21
2	72 (24)	259 (25)		54 (18)	52 (19)	0.44
3	54 (19)	233 (81)		53 (19)	52 (18)	0.82
4	59 (26)	168 (74)		51 (19)	51 (21)	0.97
5	33 (23)	108 (77)		45 (20)	50 (19)	0.22
Age (years)			0.46			
2	117 (29)	279 (71)		53 (19)	51 (19)	0.44
3	104 (23)	344 (77)		52 (20)	53 (19)	0.24
4	78 (20)	307 (80)		53 (19)	51 (19)	0.43
Gender			0.09			
Male	166 (25)	515 (75)		56 (20)	54 (20)	0.28
Female	133 (20)	515 (80)		49(18)	50 (18)	0.56
BMI (kg/m^2)‡			0.52			
Normal	242 (22)	852 (78)		54 (20)	52 (19)	0.20
Overweight	39 (22)	134 (78)		49(19)	53 (19)	0.32
Obese	14 (29)	34 (71)		42(18)	47 (19)	0.39
Skin colour			0.03			
Very fair-fair	187 (24)	580 (76)		54 (19)	53 (18)	0.19
Medium	81 (18)	364 (82)		53 (19)	53(21)	0.99
Olive-dark	31 (26)	86 (74)		41 (20)	42 (20)	0.81
Ethnicity			0.17			
Maori	44 (25)	129 (75)		47(19)	51(20)	0.23
Pacific	14 (35)	26 (65)		36(17)	42(19)	0.38
Other	35 (22)	122 (78)		48(23)	41(19)	0.06
NZ European	206 (21)	753 (79)		56(18)	54(18)	0.23
Daycare attendance			0.28			
Yes	250 (22)	887 (78)		54(20)	52(19)	0.79
No	49 (25)	143 (75)		48(17)	49(19)	0.36
Maternal education			0.01			
No secondary	22 (37)	37 (63)		38(15)	41(28)	0.71
Secondary	49 (25)	149 (75)		47(17)	52(20)	0.09
Post secondary	228 (21)	844 (79)		55(20)	52(18)	0.03
Taking supplements			0.40			
Yes	62 (24)	191 (76)		60(20)	57(20)	0.93
No	237 (22)	839 (78)		51(19)	51(19)	0.32

	n (%)			Mean 25(OH) (S.D.) in nmol/L		
Dog in house			0.16			
Yes	80 (24)	235 (76)		54(19)	54(17)	0.57
No	219 (21)	795 (79)		52(19)	51(20)	0.82
Cat in house			0.47			
Yes	125 (21)	455 (79)		53(19)	54(19)	0.12
No	174 (23)	575 (77)		53(20)	50(19)	0.45
Smoking in same room			0.15			
Ever	10 (33)	20(67)		43(14)	46(16)	0.40
Never	289 (22)	1010 (78)		53(20)	52(19)	0.62
Total children in house			0.01			
1	62 (31)	185 (69)		53(24)	54(21)	0.75
2	153 (22)	542 (78)		54(18)	51(18)	0.06
3	55 (20)	226 (80)		49(18)	51(18)	0.37
4	12 (18)	56 (82)		64 (16)	56 (25)	0.27
5	17 (45)	21 (55)		41 (18)	42 (21)	0.94

25(OH)D concentration reported as mean; S.D. standard deviation

*P value from chi squared test

†P value from Independent t-test

‡Cole et al, 2000

Table 8.8. Participant characteristics and mean dried blood spot 25-hydroxyvitamin D concentrations for children with and without doctor diagnosed food allergy

	n (%)			Mean 25(OH) (S.D.) in nmol/L		
Variable	**Food allergy**	**No food allergy**	**P value***	**Food allergy**	**No food allergy**	**P value†**
Total	153 (12)	1176 (88)		56 (21)	52 (19)	0.007
Country section			0.35			
North	71 (11)	580 (89)		55 (22)	53 (19)	0.35
Central	51 (13)	327 (87)		59 (19)	48 (19)	0.000
South	31 (10)	269 (90)		52 (18)	52 (19)	0.89
Household deprivation quintile			0.59			
1	40 (12)	303 (88)		59 (22)	53 (19)	0.04
2	36 (11)	295 (89)		59 (20)	52 (19)	0.03
3	29 (10)	258 (90)		55 (16)	52 (19)	0.45
4	33 (14)	194 (86)		53 (20)	51 (21)	0.58
5	15 (11)	126 (89)		49 (25)	49 (18)	0.93
Age in years			0.29			
2	62 (12)	434 (88)		55 (20)	51 (19)	0.08
3	55 (12)	393 (88)		57 (20)	52 (20)	0.15
4	36 (9)	349 (91)		56 (23)	51 (18)	0.15
Gender			0.07			
Male	89 (13)	592 (87)		60 (20)	49 (18)	0.01
Female	64 (10)	584 (90)		51 (21)	54 (20)	0.46
BMI (kg/m^2)‡			0.97			
Normal	125 (11)	969 (89)		56 (22)	52 (19)	0.01
Overweight	19 (11)	154 (89)		54 (15)	52 (19)	0.60
Obese	5 (10)	43 (90)		48 (15)	46 (19)	0.79
Skin colour			0.10			
Very fair-fair	100 (13)	667 (87)		55 (19)	53 (18)	0.32
Medium	40 (9)	405 (81)		61 (24)	52 (20)	0.01
Olive-dark	13 (11)	104 (89)		51 (20)	40 (20)	0.07
Ethnicity			0.60			
Maori	22 (13)	151 (87)		57 (20	49 (19)	0.06
Pacific	7 (18)	33 (82)		43 (19)	39 (18)	0.57
Other	18 (11)	139 (89)		50 (20)	41 (20)	0.08
NZ European	58 (6)	853 (94)		58 (21)	54 (18)	0.07
Daycare attendance			0.79			
Yes	132 (12)	1005 (88)		56 (22)	52 (19)	0.02
No	21 (11)	171 (89)		54 (14)	48 (19)	0.20
Maternal education			0.46			
No secondary	9 (15)	50 (85)		55 (31)	38 (22)	0.04
Secondary	19 (10)	179 (90)		55 (20)	50 (19)	0.34
Post secondary	125 (12)	947 (88)		56 (20)	52 (19)	0.04
Taking supplements			0.01			
Yes	41 (16)	212 (84)		62 (21)	57 (20)	0.11
No	112 (10)	964 (90)		54 (20)	50 (19)	0.08

	n (%)			Mean 25(OH) (S.D.) in nmol/L		
Variable	**Food allergy**	**No food allergy**	**P value***	**Food allergy**	**No food allergy**	**P value†**
Dog in house			0.88			
Yes	37 (12)	278 (88)		58 (17)	53 (18)	0.09
No	116 (11)	898 (89)		55 (22)	51 (19)	0.03
Cat in house			0.51			
Yes	63 (11)	517 (89)		58 (21)	54 (19)	0.06
No	90 (59)	659 (56)		54 (20)	50 (19)	0.04
Smoking in same room			0.75			
Ever	4 (3)	26 (2)		43 (16)	45 (15)	0.77
Never	149 (11)	1150 (89)		56 (20)	52 (19)	0.005
Total children in house			0.90			
1	32 (13)	215 (87)		54 (24)	53 (21)	0.93
2	78 (11)	617 (89)		55 (19)	52 (18)	0.14
3	32 (11)	249 (89)		56 (22)	50 (17)	0.05
4	8 (12)	60 (88)		64 (17)	57 (25)	0.41
5+	3 (8)	35 (92)		81 (23)	38 (16)	0.002

25(OH)D concentration reported as mean; S.D. standard deviation

*P value from chi squared test

†P value from Independent t-test

‡Cole et al, 2000

Table 8.9. Participant characteristics and mean dried blood spot 25-hydroxyvitamin D concentrations of children with and without allergic rhinoconjunctivitis

Variable	n (%)			Mean 25(OH)D (S.D.) in nmol/L		
	Allergic rhino-conjunctivitis	No allergic rhino-conjunctivitis	P value*	Allergic rhino-conjunctivitis	No allergic rhino-conjunctivitis	P value†
Total	139 (10)	1190 (90)		52 (23)	52 (19)	0.98
Country section			0.34			
North	60 (9)	591 (91)		52 (23)	54 (19)	0.46
Central	45 (12)	331 (88)		51 (23)	49 (19)	0.69
South	34 (11)	266 (89)		54 (21)	52 (18)	0.46
Household deprivation quintile			0.13			
1	35 (10)	308 (90)		53 (20)	53 (19)	0.80
2	28 (8)	303 (92)		52 (21)	53 (19)	0.86
3	5 (9)	262 (91)		52 (23)	52 (18)	0.91
4	33 (15)	194 (85)		54 (26)	51 (19)	0.39
5	18 (13)	123 (87)		48 (25)	49 (18)	0.78
Age in years			0.03			
2	45 (9)	451 (91)		52 (23)	52 (19)	0.97
3	61 (14)	387 (86)		52 (21)	53 (19)	0.80
4	33 (9)	352 (91)		52 (24)	51 (18)	0.86
Gender			0.39			
Male	76 (11)	605 (89)		56 (24)	54 (19)	0.60
Female	63 (10)	585 (90)		48 (21)	50 (18)	0.41
BMI (kg/m^2)‡			0.89			
Normal	114 (10)	980 (90)		52 (24)	54 (19)	0.99
Overweight	16 (9)	157 (91)		51 (16)	52 (19)	0.99
Obese	5 (10)	43 (90)		36 (17)	47 (19)	0.20
Skin colour			0.30			
Very fair-fair	79 (10)	688 (90)		54 (23)	53 (17)	0.53
Medium	43 (10)	402 (90)		53 (18)	53 (20)	0.91
Olive-dark	17 (15)	100 (85)		39 (26)	42 (19)	0.69
Ethnicity			0.03			
Maori	22 (13)	151 (87)		48 (18)	50 (20)	0.61
Pacific	4 (10)	36 (90)		41 (32)	39 (17)	0.84
Other	26 (17)	131 (83)		41 (20)	43 (20)	0.72
NZ European	87 (9)	872 (91)		57 (23)	54 (18)	0.23
Daycare attendance			0.005			
Yes	130 (11)	1007 (89)		51 (22)	53 (19)	0.31
No	9 (5)	183 (95)		67 (28)	48 (17)	0.002
Maternal education			0.93			
No secondary	7 (12)	52 (88)		29 (20)	42 (24)	0.17
Secondary	21 (11)	177 (89)		42 (15)	52 (19)	0.02
Post secondary	111 (10)	961 (90)		55 (23)	53 (18)	0.14
Taking supplements			0.92			
Yes	26 (10)	227 (90)		62 (24)	57 (20)	0.28
No	113 (11)	963 (89)		50 (22)	51 (18)	0.57

Variable	n (%)			Mean 25(OH)D (S.D.) in nmol/L		
	Allergic rhino-conjunctivitis	No allergic rhino-conjunctivitis	P value*	Allergic rhino-conjunctivitis	No allergic rhino-conjunctivitis	P value†
Dog in house			0.68			
Yes	31 (10)	284 (90)		53 (18)	54 (18)	0.72
No	108 (11)	906 (89)		52 (24)	51 (19)	0.86
Cat in house			0.76			
Yes	59 (10)	521 (90)		52 (21)	54 (19)	0.32
No	80 (11)	669 (89)		52 (24)	50 (18)	0.38
Smoking in same room			0.60			
Ever	4 (13)	26 (87)		40 (22)	46 (14)	0.44
Never	135 (11)	1164 (89)		52 (23)	52 (19)	0.91
Total children in house			0.67			
1	32 (13)	215 (87)		52 (25)	54 (21)	0.61
2	67 (10)	628 (90)		54 (20)	52 (18)	0.46
3	30 (11)	251 (89)		49 (23)	51 (17)	0.52
4	6 (9)	62 (91)		54 (36)	58 (23)	0.75
5+	4 (11)	34 (89)		48 (19)	41 (20)	0.47

25(OH)D concentration reported as mean; S.D. standard deviation

*P value from chi squared test

†P value from Independent t-test

‡Cole et al, 2000

Table 8.10. Participant characteristics and mean 25-hydroxyvitamin D concentrations in children with or without atopic asthma

	n (%)			Mean 25(OH)D (S.D.) in nmol/L		
Variable	**Atopic asthma**	**No atopic asthma**	**P value***	**Atopic asthma**	**No atopic asthma**	**P value†**
Total	162 (12)	1167 (88)		54 (19)	52 (19)	0.25
Country section			0.02			
North	75 (11)	576 (89)		54 (20)	53 (19)	0.76
Central	60 (16)	318 (84)		53 (19)	49 (18)	0.12
South	27 (9)	273 (91)		53 (18)	52 (19)	0.71
Household deprivation quintile			0.18			
1	32 (9)	311 (91)		58 (21)	53 (19)	0.17
2	44 (13)	287 (87)		54 (18)	57 (19)	0.63
3	31 (11)	256 (89)		57 (19)	52 (18)	0.19
4	35 (15)	192 (85)		53 (19)	51 (21)	0.45
5	20 (14)	121 (86)		43 (18)	50 (19)	0.10
Age in years			0.56			
2	66 (13)	430 (87)		53 (19)	51 (19)	0.41
3	54 (12)	394 (88)		54 (18)	53 (20)	0.68
4	42 (11)	343 (89)		53 (21)	51 (18)	0.46
Gender			0.001			
Male	103 (15)	578 (85)		56 (20)	54 (20)	0.35
Female	59 (9)	589 (91)		49 (18)	49 (18)	0.92
BMI (kg/m^2)‡			0.36			
Normal	126 (12)	968 (88)		55 (20)	52 (19)	0.08
Overweight	26 (15)	147 (85)		48 (15)	53 (19)	0.30
Obese	7 (14)	41 (86)		40 (18)	47 (19)	0.36
Skin colour			0.01			
Very fair-fair	108 (14)	659 (86)		59 (18)	53 (18)	0.08
Medium	37 (8)	408 (92)		54 (20)	53 (20)	0.71
Olive-dark	17 (14)	100 (86)		38 (19)	42 (20)	0.47
Ethnicity			0.04			
Maori	28 (16)	145 (84)		52 (20)	49 (19)	0.42
Pacific	9 (23)	31 (77)		35 (18)	41 (18)	0.38
Other	14 (9)	143 (91)		43 (15)	42 (20)	0.87
NZ European	111 (12)	848 (88)		57 (19)	54 (18)	0.17
Daycare attendance			0.57			
Yes	141 (12)	996 (88)		55 (20)	52 (19)	0.61
No	21 (11)	171 (89)		47 (15)	49 (19)	0.17
Maternal education			0.001			
No secondary	16 (27)	43 (73)		47 (25)	38 (23)	0.21
Secondary	21 (11)	177 (89)		49 (16)	51 (20)	0.63
Post secondary	125 (12)	947 (88)		55 (19)	52 (19)	0.12
Taking supplements			0.37			
Yes	35 (14)	218 (86)		59 (20)	58 (20)	0.33
No	127 (12)	949 (88)		52 (19)	50 (19)	0.74

Dog in house			0.09			
Yes	47 (15)	268 (85)		56 (17)	53 (18)	0.55
No	115 (11)	889 (89)		52 (20)	51 (20)	0.28
Cat in house			0.19			
Yes	63 (11)	517 (89)		58 (21)	53 (19)	0.96
No	99 (13)	650 (87)		51 (18)	50 (19)	0.05
Smoking in same room			0.003			
Yes	9 (30)	21 (70)		42 (13)	46 (16)	0.14
No	153 (12)	1143 (88)		42 (13)	46 (16)	0.45
Total children in house			0.82			
1	31 (12)	216 (88)		54 (20)	53 (22)	0.99
2	87 (13)	608 (87)		54 (17)	52 (18)	0.25
3	29 (10)	252 (90)		54 (25)	50 (17)	0.25
4	9 (13)	59 (87)		58 (15)	57 (25)	0.91
5+	6 (16)	32 (84)		36 (17)	42 (20)	0.49

25(OH)D concentration reported as mean; S.D. standard deviation

*P value from chi squared test

†P value from Independent t-test

‡Cole et al, 2000

Table 8.11. Participant characteristics and mean 25(OH)D concentrations of children with upper respiratory infection (common cold)

	n (%)			Mean 25(OH)D (S.D.) in nmol/L		
Variable	**Lower frequency common cold**	**Higher frequency common cold**	**P value***	**Lower frequency common cold**	**Higher frequency common cold**	**P value†**
Total	562 (42)	767 (58)		51 (19)	52 (19)	0.28
Country section			0.88			
North	278 (43)	373 (57)		53 (20)	54 (18)	0.32
Central	161 (43)	217 (57)		49 (18)	50 (21)	0.45
South	123 (41)	177 (59)		52 (19)	52 (18)	0.97
Household deprivation quintile			0.91			
1	144 (42)	199 (58)		54 (17)	53 (21)	0.70
2	141 (43)	190 (57)		52 (20)	53 (19)	0.93
3	116 (40)	171 (60)		51 (20)	53 (17)	0.42
4	97 (43)	130 (57)		49 (21)	53 (20)	0.17
5	64 (45)	77 (55)		48 (18)	50 (20)	0.50
Age in years			0.000			
2	182 (37)	314 (63)		50 (20)	52 (19)	0.30
3	185 (41)	263 (59)		52 (20)	54 (20)	0.29
4	195 (51)	190 (49)		52 (18)	51 (19)	0.71
Gender			0.10			
Male	273 (40)	408 (60)		53 (20)	56 (19)	0.08
Female	289 (45)	359 (55)		50 (18)	49 (18)	0.52
BMI (kg/m^2) ‡			0.97			
Normal	463 (42)	631 (58)		52 (20)	53 (19)	0.24
Overweight	73 (42)	100 (58)		52 (17)	52 (20)	0.92
Obese	20 (42)	28 (58)		46 (20)	46 (19)	0.96
Skin colour			0.24			
Very fair-fair	310 (40)	457 (60)		52 (17)	54 (19)	0.11
Medium	202 (45)	243 (55)		52 (22)	54 (19)	0.51
Olive-dark	50 (43)	67 (57)		45 (19)	55 (18)	0.31
Ethnicity			0.57			
Maori	74 (43)	99 (57)		48 (20)	51 (19)	0.22
Pacific	21 (52)	19 (48)		43 (19)	36 (16)	0.18
Other	63 (40)	94 (60)		42 (20)	42 (20)	0.93
NZ European	404 (42)	555 (58)		54 (19)	55 (18)	0.31
Daycare attendance			0.16			
Yes	472 (42)	665 (58)		52 (19)	53 (20)	0.54
No	90 (47)	102 (53)		47 (20)	50 (16)	0.25
Maternal education			0.02			
No secondary	29 (49)	30 (51)		39 (23)	41 (24)	0.80
Secondary	100 (51)	98 (49)		52 (20)	50 (18)	0.49
Post secondary	433 (40)	639 (60)		52 (19)	53 (19)	0.23
Taking supplements			0.16			
Yes	97 (38)	156 (62)		57 (22)	58 (19)	0.45
No	465 (43)	611 (57)		50 (18)	51 (19)	0.64

	n (%)			Mean 25(OH)D (S.D.) in nmol/L		
Variable	**Lower frequency common cold**	**Higher frequency common cold**	**P value***	**Lower frequency common cold**	**Higher frequency common cold**	**P value†**
Dog in house			0.28			
Yes	125 (40)	190 (60)		54 (17)	54 (18)	0.27
No	437 (43)	577 (57)		51 (20)	52 (20)	0.92
Cat in house			0.63			
Yes	241 (42)	339 (58)		55 (20)	53 (19)	0.02
No	321 (43)	428 (57)		49 (18)	52 (20)	0.30
Smoking in same room			0.53			
Ever	11 (37)	19 (63)		46 (11)	44 (17)	0.25
Never	551 (42)	748 (58)		51 (19)	53 (19)	0.76
Total children in house			0.66			
1	99 (40)	148 (60)		53 (22)	53 (21)	0.99
2	290 (42)	405 (58)		51 (18)	53 (18)	0.19
3	128 (46)	153 (54)		50 (18)	51 (18)	0.74
4	27 (40)	41 (60)		60 (27)	56 (21)	0.49
5+	18 (47)	20 (53)		39 (16)	43 (23)	0.56

lower frequency of common cold = 0-2 times in last 12 months
higher frequency of common cold = 3 or more times in last 12 months
25(OH)D concentration reported as mean; S.D. standard deviation
*P value from chi squared test
†P value from Independent t-test
‡Cole et al, 2000

Appendix 9

Presentations and Abstracts

Presentations and Abstracts

2015

Cairncross CT, Stonehouse W, Conlon CA, McDonald B, Grant CC, Eyles D, Houghton LA, Coad J, Camargo CA, von Hurst P (2015)

Factors Affecting Vitamin D Status in New Zealand Preschool Children

Poster presentation at the *18th Vitamin D Workshop*, Delft, The Netherlands.

2014

Cairncross CT (2014)

Development of a simple questionnaire to predict vitamin D deficiency in preschool children

Oral presentation at the *Auckland Nutrition Research Network* student presentations, Auckland, New Zealand.

2013

Cairncross CT (2013)

Vitamin D and New Zealand pre-schoolers – study update

Oral presentation at the *Massey University Symposium Series Number 7, Vitamin D for Mothers and Children,* Auckland, New Zealand.

2012

Cairncross CT, Conlon CA, Stonehouse W, Coad J, Houghton L, Eyles D, Camargo C, Grant CC, McDonald B, von Hurst P (2012)

Vitamin D Status of New Zealand Pre-schoolers - Te Ra Whakaora: Recruitment and data collection phase.

Poster presentation at the *Annual Nutrition Society of New Zealand Conference*, Auckland, New Zealand.

Cairncross CT (2012) Vitamin D Status of New Zealand Pre-schoolers - recruitment and data collection.

Oral presentation at the *Auckland Nutrition Research Network* student presentations, Auckland, New Zealand.

Publications in progress

Cairncross CT, Stonehouse W, Conlon CA, McDonald B, Grant CC, Eyles D, Houghton LA, Coad J, Camargo CA, von Hurst P
Vitamin D status and predictors of deficiency in New Zealand preschool children.

Cairncross CT, Stonehouse W, Conlon CA, McDonald B, Grant CC, Eyles D, Houghton LA, Coad J, Camargo CA, von Hurst P
Can a questionnaire predict vitamin D deficiency in New Zealand preschool children?

Cairncross CT, Stonehouse W, Conlon CA, McDonald B, Grant CC, Eyles D, Houghton LA, Coad J, Camargo CA, von Hurst P
The relationship of vitamin D status with prevalence of allergic and respiratory disease in New Zealand preschool children.