# University of Alberta

# Association between Asthma and Vitamin D in Children, Adolescents and Adults

by

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*[Disclaimer: The research analysis and results are based on data from Statistics Canada and the opinions expressed herein do not represent the views of Statistics Canada.]*

## Abstract

Inconsistent results have been reported in studies investigating the relationship between serum vitamin D levels and respiratory outcomes. In this thesis, data from the Canadian Health Measures Survey were used to examine the relationship between serum 25-hydroxy vitamin D [25(OH)D] levels and respiratory outcomes. Children with 25(OH)D levels less than 50 nmol/L and greater than 75 nmol/L had increased risk of current wheeze and reduced cross-sectional changes in lung function with age in comparison to those children with 25(OH)D levels between 50 and 74nmol/L suggesting a U-shaped relationship between 25(OH)D levels and these respiratory outcomes in children. The lower 25(OH)D category was also a significant risk factor for asthma attacks in adolescents and adults. Monitoring of serum vitamin D level may enhance management of respiratory conditions in children, adolescents and adults. Further exploration of this relationship is needed to identify the optimal level of vitamin D for respiratory outcomes.

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| | |
|---|---|
| $1,25(OH)_2D_3$ | Calcitriol |
| 25(OH)D | 25-hydroxy vitamin D |
| $25(OH)D_3$ | Calcidiol |
| 7-DHC | 7-Dehydrocholesterol |
| APC | antigen presenting cells |
| ATS | American Thoracic Society |
| CDC | Centers for Disease Control and Prevention |
| CHMS | Canadian Health Measures Survey |
| COPD | Chronic obstructive pulmonary disease |
| $D_2$ | Ergocalciferol |
| $D_3$ | Cholecalciferol |
| $FEF_{25\%-75\%}$ | Forced expiratory flow between 25% and 75% of forced vital capacity |
| $FEV_1$ | Forced expiratory volume in one second, |
| FVC | Forced vital capacity (FVC) |
| HRV | Human rhinovirus |
| IgE | Immunoglobulin E |
| IL | interleukin |
| IOM | Institute of Medicine |
| IU | international unit |
| KNHANES | Korean National Health and Nutritional Examination Survey |
| MEC | Mobile examination centre |
| MHC | major histocompatibility complex |
| NHANES | National Health and Nutrition Examination Survey |
| OR | Odds ratio |
| PTH | parathyroid hormone |
| PUFA | Poly-unsaturated fatty acids |
| RSV | Respiratory syncytial virus |
| $Th_1$ | T-helper type 1 cells |
| $Th_2$ | T-helper type 2 cells |

| | |
|---|---|
| TNF | tumor necrosis factor |
| T-reg cells | T-regulator cells |
| UVB | ultraviolet B waves |
| VDR | vitamin D receptor |

# Chapter 1 Introduction

## 1.1 Background

A paper format is followed in this thesis. In Chapter 1, a literature review of asthma, lung function, vitamin D and the relationship between vitamin D levels and respiratory outcomes is given. It is followed with a manuscript on the relationship between vitamin D levels and respiratory outcomes in children in Chapter 2, and a manuscript on the relationship between vitamin D levels and respiratory outcomes in and adolescents and adults in Chapter 3, respectively. Finally, a general discussion of the findings in the thesis and overall conclusions of the thesis are given in Chapter 4.

### 1.1.1 Definition of Asthma

According to the American Thoracic Society's revised definition in 1987 [ATS 1987], asthma is: "a clinical syndrome characterized by increased responsiveness of the tracheobronchial tree to a variety of stimuli. The major symptoms of asthma are paroxysms of dyspnoea, wheezing, and cough, which may vary from mild and almost undetectable to severe and unremitting (status asthmaticus). The primary physiological manifestation of this hyper-responsiveness is variable airways obstruction. This can take the form of spontaneous fluctuations in the severity of obstruction, substantial improvements in the severity of obstruction following bronchodilators or corticosteroids, or increased obstruction caused by drugs or other stimuli" (1).

Asthma is a complex heterogeneous disorder with respect to immunopathology, clinical phenotypes, response to therapies, and natural history (2). It is characterized by chronic airway abnormalities such as airway hyper-responsiveness, airway obstruction, airway remodeling, and infiltration of eosinophils and T-helper type 2 ($Th_2$) cells in the airway sub-mucosa, which leads to inflammation and edema in the bronchial mucosa, and hyper-secretion of mucus (3). Asthma often develops in childhood, but it can develop at any stage in a person's life. Factors that trigger an asthma attack may vary from person to person, and include indoor and outdoor allergens, air pollutants, respiratory tract infections, exercise, weather changes, food additives, drugs, and extreme emotions (4). Frequent exacerbation of asthma has a significant impact on quality of life. Severity of asthma is measured using the frequency and severity of symptoms, as well as response to available medications. Severe asthma can be life threatening. Corticosteroids and beta-adrenergic agonists are the most effective classes of agents used in asthma care. Mild to moderate asthma is usually well controlled by low-dose inhaled corticosteroids (5). A significant proportion of asthma patients have either no or poor response to asthma medications. This difference in response to medication may be attributable to genetic factors, and other factors at host and environment level (6).

### 1.1.2 Prevalence and risk factors of asthma

Asthma 'epidemic' was noticed in developed countries in the latter half of the 20th century. Increasing prevalence rates of asthma were noted in both developed and developing countries (7). Worldwide, over 300 million people are

thought to be affected by asthma. It is estimated that this number will increase to 400 million by 2025 (8). Although the prevalence of asthma appeared to have reached a plateau in industrialized countries, recent studies show its prevalence has continued to rise in developing countries (9). In the United States alone, the cost of health-care due to asthma care was estimated as $ 56 billion each year. Prescription medication accounted for the largest proportion of this cost (10).

The most common early life condition affecting children is wheezing (7). Parental history of asthma, male sex, exposure to indoor air pollutants, respiratory syncytial virus (RSV) and human rhinovirus (HRV) infection are some of the risk factors associated with asthma in early childhood. Allergens including domestic mites, furred animals, cockroach allergen, fungi, molds, and yeasts all appear to influence the development of asthma. Asthma is also associated with obesity, ethnicity, and living in westernized countries (3). In terms of race, Puerto Ricans, non-Hispanic blacks, and American Indians appear to have a higher prevalence of asthma compared to non-Hispanic whites (11). Asthma is common in adults too. CDC has estimated that 1 in 12 adults has asthma in United States. Obesity, female sex, atopy, and low socioeconomic status are some of the risk factors for asthma in adults (12).

There are many hypotheses put forward to explain the increase in prevalence of asthma. According to the hygiene hypothesis, decreased exposure to infections in early life leads to altered development of the immune system. Smaller families in developed countries, improved hygiene, wide-spread antibiotic usage and increasingly ‘germ’ free environments have altered exposure

to microorganisms (3). As a result, the immune system of many people has deviated towards a predominantly $Th_2$ phenotype rather than a balanced $Th_1/Th_2$ response. This inadequate up-regulation of $Th_1$ immune responses is thought to result in increased risk of asthma and allergy related conditions. In recent years, the hygiene hypothesis has been broadly accepted, although it cannot explain some inconsistent asthma findings such as the link between obesity and asthma, the high prevalence in poor and urban environments, and the concomitant rise in $Th_1$ mediated autoimmune diseases.

There are also dietary hypotheses put forward to explain the increase in asthma in recent decades. From observational studies, declining or reduced dietary antioxidants (e.g.: vitamin C, and E) and minerals (e.g.: Se) intake has been linked to increases in asthma incidence. Similarly, declining intake of long chain n-3 poly-unsaturated fatty acids (PUFA) and increase in n-6 PUFA was associated with an increase in asthma. These hypotheses suggest that declining lung and respiratory epithelial antioxidant defenses increased oxidant induced airway damage, inflammation and damage (13).

### 1.1.3 Determinants of lung function

Lung function tests are widely used standard parameters for the assessment of lung function. These parameters, typically including forced vital capacity (FVC), forced expiratory volume in 1 second ($FEV_1$), the ratio between $FEV_1$ and FVC ($FEV_1$/FVC), and forced expiratory flow between 25% and 75% of vital capacity ($FEF_{25\%-75\%}$) are obtained noninvasively using spirometry and

can inform about dynamic lung volumes, capacities, and rates of flow. Various factors have been recognized as affecting lung function parameters. It is important to identify the factors affecting pulmonary function in order to prevent impaired or suboptimal lung function. Physical exercise, ethnicity, age, sex and smoking are some of these influential factors (14). In addition to biological factors such as height and weight, socioeconomic status, exposure to pollution, malnutrition and low birth weight also have been identified as significant predictors of lung function in study reports (15). Spirometry measurements taken before and after the administrating a bronchodilator is often used to diagnose reversible airway obstruction and measure therapeutic response (16).

### 1.2.1 Vitamin D overview

Vitamin D is an essential nutrient. Although well known as one of the fat-soluble vitamins, the active form of vitamin D actually belongs to family of steroid hormones, and is called as a secosteroid which means hormone/drug derived from the cholesterol molecule (17). Vitamin D is part of a complex endocrine pathway that regulates the interactions among kidney, bone, parathyroid gland, and intestine that maintains extracellular calcium levels within physiological levels to maintain skeletal integrity. It regulates serum calcium level by suppressing the effects of parathyroid hormone (18). Cholecalciferol [$D_3$], calcidiol [$25(OH)D_3$], and calcitriol [$1,25(OH)_2D_3$] are three forms of vitamin D. The plant form of vitamin D is called ergocalciferol [$D_2$] (19). The other naturally occurring form is cholecalciferol and is found in dietary sources or synthesized in skin from 7-dehydrocholestrol (7-DHC). During this transformation previtamin $D_3$ undergoes photothermal-isomerization under ultraviolet B irradiation (290-315nm wavelength) and gives rise to cholecalciferol (20,21). Bound to vitamin D-binding protein (DBP), cholecalciferol is transferred to the liver where it is converted to 25(OH)D by 25-α-hydroxylase. This metabolism is not regulated by a feedback mechanism, so the serum level of 25(OH)D largely reflects the amount of vitamin D produced in the skin in addition to dietary intake. The active form of vitamin D [$1,25(OH)_2D_3$] is formed from 25(OH)D in the kidneys. This hydroxylation process is tightly regulated by parathyroid hormone, and serves as a major control point in the production of the active form of vitamin D. The enzyme converting inactive 25(OH)D to the active form of vitamin D is 25-

hydroxyvitamin D-1α-hydroxylase and is found in kidney and other tissues such as respiratory epithelial cells (20). This extra-renal hydroxylation results in calcitriol appearing mainly intracellularly, which is clinically unmeasurable (22). 25(OH)D and 1,25$(OH)_2$D are catabolized by 25-hydroxyvitamin D-24-hydroxylase into biologically inactive, and water-soluble calcitroic acid.

### 1.2.2 Sources of vitamin D

Sunlight is the primary source of vitamin D for humans. Because of this, low levels of vitamin D are considered in part a behavioral / lifestyle issue rather than simply being due to inadequate intake. Increased time spent indoors, increased use of sunscreen, and sun protective clothing are some behavioral changes in modern lifestyle leading to low vitamin D levels (13,21). Cutaneous synthesis of vitamin D reaches equilibrium after several minutes of sun exposure. With excess exposure to sunlight, it is not possible to develop toxic levels of vitamin D (23). On the other hand, there are recommendations that in the summer the sun should be avoided to reduce the risk of wrinkles and skin cancer. Due to this fact, it was widely accepted that dietary supplementation and short periods of sun exposure are the preferred methods of obtaining vitamin D (24). In Canada, the amount of sunlight in the fall and winter are inadequate to meet the required levels of vitamin D (25). As a result, anyone can be vitamin D deficient in winter and but have sufficient levels in the summer.

Unlike other vitamins, vitamin D doesn’t naturally occur in many foods. Dietary sources of vitamin D include fortified foods, fatty fish, fish oils, and vitamin D pills (24). Commercially distributed major preparations of vitamin D

contain vitamin $D_2$ (19). Some researchers strongly argue that dietary vitamin D is insufficient to maintain adequate vitamin D levels in the body. They strongly advocate using both fortification and supplementation to maintain optimal levels of vitamin D (26).

### 1.2.3 Determinants of serum vitamin D level

The serum level of vitamin D is influenced by several factors including the melanin content of the skin, age, factors affecting the duration and intensity of sun light exposure (latitude, season, time spent outdoors, clothing, and cloud cover), body fat, and sunscreen use (23). Over-weight or obese patients are significantly more likely to suffer from low vitamin D levels. Fat sequesters vitamin D in the body; so obese people may need a higher intake of vitamin D. The use of exogenous steroids (e.g.: those who are on glucocorticoids for asthma) tends to increase the destruction of vitamin D, thus making patients with asthma at higher risk for vitamin D deficiency. Aging is another factor affecting body's ability to synthesize vitamin D from sunlight. With aging, the amount of 7-DHC in skin decreases. About 50-60% of the older population doesn't have satisfactory vitamin levels (27). Apart from aging, urbanization, decreased outdoor activities, air pollution, and global dimming are some factors accounting for vitamin D deficiency in older population (28).

### 1.2.4 Recommended dietary intake level

Having optimal vitamin D level may help improve existing medical conditions, and protect against the development of new disease conditions (29).

Some studies showed that supplementation of vitamin D in high levels (4000-8000 IU/day) reduced autoimmune disease symptoms (20). The current recommended daily allowance of 400-600 IU is criticized as being too low by some as, while adequate for preserving bone health, it potentially ignores the important role of vitamin D in immunoregulation. In general, each additional 100 IU of vitamin D per day raises serum 25(OH)D concentration by approximately 2.5 nmol/L. Factors that call for a higher intake include dark skin color, older age, and obesity. It is suggested that 1400-2000 IU/day, and if the patient is obese, 1.5-2 times of that may be needed. Up to 10,000 IU/day appears to have no unwanted toxicity.

Since vitamin D deficiency is associated with numerous adverse outcomes, routine vitamin D testing for monitoring serum vitamin D level in individuals at risk has been recommended. A faction of researchers suggest that annual monitoring of serum 25(OH)D should be implemented to actively detect individuals at risk of vitamin D deficiency (24).

Vitamin D deficiency has no easily visible symptoms. Pregnant women, school going children, young adults, and elderly are some population subcategories that are more prone to develop vitamin deficiency. Specially, breast-fed infants, children who do not drink fortified milk, those with mal-absorption, kidney disease, severe liver disease, taking drugs that interfere with vitamin D metabolism, those living in higher latitudes, and those with decreased sun exposure due to cultural reasons are all at increased risk of developing vitamin D deficiency (20).

### 1.2.5 Measurement of vitamin D level in population-based studies

Serum 25(OH)D is the major circulating metabolite of vitamin D and used to determine body vitamin D status. Measured serum 25(OH)D is a total quantity of both $25(OH)D_2$ and $25(OH)D_3$ (30). The half-life of serum 25(OH)D is 2-3 weeks; serum $1,25\text{-}(OH)_2D$ has a half-life of 4 hours (31). Several assays are available to measure levels of 25(OH)D and $1,25\text{-}(OH)_2D$. The gold standard reference method to measure serum 25(OH)D is liquid chromatography tandem mass spectrometry. Immunoassay and chemiluminescence assay methods are becoming popular and are commonly used due to their low cost, and ability to handle high-volume throughputs (32). A recent study reported that the agreement between LIAISON chemiluminescence assay and liquid chromatography-tandem mass spectrometry for measuring 25(OH)D levels was excellent and superior in comparison to other automated immunoassays that are currently used in laboratories (33).

### 1.2.6 Vitamin D action relevant to allergic diseases

Vitamin D has a significant impact on immune homeostasis, especially modifying inflammatory responses and affecting differentiation of immune cells. Vitamin D directly affects dendritic cell, monocyte, macrophage, B cell, T cell, and airway epithelial cell functions (34). These cells express vitamin D receptors (VDR) and vitamin D metabolizing hormones (35). The active form of vitamin D directly acts on T lymphocytes and helps activate them. Activation of $CD_4^+$ T cells results in a five-fold increase in VDR expression, which enables calcitriol to

regulate over 200 identified genes (36). $Th_1$ cells typically secrete interferon-γ, interleukin (IL)-2, IL-12, TNF-α, all of which augment the cell-mediated defense against intracellular pathogens. $Th_2$ cells express IL-4, IL-5, and IL-13 which participate in allergic responses. A strong suppression of $Th_1$ cells is effected by vitamin D through expression of $Th_2$ cytokines. By this mechanism, $Th_1$ T-cell proliferation is inhibited. Vitamin D also enhances $Th_2$ responses through enhancement of IL-4 production. This effect is further facilitated through the effect of vitamin D on Antigen presenting cells (APC). Similarly, activated macrophages release TNF-α, IL-6, and IL-17 which are involved in allergic inflammation (35). Vitamin D also acts as a potent suppressor of interferon-γ-mediated macrophage activation. Likewise, higher serum levels of vitamin D are associated with increased numbers of circulating T-regulator cells which might be stimulating the secretion of the anti-inflammatory cytokine, IL-10, resulting in a reduction in the levels of many pro-inflammatory cytokines involved in pathogenesis of asthma.

Experimental studies provide evidence suggesting alternative mechanisms as to how low levels of vitamin D might be involved in the pathogenesis of asthma. Agrawal *et al*., had shown that calcitriol decreases TNF-α (37). Decreases in the levels of various pro-inflammatory cytokines, including TNF- α, and IL-1 beta deactivate NF-kB, a potential mechanism where the active form of vitamin D protects against airway hyper-responsiveness and airway neutrophilia (36). NF-kB is a transcriptional factor that acts as a central mediator of human immune response by regulating and coordinating the expression of various inflammatory

genes for inflammatory mediators. This provides a cellular level mechanism through which deficiency of vitamin D could lead to the development of asthma (38).

Vitamin D is involved in innate immunity (39). One mechanism by which vitamin D involves is via its inhibiting effect on dendritic cells which have important functions in maintaining both protective immunity and self-tolerance. By down-regulating the expression of MHC class II molecules, vitamin D inhibits the differentiation, maturation, and immune-stimulating ability of dendritic cells. Vitamin D inhibits the maturation of dendritic cells, which in turn maintain an immature, and tolerogenic phenotype with inhibition of activation markers such as MHC class II CD40, CD80, and CD86 and up-regulation of inhibitory molecules. Concurrently, vitamin D also suppresses IL-12 and enhances IL-10 production in these dendritic cells (35).

Vitamin D potentially modifies innate immunity also through induction of the antimicrobial peptide cathelicidin. Cathelicidin is an antimicrobial peptide produced by neutrophils, macrophages, and the cells lining epithelial surfaces of the skin, respiratory tract, and gastrointestinal tract in humans. It has a broad antimicrobial activity against Gram-positive and Gram-negative bacteria, as well as certain viruses and fungi (40). Vitamin D up-regulates cathelicidin mRNA in several cell lines and primary cultures including keratinocytes, neutrophils and macrophages. Direct correlation of vitamin D levels with systemic levels of cathelicidin suggests that vitamin D in the control of infections (41).

### 1.2.7 Vitamin D and respiratory outcomes in observational studies

Observational studies have suggested that vitamin D plays a role in allergic airway diseases and that low serum vitamin D is associated with poor asthma control, frequent and severe asthma exacerbations, reduced lung function, and increased medications use. It has also been reported that the asthma epidemic and vitamin D deficiency follow the same epidemiological patterns of association with obesity, African American race, socioeconomic status, and living in westernized countries (29). These findings led researchers to extensively study the role of vitamin D in asthma.

Concerning the role of vitamin D on asthma, two distinct schools of thought exist. One thought is that widespread use of vitamin D as rickets prophylaxis resulted in an increase in asthma and allergy. This hypothesis has been favored by the effects of vitamin D in promoting differentiation of naïve $Th_0$ cells towards the $Th_2$ phenotype (42). The other line of thought suggests that there is a widespread vitamin D deficiency despite the fortification of vitamin D in food items, which resulted from lifestyle changes such as the increasing tendency to stay indoors, sunshine avoidance to prevent skin cancer, and inadequate intake of vitamin D, and this vitamin D deficiency impacted the regulatory T-cell population, and reduced the inhibitory effect of regulatory T-cells on $Th_2$ immune differentiation (43).

A study by Gupta *et al*, in 86 British children aged 6-16 years with varying asthma severity found that vitamin D levels were significantly lower in severe

therapy resistant asthmatics, positively related to $FEV_1$, FVC, and negatively associated with airway smooth muscle mass. The authors concluded that vitamin D supplementation may be helpful in management of steroid therapy resistant asthma in pediatric population (44). A recent study by Sutherland *et al.*, in asthmatic adults observed an association between reduced vitamin D levels and impaired lung function, increased airway hyper-responsiveness and reduced glucocorticoid response. It showed low vitamin D levels affect long-term prognosis of asthma by increasing expression of pro-inflammatory TNF-α and accelerating deterioration of lung function (45). In a case-control study conducted on Qatari children below 16 years, vitamin D levels below 50 nmol/L were found to be the strongest predictor of asthma with an odds ratio of 4.8 (46). Similarly, in a case-control study conducted on Iranian children between 6 and 18 years, serum 25(OH)D levels were linearly associated with $FEV_1$ and $FEV_1$/FVC, and low levels of vitamin D were associated with asthma (47).

In the PIAMA cohort study, in a subgroup with measurement of serum vitamin D levels at age 4 were inversely associated with asthma at ages 4 and 8 years, while in another subgroup serum levels measured at age 8 were positively associated with asthma at age 8. This study suggested that vitamin D exerts a complex and age-dependent effect on respiratory outcomes (48).

A randomized control study conducted on Japanese school children aged 6-15 years reported that a 1200 IU/day vitamin D supplement taken during the winter and early spring helped prevent influenza and asthma attacks (49). An ecological study using asthma prevalence data in adults from United States and

Australia reported that the prevalence of asthma was associated with geographical latitude, implying that latitudinal difference in insolation, and subsequently serum vitamin D as an environmental factor influences the prevalence of asthma (50). There are also studies that reported no association between vitamin D supplementation and asthma. In a small study of children aged 6-17 years from America with physician diagnosed asthma, supplementation of 1000 IU/daily vitamin D did not affect asthma control test (ACT) score or $FEV_1$ at 6 months or 1-year of follow-up (51).

It has been suggested that vitamin D may slow the progressive decline in lung function seen in some asthmatics, a characteristic of airway remodeling. By inhibiting the formation of matrix metalloproteinase as well as fibroblast proliferation and influencing collagen synthesis, vitamin D may affects the tissue remodeling and probably lung function (52). In airway remodeling, a certain type of smooth muscle growth occurs prominently which reflects airway inflammation and leads to the impaired lung function. In addition it may also modulate protease-antiprotease imbalance and oxidative stress (53).

Initially, from a population-based cross-sectional data, Black *et al.,* reported a positive association of serum vitamin D levels and lung function in adults (14). Likewise, a cross-sectional analysis of British birth cohort participants at the age of 45 years reported that increasing serum 25(OH)D levels were linearly associated with greater $FEV_1$ and FVC (54). Similar findings were observed in an Iranian study too (47). According to a cohort study conducted on Danish adults, lower plasma 25(OH)D levels were not only associated with lower

$FEV_1$ and FVC, but also with faster decline of lung function in COPD patients (53). In a cross-sectional study conducted on Egyptian adults, serum levels of 25(OH)D were significantly lower among COPD patients, and positively associated with $FEV_1$ (55). In a review, Zarogoulidis *et al.* reported that between 2007 to 2012, 11 out of 15 observational studies report an inverse association between serum 25(OH)D and asthma (56).

Early-life events, especially exposures during pregnancy, have been increasingly recognized as the determinants of early programming of several clinical conditions. Particularly, these events activate or silence genes which are associated with disease conditions. Prematurity, fetal growth restriction, and mode of delivery are some of the known risk factors associated with childhood asthma (57). Along this line of research, an inverse association between wheezing and maternal vitamin D levels has been reported.

An Australian cohort study of singleton children reported that an increased risk of asthma was observed in children born in winter and autumn compared to summer, suggesting that reduced sun exposure levels during pregnancy had resulted in low levels of vitamin D in mothers, and resulted in asthma in their offspring (57).

While most studies have suggested low levels of vitamin D are associated with poorer respiratory health, some have also raised concerns about higher levels. From a prospective cohort of 219 children from Tucson, Arizona, Rothers *et al.,* reported that both low and high levels of maternal vitamin D were

associated with increased allergic markers in their offspring at age 5 years (58). Similarly, another cohort study of Caucasian children from the UK reported that higher maternal vitamin D levels during late pregnancy were associated with increased risk of atopic asthma in children at 9 months of age compared to levels below 30 nmol/L (59). In a cohort of 5 year old children from Finland with susceptibility for type 1 diabetes mellitus, a negative association between maternal intake of vitamin D during pregnancy and asthma, allergic rhinitis and atopic asthma was observed (60). A cohort study of Swedish children aged 6 years reported that higher vitamin D intake was associated with more prevalent atopic manifestations, suggesting that vitamin D intake during infancy may cause atopic allergies later in childhood (61).

### 1.2.8 Non-linear relationship between vitamin D and Health outcomes

As an elevated level of parathyroid hormone is an indicator of vitamin D deficiency, levels of 25(OH)D that suppress the parathyroid hormone have traditionally been utilized as signifying optimal vitamin D levels. From a group of healthy normal adults the mean ± 2SD values were used to define a normal serum 25(OH)D reference range (20). Current normal ranges were determined from a north American reference population, although these values may vary according to age and geographic location (62). However, the use of serum PTH hormone to define an optimal level of serum 25(OH)D has been criticized as giving a false picture of a linear and inverse association between the two (63). Historically, the lower limit of the normal range was much lower than currently accepted levels. Following the establishment of vitamin D requirements by the Food and Nutrition

Board of the Institute of Medicine in 1989 (64) and 1997 (65), vitamin D deficiency was redefined as serum 25(OH)D levels below 50 nmol/L in 2011. Furthermore, it has been suggested that levels above 75 nmol/L are not consistently associated with benefits and levels greater than 125 nmol/L may be associated with toxicity. Of note, the IOM considered hypercalcemia as an indicator of vitamin D toxicity in setting these recommendations (66). In the clinical practice guidelines of the Endocrine Society levels below 50 nmol/L are considered as deficiency, levels between 50-75 nmol/L as insufficiency, and levels above 75nmol/L were recommended for optimal health (67).

While the number of studies examining the role of vitamin D on a wide range of clinical diseases has exponentially increased in recent years, there has been no unanimity on levels that can be considered as optimal. Traditionally serum levels below 13-17 nmol/L were believed to induce osteomalacia, serum levels below 25-30 nmol/L induced secondary hyperparathyroidism and osteoporosis, while serum levels above 45-50 nmol/L were usually considered physiologically adequate levels (62). It is noted that vitamin D levels vary significantly between people from different geographic locations and races. Vitamin D concentrations were generally higher among people living in northern latitudes compared to those living near the equator (50).

Hypponen *et al.,* reported that both <25 and >135 serum 25(OH)D levels were associated with respect to high allergic marker (IgE) concentrations in a cohort of 45-yr old British adults (69). A small cohort study from Tucson, Arizona looked at the association between maternal cord blood 25(OH)D

concentrations and total IgE levels in offsprings measured serially at 1, 2, 3, and 5 years. Their study categorized vitamin D into 4 categories <50, 50-74, 75-99, and ≥100 nmol/L. That study reported that levels <50 and ≥100 were associated with adverse effects, reflecting an U-shaped relationship between maternal cord 25(OH)D levels and risk of increased allergic markers in early childhood. It is possible to get levels ≥100 nmol/L in a sun-replete area like Arizona, and as a result they may have an adapted immune system with respect to tolerated high serum 25(OH)D levels and may pose a wider optimal range of serum 25(OH)D (58).

The Southampton women's cohort study looked at association between maternal 25(OH)D measured at the 34th week of pregnancy and asthma, wheeze or lung function in offspring at 6-years. This study reported no linear or non-linear association with outcomes considered (70). Another cross-sectional study conducted on two samples of adolescents data reporting no association between linear, or dichotomized 25(OH)D at 50 nmol/L (71). Another study in asthmatic adults from Costa Rica, reported a significant association of serum 25(OH)D between 50-75 nmol/L with severe asthma, and levels >75 nmol/L with lower risk of hospitalization related to asthma. This study also reported a marginally significant linear correlation between serum 25(OH)D and FEV1 in asthmatic adults (72).

In a nested case-control study on Nordic Men aged 40-58 years, Tuohimaa *et al.*, looked at the association between serum 25(OH)D levels and prostate cancer. This study used 25(OH)D measured pre-diagnosis (10 years ago) of

cancer and categorized them into <20 nmol/L, 20-30 nmol/L, 20-59 nmol/L, 60-79 nmol/L, and >79 nmol/L, and reported both levels <40 nmol/L and >60 nmol/L were associated with increased risk of prostate cancer (68). Another similar study on pooled data from multiple cohorts reported that serum levels >100 nmol/L were associated with increased risk of pancreatic cancer, while no significant association was observed with lower levels (73). Dror *et al.*, reported a non-linear association between acute coronary syndrome and serum 25(OH)D level, and suggested that levels between 50-90 nmol/L were protective in adults >45 years of age (74). A study on population-based Korean National Health and Nutritional Examination Survey data reported a similar non-linear relationship between serum 25(OH)D levels and hemoglobin levels, and the maximal level of hemoglobin was observed at 65 nmol/L serum 25(OH)D concentration. This study also pointed out this threshold level varied according to sex and menstrual status in females (75). In a study conducted in hip fracture cases and hospital-based controls, the linear and inverse relationship between serum 25(OH)D level and risk fracture was only evident at levels between 0-70 nmol/L. At levels above 70 nmol/L the association was not significant and may even be a risk factor for hip fracture (76). Likewise, a study conducted on Chinese children aged 0-6 years reported an odds ratio of 5.5 of low bone mineral density for <50 nmol/L serum 25(OH)D levels, with the threshold for optimal bone mineral density being 50nmol/L for Chinese children (77). Another study conducted on NHANES data, found that the beneficial effect of increasing serum 25(OH)D levels reached a plateau at 53 nmol/L with respect to ankle-brachial index, a marker of peripheral arterial disease (78).

### 1.2.9 Summary:

Asthma is a disease with altered immune responses in the lungs. Vitamin D is a significant immune modulator, which exerts its biological actions at the cellular and subcellular level of immune cells. Vitamin D possibly affects the pathogenesis and morbidity of asthma through prevention of viral infections, and enhancing steroid responsiveness. Serum vitamin D level is highly variable depending on biological, behavioral, and environmental factors. Current experimental and epidemiologic evidence provides an inconsistent and incomplete picture of the association between vitamin D status and asthma. Both low and high levels of serum vitamin D may be associated with an increased risk of adverse outcomes, especially asthma. Nevertheless, the threshold used to define low and high vitamin D status has not been consistent between studies. Effects of low and high serum vitamin D levels may impact health differently in children and adults. Overall, the optimal serum vitamin D level, the mode to achieve this optimal level, and the safety of vitamin D intake to prevent from developing asthma or asthma exacerbations still needs to be assessed. Guidelines on recommendations for screening and supplementation of vitamin D need be revisited.

### 1.2.10 Research questions

The primary objective of this study was to investigate the much debated relationship between serum 25(OH)D level and respiratory outcomes using data from a Canadian population. The following are the list of specific objectives:

1.1 To investigate the association between serum 25(OH)D level and current wheeze, ever asthma, and lung function parameters in children after controlling for potential confounders.

1.2 To identify lower and upper thresholds of optimal range of serum 25(OH)D level in which the lowest risk of current wheeze was observed in children.

2.1 To identify the association between current and ever asthma, and lung function outcomes and serum 25(OH)D level in adolescents and adults after controlling for potential confounders.

2.3 To identify whether the association between ever asthma and serum 25(OH)D status vary between early-onset and late-onset asthma phenotypes in adults.

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# Chapter 2 Association of vitamin D with respiratory outcomes in Canadian children

## 2.1 Introduction:

Asthma is the most common chronic condition affecting children in developed countries (1, 2). Asthma prevalence has been increasing during recent decades in many parts of the world (3- 6). It is likely that this increase in asthma prevalence is influenced by many environmental (4) and behavioral factors (7-9).

Vitamin D has been recognized to play an important role in innate and adaptive immune responses for some years (10). It has been shown to be a potent stimulator of mechanisms associated with pathogen elimination (11). Recent animal studies have raised the possibility of vitamin D exerting some of its effect on inflammation and autoimmune diseases through the regulation of Th17 cells (a T-cell lineage distinct from Th1 or Th2 cells) (10). Initially, Bäck *et al.*, demonstrated a significant association of vitamin D with asthma related parameters, including lung function, from a representative adult population from the US (8). However, despite ongoing research the evidence remains conflicting with studies reporting protective effects (12-14), adverse effects (8, 15 16), as well as an absence of any effect (17, 18) of vitamin D on asthma. There are also suggestions that maternal vitamin D status (19-22), and early infant supplementation (8, 15) may influence the early programming of the immune system and so the development of asthma and allergic diseases in offspring.

A committee of the Institute of Medicine (IOM) reported that a serum vitamin D level of 50 nmol/L or more for skeletal health, and higher values were

not consistently associated with increased health benefit (23). For extraskelatal outcomes such as cancer, cardivascular disease, diabetes and autoimmune disorder, no recommendations were made by the committee due to the inconsistent and inconclusive evidence (23). In the conclusion of manuscript the authors reported "serum concentrations of 25(OH)D above 30 ng/ml (75 nmol/L) are not consistently associated with increased benefit, and risks have been identified for some outcomes at 25(OH)D levels above 50 ng/ml (125 nmol/L)". Interestingly, the relationship between at 25(OH)D levels with some of the extraskeletal oucomes has been reported to be U-shaped with increased risk at both low and high levels of 25(OH)D (24). Only a few studies published to date have investigated the possibility of a non-linear relationship between 25(OH)D levels and respiratory outcomes. In this study, the data from the Canadian Health Measures Survey (CHMS) were used to further examine whether there might be a non-linear relationship between serum 25(OH)D concentration and current wheeze, ever asthma and lung function parameters in children of ages 6 to 12 years.

## 2.2 **Methods**:

The CHMS was developed by Statistics Canada in partnership with Health Canada and the Public Health Agency of Canada (25). It was conducted in the provinces of British Columbia, Alberta, Ontario, Quebec and New Brunswick. A detailed description of data collection in CHMS is given elsewhere (26). Briefly, the participants were chosen using a multi-stage sampling process with initial sampling of sites based on population size at the geographic region and census metropolitan areas, followed by random sampling of dwellings and then stratified sampling of participants within the dwelling based on age-groups. The survey included two main components: an in-home interview and a clinic visit. The in-home interview gathered information related to personal and family characteristics. Upon completion of the household interview, participants were asked to attend a mobile examination centre (MEC) (27). The measurements taken at this centre included a questionnaire, anthropometry and spirometry. Blood samples were also taken at the MEC for laboratory analysis.

Of the 7,478 individuals who agreed to participate, 6,604 (88.3%) completed the household questionnaire and 5,604 (84.9%) attended the MEC. Of those attending the MEC 5,373 (95.9%) provided a blood sample. After excluding adolescents and adults, a total of 1,030 children aged 6 to 12 years had vitamin D measurements and consequently could be considered for this report. Of the 1,030 children, 951 had valid lung function measurements. The study was approved by the University of Alberta Health Research Ethics Board.

In the CHMS, the presence of current wheeze was established from the question “Has your child had wheezing or whistling in the chest in the last 12

months?" from the clinic questionnaire. The presence of ever asthma was determined from the question "We are interested in long-term conditions which are expected to last or have already lasted 6 months or more and that have been diagnosed by a health professional: Do you have asthma?". Spirometric measurements of lung function comprised forced expiratory volume in one second ($FEV_1$), forced vital capacity (FVC), $FEV_1$/FVC ratio, and forced expiratory flow between 25% and 75% of forced vital capacity ($FEF_{25\%-75\%}$). Spirometry was performed by a trained technician using Respironics KoKo spirometer (PDS Instrumentation, Louisville, CO) according to the American Thoracic Society guidelines. All spirometry results were reviewed by an external reviewer to ensure the quality of the measurements. Predicted lung function values used for bivariate analyses were based on the equations proposed by Corey et al. for 6 to 7 years (28) and Hankinson et al. for 8 years and older (29).

Venous blood was drawn using routine methods and stored at -20˚C in a freezer within the MEC laboratory. Subjects with significant relevant co-morbidities (e.g. hemophilia, significant edema, burned or scarred arms) were excluded. Frozen samples of blood were sent weekly to the Health Canada reference laboratory in Ottawa, where the serum 25(OH)D was measured using the LIAISON 25-hydroxyvitamin D total assay (Diasorin Ltd, Stillwater, MN). The lower and upper detection limits were 10 nmol/L and 375 nmol/L, respectively.

## 2.3 Statistical analysis

Age, weight, height and lung function were considered as continuous variables. The 25(OH)D levels were divided into three categories : 13.7 to ≤ 49 nmol/L (low), 50 to ≤ 74 nmol/L (moderate) and ≥75 to ≤ 195.1 nmol/L (high) where 13.7 nmol/L and 195.1 nmol/L are the minimum and maximum values of the 25(OH)D levels . Four categories based on the quartiles of 25(OH)D levels (13.7 to ≤ 57.9 nmol/L, 58.0 to ≤ 72.5 nmol/L, 72.6 to ≤ 86.8 nmol/L, 72.6 to ≤ 86.8 nmol/L) were also considered for comparison with the results obtained with three categories. . The body mass index was categorized into normal weight, overweight and obesity using the CDC classification. Other factors included in the statistical analyses were racial origin (Caucasian or not), whether breastfed as an infant, family history of asthma, household income, number of children over 12 years of age at home, duration of weekly physical activity, duration of daily sun exposure, frequency of use of sunscreen, frequency of daily consumption of milk, duration of daily sedentary activities, use of gas furnace, use of electric heating system, and study period (summer and winter).

Analysis of variance and Pearson chi-square tests were used to determine the significance of the association between 25(OH)D levels and outcome measures (lung function parameters current wheeze and ever asthma). Multiple logistic regression analysis was used to test the association between 25(OH)D levels, and current wheeze and ever asthma after controlling for other factors. Multiple linear regression analysis was used to determine the relationship between the 25(OH)D levels and lung function controlling for age, sex, weight, height,

and height-squared. Interactions between vitamin D level and other covariates were examined in all the models. Survey design weight and bootstrap weights provided by Statistics Canada were incorporated in all statistical analyses. Analyses were conducted using STATA statistical software (*Stata Statistical Software: Release 12*. College Station, TX: StataCorp LP). The package *mgcv* in R (R Core Team, 2013) was used to conduct logistic regression analysis using the generalized additive model (GAM) procedure (30) to explore the non-linear relationship between 25(OH) levels, considered as a continuous variable and current wheeze (dichotomous outcome). Cubic regression splines was used as the smooth function in GAM and the unbiased risk estimator score was used to determine the most appropriate model among models with varying number of knots and degrees of smoothness.

## 2.4 Results

The mean age of the children included in the study was 9.1 years. The prevalence of current wheeze and ever asthma was 7.4% and 10.3%, respectively. Among the children with ever asthma, 53.4% children had current wheeze. Among the children reporting current wheeze, 65.9% reported less than 4 episodes of wheeze while 34.1% reported 4 or more episodes of wheeze in the last 12 months.

As shown in Table 2.1, the proportions of children in the low, moderate, and high 25(OH)D categories were 15.6%, 37.5%, and 46.9%, respectively. The prevalence of current wheeze was 11.4%, 4.1% and 7.7% in the low, moderate and high 25(OH)D categories, respectively. Children in the low and high 25(OH)D categories were more likely to report current wheeze in comparison to those children in the moderate category. Children with a family history of asthma had a significantly greater prevalence of current wheeze (16.0%) than those without a family history of asthma (5.0%). Use of gas furnace-heating and electric-heating systems had opposite effects on the prevalence of current wheeze with symptom prevalence being higher in homes using a gas furnace-heating system, and lower in homes using electric-heating systems in comparison to homes using other heating systems.

As shown in Table 2.2, the prevalence of ever asthma was 14.5%, 8.2% and 10.2% in the low, moderate and high 25(OH)D categories, respectively. Children in the low and high 25(OH)D categories were more likely to have a report of ever asthma in comparison to those children in the moderate category.

Children with a family history of asthma had a significantly greater prevalence of ever asthma (25.9%) than those without a family history of asthma (5.6%).

The factors included in the Tables 2.1 and 2.2 were initially considered for the multiple logistic regression analysis of current wheeze and ever asthma, respectively. The results from the final multiple logistic regression of current wheeze or ever asthma are shown in Table 2.3. After controlling for confounders, children in both the low and high 25(OH)D categories had an increased odds of reporting current wheeze in comparison to those in the moderate category (OR: 3.26; 95% CI: 1.16-9.17 for low vs. moderate and OR: 2.14; 95% CI: 1.07-4.28, for high vs. moderate). Children in the low 25(OH)D category had an increased odds of ever asthma in comparison to those in the moderate category (OR: 1.86, 95% CI: 1.15, 3.01). Children in the high 25(OH)D category had a non-significant increased risk of ever asthma in comparison to the moderate category (OR: 1.46, 95% CI: 0.76, 2.80).

The mean values of age, height, weight and lung function outcomes were significantly different between the three 25(OH)D categories (Table 2.4). The mean percent predicted $FEV_1$, FVC, $FEV_1$/FVC ratio, and $FEF_{25\%-75\%}$ were greatest in the moderate 25(OH)D category in comparison to the low and high 25(OH)D categories but the differences between categories were not statistically significant. The mean values of age, height and weight were not significantly different between children with and without current wheeze (data not shown).

Table 2.5 displays the relationship between 25(OH)D levels and $FEV_1$, FVC, $FEV_1$/FVC ratio, and $FEF_{25\%-75\%}$ after adjusting for age, sex, race

(Caucasian or not), height, height-squared (for $FEV_1$ and FVC) and weight. The interaction effect between 25(OH)D levels and children's age was significant in the relationship of 25(OH)D levels with FVC (p=0.02) and $FEV_1$ (p=0.02). To elucidate the interaction effects, the estimated mean $FEV_1$ and FVC values were plotted against the children's age, for male Caucasian children with height and weight adjusted to the mean values in Figures 2.1 and 2.2, respectively. Figure 2.1 shows that the increase in $FEV_1$ with children's age, indicated by the slopes of the fitted lines, was greater in the moderate 25(OH)D category than in the low and high 25(OH)D categories The relationship between $FEV_1$ and age for females and for other race categories was similar to Figure 2.1 except for a shift in the intercepts. As shown in Figure 2.2, the results for FVC were similar to those observed for $FEV_1$ with the slopes greater in the moderate 25(OH)D category than in the low and high 25(OH)D categories. After controlling for the factors in Table 2.5, mean $FEV_1$/FVC ratio was greater for the low and high 25(OH)D categories than the moderate category with the difference being statistically significant only between moderate and high 25(OH)D categories. As shown in Table 2.5, there was a significant interaction between weight and 25(OH)D categories in the regression model for $FEF_{25\%-75\%}$ with the increase in $FEF_{25\%-75\%}$ with children's weight being greater in the moderate 25(OH)D category than in the low and high 25(OH)D categories.

In the GAM analysis, the 25(OH)D level was considered as a continuous variable and its relationship with current wheeze was examined. To characterize the interpretation of the results from GAM procedure, the predicted risk of current

wheeze was plotted in Figure 2.3 against the 25(OH)D levels for a male child of 9 years with a positive family history of asthma and absence of electric heating at home. After controlling for the factors considered previously in Table 2.3, there was an increased risk of current wheeze for 25(OH)D levels from 13.7 nmol/L to about 50 nmol/L, followed by minimal risk of current wheeze until about 75 nmol/L and then an increase in the risk of current wheeze until from 75 nmoll/L to about 100 nmol/L. As indicated by the wide 95% confidence intervals in Figure 3, the risk estimates were not stable above 100 nmol/L due to the small number of observations. When four categories were considered using the 25(OH)D level quartiles, relative to the second category (58.0 to ≤ 72.5 nmol/L), odds ratios of current wheeze were 2.75 (95% CI: 1.20, 6.31; p=0.02) in the first category (13.7 to ≤ 57.9 nmol/L), 3.02 (95% CI: 1.26,7.25; p=0.018) in the third category (72.6 to ≤ 86.8 nmol/L ) and 1.59 (95% CI: 0.85,2.98; p=0.13) in the fourth category (86.9 to ≤ 195.1 nmol/L) after controlling for the factors considered in Table 2.3.

## 2.5 Discussion

In this study of children of ages 6 to 12 years, a U-shaped relationship was observed between 25(OH)D levels and current wheeze and lung function, indicators of current respiratory health. The 25(OH)D levels below 50 nmol/L, and above 75 nmol/L were both associated with a greater than two fold increased risk of current wheeze and reduced lung function. The significant interaction effect between age and 25(OH)D levels on FVC and $FEV_1$ indicated that the rate of changes in lung function with children's age was dependent on25(OH)D levels, with increases being greatest in the moderate 25(OH)D category (50 to 74 nmol/L). The U-shaped relationship was also observed between 25(OH)D levels and ever asthma but the odds ratio for the high 25(OH)D category was not statistically significant.

One of the conclusions in the IOM report was that 25(OH)D levels above 75 nmol/L were not consistently associated with increased benefit and the risks have been identified for some outcomes at 25(OH)D levels above 125 nmol/L (23). Based on this report, three categories were chosen, 13.7 to 49 nmol/L (low), 50 to 74 nmol/L (moderate), and 75 to 195.1 nmol/L (high) in this study and found an increased risk for adverse respiratory health outcomes in low and high categories in comparison to the moderate category. This U-shaped non-linear relationship between 25(OH)D levels and current wheeze was further examined in two independent analyses by considering 25(OH)D levels as a continuous variable and a four-level categorical variable based on quartiles, respectively. The results from these analyses provided further support to the findings that lower and

higher 25(OH)D levels were associated with increased risk of current wheeze in comparison to the moderate 25(OH)D levels. A birth cohort study from Tucson, Arizona similarly found a non-linear relationship between cord blood 25(OH)D levels and allergic outcomes. Relative to the reference category (50 to 74.9 nmol/L), 25(OH)D levels < 50 nmol/L and ≥ 100 nmol/L were associated with increased total IgE and inhalant allergen-specific IgE measured at ages 1 to 5 years in a longitudinal analysis , but no association was observed with asthma prevalence at 5 years (16). In a study from Southampton, UK, increased maternal 25(OH)D levels (> 75 nmol/L) during late pregnancy had an increased risk of childhood eczema at 9 months and asthma at 9 years (21).

The findings from previous studies have not been consistent in terms of characterizing the relationship between 25(OH)D levels and respiratory outcomes. Similar to the finding in this study, several studies have suggested lower 25(OH)D levels are associated with a higher risk of asthma. In a study of asthmatic children age 6 to 14 years from Costa Rica, circulating levels of vitamin D were inversely associated with hospitalization rates for asthma, bronchodilator responsiveness, and allergic markers such as IgE levels and eosinophil counts (12). In another study of children below 16 years from Qatar, those with asthma had lower serum vitamin D levels than the children without asthma (14). The PIAMA cohort study from Netherlands reported a more complex age-dependent association between serum vitamin D levels and asthma in two independent groups of children of ages 4 and 8 years (13). Serum vitamin D levels measured at age 4 had an inverse

association with asthma at ages 4 and 8 and vitamin D levels measured at age 8 had a positive association with asthma at age 8 (13).

In a prospective pilot study examining whether increased serum 25(OH)D levels, affected asthma control in children of ages 6 to 17 years with chronic persistent asthma, no significant effect was observed on asthma control test score or spirometry measurements (18). In contrast, in a Polish prospective study of children with newly diagnosed asthma, vitamin D supplementation improved the control of asthma exacerbations triggered by acute respiratory infections (31). A Swedish birth cohort study reported that a higher intake of vitamin D during infancy was associated with an increased cumulative incidence of atopic dermatitis, allergic rhinitis and asthma from infancy to 6 years (8). A further study of a birth cohort form the USA with early infant vitamin D supplementation also noted an increased risk of asthma by 3 years of age among black children (15).

The results from the studies examining the relationship between vitamin D levels and lung function have also not been consistent. A positive significant linear relationship was observed between predicted $FEV_1$ and $FEV_1$/FVC ratio and 25(OH)D levels in asthmatic children in a study conducted in Iran (32). In this study of 50 asthmatic children of ages 6 to 18 years, 25(OH)D levels were also categorized using the cut-offs similar to this study but a continuous scale was used for 25(OH)D levels in the multivariate analyses reported in the manuscript (32). In a study examining the serum 25(OH)D levels and exercise induced bronchoconstriction in children with intermittent asthma, a positive relationship was observed between serum 25(OH)D levels and FVC, and $FEV_1$

(33). The mean 25(OH)D level was significantly lower in children with a positive response to the exercise challenge, as indicated by a change of 10% or greater in $FEV_1$, than those with a negative challenge (33). There was no significant association between 25(OH)D levels and lung function in children using regular corticosteroids, who were part of a multi-center childhood asthma management program (34).

There may also be some confusion in interpreting these studies because serum 25(OH)D consists of both the more potent $25(OH)D_3$ and the less potent $25(OH)D_2$ (35). In the ALSPAC prospective birth cohort study from South West England, $25(OH)D_2$ levels measured at the mean age 9.8 years were associated with a lower risk of wheezing at the mean age 15.5 years while $25(OH)D_3$ levels were associated with an increased risk of wheezing (36). In addition, $25(OH)D_2$ levels were weakly positively associated with FVC and $FEV_1$, while no association was found with $25(OH)D_3$ (36).

The reasons for these inconsistent findings in the studies examining the relationship between 25(OH)D levels and respiratory outcomes in children are unclear but they might be related with the age group of children considered and differences between the assays used to measure the serum 25(OH)D. In this study, LIAISON assay, an automated chemiluminescent immunoassay, was used to measure serum 25(OH)D. Liquid chromatography-tandem mass spectrometry is considered as the gold standard for measuring 25(OH)D levels. The use of different assays might well have contributed to the observed inconsistencies between the studies (37, 38). However, a recent study reported that the agreement

between LIAISON assay and liquid chromatography-tandem mass spectrometry for measuring 25(OH)D levels was excellent and superior in comparison to other automated immunoassays that are currently used in laboratories (39).

Vitamin D plays an active role in regulating specific phases of human immunity and has been shown to be associated with innate and adaptive immunity. The vitamin D receptor (VDR) gene and the gene for 1α-hydroxylase (CYP27B1) have been shown to be induced following activation of toll-like receptor 2/1(TLR2/1) gene, the principal pathogen recognition receptor for Mycobacterium tuberculosis (40). However, the role of CYP27B1 gene in the expression of VDR gene following activation of toll-like receptor 2/1(TLR2/1) gene is not very clear (10).

The possibility of Vitamin D acting as regulator of Th17 cells, which have the capacity to synthesize the pro-inflammatory cytokine interleukin-17 (IL-17) (41), has been suggested from animal models of gastrointestinal inflammatory disease (colitis) in which treatment with 1, 25(OH)$D_3$ was shown to reduce the IL-17 expression (42). A recent review article proposed asthma as "a chronic disease of the innate and adaptive immune systems responding to viruses and allergens," (43) providing the relevance for examining relationships between vitamin D and respiratory outcomes in this study.

Other potential mechanisms of action by which vitamin D may protect against asthma include modification of the expression of TNF-α cytokine (44), induction of T-reg cells and expression of inhibitory cytokines (IL-10 ,TGF-β). and modification of control of CD4-positive T-lymphocytes (45,46). Vitamin D

might feasibly also increase the risk of asthma exacerbations through mechanisms such as increasing allergen-induced T-cell proliferation, IL-4, IL-12 cytokine levels, and serum IgE production (47). Measured serum 25(OH)D level includes contributions from both cutaneous production and dietary intake (e.g.: fortified milk) (2, 13). The half-life of vitamin D is approximately three weeks and is affected by serum calcium levels (48-50). Cutaneous synthesis of vitamin D is dependent on skin pigmentation, age, behavioral and lifestyle factors (38, 51). The study reported in this manuscript was conducted in Canada which is located in the northern hemisphere (43 ˚ N latitude, and above) (52), with children living in some of the data collection sites receiving sun light only for a short duration, as little as 3 hours per day during the winter months (53). Consequently very limited cutaneous vitamin D synthesis occurs during this period (54,55). Seasonal variation of serum vitamin D may vary by 10-25 nmol/l between winter and summer months (56). However, season of blood collection, skin pigmentation, use of sunscreen, and duration of daily sun exposure were not significant factors in this study. As reported in other studies, the confounding effect of these variables may be minimal among children (57, 58).

One of the strengths of this study was that it was conducted in a large population and adequately powered to detect the association in age–specific groups. In addition, it had objective measures for lung function and serum vitamin D levels. Nonetheless this study did have some limitations. Due to the cross-sectional study design, reverse causality cannot be excluded. In addition, as the data were collected from 15 sites in 4 provinces, the degrees of freedom available

for statistical analysis was 11 which restricted the number of variables chosen for the final regression models.

In summary, the observed association in these data between vitamin D levels with current wheeze and lung function supports a U-shaped dose-response relationship. Preventative strategies targeting children are warranted to avoid highly prevalent vitamin D deficiency. Whether advice is need to avoid vitamin D over provision remains more controversial but these results suggest that may be so. In conclusion, improved guidelines regarding vitamin D in childhood are needed, and monitoring of serum vitamin D level may enhance management of respiratory conditions in children.

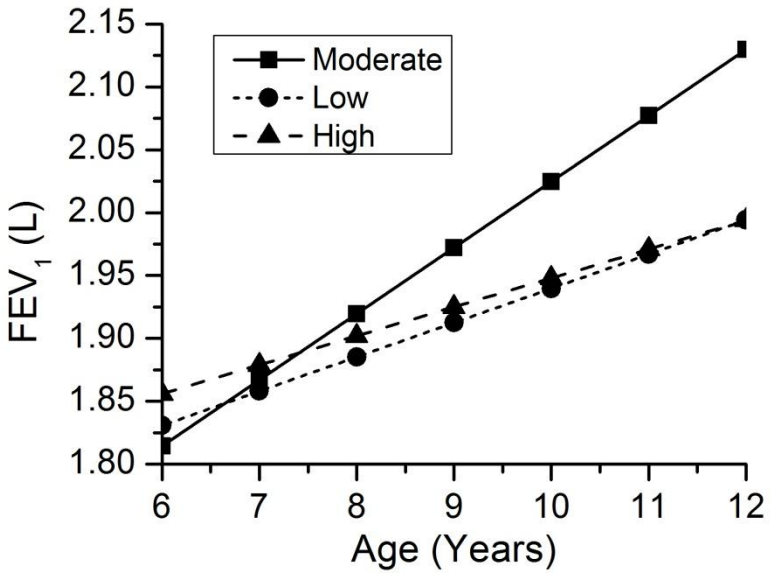


Figure 2.1 Relationship between forced expiratory volume in one second ($FEV_1$) with children's age for the vitamin D categories (low: ≤ 49 nmol/L, moderate: 50 to ≤74 nmol/L and high: ≥75 nmol/L) for male Caucasian children with height and weight adjusted at the mean values.

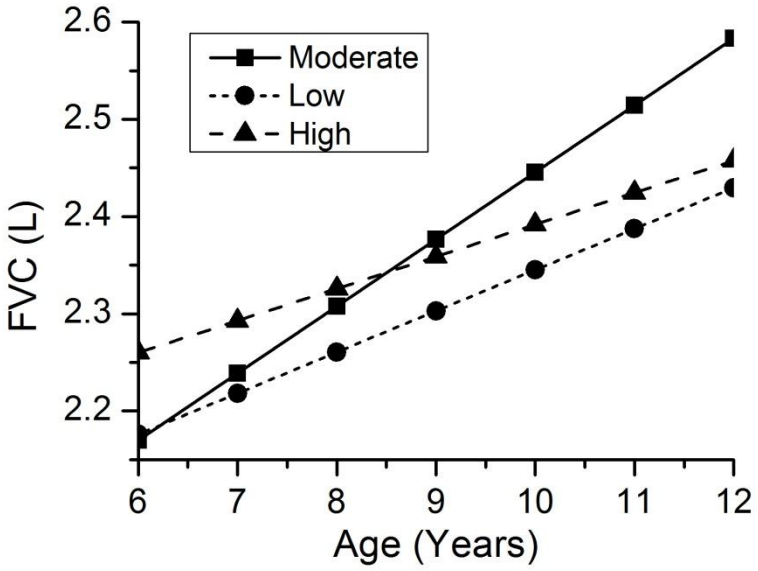

Figure 2.2 Relationship between forced vital capacity (FVC) with children's age for the vitamin D categories (low: ≤ 49 nmol/L, moderate: 50 to ≤74 nmol/L and high: ≥75 nmol/L) for male Caucasian children with height and weight adjusted at the mean values.

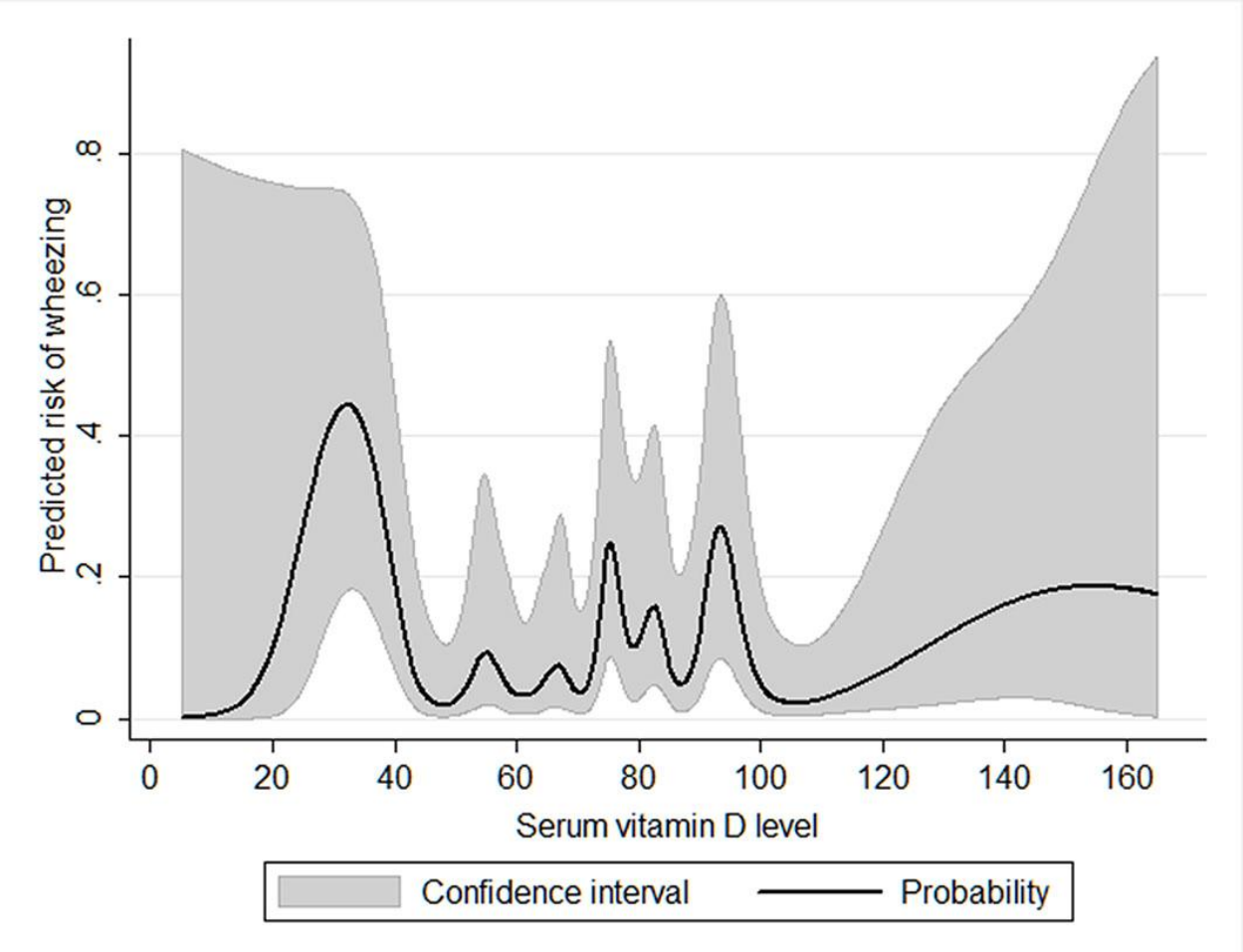


Figure 2.3 Relationship between predicted risk of current wheeze with serum vitamin D level from generalized additive model. The predicted risk is plotted for a male child at age 9 years with positive family history of asthma and absence of electric heating at home.

**Table 2.1 Characteristics of the study sample, proportion of current wheeze by factors and odds ratio for the relationship of factors with current wheeze**

| Factors | Distribution of characteristics in the sample (% ) | Proportion of current wheeze within factor (%) | Unadjusted OR (95% CI) | p-value* |
|---|---|---|---|---|
| Vitamin D level (nmol/L) | | | | 0.08 |
| ≤ 49 | 15.6 | 11.4 | 3.02 (1.04, 8.78) | |
| 50-74 | 37.5 | 4.1 | 1.0 | |
| ≥75 | 46.9 | 7.7 | 1.96 (0.91, 4.22) | |
| Sex | | | | 0.38 |
| Female | 47.5 | 6.7 | 1.00 | |
| Male | 52.5 | 8.1 | 1.21 (0.75, 1.98) | |
| Body mass index | | | | 0.37 |
| Under or normal weight | 67.8 | 7.9 | 1.00 | |
| Overweight or Obese | 32.2 | 5.8 | 0.72 (0.32, 1.63) | |
| Caucasian | | | | 0.99 |
| Yes | 77.9 | 7.5 | 1.00 | |
| No | 22.1 | 7.4 | 0.99 (0.19, 5.21) | |
| No. of children > 12 years | | | | 0.004 |
| ≤ 1 | 86.6 | 6.2 | 1.00 | |
| ≥ 2 | 13.4 | 15.5 | 2.79 (1.38, 5.64) | |
| Family history of asthma | | | | 0.002 |
| No | 76.3 | 4.9 | 1.00 | |
| Yes | 23.7 | 15.9 | 3.64 (1.67, 7.95) | |
| Household income | | | | 0.83 |
| Middle | 27.2 | 6.8 | 1.00 | |
| Low | 26.9 | 7.1 | 1.05 (0.31, 3.64) | |
| High | 45.9 | 8.0 | 1.21 (0.35, 4.11) | |
| Smoking inside home | | | | 0.25 |
| No | 88.6 | 6.9 | 1.00 | |
| Yes | 11.4 | 11.3 | 1.71 (0.41, 7.17) | |
| Use of gas furnace | | | | 0.02 |
| No | 39.0 | 4.3 | 1.00 | |
| Yes | 61.0 | 9.3 | 2.28 (1.11, 4.67) | |
| Use of electric heating | | | | <0.001 |
| No | 71.8 | 9.3 | 1.00 | |
| Yes | 28.2 | 2.5 | 0.25 (0.15, 0.43) | |
| Region | | | | 0.08 |
| Ontario | 41.4 | 8.7 | 1.00 | |
| Quebec, New Brunswick | 28.4 | 4.5 | 0.49 (0.25, 0.97) | |
| British Columbia, Alberta | 30.2 | 8.4 | 0.96 (0.51, 1.84) | |

| | | | | |
|---|---|---|---|---|
| Season | | | | 0.36 |
| Summer | 53.2 | 6.7 | 1.00 | |
| Winter | 46.8 | 8.2 | 1.25 (0.74-2.11) | |
| Daily sun exposure | | | | 0.58 |
| 1-3 hours | 36.3 | 7.8 | 1.00 | |
| < 1 hour | 11.4 | 10.5 | 1.39 (0.22, 8.62) | |
| >3 hours | 52.3 | 6.5 | 0.82 (0.39, 1.70) | |
| Use of sunscreen | | | | 0.07 |
| Always/often | 66.3 | 6.4 | 1.00 | |
| Sometimes | 16.7 | 5.9 | 0.92 (0.38, 2.23) | |
| Rarely/never | 17.0 | 11.8 | 1.96 (1.05, 3.66) | |
| Daily consumption of milk | | | | 0.12 |
| At least once | 58.2 | 5.8 | 1.00 | |
| Less than once | 10.6 | 13.4 | 2.52 (0.77, 8.23) | |
| More than twice | 31.2 | 8.5 | 1.52 (0.92, 2.51) | |
| Daily sedentary activities | | | | 0.17 |
| 0-2 hours | 64.9 | 5.9 | 1.00 | |
| 3-4 hours | 23.9 | 6.9 | 1.18 (0.66, 2.10) | |
| 5 and above hours | 11.1 | 9.9 | 1.74 (0.88, 3.44) | |

*p-value from chi-squared test indicating the significant difference in the characteristics between children with and without current wheeze

**Table 2.2 Proportion of ever asthma by factors and odds ratio for the relationship of factors with ever asthma**

| Factor | | Proportion of ever asthma within factor | Unadjusted OR (95% CI) | p-value* |
|---|---|---|---|---|
| Vitamin D level (nmol/L) | | | | 0.13 |
| | ≤ 50 | 14.5 | 1.9 (1.27, 2.84) | |
| | 50-75 | 8.2 | 1.00 | |
| | ≥ 75 | 10.2 | 1.28 (0.65, 2.50) | |
| Sex | | | | 0.89 |
| | Female | 10.2 | 1.00 | |
| | Male | 10.5 | 1.03 (0.6, 1.78) | |
| BMI | | | | 0.09 |
| | Under or normal weight | 12.6 | 1.00 | |
| | Overweight/obese | 8.6 | 0.65 (0.39, 1.10) | |
| Caucasian | | | | 0.01 |
| | Yes | 9.2 | 1.00 | |
| | No | 14.4 | 1.67 (1.11, 2.50) | |
| No. of children > 12 years | | | | 0.38 |
| | ≤ 1 | 9.7 | 1.00 | |
| | ≥ 2 | 14.0 | 1.51 (0.43, 5.37) | |
| Family history of asthma | | | | <0.001 |
| | No | 5.6 | 1.00 | |
| | Yes | 25.9 | 5.94 (3.55, 9.95) | |
| Household income | | | | 0.14 |
| | Middle | 8.9 | 1.00 | |
| | Low | 14.3 | 1.7 (0.78, 3.72) | |
| | High | 9.2 | 1.03 (0.48, 2.18) | |
| Smoking inside home | | | | 0.05 |
| | No | 9.2 | 1.00 | |
| | Yes | 18.7 | 2.26 (0.77, 6.66) | |
| Use of gas furnace | | | | 0.61 |
| | No | 11.0 | 1.00 | |
| | Yes | 9.7 | 0.88 (0.48, 1.59) | |
| Use of electric heating | | | | 0.81 |
| | No | 10.0 | 1.00 | |
| | Yes | 10.6 | 1.07 (0.57, 2.02) | |
| Region | | | | 0.87 |
| | Ontario | 11.1 | 1.00 | |
| | Quebec, New Brunswick | 9.8 | 0.87 (0.46, 1.66) | |
| | British Columbia, Alberta | 9.7 | 0.87 (0.3, 2.48) | |

| | | | |
|---|---|---|---|
| Season | | | 0.03 |
| Summer | 8.8 | 1.00 | |
| Winter | 12.0 | 1.4 (1.00, 1.98) | |
| Daily sun exposure | | | 0.068 |
| 1-3 hour | 7.9 | 1.00 | |
| <1 hour | 16.2 | 2.27 (1.35, 3.84) | |
| >3 hours | 10.7 | 1.41 (0.79, 2.53) | |
| Use of sunscreen | | | 0.33 |
| Always / often | 9.3 | 1.00 | |
| Sometimes | 9.8 | 1.06 (0.54, 2.08) | |
| Rarely / never | 13.3 | 1.51 (0.69, 3.3) | |
| Daily consumption of milk | | | 0.69 |
| At least once | 9.9 | 1.00 | |
| Less than once | 12.6 | 1.31 (0.42, 4.05) | |
| More than twice | 10.2 | 1.03 (0.70, 1.53) | |
| Daily sedentary hours | | | 0.66 |
| 0 - 2 hours | 9.3 | 1.00 | |
| 3 - 4 hours | 8.6 | 0.91 (0.57, 1.47) | |
| 5 and above | 11.7 | 1.29 (0.36, 4.57) | |

*p-value from chi-squared test indicating the significant difference in the characteristics between children with and without ever asthma

**Table 2.3 Results from the multiple logistic regression of current wheeze and ever asthma with vitamin D levels**

| | Current wheeze | | Ever asthma | |
|---|---|---|---|---|
| Factors | Odds ratio (95% CI) | p-value | Odds ratio (95% CI) | p-value |
| Age (yr) | 1.10 (0.87, 1.37) | 0.38 | 1.15 (0.97, 1.37) | 0.09 |
| Sex | | | | |
| Female | 1.00 | | 1.00 | |
| Male | 1.14 (0.67, 1.93) | 0.59 | 0.91 (0.47, 1.77) | 0.76 |
| Family history of asthma | | | | |
| No | 1.00 | | 1.00 | |
| Yes | 4.09 (1.55, 10.76) | 0.008 | 6.05 (3.62, 10.11) | <0.001 |
| Number of children > 12 years | | | | |
| ≤ 1 | 1.00 | | - | |
| ≥ 2 | 2.21 (1.06, 4.57) | 0.04 | - | |
| Use of electric heating | | | | |
| No | 1.00 | | - | |
| Yes | 0.29 (0.14, 0.60) | 0.003 | - | |
| Daily sun exposure | | | | |
| < 1 hours | - | | 4.2 (2.50, 7.07) | <0.001 |
| 1-3 hours | - | | 1.00 | |
| >3 hours | - | | 1.62 (0.93, 2.82) | 0.08 |
| Vitamin D level | | | | |
| ≤ 49 nmol/L | 3.26 (1.16, 9.17) | 0.03 | 1.86 (1.15, 3.00) | 0.02 |
| 50-74 nmol/L | 1.00 | | 1.00 | |
| ≥ 75 nmol/L | 2.14 (1.07, 4.28) | 0.03 | 1.46 (0.76, 2.80) | 0.22 |

**Table 2.4 Distribution of demographic factors and lung function by vitamin D categories**

| Factor | Vitamin D categories | | | |
|---|---|---|---|---|
| | ≤ 49 nmol/L | 50 - 74 nmol/L | ≥ 75 nmol/L | |
| | Mean (SD) | Mean (SD) | Mean( SD) | p-value* |
| Age (year) | 9.92 (2.85) | 9.3 (3.19) | 9.0 (3.07) | 0.002 |
| Height (cm) | 144.73 (20.43) | 139.36 (21.95) | 137.83 (20.32) | 0.002 |
| Weight (kg) | 43.58 (24.92) | 36.42 (18.71) | 34.24 (17.64) | 0.004 |
| FVC (L) | 2.59 (1.11) | 2.43 (1.14) | 2.33 (0.96) | 0.03 |
| $FEV_1$ (L) | 2.17 (0.86) | 2.05 (0.95) | 1.94 (0.77) | 0.005 |
| $FEV_1$/FVC | 0.85 (0.1) | 0.85 (0.1) | 0.84 (0.09) | 0.06 |
| $FEF_{25\%-75\%}$ (L/s) | 2.31 (1.07) | 2.22 (1.25) | 2.01 (1.05) | 0.001 |
| Percent predicted FVC | 101.9 (18.14) | 103.09 (20.22) | 102.3 (17.61) | 0.45 |
| Percent predicted $FEV_1$ | 98.86 (18.56) | 100.89 (20.67) | 98.73 (17.99) | 0.06 |
| Percent predicted $FEV_1$/FVC | 96.84 (11.71) | 97.8 (11.26) | 96.49 (10.69) | 0.06 |

* p-values are from analyis of variance for comparison between vitamin D categories

**Table 2.5 Results from the multiple linear regression analysis of lung function outcomes**

| **Factor** | **FVC** (ml) B** (SE) | **$FEV_1$** (ml) B** (SE) | **$FEV_1$/FVC** (%) B** (SE) | **$FEF_{25\%-75\%}$** (ml/s) B** (SE) |
|---|---|---|---|---|
| Age* | 68.93 (15.88)¶ | 52.49 (14.4)‡ | -0.197 (0.198) | 57.62 (14.2)‡ |
| Sex (ref - Male) | -109.49 (16.06)§ | -33.05 (15.85) | 2.198 (0.485)¶ | 103.49 (36.73)† |
| Caucasian (ref -Yes) | -143.35 (53.05)† | -102.25 (38.6)† | - | - |
| Height* | 27.48 (3.35)§ | 26.21 (2.94)§ | 0.08 (0.062) | 23.74 (4.22)§ |
| Height2* | 0.29 (0.06)¶ | 0.28 (0.07)‡ | - | 0.41 (0.14)† |
| Weight* | 12.91 (1.62)§ | 5.83 (1.94)† | -0.156 (0.061)† | 7.69 (5.98) |
| Vitamin D level | | | | |
| ≤ 49 nmol/L | -73.88 (32.19)† | -59.61 (25.57)† | 0.436 (0.793) | -52.27 (57.91) |
| 50-74 nmol/L (ref) | - | - | - | - |
| ≥75 nmol/L | -17.88 (21.49) | -47.12 (12.79)‡ | -1.46 (0.398)‡ | -142.48 (48.86)† |
| Age and vitamin D level interaction | | | | |
| ≤ 49 nmol/L | -26.65 (22.79) | -25.28 (15.35) | - | - |
| 50-74 nmol/L (ref) | - | - | - | - |
| ≥75 nmol/L | -36 (10.32)‡ | -29.5 (8.08)‡ | - | - |
| Weight and vitamin D level interaction | | | | |
| ≤ 49 nmol/L | - | - | - | -10.26 (3.31)‡ |
| 50-74 nmol/L (ref) | - | - | - | - |
| ≥75 nmol/L | - | - | - | -8.58 (3.42)† |
| Intercept | 2376.73 (25.66)§ | 1972.11 (16.58)§ | 83.974 (0.397)§ | 2039.94 (44.59)§ |

* Variables were centralized

B** (SE) - Regression coefficient (Standard error)

†p-value ≤ 0.05

‡p-value ≤ 0.01

¶p-value ≤0.001

§p-value ≤ 0.0001

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# Chapter 3 Association between vitamin D level and asthma in Canadian adolescents and adults

## 3.1 Introduction

The World Health Organization estimated that 300 million people worldwide currently suffer from asthma (1). Not only high-income countries, but all countries regardless of the level of development or their geographical location experience a substantial burden due to asthma morbidity (2). The prevalence of asthma among adults has been increasing over the last 20 years in Canada (3) and was reported as 8.1% in 2012 (4). An asthma attack is one of the most common conditions reported in primary care and emergency visits (5). The Conference Board of Canada reported that the economic impact due to asthma was $3.4 billion in direct healthcare costs and $8.6 billion in indirect costs including work loss (6). Environmental, biological and genetic factors have been shown to be important factors associated with asthma (7).

Although the classic vitamin D deficiency disease of rickets was first described in the 17$^{th}$ century (8), vitamin D was first discovered by Sir Edward Mellanby in 1922 (9). Vitamin D has long been known for its role in the prevention of rickets, and in the intestinal absorption of dietary calcium (9,10). In recent decades, it has been recognized as a vital nutrient in protecting the body from a wide range of diseases (11). The link between vitamin D and asthma has been the subject of considerable scientific debate in recent years (12). The hypothesis that vitamin D plays central role as an environmental and epigenetic contributor to the pathogenesis of asthma and its exacerbation, allergy and

respiratory infections, termed the "vitamin D hypothesis", was proposed based on various experimental and epidemiological findings (13). Vitamin D deficiency is reported to be prevalent among asthmatics, and results in poor control of symptoms, and steroid insensitivity (14). By affecting the functions of subsets of immune cells, vitamin D potentially impacts on the levels of circulating chemokines and cytokines involved in cellular immune responses and inflammation reduction (15). It is also known that vitamin D synergistically enhances anti-inflammatory effects of steroids administered in asthmatics (16,17). Despite these findings, data on a possible relationship between asthma and vitamin D, and the optimal range of vitamin D for patients with asthma remains unclear.

Initially, normal levels of serum vitamin D were defined by the amount needed to keep parathyroid hormone from becoming abnormally high (18), and the optimal range was decided from mean serum 25(OH)D ± 2 standard deviation in a group of healthy individuals (19). However, what is considered the normal range has varied considerably (19,20). In the early 1990's 25(OH)D levels between 25 -137.5 nmol/L measured in the wintertime were considered as normal (21). Due to the recent advances in understanding of the immunological functions of vitamin D, a panel from the Institute of Medicine concluded that a serum level of 50 nmol/L is sufficient for the bone health of 97% of the population (22). In this chapter, the association between 25(OH)D level and respiratory outcomes is examined among Canadian adolescents and adults using the data from CHMS.

## 3.2 Methods

A secondary analysis was conducted using the data for subjects aged 6-79 years of age included in population-based data available from the CHMS. The data was considered representative of 96.3% of Canadian population. This study was approved by the Health Research Ethics Board of the University of Alberta.

The details of the CHMS are fully described elsewhere (23). In brief, the survey used a multistage sampling design to collect data on self-reported and direct measures of health from a representative sample of Canadians aged 6 to 79 years living in private households at the time of the survey. Residents of Indian Reserves or Crown lands, institutions and certain remote regions, and full-time members of the Canadian Forces were excluded. The survey was conducted between March, 2007 and February, 2009. Trained staff of Statistics Canada conducted household interviews using the questionnaire. The content of the questionnaire was developed by Statistics Canada in partnership with Health Canada and the Public Health Agency of Canada. From the household interview information on respiratory symptoms and diseases, personal and family characteristics were obtained. Within 14 days of the household interview, participants were invited to visit a Mobile Examination Clinic (MEC) for measurement of a number of relevant characteristics. In total, 5604 individuals randomly selected from 15 sites from British Columbia, Alberta, Ontario and Quebec participated in the survey. The overall combined response rate for this survey was 51.7%. Of the 4,385 subjects of ages 13 to 79 years who participated in the CHMS, 3,762 subjects were included in these analyses after removing the

subjects with missing 25(OH)D measurements and those who were older than 69 years.

As part of the visit to the MEC, venous blood samples were collected using routine methods, processed, and stored at -18C in a freezer within the MEC laboratory. Frozen samples of blood were sent weekly to the Health Canada reference laboratory in Ottawa, where the serum 25(OH)D was measured.

Spirometry was performed using the Respironics KoKo spirometer (PDS Instrumentation, Louisville, CO) according to American Thoracic Society guidelines by a health measurement specialist. A minimum of 3 and a maximum of 8 acceptable trials were performed. From the best test effort, the following parameters were obtained: forced expiratory volume in one second ($FEV_1$), forced vital capacity (FVC), $FEV_1$/FVC ratio, and forced expiratory flow between 25% and 75% of forced vital capacity ($FEF_{25\%-75\%}$). All spirometry results were reviewed by an external reviewer to ensure quality of the measurements. Participants were given accelerometers to monitor and measure their physical activity for one week.

Serum 25(OH)D was selected as the measure of vitamin D status because levels of this indicator reflect total vitamin D exposure from foods, supplements, and synthesis in the skin. Serum levels of 25(OH)D were assessed by a chemiluminescence assay using the LIAISON 25-Hydroxyvitamin D total assay (Diasorin Ltd, Stillwater, MN). The lower and upper detection limits were 10 nmol/L and 375 nmol/L, respectively. For the analysis, serum 25(OH)D levels

were categorized into ≤49 nmol/L (low), 50-74 nmol/L (moderate), and ≥75 nmol/L (high).The cut-points used were based on IOM guidelines, and a number of observational studies.

Ever asthma was determined from the household questionnaire from responses to the question “in conditions lasted 6 months or more and that have been diagnosed by a health professional, do you have asthma". Age of onset was obtained from the question "How old was the participant when this was first diagnosed?" Current asthma was obtained from the responses to the responses to the question "whether or not the participant had any asthma symptoms of asthma attacks in the past 12 months?"

### 3.3 Statistical analysis

To test for differences in continuous outcomes between those with and without current asthma, and between vitamin D groups, analysis of variance (ANOVA) was used. To test for association of categorical variable with current asthma or ever asthma, Pearson's chi-squared test was used. Multiple logistic regression was used to identify the predictors of current asthma and ever asthma. Similarly, multiple linear regression was used to identify the predictors of lung function parameters. Probability weights derived from the 2006 census-based population estimates were used to allow for the sampling design and response rates. Five hundred bootstrap weights of probability weight provided by Statistics Canada were used to estimate the standard errors of the estimated parameters.

Predictor variables of interest were sex, age, body mass index, household size, household income, attending school, family history of asthma, daily sun exposure, number of bedrooms in the house, use of gas furnace, use of electric heating system, racial origin, number of weekly sedentary activities, region, smoking status, and use of sun-screen. Racial origin was obtained from respondent's self-reported cultural and racial background.

From the graphical descriptive analysis, a non-linear association of age with lung function parameters was noticed. As a result, restricted cubic spline terms (24) of age were used in all multiple regression analysis of lung function outcomes. For restricted cubic splines, the position of three knots at 18, 29 and 50 was determined from the distribution of age in the association with FVC. Tests for non-linear association of age with lung functions used the likelihood ratio test comparing the model with only the linear term to the model with the linear and the cubic spline term. Post hoc testing was done using Wald's t-statistic to detect the overall significance of a variable in the model. Variables were included in the regression models if there was evidence of association with dependent variable in the univariate analysis (p-value $< 0.2$). As the data were collected from 15 sites in 4 provinces, the degrees of freedom available for statistical analysis was 11 which restricted the number of variables chosen for the final regression models. All p-values were two-tailed and $p \leq 0.05$ was considered significant.

## 3.4 Results

Overall prevalence of ever asthma and current asthma in the study was 8.1%, and 4.7%, respectively. The mean 25(OH)D level (SD) was 66.75 (24.11) nmol/L. As seen in Table 3.1, the mean 25(OH)D level was lower in those with current asthma than those without current asthma but the difference was not statistically significant. Similarly, no significant differences were observed in mean 25(OH)D levels between those with and without ever asthma. There were no significant differences in height or weight between those with and without current asthma or ever asthma categories. Those with current asthma, and ever asthma were significantly younger than those without.

Characteristics of the cohort, including the proportion with current asthma and the unadjusted odds ratios of the association between current asthma and the demographic characteristics of the population are shown in Table 3.2. In this study, 27.2% of the subjects had low 25(OH)D levels ( ≤49 nmol/L), 39.3% had moderate levels (50-74 nmol/L), and 33.6% had high levels (≥75 nmol/L). Among the subjects with low 25(OH)D levels, the proportion of males was greater than females, while in the moderate and high 25(OH)D levels proportion of females was greater than males (p=0.05). The prevalence of ever asthma was 9.5% in subjects with low 25(OH)D levels, 7.5% in subjects with moderate 25(OH)D levels, and 7.7% in subjects with high 25(OH)D levels with differences not being statistically significant (p=0.28). The prevalence of current asthma was not significantly different between low (6.4%), moderate (4.1%), and high (4.2%) 25(OH)D categories, respectively (p=0.09). In the univariate analysis, current

asthma was associated with younger age, attending school, and having family history of asthma, and greater number of hours spent in sedentary activities (Table 3.2).

Characteristics of those with ever asthma and the unadjusted odds ratios for the association between ever asthma and the characteristics are shown in Table 3.3. In the univariate analysis, female sex, low household income, attending school, and having a family history of asthma were positively associated with ever asthma (Table 3.3). Among subjects with asthma, around two-thirds (62.9%) developed asthma before 20 years (early-onset), and the rest developed asthma after 20 years (late-onset).

Although the highest mean values of FVC, $FEV_1$, and $FEF_{25\%-75\%}$ were observed in subjects in the moderate 25(OH)D category, these differences were not statistically significant (Table 3.4). In contrast, the highest mean values of percent predicted FVC and $FEV_1$ were observed in the high 25(OH)D category, but again these differences were not statistically significant. There were significant increases in age (p=0.05), decreases of weight (p=0.001), and $FEV_1$/FVC ratio (p=0.002) from low to high 25(OH)D categories

As seen in Table 3.5, after controlling for sex, age, family history of asthma, and daily energy expenditure, subjects in the low 25(OH)D category had a 54% increased odds of current asthma in comparison to those in the moderate category (OR: 1.54, 95% CI: 1.01-2.36). In a multiple logistic regression analysis of ever asthma, the interaction between 25(OH)D categories and family history of

asthma was significant. After allowing for the interaction, and adjustment for age, race and sedentary activity, subjects in the low 25(OH)D category with a family history of asthma has a more than two fold increase in odds of ever asthma in comparison to the moderate category (OR: 2.11, 95% CI: 1.40-3.21). The association between low 25(OH)D level and ever asthma in subjects without a family history of asthma was not statistically significant (p=0.45). Subjects with 35 or greater hours of weekly sedentary activity had an increased odds of asthma attacks and ever asthma in comparison to those who had 19 or lesser hours of weekly sedentary activity.

The results from the stratified analysis according to early and late onset of asthma (≤ 20 years and > 20 years) among subjects with asthma are shown in Table 3.6. An increase of one year in age was associated with a 4% decrease in likelihood of developing asthma in those with early onset asthma ($p<0.001$). Among subjects with early onset of asthma, those in the low 25(OH)D category with a family history of asthma had an almost 3-fold increase in the odds of ever asthma in comparison to those in the moderate category (OR: 2.84, 95% CI: 1.35-6.00). Among subjects with late onset of asthma, one year increase in age was associated with a 3% increase in the odds of ever asthma. There was no significant association between 25(OH)D categories and ever asthma among subjects with late-onset of asthma.

Table 3.7 shows the association of 25(OH)D categories with lung function parameters from the multiple linear regression analysis. The intercept indicates the mean value of respective lung function of a Caucasian female of mean age,

height and weight. After adjusting for age, gender, height, weight, smoking status, and race, subjects in the low 25(OH)D category had lower mean values of FVC, $FEV_1$, $FEV_1$/FVC ratio, and $FEF_{25\%-75\%}$ compared to those in the moderate category, however these differences were not statistically significant. Subjects in the high 25(OH)D category had significantly lower mean values of $FEV_1$/FVC ( p=0.02), in comparison to those in the moderate 25(OH)D category. Increasing age had an inverse non-linear association with lung function parameters; in particular cubic spline non-linear term of age was highly significantly associated.

## 3.5 Discussion

In this study, low vitamin D level was associated with a more than two-fold increased odds of risk of ever asthma in those with family history of asthma, and an almost 50% increased risk of current asthma compared to a moderate vitamin D level. Importantly, the association of lower vitamin D level with ever asthma was only evident in those who developed asthma before 20 years of age and had a family history of asthma. The cross-sectional decline of $FEV_1$/FVC with age was dependent on vitamin D levels. These results suggest that low vitamin D levels have an adverse effect on ever asthma, and high vitamin D level had an adverse effect on $FEV_1$/FVC ratio.

Several studies have assessed vitamin D status in Canada (25). It is acknowledged that the prevalence of low vitamin D level varies, and is much higher than previously thought (26). Using the 50 nmol/L value as cutoff, this study reports a prevalence of vitamin D deficiency of 27.2%, which is similar to

other reports. Of note, vitamin D deficiency is mainly a consequence of dietary, lifestyle, and behavioral factors (12). A study of the economic burden of vitamin D deficiency reported that the burden could be reduced by 7.3% in Canada if mean serum vitamin D levels were increased to 105 nmol/L (27). Although the optimal range of 25(OH)D varies widely depending on ethnic background, age and presence of conditions affecting serum vitamin D level, there is an urgent need for efforts to increase vitamin D levels in those with deficiency. Studies need to identify the best method to increase vitamin D status either by recommending vitamin D supplementation, or by recommending sun-exposure, or both.

Family history of asthma is recognized to be an important risk factor for asthma in children (28) and adults (29,30). Individuals having a family history of asthma have a stronger genetic predisposition to develop asthma compared to those without a family asthma (31). This study found that low vitamin D levels synergistically increased the risk of ever asthma among those with familial asthma. This is a novel finding and suggests that individuals with family history of asthma might have a unique immune and/or genetic phenotype, in which vitamin D deficiency increases the likelihood of the development of asthma. The synergistic effect of vitamin D with family history asthma for ever asthma was evident only in those who developed asthma before 20 years of age (early-onset) but not in those who developed asthma after 20 years of age (late-onset). The lack of synergistic effect between vitamin D and family history of asthma among those with late-onset asthma in this study raises the possibility that late-onset asthma may have a different mechanism to early-onset asthma. These findings put

together suggest that maintaining vitamin D at optimal physiologic level may be useful in preventing those with a family history of asthma from developing asthma in childhood, but the effect may be lost by adulthood. In a clinical context, this finding will be useful in early detection of those at most risk to develop asthma and asthma exacerbations.

The exact mechanism by which vitamin D modulates the risk of asthma is not completely understood. However, there are experimental data, which elucidate the mechanism for the association of vitamin D with the pathogenesis and course of asthma. The active form of vitamin D is involved in the regulation of expression of the antimicrobial peptide cathelicidin in bronchial epithelial cells, which is important in the defense against respiratory infections. Through this mechanism vitamin D likely protects against asthma exacerbation (32). Vitamin D likely also acts as a physiological regulator modifying inhibition and enhancement of processes potentiating $Th_2$ responses (33). Furthermore, vitamin D receptors (VDR) have been localized in both respiratory epithelial cells and in bronchial smooth muscle. VDR bound with the active form of vitamin D controls the transcription of target genes involved in signaling pathways (34). Furthermore, there have been reports of an association of some single nucleotide polymorphisms of the VDR gene with asthma and atopy (35,36), which suggests a plausible explanation for the novel findings in this study.

In this study, lung function parameters did not significantly vary across vitamin D categories in multiple linear regression models after adjusting for potential confounders, except for the association of high vitamin D level with

$FEV_1$/FVC ratio. The inability to find an association of vitamin D with other parameters of lung function in this study cannot altogether exclude an effect of vitamin D on lung function, since there have been several studies reporting a significant association (37–39). Given that the mean age of the sample was 40 years, it is possible that the role of vitamin D on lung function may be marred by other influential factors on lung function. A cubic spline method was used to control for the non-linear effect of age on lung parameters, which was important as the sample comprised a relatively heterogeneous group (adolescents, middle age, and above) of individuals who were at different points with respect to lung development and maturity. A study which included individuals of ages 18 years or greater from the CHMS data found a significant association of FVC, and $FEV_1$/FVC with log-transformed 25(OH)D level in male and obese subgroups (40). It is also known that airway remodeling, a process where structural airway changes including airway wall thickness, subepithelial fibrosis, airway lumen stenosis, hyperplasia and hypertrophy of myofibroblasts and epithelial cells in airway lumen, is more prominent in adults (41). By down-regulating the expression of genes associated with airway remodeling, and enhancing anti-proliferative effects on airway smooth muscles cells, vitamin D potentially reduces airway remodeling (42).

Based on a recent review of the literature about vitamin D, the Institute of Medicine recommended individuals should have a serum vitamin D level of 50 nmol/L or greater and levels >50nmol/L met the needs of 97.5% of the population (22). This review also suggested that levels more than 75 nmol/l were

not consistently associated with increased health benefits and concluded that there were insufficient data to recommend supplementation with vitamin D for the prevention of non-bone related diseases (22). This study used the cut-points suggested by IOM, which have been used in several studies previously. Some studies suggested that the optimal level of vitamin D in adults needed to be ≥75nmol/L, a level associated with maximal suppression of parathyroid hormone and reduced fracture risk (43,44,26). From the study findings, vitamin D level ≥75nmol/L nmol/L may not provide an increased health benefit. Rather a vitamin D level ≥75nmol/L was associated with a slightly higher prevalence of current asthma and ever asthma. In simple and multiple regression analyses, the lack of a protective association of high vitamin D levels with current asthma or ever asthma also argues against this option. Additionally, the highest mean $FEV_1$/FVC ratio was observed in moderate vitamin D level in univariate analysis and this association remained significant in multiple regression analysis.

In Chapter 2, low and high vitamin D levels were adversely associated with current respiratory parameters such as current wheeze, and lung function in children. In this chapter, an adverse association of low vitamin D level with respiratory outcomes continuing in adulthood was observed. The present study has several strengths. First, the data for this study came from a nationally representative, large and population-based sample. Second, the outcome and covariates were derived from a standardized questionnaire, objectively measured health measures and bio-specimen obtained using trained technicians. Unfortunately, as this study used a cross-sectional study design, the temporal

sequence of events cannot be determined and a causal relationship between respiratory outcomes and vitamin D cannot be ascertained. In conclusion, vitamin D may be playing a role in optimal respiratory health, in particular moderate levels of vitamin D protect against asthma development and exacerbation. Exploration of this relationship is needed to inform optimal level of vitamin D in regard to respiratory comes.

**Table 3.1 Distribution of sample characteristics by current and ever asthma**

| Factor | Current asthma¶ | | | Ever asthma¶ | | |
|---|---|---|---|---|---|---|
| | No Mean (SD) | Yes Mean (SD) | p-value* | No Mean (SD) | Yes Mean (SD) | p-value* |
| Age (years) | 40.05 (13.97) | 36.98 (14.55) | 0.03 | 40.15 (13.84) | 37.08 (15.72) | 0.008 |
| Height (cm) | 168.83 (8.57) | 168.66 (9.01) | 0.88 | 168.93 (8.51) | 167.66 (9.43) | 0.22 |
| Weight (kg) | 76.27 (16.53) | 77.28 (18.16) | 0.55 | 76.34 (16.4) | 76.03 (18.98) | 0.81 |
| Vitamin D (nmol/L) | 66.91 (24.13) | 63.44 (23.29) | 0.28 | 66.94 (24.13) | 64.60 (23.31) | 0.31 |

*p-value from ANOVA

**Table 3.2 Characteristics of the study sample, proportion of current asthma by factors and odds ratio for the relationship of factors with current asthma**

| Factor | Distribution in the sample (%) | Proportion of current asthma (%) | Unadjusted OR (95% CI) | p-value* |
|---|---|---|---|---|
| Vitamin D Level | | | | 0.075 |
| Low (≤ 49 nmol/L) | 27.2 | 6.4 | 1.6 (1.09, 2.36) | |
| Moderate (50-74 nmol/L) | 39.3 | 4.1 | 1.00 | |
| High (≥ 75 nmol/L) | 33.6 | 4.2 | 1.03 (0.63, 1.70) | |
| Sex | | | | 0.21 |
| Female | 50.2 | 5.3 | 1.00 | |
| Male | 49.8 | 4.2 | 0.78 (0.51, 1.18) | |
| Body mass index | | | | 0.31 |
| Normal or under weight | 45.3 | 4.0 | 1.00 | |
| Overweight | 34.2 | 4.3 | 1.09 (0.56, 2.12) | |
| Obese | 20.5 | 5.7 | 1.48 (0.89, 2.46) | |
| Family history of Asthma | | | | <0.001 |
| No | 77.1 | 3.1 | 1.00 | |
| Yes | 22.9 | 10.7 | 3.8 (2.38, 6.06) | |
| Household income | | | | 0.22 |
| Low | 18.8 | 8.7 | 2.22 (0.81, 6.1) | |
| Middle | 31.5 | 4.1 | 1.00 | |
| High | 49.7 | 3.5 | 0.85 (0.52, 1.4) | |
| Attending school | | | | 0.03 |
| No | 80.2 | 4.1 | 1.00 | |
| Yes | 19.8 | 7.2 | 1.84 (1.07, 3.16) | |
| Daily sun exposure | | | | 0.58 |
| <1 hour | 29.0 | 5.3 | 1.35 (0.74, 2.47) | |
| 1-3 hour | 38.7 | 4.0 | 1.00 | |
| >3 hour | 32.3 | 5.2 | 1.32 (0.67, 2.59) | |
| Use of electric heating system | | | | 0.67 |
| No | 65.6 | 4.4 | 1.00 | |
| Yes | 34.4 | 5.0 | 1.15 (0.57, 2.33) | |
| Use of gas furnace | | | | 0.64 |
| No | 47.5 | 4.8 | 1.00 | |
| Yes | 52.5 | 4.3 | 0.89 (0.51, 1.55) | |
| Weekly sedentary hours | | | | 0.02 |
| <= 19 | 45.7 | 3.7 | 1.00 | |
| 20-34 | 40.9 | 4.6 | 1.23 (0.52, 2.9) | |
| >35 | 3.4 | 8.6 | 2.43 (1.41, 4.21) | |

| | | | | |
|---|---|---|---|---|
| Consumption of milk (daily) | | | | 0.35 |
| Less than once | 43.1 | 4.8 | 1.14 (0.72, 1.8) | |
| At-least once | 48.2 | 4.2 | 1.00 | |
| More than twice | 8.7 | 7.5 | 1.86 (0.76, 4.58) | |
| Smoking status | | | | 0.54 |
| Never | 52.4 | 4.4 | 1.00 | |
| Current | 21.5 | 5.9 | 1.39 (0.76, 2.53) | |
| Former | 26.1 | 4.5 | 1.02 (0.53, 1.97) | |
| Liver or kidney diseases | | | | 0.39 |
| No | 95.6 | 4.6 | 1.00 | |
| Yes | 4.4 | 6.8 | 1.51 (0.54, 4.25) | |
| Race | | | | 0.19 |
| Caucasian | 81.4 | 4.3 | 1.00 | |
| Other | 18.6 | 6.6 | 1.58 (0.77, 3.25) | |
| Smoking inside home | | | | 0.38 |
| No | 84.4 | 4.5 | 1.00 | |
| Yes | 15.6 | 6.1 | 1.39 (0.62, 3.13) | |
| Seasonality | | | | 0.29 |
| May - Oct | 51.8 | 4.2 | 1.00 | |
| Nov - Apr | 48.2 | 5.3 | 1.27 (0.79, 2.05) | |
| Use of sunscreen | | | | 0.19 |
| Never | 30.3 | 4.3 | 1.00 | |
| Always/often | 31.5 | 5.2 | 1.23 (0.75, 2) | |
| Sometimes/rarely | 38.2 | 3.9 | 0.9 (0.38, 2.13) | |
| Region | | | | 0.29 |
| Ontario | 38.9 | 3.7 | 1.00 | |
| Quebec, New Brunswick | 30.5 | 5.0 | 1.39 (0.67, 2.87) | |
| British Columbia, Alberta | 30.5 | 5.7 | 1.59 (0.88, 2.86) | |

*p-value from univariate logistic regression of current asthma

**Table 3.3 Proportion of ever asthma by factors and odds ratio for the relationship of factors with ever asthma**

| Factor | Proportion of ever asthma (%) | Unadjusted OR (95% CI) | p-value* |
|---|---|---|---|
| Vitamin D categories | | | 0.28 |
| Low (≤ 49 nmol/L ) | 9.5 | 1.29 (0.93, 1.79) | |
| Moderate (50-74 nmol/L) | 7.5 | 1.00 | |
| High (≥ 75 nmol/L ) | 7.7 | 1.02 (0.7, 1.51) | |
| Sex | | | 0.04 |
| Female | 9.5 | 1.00 | |
| Male | 6.7 | 0.68 (0.47, 0.99) | |
| Body mass index | | | 0.25 |
| Normal or under weight | 7.6 | 1.00 | |
| Overweight | 6.6 | 0.85 (0.51, 1.44) | |
| Obese | 9.4 | 1.25 (0.84, 1.85) | |
| Family history of asthma | | | <0.001 |
| No | 5.8 | 1.00 | |
| Yes | 16.2 | 3.15 (2.1, 4.72) | |
| Household income | | | 0.004 |
| Low | 14.6 | 2.27 (1.24, 4.14) | |
| Middle | 7.0 | 1.00 | |
| High | 6.2 | 0.87 (0.55, 1.38) | |
| Attending school | | | 0.004 |
| No | 7.1 | 1.00 | |
| Yes | 12.2 | 1.82 (1.26, 2.65) | |
| Daily sun exposure | | | 0.48 |
| <1 hour | 8.4 | 1.17 (0.75, 1.82) | |
| 1-3 hour | 7.3 | 1.00 | |
| >3 hours | 8.8 | 1.22 (0.82, 1.83) | |
| Use of electric heating system | | | 0.37 |
| No | 7.4 | 1.00 | |
| Yes | 8.7 | 1.19 (0.78, 1.82) | |
| Use of gas furnace | | | 0.63 |
| No | 8.2 | 1.00 | |
| Yes | 7.6 | 0.92 (0.64 1.33) | |
| Weekly sedentary hours | | | 0.054 |
| <=19 | 6.8 | 1.00 | |
| 20-34 | 8.1 | 1.2 (0.74, 1.94) | |
| >35 | 12.4 | 1.93 (1.22, 3.04) | |

| | | | |
|---|---|---|---|
| Consumption of milk (daily) | | | 0.09 |
| Less than once | 8.1 | 1.1 (0.86, 1.41) | |
| At least once | 7.4 | 1.00 | |
| More than twice | 11.7 | 1.64 (0.95, 2.84) | |
| Smoking status | | | 0.19 |
| Never | 7.6 | 1.00 | |
| Current | 10.3 | 1.4 (0.95, 2.06) | |
| Former | 7.4 | 0.97 (0.64, 1.47) | |
| Liver or kidney disease | | | 0.49 |
| No | 8.0 | 1.00 | |
| Yes | 9.9 | 1.26 (0.57, 2.82) | |
| Race | | | 0.5 |
| Caucasian | 7.8 | 1.00 | |
| Other | 9.3 | 1.2 (0.67, 2.14) | |
| Smoking inside home | | | 0.48 |
| No | 7.9 | 1.00 | |
| Yes | 9.4 | 1.21 (0.66,, 2.23 | |
| Seasonality | | | 0.11 |
| May - Oct | 7.1 | 1.00 | |
| Nov - Apr | 9.2 | 1.33 (0.88, 2.02) | |
| Use of sunscreen | | | 0.9 |
| Never | 7.6 | 1.00 | |
| Always/often | 8.2 | 1.09 (0.72, 1.65) | |
| Sometimes | 7.9 | 1.04 (0.69, 1.57) | |
| Region | | | 0.52 |
| Ontario | 7.2 | 1.00 | |
| Quebec, New Brunswick | 8.1 | 1.13 (0.63, 2.01) | |
| British Columbia, Alberta | 9.3 | 1.32 (0.71, 2.46) | |

*p-value from univariate logistic regression of ever asthma

**Table 3.4 Distribution of sample characteristics and lung function by vitamin D categories**

| Factor | Overall | Vitamin D categories | | | |
|---|---|---|---|---|---|
| | | **Low** | **Moderate** | **High** | p-value |
| | | Mean (SD) | Mean (SD) | Mean (SD) | |
| Age (years) | 39.91 (14.01) | 38.70 (12.97) | 39.83 (13.63) | 41.58 (14.93) | 0.09 |
| Height (cm) | 168.82 (8.59) | 168.97 (8.5) | 168.72 (8.66) | 168.92 (8.48) | 0.91 |
| Weight (kg) | 76.32 (16.61) | 78.73 (18) | 76.96 (16.14) | 73.69 (15.17) | 0.001 |
| Percent predicted FVC (%) | 99.26 (11.27) | 98.22 (11.74) | 99.65 (11.02) | 99.87 (10.93) | 0.45 |
| Percent predicted $FEV_1$ (%) | 95.94 (12.19) | 95.32 (12.22) | 96.18 (12.03) | 96.25 (12.16) | 0.72 |
| Percent predicted $FEV_1$/FVC (%) | 96.54 (7.64) | 97.01 (7.12) | 96.37 (7.72) | 96.20 (8.0) | 0.28 |
| FVC (L) | 4.21 (0.95) | 4.17 (0.97) | 4.25 (0.97) | 4.23 (0.92) | 0.74 |
| $FEV_1$ (L) | 3.30 (0.77) | 3.28 (0.78) | 3.32 (0.78) | 3.28 (0.74) | 0.77 |
| $FEV_1$/FVC (%) | 78.33 (6.89) | 78.95 (6.55) | 78.21 (6.9) | 77.73 (7.12) | 0.01 |
| $FEF_{25\%-75\%}$ (L/s) | 3.05 (1.05) | 3.07 (1.06) | 3.08 (1.03) | 3.00 (1.04) | 0.36 |

* p-values are from analysis of variance for comparison between vitamin D categories

**Table 3.5 Results from the multiple logistic regression of current asthma and ever asthma**

| Factor | Current asthma | | Ever asthma | |
|---|---|---|---|---|
| | Odds Ratio (95% CI) | p-value | Odds Ratio (95% CI) | p-value |
| Vitamin D levels | | | | |
| ≤ 49 nmol/L | 1.54 (1.01, 2.36) | 0.047 | - | |
| 50-74 nmol/L | 1.00 | | - | |
| ≥ 75 nmol/L | 1.03 (0.6, 1.75) | 0.90 | - | |
| Age (1 year) | 0.99 (0.98, 1.00) | 0.082 | 0.99 (0.98, 1.00) | 0.034 |
| Family history of asthma | | | | |
| No | 1.00 | | - | |
| Yes | 3.63 (2.17, 6.07) | <0.001 | - | |
| Sex | | | | |
| Female | 1.00 | | 1.00 | |
| Male | 0.81 (0.56, 1.18) | 0.25 | 0.68 (0.47, 0.99) | 0.044 |
| Sedentary activities | | | | |
| ≤ 19 hours | 1.00 | | 1.00 | |
| 20-34 hours | 1.25 (0.54, 2.87) | 0.57 | 1.27 (0.80, 2.03) | 0.28 |
| ≥ 35 hours | 2.27 (1.27, 4.08) | 0.01 | 1.85 (1.21, 2.82) | 0.008 |
| With Vitamin D at 50-74 nmol/L | | | | |
| No Family history | - | | 1.00 | |
| Family History | - | | 2.49 (1.35, 4.60) | 0.008 |
| With family history of asthma | | | | |
| ≤ 49 nmol/L | - | | 2.11 (1.44, 3.21) | 0.002 |
| 50-74 nmol/L | - | | 1.00 | |
| ≥ 75 nmol/L | - | | 0.80 (0.41, 1.55) | 0.024 |
| Without family history of asthma | | | | |
| ≤ 49 nmol/L | - | | 0.85 (0.54, 1.35) | 0.45 |
| 50-74 nmol/L | - | | 1.00 | |
| ≥ 75 nmol/L | - | | 1.20 (0.68, 2.12) | 0.50 |

**Table 3.6 Relationship between ever asthma and vitamin D categories for early and late onset of asthma.**

| Factor | Age of onset <=20 years | | Age of onset >20 years | |
|---|---|---|---|---|
| | Odds Ratio (95% CI) | p-value | Odds Ratio (95% CI) | p-value |
| Vitamin D | | | | |
| ≤ 49 nmol/L | - | | 1.04 (0.47, 2.28) | 0.9 |
| 50-74 nmol/L | - | | 1.00 | |
| ≥ 75 nmol/L | - | | 0.72 (0.32, 1.65) | 0.4 |
| Age (years) | 0.96 (0.94, 0.97) | <0.001 | 1.03 (1.02, 1.05) | 0.001 |
| Sex | | | | |
| Female | 1.00 | | 1.00 | |
| Male | 0.71 (0.44, 1.13) | 0.13 | 0.7 (0.36, 1.37) | 0.26 |
| Family history of asthma | | | | |
| No | - | | 1.00 | |
| Yes | - | | 2.11 (0.83, 5.37) | 0.10 |
| With vitamin D at 50-74 nmol/L | | | | |
| No family history | 1.00 | | - | |
| Family history | 3.20 (1.49, 6.86) | 0.006 | - | |
| With family history of asthma | | | | |
| ≤ 49 nmol/L | 2.85 (1.35, 6.00) | 0.01 | - | |
| 50-74 nmol/L | 1.00 | | - | |
| ≥ 75 nmol/L | 0.78 (0.42, 1.44) | 0.40 | - | |
| Without family history of asthma | | | | |
| ≤ 49 nmol/L | 0.90 (0.40, 2.01) | 0.70 | - | |
| 50-74 nmol/L | 1.00 | | - | |
| ≥ 75 nmol/L | 1.74 (0.88, 3.46) | 0.10 | - | |

**Table 3.7. Results from the multiple linear regression analysis of lung function outcomes**

| Factor | FVC (ml) | $FEV_1$ (ml) | $FEV_1$/FVC (%) | $FEF_{25\%-75\%}$ (ml) |
|---|---|---|---|---|
| | β (SE)† | β (SE)† | β (SE)† | β (SE)† |
| Vitamin D (ref - moderate) | | | | |
| ≤ 49 nmol/L | -86.06 (52.45) | -60.94 (37.59) | -0.3 (0.57) | -71.75 (72.6) |
| ≥ 75 nmol/L | 6.59 (30.51) | 12.41 (18.37) | -0.95 (0.35)‡ | 20.81 (38.17) |
| Age* | | | | |
| Linear | 22.23 (3.29)¶ | 7.08 (3.02)‡ | -0.12 (0.03)§ | -5.02 (4.64) |
| Non-linear | -51.57 (3.61)¶ | -37.82 (3.22)¶ | -0.13 (0.05)‡ | -38.48 (4.97)¶ |
| Sex | | | | |
| Male (ref - female) | 574.32 (34.25)¶ | 459.82 (29.64)¶ | 0.08 (0.41) | 427.24 (79.2)¶ |
| Height* | 63.2 (1.37)¶ | 42.15 (1.01)¶ | -0.14 (0.02)¶ | 21.32 (3.08)¶ |
| Weight* | -2.39 (1.01)‡ | -0.26 (0.69) | 0.03 (0.01§ | 4.88 (1.42)§ |
| Smoking status (ref - Never) | | | | |
| Current | - | -154.45 (37.2)§ | -4.25 (0.41)¶ | -426.26 (49.04)¶ |
| Former | - | -40.56 (29.68) | -1.1 (0.38)‡ | -140.69 (61.32)‡ |
| Race | | | | |
| Others (ref - Caucasian) | -385.25 (64.09)¶ | -219.31 (49.47)¶ | 1.99 (0.48)§ | - |
| Vitamin D and Age interaction | | | | |
| ≤ 49 nmol/L and linear | - | - | -0.11 (0.07) | - |
| ≥ 75 nmol/L and linear | - | - | -0.23 (0.05)¶ | - |
| ≤ 49 nmol/L and non-linear | - | - | 0.15 (0.1) | - |
| ≥ 75 nmol/L and non-linear | - | - | 0.3 (0.06)¶ | - |

| | | | | |
|---|---|---|---|---|
| Intercept | 4187.41 (29.78)¶ | 3284.94 (16)¶ | 79.45 (0.44)¶ | 3110.6 (61.28)¶ |
| Adj. R-squared (%) | 74.1 | 70.9 | 27.8 | 40.1 |

* Variables were centralized

†β (SE) - Regression coefficient (Standard error)

‡p-value ≤ 0.05

§p-value ≤ 0.01

¶p-value ≤ 0.001

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# Chapter 4 Discussion and Conclusions

## 4.1 Summary of the findings

Asthma and wheezing / current asthma poses a significant burden in Canada (1). The primary objective of this study was to investigate a possible link between vitamin D and asthma using data from the Canadian population. Over the last two decades, several observational studies have investigated this link. Most of the studies published to date have supported a protective effect of vitamin D on asthma (2–5), but a few studies have suggested vitamin D was a risk factor for asthma (6,7). Studies have used different cut-off points of vitamin D levels to assess the relationship with asthma outcomes and often report only a linear relationship.

To date there have been only a few studies of vitamin D and asthma conducted in a Canadian population. One of the objectives of this thesis was to determine the pattern of the relationship between vitamin D and asthma, and so to establish the range of vitamin D levels at which the adverse risk of asthma is minimized and lung function is optimal. This question was addressed in children in Chapter 2. Regardless of the presence of asthma, a significant proportion of children in this study had lower levels of vitamin D, indicating that vitamin D deficiency is a substantial problem in Canadian children. Serum 25(OH)D level was associated with current respiratory health, with both low and high serum levels posing an increased risk of current wheeze, lower lung function, and smaller increases in lung function with age compared to the moderate category,

supporting a U-shaped association. Serum 25(OH)D levels ≤49 nmol/L were associated with increased risk of ever asthma, but no such association could be demonstrated for levels ≥ 75 nmol/L. Although some researchers have concluded that vitamin D levels between 50 and 75nmol/L are insufficient, these data supports that the optimal range with respect to respiratory health lies within this range. In these data, less than one hour of sun exposure was associated with an increased risk of ever asthma, independent of serum vitamin D status. It appears that decreased sun exposure, a modifiable behavioral factor, may be related to some other factor such as physical activity and could be an influential factor in the development of ever asthma.

In Chapter 3, an attempt was made to identify determinants affecting the relationship between asthma and vitamin D in adolescents and adults. The hypothesis examined in this chapter was that vitamin D deficient was associated with asthma in adults prone to develop current and ever asthma. In addition, the relationship between vitamin D and ever asthma was investigated among subjects with early-onset and late onset asthma phenotypes. Lower levels of vitamin D were observed in a significant proportion of adolescents and adults. Although not significant, lower serum 25(OH)D levels were observed in those with current or ever asthma, and people with serum 25(OH)D between 50 and 75 nmol/L level had the highest mean lung function. Individuals with serum levels ≤49 nmol/L had 185% increased risk of ever asthma in those with family history of asthma and 50% increased risk of current asthma. The association between ever asthma and vitamin D was only evident in those who developed asthma before 20 years

of age. Serum 25(OH)D levels ≥ 75 nmol/L were associated with lower mean and higher rate of decrease of $FEV_1$/FVC with increasing age. In these data, a positive correlation between weekly sedentary hours and current asthma was observed. It provides some evidence against the argument that physical activity increases the risk of asthma by increasing exposure to asthma triggers.

## 4.2 Discussion

This thesis has identified a number of modifiable determinants associated with respiratory outcomes, including sun exposure, use of electric heating systems, and vitamin D status in children and sedentary lifestyle, weight, and vitamin D status in adults. There were non-modifiable factors such as male sex, family history of asthma, associated with increased risk of asthma, which can help to increase the understanding.

A case-control study of urban African American children aged 6-20 years from Washington showed serum vitamin D levels were significantly lower in those with asthma and serum 25(OH)D levels ≤50 and 50-75 nmol/L were associated with asthma (8). Another case-control study on children (≤16 years) from Qatar also showed that asthmatic children had lower serum vitamin D levels, exposure to sunlight and physical activity compared to controls (9). Likewise, low vitamin D levels were associated with lower lung function and asthmatic state in Iranian children aged 6-18 years (10). In a study included children with moderate and steroid resistant asthma (STRA), very low serum 25(OH)D levels were associated with STRA, reduced lung function, increased corticosteroid use and

asthma exacerbations (11). In children's data, the finding that low vitamin D levels were associated with an increased predisposition to ever asthma and current wheeze was consistent with the above studies. On the contrary, while the above studies suggest that serum levels ≥75 nmol/L may protect against asthma, this data suggest serum levels ≥75 nmol/L may also pose significant adverse effect.

In a cross-sectional study conducted on adults aged 20 and above years using NHANES data between 1988-1994, the highest quintile of serum vitamin D level (≥85 nmol/L) had significantly higher $FEV_1$ and FVC compared to the lowest quintile (≤40 nmol/L) (4). Similarly, another cross-sectional study conducted on 6,857 subjects of age 6 and above years from NHANES data of 2005-2006, serum vitamin D levels were inversely associated with current wheeze, and ever asthma (2). In another small cross-sectional study of asthmatic adults (18-60 years), reduced vitamin D levels were associated with impaired lung function, increased airway hyper-responsiveness, and reduced glucocorticoid response (3). However, another study did not find a significant difference in serum 25(OH)D levels or a correlation with asthma severity in 80 asthmatic adults and 80 controls arising from two separate cities in Europe (12). Goleva *et al.*, in a case-control study of both pediatric and adult patients with asthma reported no difference in serum 25(OH)D levels, but vitamin D level had inverse correlation with serum IgE levels, and daily inhaled corticosteroid dose in only the pediatric asthma group, suggesting an age-specific effects of vitamin D in asthma (13).

In an interesting study, Luxwolda *et al.,* looked at serum 25(OH)D levels in two traditionally living adult populations in East Africa with lifelong exposure

to abundant tropical sunlight. In these populations of traditional tribes living in the cradle of mankind, who might be comparable to our African ancestors before the out-of-Africa diaspora, mean serum 25(OH)D concentration was 115 nmol/L(SD: 26). In this study, none had serum 25(OH)D levels below 50 nmol/L, and more than 85% of the sample had serum 25(OH)D greater than 80 nmol/L. However, the results from this study may not be generalized to the current western society since many other factors related with vitamin D homeostasis have changed during the human evolution.(14).

In conclusion, consistent with other studies, this study found that lower serum vitamin D levels were associated with an increased predisposition to asthma and asthma attacks in both children and adults. Serum levels ≥75 nmol/L imposed significant adverse effect on respiratory outcomes in children while such effect was not identified in adolescents and adults. It suggests that the action of vitamin D with respect to respiratory outcomes is somewhat different between children and adults. This could be partially due to a developing immune and respiratory systems and differential sensitivity of vitamin D receptor found between children and adults (15). Asthma in children is almost always allergic in origin (16), whereas in adults other mechanisms also seem important. Current data support the claim that guidelines regarding optimal vitamin D level should consider this difference between children and adolescents/adults.

## 4.3 Limitations

The main limitations of this study were use of self-reported outcomes, missing data for outcomes and vitamin D measurements, possibility of residual confounding and t relatively high overall high non-response rate.

Serum 25(OH)D has a half-life of 10-25 days. Serum levels of 25(OH)D are the best available marker of total vitamin D synthesized from sun exposure and dietary intake occurring in the preceding 2-3 weeks (17). The variability of serum concentration of vitamin D is high, likely due to its association with many lifestyle and nutritional factors in a given period of time. As a result, a single determination of vitamin D may be imprecise and provide an inadequate picture of the association in any cross-sectional studies.

Although a large number of potential confounders were considered in this study several confounders including non-nutritional factors (clothing, geographic latitude, institutionalization, etc.) and physiological factors (concentration of vitamin D binding protein) were not considered (18). Due to this, it is not possible to rule out that the associations observed in this study resulted from confounding.

The main outcomes used in the study, ever asthma, current asthma in adults, and current wheeze in children, were self-reported. The presence of ever asthma, and asthma attack in the past 12 months were derived from household questionnaires and used as outcomes in Chapter 3. The use of self-reported asthma and asthma attack, in-lieu of physician confirmed asthma, have been assessed and accepted as valid and reliable measures (19–21).

The data from CHMS were collected using a multi-stage sampling design to obtain data from a representative sample of Canadians. The overall response rate was 51.7% for the Canadian Health Measures Survey. Although sampling weights were adjusted to compensate for the various levels of non-response, estimates could nonetheless be biased if respondents' characteristics differed significantly from non-respondents.

The strength of associations reported in this study may have been under- or overestimated due to missing data. There was over 10% data missing or incomplete with regard to lung function parameters in children. In adults, missing data on outcomes varied between 3-5%. The reasons the data were not included were: exclusion for safety reasons; spirometric testing of insufficient quality; and spirometric testing not performed. With regard to vitamin D measurements and other covariates included in the models, there was over 15% missing data in children and approximately 3% in adults. It is unclear how the missing data might have affected the effect and strength of associations.

There are several assays available to determine serum concentration of vitamin D. Among them, liquid chromatography-tandem mass spectrometry (LCMS) is considered as gold-standard reference method. The performance of some commercially available assays are somewhat less good in their ability to estimate 25(OH)D due to differences in the affinity between vitamin D binding antibodies or D-binding proteins employed. In CHMS, vitamin D levels were determined using the Diasorin Liaison chemiluminescent assay. This assay was

found to recognise $25(OH)D_2$ more than $25(OH)D_3$ (22). However, it has been shown that this assay has a good agreement with LCMS measurements.

The CHMS was designed to produce national estimates only. Due to logistical reasons, sampling was confined to 15 sites within four major provinces (British Columbia, Alberta, Ontario, and Quebec). As a result of this design, the degrees of freedom available to any analysis using CHMS data was 11 (23). This constraint limited the number of variables that could be included in the model. Notably, it limited the ability to examine interactions and confounding effects.

Mean serum vitamin D levels, and prevalence of asthma vary in different ethnic groups. The sample from CHMS had an ethnicity mix similar to what has been reported in the Canadian census. Over 80% were Caucasians, but other groups were represented. However, the sample size was not large enough to conduct analyses stratified by race, in particular, on those who are known to have lower vitamin D levels.

## 4.4 Conclusions

Most Canadian cities are located north of 43° N latitude (24). During late fall to winter months, vitamin D synthesis from sun exposure is compromised (24,25). According to Webb *et al.,* the Canadian diet provides about 5 mcg (200 IU) of vitamin D which is not adequate to maintain an optimal serum levels (26–28). As a result, vitamin D levels are likely to be too low in many Canadians. In this study, serum vitamin D levels of 50-74 nmol/L attained optimal respiratory outcomes. To achieve adequate vitamin D levels in sunlight-deprived seasons, a

number of viable options are available. The first option to increase vitamin D levels is through supplements. An intake of 1000 IU/day of a commercially distributed vitamin D supplement for a specified period might raise serum level by 15-25 nmol/L (24,29,30). However, there have been reports suggesting that there is considerable variability in responses to supplementation, and bioavailability in different population groups (18). A second option is fortifying food with vitamin D. Food items such as milk, orange juice, cereal have been fortified (18,30,31). In Canada, only two food staples, milk and margarine are fortified (32). After World War II, due to massive fortification of food items, an epidemic of hypercalcemia occurred in some European countries, which eventually lead to banning of fortification (31,33). Considering that, there would need to be a clear policy regulating fortification practices such as mandating nutrition labels provide information about vitamin D content, so as to try to prevent a recurrence of this. A third option is the use of artificial solar UVB which can be used to synthesize vitamin D. Artificial solar UVB exposure has been shown to be more efficient in increasing serum vitamin D level than solar UVB exposure, however, it is associated with a cost (34).

From a study conducted on asthmatic adults, increasing vitamin D levels were associated with greater lung function, reduced airway hyper-responsiveness, and greater glucocorticoid sensitivity, and the authors recommended monitoring & supplementation of vitamin D for those who were sub-optimally responding to inhaled corticosteroids (3). Intoxication secondary to an excess intake of vitamin D is an extremely rare condition (35). It is also not possible to develop vitamin D

intoxication from excess exposure to sunlight (25). Given these facts and the finding that vitamin D deficiency is associated with an increased risk of asthma and asthma exacerbations, increasing serum levels in asthmatic adolescents and adults is unlikely to result in harm to the population. Supplementation and/or fortification, coupled with sensible sunlight exposure, could result in adequate vitamin D levels. The associated cost, to fortify food with vitamin D or to increase supplementation, would likely be cheaper than the cost of drug treatments for the many chronic diseases associated with vitamin D status (32).

There are considerable deficiencies in the knowledge and understanding of the role of vitamin D on optimal respiratory health. The longitudinal relationship between serum 25(OH)D and respiratory outcomes and how they vary between children and adults over time is still unknown. The optimal level of vitamin D with respect to both skeletal and non-skeletal outcomes needs to be ascertained. Since race is an important predictor of serum vitamin D level, racial differences in outcomes and safety of vitamin D interventions also need to be investigated. Other than association between low vitamin D levels and risk of falls, no data from randomized control trials exist. The use of vitamin D as an add-on for asthma management needs to be assessed further, possibly using randomized trials.

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# Approval

| | |
|---|---|
| Date: | January 7, 2014 |
| Study ID: | Pro00045399 |
| Principal Investigator: | Ambikaipakan Senthilselvan |
| Study Title: | Association of Vitamin D and Respiratory Outcomes in Canadian Children, Adolescents and Adults |
| Approval Expiry Date: | January 6, 2015 |

Thank you for submitting the above study to the Health Research Ethics Board - Health Panel. Your application has been reviewed and approved on behalf of the committee.

This project involves the secondary analysis of anonymous StatsCan data.

A renewal report must be submitted next year prior to the expiry of this approval if your study still requires ethics approval. If you do not renew on or before the renewal expiry date, you will have to re-submit an ethics application.

Approval by the Health Research Ethics Board does not encompass authorization to access the patients, staff or resources of Alberta Health Services or other local health care institutions for the purposes of the research. Enquiries regarding Alberta Health Services approvals should be directed to (780) 407-6041. Enquiries regarding Covenant Health should be directed to (780) 735-2274.

Sincerely,

Anthony S. Joyce, Ph.D.
Chair, Health Research Ethics Board - Health Panel

*Note: This correspondence includes an electronic signature (validation and approval via an online system).*