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Vitamin D status in a Norwegian population: any link to lung function?

Thesis for the degree of Philosophiae Doctor

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This doctoral dissertation in medicine is dedicated to my Family

and defended

in loving memory of

Uncle Larry Larose

08 February 1946 – 10 November 2012

Little Cousin Ryley Regent Larose

31 August 2000 – 05 December 2012

Grandmother Lois Marilyn Satherstrom (née Cornthwaite)

10 October 1932 – 09 September 2013

Bonus-Pappa Louis Bélanger

29 July 1956 – 10 May 2014

may you rest in peace

Vitamin D-status i en norsk befolkning: assosiert med lungefunksjon?

Vitamin D syntetiseres i huden ved direkte UVB-eksponering fra solen. Vitamin D kan også tilføres kroppen ved inntak av vitamin-D-rik mat, eller ved bruk av kosttilskudd som inneholder vitamin D. Lav vitamin-D-status har blitt rapportert på verdensbasis, og kjente risikofaktorer inkluderer ueksponert hud, høye breddegrader, vintersesong og høy kroppsmasseindeks (BMI). Serum 25-hydroxyvitamin D (25(OH)D) er den viktigste sirkulerende metabolitt av vitamin D og regnes som den beste markøren for vitamin D-status. Sammenhengen mellom vitamin D-status og beinhelse er godt dokumentert, og økende bevis tyder på en sammenheng mellom lavt serum 25(OH)D nivå og et spekter av tilstander som ikke er knyttet til skjelettet, inkludert luftveislidelser som astma.

Denne avhandlingen bruker data fra Helseundersøkelsene i Nord-Trøndelag, en av de største befolkningsbaserte helseundersøkelsene i Norge, hvor klinisk-, livsstils- og miljøinformasjon for flere enn 130,000 mennesker har blitt samlet inn over en 25 års periode. Ved hjelp av data fra HUNT2 (1995-1997) og HUNT3 (2006-2008), undersøkte vi: 1) forekomsten av vitamin-D-mangel (serum 25(OH)D<50nmol/L) i en generell voksen befolkning og faktorer forbundet med vitamin-D-mangel; 2) sammenhengen mellom serum 25(OH)D nivå og lungefunksjon (LF) hos voksne med astma; og 3) sammenhengen mellom serum 25(OH)D nivå, røyking og LF endringer i en generell voksen befolkning.

Vi fant høy forekomst av vitamin-D-mangel i denne norske befolkning (40% totalt), spesielt i vintermånedene (64 %). Foruten om sesong og høy BMI, ble flere potensielt påvirkningsmulige livsstilsfaktorer forbundet med vitamin-D-mangel. Vi fant også at lavt serum 25(OH)D nivå ikke var assosiert med luftveisobstruksjon hos de

fleste voksne med astma, med unntak av menn med astma, men uten allergi. Vi fant t ingen klare assosiasjoner mellom serum 25 (OH) D-nivåer og lungefunksjonsendringer hos ikke-røykere, mens vi derimot observerte betydningsfulle sammenhenger hos røykere.

Ytterligere forskning er nødvendig for å avgjøre om vitamin-D-tilskudd kan være en kostnadseffektiv, sikker og enkel måte å kontrollere astmaplager på eller for å lindre symptomer hos røykere.

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Summary

Vitamin D is predominantly synthesized from precursors in the skin after direct exposure to ultraviolet B radiation from the sun. Vitamin D can also be obtained by dietary intake of vitamin D rich food, or by supplement intake. Poor vitamin D status has been reported worldwide. Determinants include unexposed skin, high latitude, winter season, and high body mass index (BMI). Serum 25-hydroxyvitamin D (25(OH)D) is the major circulating metabolite of vitamin D and considered the best marker of vitamin D status. The association between vitamin D status and bone health is well studied, and increasing evidence suggests an association between low serum 25(OH)D level and range of non-skeletal health outcomes including respiratory disorders, such as asthma.

This dissertation uses data from the Nord-Trøndelag Health Study (HUNT), one of the largest population based health studies in Norway, with clinical, lifestyle and environmental information on more than 130,000 people collected over 25 years. Using data from HUNT2 (1995-1997) and HUNT3 (2006-2008), we investigated: 1) the prevalence of vitamin D deficiency (serum 25(OH)D<50nmol/L) in a general adult population and factors associated with vitamin D deficiency; 2) the association between serum 25(OH)D level and lung function (LF) in adults with asthma; and 3) the interrelationship between serum 25(OH)D level, smoking and LF changes in a general adult population.

We found a high prevalence of vitamin D deficiency in this Norwegian population (40% overall), particularly in winter months (64%). Besides season and high BMI, several potentially modifiable lifestyle factors were associated with vitamin D

deficiency. We also found that low serum 25(OH)D level was not associated with airway obstruction in most adults with asthma, with the exception of men with asthma but without allergy status. Finally, we found no clear associations between serum 25(OH)D levels and LF changes in never smokers, whereas we did observe significant associations in ever smokers.

Replication and confirmation of these findings via well-designed prospective and intervention studies is required to determine if vitamin D supplementation is a potentially cost effective, safe and straightforward means of asthma control or disease mitigation in ever smokers.

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I used data from the second and third surveys of the Nord-Trøndelag Health (HUNT) Study for three original scientific works included in this doctoral dissertation. The HUNT Study is a collaboration between the HUNT Research Centre (Faculty of Medicine, NTNU), the Nord-Trøndelag County Council, and the Norwegian Institute of Public Health. I especially thank the individual HUNT participants and HUNT laboratory personnel for the measurement of serum 25(OH)D levels.

Paper I is reproduced with permission from the BMJ Publishing Group Ltd: Larose TL, Chen Y, Camargo CA, Langhammer A, Romundstad P and Mai XM. Factors associated with vitamin D deficiency in a Norwegian population: the HUNT Study. *J Epidemiol Community Health* 2014;68: 165-170. Paper II is reproduced with permission of the European Respiratory Society © Eur Respir J: Larose TL, Langhammer A, Chen Y, Camargo CA, Romundstad P and Mai XM. Serum 25-

hydroxyvitamin D levels and lung function in adults with asthma: the HUNT Study. 2014. Advance online publication. doi: 10.1183/09031936.00069714.

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Finally, to my family and friends, thank you for believing in me. I am grateful to you every day for reasons both big and small.

List of papers

This doctoral dissertation consists of the following three original works. I will refer to each paper in text by their roman numerals.

I. Larose TL, Chen Y, Camargo CA, Langhammer A, Romundstad P and Mai XM. **Factors associated with vitamin D deficiency in a Norwegian population: the HUNT Study.** *J Epidemiol Community Health* 2014;**68**: 165-170.

II. Larose TL, Langhammer A, Chen Y, Camargo CA, Romundstad P and Mai XM. **Serum 25-hydroxyvitamin D levels and lung function in adults with asthma: the HUNT Study.** *Eur Respir J* 2014. Advance online publication. doi: 10.1183/09031936.00069714

III. Larose TL, Brumpton BM, Langhammer A, Camargo CA, Chen Y, Romundstad P and Mai XM. **Serum 25-hydroxyvitamin D level, smoking and lung function in adults: the HUNT Study.** 2014. Manuscript submitted for publication and under second review.

List of abbreviations

25(OH)D	25-hydroxyvitamin D
AHR	airway hyper-responsiveness
ASM	airway smooth muscle
BMI	body mass index
CI	confidence interval
COPD	chronic obstructive pulmonary disease
D3	vitamin D3
D2	vitamin D2
FEV_1	forced expiratory volume in 1 second
FEV_1 % pred.	forced expiratory volume in 1 second percent predicted
FVC	forced vital capacity
FVC % pred.	forced vital capacity percent predicted
HUNT	Nord-Trøndelag Health Study
HUNT2	Second Nord-Trøndelag Health Study, 1995-1997
HUNT3	Third Nord-Trøndelag Health Study, 2006-2008
NHANES	National Health and Nutrition Examination Survey

ICS	inhaled corticosteroids
IgE	immunoglobulin E
KHANES	Korean National Health and Nutrition Examination Survey
LF	lung function
LHS	Lung Health Study
MICE	multivariate imputations by chained equations
OR	odds ratio
PEFR	peak expiratory flow rate
PR	prevalence ratio
PTH	parathyroid hormone
SES	socio-economic status
SZA	solar zenith angle
T_H	T helper cell
UK	United Kingdom
US	United States

1 Introduction

1.1 Vitamin D status and serum 25-hydroxyvitamin D

Vitamin D is predominantly synthesized from precursors in the skin after direct exposure to ultraviolet B (UV-B) radiation from the sun[1]. UVB radiation from the sun activates 7-dehydrocholesterol (7-DHC) in the epidermal and dermal layers of the skin to form previtamin D_3 which is then transformed to vitamin D_3 (VD3) via thermally induced isomerization[2]. To a lesser extent, vitamin D can also be obtained by dietary intake from plants (ergocalciferol, vitamin D_2 (VD2)) or oily fish (cholecalciferol, VD3)[3]. Whether activated in the skin after UVB exposure from the sun, or obtained from food or supplements, VD is transported by the vitamin-D binding protein to the liver where it is hydroxylated to form 25-hydroxyvitamin D (25(OH)D). Serum 25(OH)D is the major circulating metabolite of vitamin D. Next, 25(OH)D circulates to the kidney and undergoes a second hydroxylation to give the biologically active form 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D$)[2]. The half-life of serum 25(OH)D is approximately 3 weeks, whereas the half-life of $1,25(OH)_2D$ is approximately four hours[4]. Serum or plasma 25(OH)D levels give the best single assessment of total body vitamin D status derived from the combination of cutaneous synthesis of VD3, dietary intake, and supplement use[4-7]. The papers included in this dissertation (Papers I-III) use serum 25(OH)D measurements to determine body vitamin D status (Methods Section 3.5).

Vitamin D status is a topic of increasing clinical relevance and public health importance due to the resurgence of nutritional rickets in Europe and North America[8-10], the high prevalence of hypovitaminosis D worldwide[11-15], and due to the

possible effect of vitamin D status on a number of chronic diseases[16]. The actions of vitamin D extend beyond bone health and mineral homeostasis[17] to include non-classical molecular mechanisms that may play an important role in a range of diseases and non-skeletal health outcomes including cancer[18], auto-immune disease[19], cardiovascular disease[20], all-cause mortality[21], diabetes[22], and respiratory disorders[23]. While it is widely accepted that a serum 25(OH)D level greater than or equal to 30nmol/L[24] is required for good bone health and the prevention of rickets, osteoporosis and osteomalacia[24-26], the minimum level of serum 25(OH)D needed for normal immune function[24, 27] or non-skeletal health outcomes such as asthma or allergy[28], has not yet been determined. Comparisons on the prevalence of vitamin D deficiency are difficult due to the lack of general consensus regarding cut-points used to determine a deficient level, and the inconsistent use of terminology to define these cut-points. For example, a serum 25(OH)D level between 30-50nmol/L has been defined as "inadequate" [24], and levels below 50nmol/L have been defined as "deficient"[27], or "insufficient"[29]. Widely recommended cut-points to define vitamin D deficiency and insufficiency are serum 25(OH)D levels <50nmol/L, and <75nmo/L, respectively[17]. The cut-points used in this dissertation (Papers I-III) are <25nmol/L, <50nmol/L, <75nmol/L and ≥75nmol/L, where <50nmol/L was considered deficient (Methods section 3.5).

1.2 Factors associated with vitamin D status

Low vitamin D status is prevalent world-wide[30], including sun-abundant countries[31-36] where urban living[31], gender[32, 33] ethnicity[34], and protective clothing associated with religious practice[35] or sun avoidance[36] are prominent.

Other determinants of vitamin D status include an individual's location (latitude and altitude) and the duration and timing of outdoor activities dependent on season and time of day [37, 38]. The amount of UVB transmission capable of reaching the Earth's surface is dependent on radiation path-length which is determined by solar zenith angle (SZA) [1, 38]. The SZA is a function of the Earth's motion around the Sun on it's own axis, in combination with the Sun's position on the Earth's surface, such that small SZAs occur during summer, at noon and at low latitudes, whereas large SZAs occur in winter, early morning/late afternoon and at high latitudes[1, 38]. At small SZAs the sun is high in the sky and solar radiation has a short path-length resulting in less chance of attenuation and greater opportunity for UVB radiation to reach the Earth's surface[1]. At high latitudes, UVB radiation capable of cutaneous synthesis of vitamin D occurs during summer months at solar noon. The atmosphere (which decreases at high altitude), cloud cover, aerosol particles (related to urbanization and pollution), and ozone absorb UVB radiation which decreases the amount of available UVB for cutaneous synthesis of vitamin D[1, 38]. Another efficient absorber of UVB radiation is melanin content, which also determines human skin pigmentation[39]. Persons with increased melanin content (darker skin) require longer exposure to UVB radiation from the sun in order to synthesize the same amount of cutaneous vitamin D compared to persons with lighter skin[39]. Consequently, immigration from sunny countries to new areas of higher latitude has increased public health concern regarding low vitamin D status of immigrant populations in some northern European countries, like Denmark[40] and Norway[41]. For example, the mean serum 25(OH)D level in Pakistani immigrants living in Oslo was 25.0±14 nmol/L compared to a mean serum 25(OH)D level of 74.8±23.7 found in ethnic Norwegians[42]. In this study, the prevalence of serum

25(OH)D level <50nmoL was four times higher in Pakistani immigrants compared to ethnic Norwegians[42]. Diet and supplement use are particularly important when cutaneous synthesis of D3 is hampered or not possible[43-46]. Dietary sources of D3 include oily fish and fish liver oil, egg yolk, shitake mushrooms, offal – such as liver, nutritional supplements, and fortified products such as dairy, juice, bread, cereal or infant formula[47-49]. In Norway only extra light milk and margarine are fortified with vitamin D[50], and a change away from more traditional dietary practices such as regular consumption of oily fish or regular intake of cod liver oil may be cause for concern[43, 51].

Due to the fat soluble nature of 25(OH)D, body fat or high body mass index (BMI) is considered another factor associated with serum 25(OH)D level[52-54]. However, the direction of the association between serum 25(OH)D level and BMI is still debated in the literature[55], and this will be further discussed in relation to findings from Paper I. Other behavioral factors related to vitamin D status include a sedentary lifestyle[56] and physical activity as either a proxy for leisure time spent outdoors[57], or as a factor independent of sun exposure[53]. Some studies have shown a positive association between smoking and vitamin D deficiency[58, 59] while others have found no association between smoking and vitamin D status[53, 60]. Finally, alcohol consumption has not been extensively studied and may also determine serum 25(OH)D level[61].

A recent systematic review of literature on vitamin D status in the Norwegian population based on 30 years of prior available data[13] summarized ten studies that gave direct estimates on the prevalence of low vitamin D status, most of which were

conducted in infants, children, the elderly, or non-ethnic Norwegians. Only one study from northern Norway provided a direct estimate for the prevalence of low vitamin D status in a general adult population[62]. However, this study was restricted to women aged 44 to 59 years, and the plasma 25(OH)D variable was dichotomized as: <37.5nmo/L vs ≥37.5nmol/L, which greatly reduces comparability of findings. Noticeably missing from the literature is a well-designed prevalence study in a general population of Norwegian adults (men and women).

Beyond filling a gap in the literature, an accurate estimate of the prevalence of vitamin D deficiency in a Norwegian general population is important for several reasons. First, the majority of Norwegians reside at high latitudes where UVB light capable of activating the cutaneous synthesis of vitamin D is absent for a large part of the year. Also, there are very few food products fortified with vitamin D in Norway, and a change away from more traditional diet seems to be underway in certain areas of the country[51]. Finally, increasing evidence suggests an association between vitamin D status and various health outcomes, including respiratory diseases such as asthma.

1.3 Asthma and allergies

Asthma is a heterogeneous disease with different underlying processes that is usually associated with airway hyper-responsiveness (AHR), and further characterized by chronic airway inflammation[63]. As a common chronic disease of the lungs, asthma is characterized by a history of respiratory symptoms which include recurrent attacks of wheezing, shortness of breath, tightness of the chest and cough, together with variable expiratory airflow limitation[63, 64]. An estimated 300 million people suffer from asthma worldwide[65], and an estimated 346,000 asthma-related deaths occur each

year[66], although most asthma-related deaths occur in low- and lower-middle income countries[64]. The prevalence of asthma is over 10% in Norwegian children and approximately 8% in Norwegian adults[67]. The socioeconomic burden of asthma is high including indirect costs, such as absenteeism or reduced performance at work, and direct medical costs associated with health services and medication use[68]. In 2011, asthma medicine dominated the list of medicines with the highest sales in Norway, topped only by medicines for rheumatoid arthritis[69].The daily cost for consumption of asthma medication in Norway was 3-16 NOK per patient in 2004[69]. Thus, asthma is not only a significant public health concern, but also a socioeconomic burden.

Several asthma phenotypes have been identified including allergic asthma and non-allergic asthma[70, 71]. Allergic asthma is considered the most commonly identified asthma phenotype in children and can readily be associated with a history of allergic disease such as food or pollen allergy, or allergic dermitis[63, 71]. More than 50% of asthma in adultsis also allergy-related. Meanwhile, 10-40% of adult patients with allergic rhinitis are estimated to have asthma[72]. Rhinitis is defined as irritation and inflammation of the mucous membrane of the nose[63]. Most patients with allergic asthma respond well to inhaled corticosteroid (ICS) treatment, whereas patients with non-allergic asthma seem to respond less well to ICS treatment[71]. Risk factors for the development or severity of asthma may include a range of host and environmental factors such as genetic predisposition, obesity, sex, allergens, infections, exposure to tobacco smoke, air pollution, diet, and stress[73]. To be noted, most knowledge on risk factors for asthma is based on studies from children, whilst risk factors for asthma in adults are less well known[63]. Suggested reasons for worldwide increases in asthma

and allergy include increased industrialization in developing nations and greater urbanization which contribute to increased air pollution and more time spent indoors. Consequently, vitamin D status might also play a role in the development of asthma and allergies.

1.4 Vitamin D in relation to asthma and allergy

Due to its regulatory effect on the immune system, vitamin D may play an important role in the development and treatment of asthma and allergies[28]. To this end, several potential mechanisms on vitamin D and the risk of asthma and allergy have been suggested. In murine and humans models, vitamin D has been shown to enhance the generation of regulatory T cells[74, 75]. Regulatory T cells express inhibitory cytokines which can in turn inhibit T-helper(T_H)1 and T_H2 immune responses[76, 77]. Thus, vitamin D deficiency may be associated with reduced expression of the inhibitory cytokines which in turn increases risks of both T_H1 mediated autoimmune diseases and Th2 mediated asthma or allergic diseases. Furthermore, low vitamin D status has been linked to enhanced oxidative stress[78] and increased airway smooth muscle (ASM) mass[79]. Additional mechanisms may include the regulation and expression of vitamin D pathway genes[80], production of cathelicidin, an antimicrobial polypeptide, in response to infections which may predispose children to develop asthma[81], and other effects on lung development and lung function that will be elaborated upon later.

Scientific positions regarding the likely association between vitamin D status and the development of asthma or allergy are contradictory. On one side, Wjst and Dold have suggested the increased prevalence of asthma coincides with the fortification of food with vitamin D, and further assert that asthma is caused by vitamin D

supplementation[82]. Conversely, Litonjua and Weiss suggest asthma is caused by vitamin D deficiency given: (i) as populations grow and become more prosperous, more time is spent indoors leading to reduced exposure to UVB radiation; and (ii) inadequate intake of vitamin D from food or supplements results in vitamin D deficiency, particularly in pregnant women; which (iii) leads to more asthma and allergy in their offspring[28]. Several studies on maternal intake of vitamin D or infant supplementation with vitamin D in association with onset of wheeze, asthma or allergic conditions, have yielded conflicting results[83-88]. These studies relied on self-reported dietary intake of vitamin D and are limited by the lack of a direct measurement of 25(OH)D. A recent review of vitamin D deficiency as a risk factor for childhood allergic disease and asthma[89] concluded that most cross-sectional and prospective studies have shown that low vitamin D levels increase the risk of asthma and allergy in children[89], which supports the position that vitamin D deficiency may lead to asthma[28]. On the contrary, some studies presented in this review suggest an association between high 25(OH)D and increased risk of childhood asthma and allergy in children[89], which supports the position held by Wjst and Dold[82]. Data from small clinical trials included in this review suggest that vitamin D supplementation may decrease the risk for asthma exacerbations and severity of eczema in children[89]. Before firm recommendations can be made on vitamin D supplementation for children with asthma or allergy, results from large clinical trials of long duration must be considered.

To date, very few studies on vitamin D status and asthma development in adults have been published[90-92]. A cross-sectional study using data from the National

Health and Nutrition Examination Survey (NHANES 2005-2006) reported an association between low serum 25(OH)D level and greater odds of asthma diagnosis in non-atopic individuals[90]. This was in line with a previous finding from our research group of an association between low serum 25(OH)D level and incident asthma in men without allergy[91]. However, a more recent study using data from the Korean National Health and Nutrition Examination Survey (KHANES 2008-2010) found subjects with lower serum 25(OH)D level had increased likelihood of atopic dermatitis, but not asthma or allergic rhinitis[92]. Despite the large sample size of the KHANES study, the cross-sectional design is a limiting factor. The evidence for an association between vitamin D status and allergy in adults is also lacking. One study using NHANES (1988-1994) data, found an increase in the prevalence of allergic rhinitis across 25(OH)D quartile groups in all ages, but with more prominent effects in children[93]. Findings from our research group showed that serum 25(OH)D level<50nmol/L was associated with increased risk of allergic rhinitis in men, but a reduced risk in women to suggest vitamin D status may play different roles in the development of allergic rhinitis among men and women[94]. Data from the UK showed a U-shape association between serum 25(OH)D levels and immunoglobulin (Ig)E levels to suggest a threshold effect of both upper (>135 nmol/L) and lower (<25nmol/L) serum 25(OH)D with increasing levels of IgE concentration[95]. This threshold effect may have important implications regarding adverse allergy risk for ongoing trials with high vitamin D supplementation. Still, due to the paucity of evidence in general adult populations, it is not possible to draw a conclusion on the relationship between vitamin D status and development of asthma or allergy.

To date, few studies on vitamin D status and asthma severity have been published, and most were done in children[96-100]. Brehm et. al, first studied the association between measured 25(OH)D and markers of asthma severity in children[96]. In 616 Costa Rican children aged 6 to 14 years, lower levels of 25(OH)D were associated with increased markers for allergy and asthma severity such that a log 10 unit increase of 25(OH)D was associated with reduced odds of any hospitalization in the previous year, any use of anti-inflammatory medication in the previous year, and increased airway responsiveness[96]. Brehm et.al, further assessed the relationship between serum 25(OH)D and severe asthma exacerbations in North American children with mild-to-moderate persistent asthma and found similar results[97]. In this population, vitamin D insufficiency (serum 25(OH)D<75nmol/L) was associated with higher odds of any hospitalization or emergency department visit over a 4 year period[97]. Another North American study in children with asthma (n=100) found that lower serum 25(OH)D levels in asthmatic patients were associated with increased corticosteroid use and worsening airflow limitation[98]. One additional study on the relationship between serum 25(OH)D level, disease severity, and airway remodeling in children with asthma found a higher prevalence of lower serum 25(OH)D levels in children with therapy-resistant asthma[99]. In this study, low vitamin D status was also associated with increased acute asthma exacerbations in the previous 6 months[99]. Despite the mounting evidence on vitamin D status and asthma severity in children, few studies in adults with asthma have been conducted. Regarding asthma severity, lung function measures are associated with future exacerbations of asthma symptoms and can therefore partly determine asthma control[63]. Studies on vitamin D status and lung function in general and asthma populations will be further discussed below.

1.5 Vitamin D in relation to lung function

Asthma is characterized by expiratory lung function (LF) that varies over time[63]. In any given patient with asthma, LF may vary from well-controlled to severely obstructed, in a reversible manner. The use of spirometry to measure LF, particularly forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC) and FEV_1/FVC ratio is an important method to assess airflow limitation in obstructive asthma. LF measures are associated with future exacerbations of asthma symptoms apart from current smoking and poor medication adherence[63, 101]. In healthy adults, the FEV_1/FVC ratio is normally greater than 0.75-0.80, and values less than this indicate airflow limitation[63]. Chronic obstructive pulmonary disease (COPD), which is further discussed below, is characterized by a fixed airflow obstruction with FEV_1/FVC<0.70[102]. It has been suggested that serum 25(OH)D levels may influence LF or LF changes through modulation of fibroblasts in respiratory epithelial cells[103], and by mediating the contraction, inflammation and remodeling of ASM function[79]. Several studies have shown an association between low vitamin D status and lower LF in general adult populations[104-108], among which, two studies have suggested a potentially stronger association in men compared to women[106, 108]. Most of these previous studies found associations between serum 25(OH)D level <50nmol/L and lower FEV_1 and FVC, but not FEV_1/FVC ratio, as might be expected in studies based on adult general populations rather than asthma or COPD cohorts. Studies on vitamin D status and LF in asthma populations are limited. One cross-sectional study of Costa Rican children with asthma (n=287) showed an association between serum 25(OH)D level<75nmol/L and lower FEV_1/FVC ratio[109]. An observational study of 54 adult patients with persistent asthma in the US, reported an association of reduced continuous

serum 25(OH)D with impaired FEV_1 and increased AHR[110]. In this study, adult asthma patients not treated with ICS seemed to be more responsive to the beneficial effects of a higher serum 25(OH)D level[110]. A recent cross-sectional study of Chinese adults with asthma (n=435) found a significant association between vitamin D deficiency (<50nmol/L) and low values for FEV_1/FVC ratio and FEV_1[111]. However, this study did not report sex-specific results.

As previously mentioned, sex is one risk factor for asthma[73]. In children, the prevalence of asthma is nearly twice as high in boys compared to girls[112]. With increasing age, the sex difference in the prevalence of asthma narrows until adulthood when the prevalence of asthma is greater in women than in men[63]. One possible explanation for a sex-differentiated prevalence of asthma is due to lung size and the potential for airway volume which is smaller in male compared to female infants[113], but larger in male compared to female adults[101]. At least one study showed an association between low serum 25(OH)D and incident asthma in adult men but not women[91]. Further sub-group analysis revealed this association to be in men without allergy status[91]. In addition, results derived from NHANES data (2005-2006) showed an association between lower serum 25(OH)D levels and greater odds of asthma diagnosis in non-atopic individuals [90]. Taken together, vitamin D status seems to be associated with asthma onset via a non-allergic pathway. However, evidence regarding the possible pathway by which vitamin D deficiency may be related to asthma severity and control in adults is lacking. Furthermore, the temporal relationship between vitamin D status and LF changes in adults, with or without asthma, requires further investigation.

To date, only three prospective studies on vitamin D status and LF changes in general adult populations have been published[105, 114, 115], and results from these studies do not concur with each other. One Danish study by Thuesen et al., (n=4,999) found a significant cross-sectional association between low serum 25(OH)D levels and a higher proportion of low FEV_1 percent predicted (% pred.) defined as less than (<) 80%, but a prospective association between high serum 25(OH)D levels and adverse LF changes[114]. In contrast, a second Danish study by Afzal et al., found a prospective association between lower plasma 25(OH)D and more LF decline (FEV_1 % pred., FVC % pred., but not FEV_1/FVC ratio)[115]. Results from this study were independently replicated in two general populations (n=10,116 and n=8,391). Some of the differences in results between these two Danish studies may be explained by method-related differences based on different assays used for measurement of serum 25(OH)D levels[116]. Finally, a smaller prospective study of elderly men (n=626) in the US found an association between vitamin D deficiency (<50nmol/L) and lower LF as well as increased LF decline in current smokers[105]. In all three prospective studies, smoking status (daily, current or continuous vs. never) showed a tendency toward a larger effect estimate of the association between serum 25(OH)D levels and LF changes[114], or modified the association between serum 25(OH)D and LF decline[105, 115]. Based on the current state of evidence, the association between serum 25(OH)D level and LF changes in the general population remains unclear, but smoking status seems to be an important covariate for consideration.

1.6 Chronic Obstructive Pulmonary Disease (COPD)

COPD is a common and preventable disease in adults that is characterized by persistent airflow limitation according to a fixed ratio of post-bronchodilator $FEV_1/FVC<0.70$[102]. Some suggest that $FEV_1/FVC<LLN$ may be an alternative diagnostic criteria for COPD[117]. However, expert-diagnosis in clinical practice is best, as the fixed ratio may over-diagnose COPD, while LLN definitions will likely under-diagnose COPD, particularly in elderly persons[118]. COPD is further characterized by a history of respiratory symptoms which include dysponea (breathing discomfort), chronic cough, chronic bronchitis, wheezing, and tightness of chest[102]. Diagnostic differentiation between asthma and COPD may be difficult and these conditions may overlap (asthma-COPD overlap syndrome, or ACOS), particularly in older persons or current and former smokers[63]. COPD is usually progressive and is associated with enhanced chronic airway inflammation in response to noxious particles or gases[102]. Smoking is the main risk factor for COPD[119], and COPD continues to be a global burden in terms of morbidity, mortality, and health-care costs[120].

1.7 Vitamin D in relation to smoking and COPD

The harmful consequences of smoking on lung health are well understood, and mechanisms by which cigarette smoke contributes to lung disease include oxidative stress and pro-inflammatory responses in lung cells[121]. Both of these mechanisms may be modulated by vitamin D[122, 123]. Lung epithelial cells generate active vitamin D which influences the expression of the vitamin D receptors (VDR) to mediate gene expression and modulate these biological effects[124]. Experimental design has shown that cigarette smoke extract can decrease conversion of inactive to active vitamin D in

the lungs[125]. Reduced local activation of vitamin D can impact the maintenance of barrier cell function in the lungs[126]. More specifically, vitamin D can regulate prostaglandin E_2 to modulate lung fibroblasts which play a critical role in lung tissue repair and remodeling[127]. Lower levels of active vitamin D in the lungs of smokers will consequently reduce barrier function by decreasing repair and remodeling of epithelial cells in the airway, increasing chemokine expression, and increasing local inflammation. Cigarette smoke can also directly induce systemic inflammation[128] and increased systemic inflammation has been linked to greater LF decline[129]. On the other hand, higher serum 25(OH)D levels may decrease circulating levels of chemokines and cytokines to reduce systemic inflammation[130]. Whereas, attenuation of airway inflammation via vitamin D is impaired in persons with lower serum 25(OH)D levels[131].

Beyond the risk of smoking, and the molecular mechanisms of vitamin D in relation to smoking and lung function decline, other factors that could explain low vitamin D status in COPD patients include but may not be limited to, poor diet, reduced outdoor activity and therefore less sun exposure, and reduced capacity for cutaneous synthesis of vitamin D due to aging skin[132].

An out-patient study in Denmark (n=62) reported a 68% prevalence of osteoporosis or osteopenia in COPD patients[133]. A high prevalence of bone disease suggests an equally high prevalence of vitamin D deficiency in this COPD population. In a more recent genotyping study, vitamin D deficiency (serum 25(OH)D<50nmol/L) was highly prevalent in COPD patients (60% to 77%, depending on disease severity) and was correlated with variants in the vitamin D binding protein[134]. It seems that

patients with severe COPD may be a good target for vitamin D supplementation, but the evidence on vitamin D supplementation or vitamin D status and disease severity or exacerbations, is conflicting. For example, high doses of vitamin D supplementation (100,000 IU every 4 weeks for 1 year) did not reduce the incidence of COPD exacerbations in a randomized trial of participants (n=182) with moderate to very severe COPD and a history of recent exacerbations[135]. However, a post hoc analysis of 30 trial participants with serum 25(OH)D level<25nmol/L at baseline did show a decrease in incidence of COPD exacerbations in those that received vitamin D supplementation compared to placebo[135]. The single-centre design of this trial may be a limiting factor. In addition, results from a nested, matched case-control study of continuous smokers (n=196), found no association between baseline serum 25(OH)D levels and subsequent lung function decline in slow versus rapid decliners[136]. However, due to the small sample size, this study may have limited variation in serum 25(OH)D levels.

The state of evidence is further confused given results from a UK population-based study[137]. The authors found high dietary intake of vitamin D to be associated with better lung function and decreased prevalence of COPD. However, no associations between serum 25(OH)D level (the gold standard to determine total body vitamin D status) and lung function were observed. The authors finally conclude that vitamin D status is not an important determinant of lung function or COPD in the general UK population[137]. A most recent prospective study by Afzal et al., reported a significant finding amongst smokers with an association between lowest quintiles of plasma 25(OH)D and higher risk for development of COPD[115]. Although these findings were

replicated in two general populations with large sample sizes (n=10,116 and n=8,391), this study has been critiqued by Young and Hopkins[138] who believe the findings may be spurious. First, they suggest the association between serum 25(OH)D level and lung function may be subject to a threshold effect[138]. Second, they cite concern with the fact that the overall estimates for an association between vitamin D status and FEV_1 % pred. were consistently mirrored by a corresponding association between vitamin D status and FVC % pred., but no effect was observed for FEV_1/FVC ratio[138, 139]. In response to this critique, Afzal et. al purport that the observed significant interaction suggests a stronger association between vitamin D status and FEV_1% pred. in individuals with airway obstruction[139]. Additional prospective and intervention studies on vitamin D status and lung function with regards to onset and severity of COPD are needed. If better vitamin D status could improve accelerated lung function decline amongst smokers, the burden of COPD may be reduced.

2 Research aims

The research aims for Papers I-III included in this doctoral dissertation are:

1) To estimate the prevalence of, and identify factors associated with vitamin D deficiency (serum 25(OH)D level <50nmol/L) in a Norwegian adult population;

2) To investigate the associations between serum 25(OH)D level and lung function in an adult asthma cohort, and to explore potential effect modification by sex and allergy status; and

3) To investigate the interrelationship between serum 25(OH)D level, smoking and lung function or lung function changes in a general Norwegian adult population.

3 Methods

3.1 The Nord-Trøndelag Health (HUNT) Study

Nord-Trøndelag county is one of 19 counties in Norway that is further divided into 23 municipalities. The county is centrally located at latitude 64° North. County population statistics (1995-2008) report a stable population of approximately 134,000 inhabitants with low net migration of 0.3%[140]. The county population is mostly Caucasian (97%), with socio-demographic characteristics, as well as morbidity and mortality profiles considered generally representative of Norway[141]. The HUNT Study is one of the largest population health studies conducted in Norway to date. Three adult HUNT surveys have been completed: HUNT1 (1984-1986), HUNT2 (1995-1997), and HUNT3 (2006-2008)[141, 142]. Planning for HUNT4 is currently underway, and data collection is expected to begin in 2017[143].

3.1.1 The HUNT2 Survey

Between August 1995 and June 1997, all residents of Nord-Trøndelag County aged 19 years and older, were invited to participate in HUNT2 via invitation letter sent to the home address of each potential participant[141, 142]. The invitation file was created from periodically updated census information provided by Statistics Norway. The first questionnaire (H2-Q1) was attached to the invitation letter and each potential participant was asked to self-administer and complete H2-Q1 prior to attending a clinical examination. H2-Q1 data included information on socio-demographics, lifestyle factors, history of asthma and allergy, as well as medication use. At the clinical examination, anthropometric data was collected including height and weight. A blood sample was also collected and stored at -70° Celsius for future use. At the clinical

examination, participants received a second questionnaire (H2-Q2) to be self-administered, completed at home and returned by mail in a pre-stamped envelope. The target population for HUNT2 consisted of 93,898 adults and the participation rate was 69.5% (n=65,237)[141, 142].

3.1.2 The HUNT2 Non-Responder Survey

A random sample of 2.5% of HUNT2 non-responders (n=26, 000) were invited to participate in a telephone survey[144]. Potential participants to the HUNT2 non-responder survey were contacted by telephone. If no telephone contact was made after three attempts on three separate dates, the H2-Q1 was mailed by post to the potential participant. Of the 685 potential participants for the HUNT2 non-responder survey, approximately 50% (n=326) gave consent to the telephone interview or completed and returned H2-Q1 which was received by mail. In the 20-69 year age group, the most cited reason for non-response to HUNT2 was lack of time or moved away (54%)[144]. Other reasons given for non-response included time consuming examinations and absence from work. A significantly higher proportion of current smokers was found in the non-responder group compared to current smokers in the responder group (current smokers: 36% of non-responders vs. 30% of responders). However, no significant difference in prevalence of self-reported asthma or use of asthma medication was found between non-responders and responders in the HUNT2 survey[144].

3.1.3 The Lung Health Study

Among those who participated in the adult HUNT2 survey, two sub-groups were invited to participate in the Lung Health Study (LHS), including: (1) a 5% random sample of total responders from the HUNT2 survey; and (2) a symptom group of HUNT2 participants who answered affirmatively to ever asthma, ever use of asthma medication, or attacks of breathlessness or wheezing in the past 12 months[145]. The LHS consisted of flow/volume spirometry and a structured personal interview on respiratory symptoms, diagnosis and treatment[145]. As part of HUNT3, a second LHS was conducted, where persons who participated in HUNT2-LHS were followed up, and a new 5% random sample of total responders from HUNT3 was invited to participate in the HUNT3-LHS.

3.1.4 The HUNT3 Survey

Between October 2006 and June 2008, all residents of Nord-Trøndelag County aged 19 years and older were invited to participate in HUNT3. The methods of data collection were similar to HUNT2. Data collected from H3-Q1 included information on socio-demographics, lifestyle factors, history of asthma and allergy, medication use, but also included questions related to culture and religion[142]. At the clinical examination, anthropometric data was collected including height and weight. Blood and urine samples were collected and given high priority as part of the establishment of the HUNT Biosciences bio-bank[142]. The target population for HUNT3 consisted of 93,860 adults and the participation rate was 54.1% (n=50,807). Among the adult HUNT2 participants, 57% (n= 37,059) were followed up to HUNT3[142].

3.1.5 The HUNT3 Non-Responder Survey

Approximately nine months after completion of the HUNT3 survey, a short two-page questionnaire was sent to all HUNT3 non-responders (n=42,024) amongst whom 17% completed the nonparticipation questionnaire[146]. The questionnaire included several of the core H3-Q1/H3-Q2 questions as well as questions related to reasons for non-response. Nearly 10% of non-responders reported they had not received the HUNT3 questionnaire. Otherwise, the dominant reason for non-participation of persons aged 20-59 years was lack of time or inconvenient session (55%-65% depending on age and sex). A significantly lower proportion of never smokers was found in the non-responder group compared to never smokers in the responder group (never smokers: 43% of non-responders vs. 46% of responders)[146]. Regarding prevalence of self-reported asthma, there was no significant difference between non-responders and responders in the HUNT3 survey. However, ever COPD was reported more frequently in male non-responders compared to male responders, whereas no substantial differences in women with COPD were found between non-responders and responders[146].

3.2 Analysis cohorts

This doctoral dissertation and papers herein are based on secondary analyses of primary data collected from HUNT2, HUNT2-LHS, HUNT3, and HUNT3-LHS. Figure 1 is a pictorial representation of the analysis cohorts used in Papers I-III which are further described in the text below.

3.2.1 Paper I

The analysis cohort for Paper I is based on a 10% random sample of participants (n=2584) from a cohort of adults aged 19-55 years who participated in both HUNT2 and HUNT3 (n=25, 616). These participants were selected for measurement of serum 25(OH)D from blood samples collected in HUNT2. In total, 97% of subjects (n=2505) had sufficient serum volume for analysis. This 10% random sample represents the general HUNT population[147].

3.2.2 Paper II

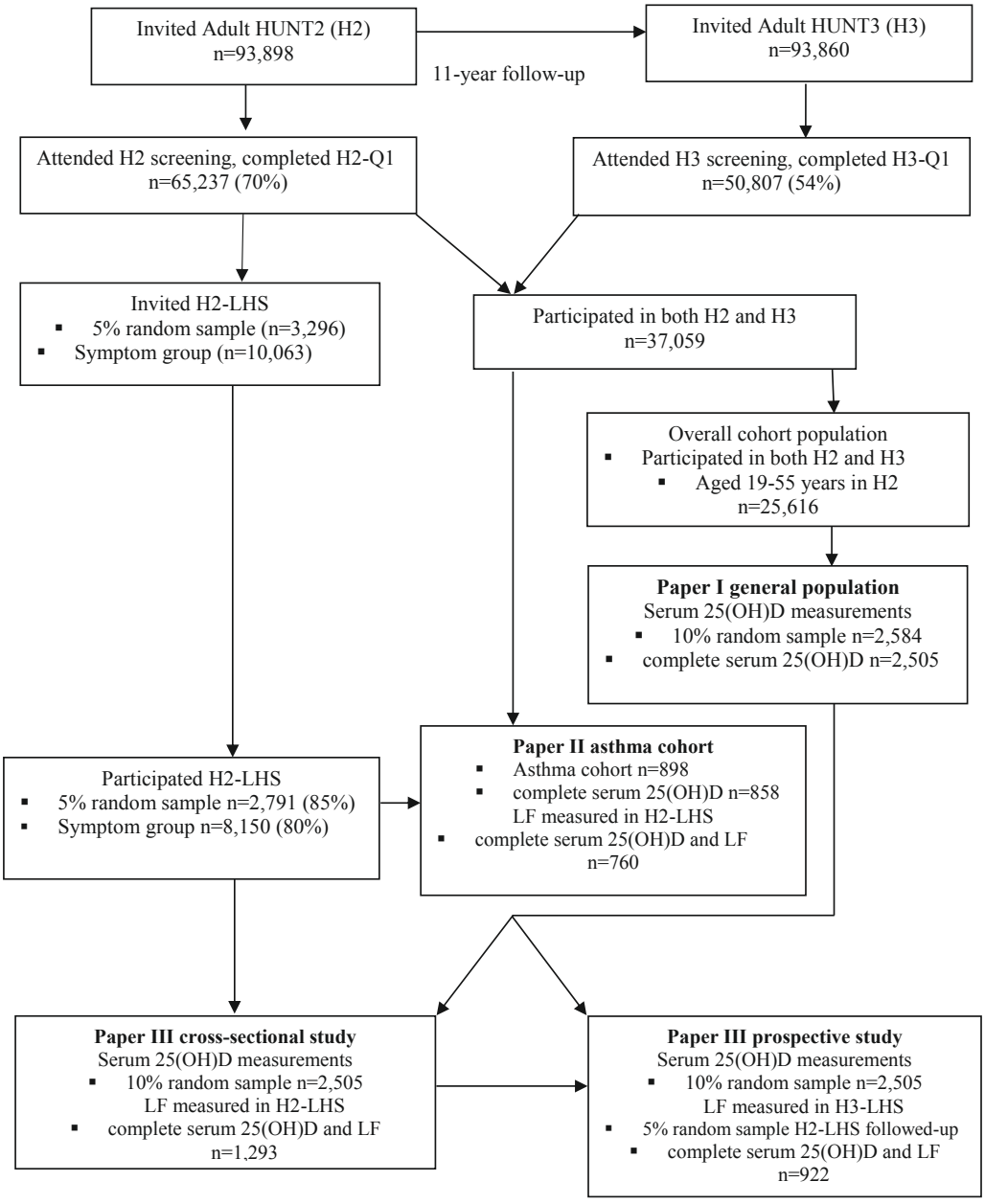
The analysis cohort for Paper 2 is based on an asthma cohort selected from the total cohort who participated in both HUNT2 and HUNT3 (n=25,616). The asthma cohort of adults aged 19-55 years in HUNT2 (n=898) was selected based on persons who answered affirmatively to both of the following two questions in HUNT2: "Have you had attacks of wheezing or breathlessness during the last 12 months?" and "Do you have or have you ever had asthma?" In order to strengthen the classification of their asthma status, persons selected into the asthma cohort also provided an affirmative response to the ever asthma question in HUNT3. Serum 25(OH)D levels were measured in this asthma cohort from blood samples collected during HUNT2. Lung function measures for Paper II were based on spirometry data collected from the symptom group during the HUNT2-LHS (Figure 1).

3.2.3 Paper III

The analysis cohort for Paper 3 is based on the 10% random sample of serum 25(OH)D measurements from HUNT2, as in Paper I. Lung function measures for Paper III were available from the 5% random sample group during HUNT2-LHS. Follow-up

spirometry in this 5% random sample group was also available from HUNT3-LHS. Persons aged 19-55 years with complete data on serum 25(OH)D level and lung function in HUNT2 were included in the cross-sectional study (n=1293). Seventy percent of participants from the cross-sectional study (n=922) were followed up with complete lung function data in HUNT3 (figure 1).

Figure 1 Analysis cohort selection, the HUNT Study, 1995-1997 to 2006-2008.



HUNT: Nord-Trøndelag Health Study; HUNT 2, 1995-1997; HUNT 3, 2006-2008; 25(OH)D: 25-hydroxyvitamin D; LHS: Lung Health Study; LF: lung function.

3.3 Serum 25(OH)D measurements

Blood samples were collected in HUNT 2 and stored at -70°C for later use. Serum 25(OH)D levels were measured using a fully automated antibody-based chemiluminescence assay (LIASON 25-OH Vitamin D TOTAL; DiaSorin, Saluggia, Italy) with detection range 10-375nmol/L, intraassay coefficient of variation (CV) 4%, and interassay CV 8%. Assay imprecision was evaluated and in compliance with standards according to CLSI EP5-A2[148, 149]. The stability in different markers in serum from HUNT2 has been questioned due to high probability of longer stay in room temperature than recommended for blood handling[150]. However, the pre-analytical stability of 25(OH)D in blood samples at room temperature is said to be stable[151].

From the 10% random sample of the overall cohort participants (n=2584), 97% of subjects (n=2505) had sufficient serum volume for analysis (Figure 1). Serum 25(OH)D levels ranged from 10 to 251 nmol/L. Circulating levels of serum 25(OH)D are indicative of bioavailable vitamin D status[4, 7]. A serum 25(OH)D level between 30-50nmol/L has been described as inadequate[24] and levels below 50nmol/L have been defined as deficient[27] or insufficient[29]. The cut-points for serum 25(OH)D levels used in this dissertation (Papers I-III) are <25nmol/L, <50nmol/L, <75nmol/L and ≥75nmol/L, where <50nmol/L was considered deficient.

3.4 Lung function measurements

Lung function measures were collected during the HUNT2 Lung Health Study (LHS) and HUNT3-LHS, and consisted of flow/volume spirometry recorded using two MasterScope Jaeger v.5.1 by trained professionals at screening stations. Instrument quality control included twice daily calibration. Biological control was conducted once

daily via staff lung function assessment. Participants were made to sit upright and use a nose-clip[152]. Recommendations and criteria from the American Thoracic Society (ATS) were followed and applied[153]. Participants were required to give three to five acceptable and reproducible trials during which expiration continued for at least six seconds. The best trial was determined by identification of the flow/volume curve using the highest sum of FEV_1 and FVC. The acceptability and reproducibility of results were reviewed by expert technicians. In the HUNT surveys, the curves with the highest sum of FEV_1 and FVC, and the best FEV_1/FVC ratio were used. Predicted reference values were derived from the prediction equations of spirometry based on the same HUNT population[152], and these predicted values were used to calculate FEV_1 % pred., FVC % pred., and lower limit of normal (LLN). Lung function impairment was defined as FEV_1/FVC ratio<70% or FEV_1/FVC ratio<LLN based on prior literature and on recommendation by the ATS[101, 153].

3.5 Other study variables

Data on all other study variables were collected by questionnaire and clinical examination in HUNT2 or HUNT3, with the majority of data used from HUNT2. Categorization of these study variables was consistent across Papers I-III unless otherwise stated. Missing data was categorized as unknown and included in the analyses unless otherwise stated in statistical methods (Section 3.8).

3.5.1 Socio-demographic variables

Age at time of invitation was included as a categorical (19-29, 30-39, 40-49 or 50-55) (Paper I) or continuous (Papers II and III) variable. Sex was dichotomized as male or female. Socio-economic variables included years of education (Paper I: <10,

10-12, ≥13 or unknown; Paper II: <10, ≥10 or unknown), receipt of social benefits (Papers I and II: yes, no or unknown), and economic difficulty in the last year (Papers I and II: yes, no or unknown). For Paper III, we generated a new variable for socio-economic status (SES) (high, low or unknown). High SES included non-recipients of social benefits and/or persons with no economic difficulties in the last year, while low SES included recipients of social benefits and/or persons with economic difficulties in the last year. Receipt of social benefits was determined based on participant response to the following HUNT2 question, "Do you receive any of the following public welfare benefits? Sick pay, rehabilitation benefits, retraining benefits, disability pension, retirement/old age pension, family income supplement, unemployment benefits, transitional benefits, widow's pension, other benefits." Economic difficulty in the last year was defined by participant response to the following HUNT2 question, "During the last year, has it at any time been difficult to meet the costs of food, transportation, housing and such?"

3.5.2 Season

Season of blood sample collection was categorized according to the Norwegian Meteorological Institute standard: summer (June through August), autumn (September through November), winter (December through February) or spring (March through May)[154] (Paper I). For Papers II and III, season was dichotomized as December through May or June through November.

3.5.3 Body mass index (BMI)

Body weight and height were measured in clinical examination by trained personnel at HUNT2 screening stations. Height was measured to the nearest centimetre

(cm) and weight to the nearest half-kilogram (kg)[141]. BMI was calculated as weight in kilograms divided by height in meters squared (kg/m^2). For Paper I, BMI was categorised according to the World Health Organization standard (<25.0, 25-29.9, ≥30.0 or unknown)[155]. For Papers II and III, BMI was included in the analyses as a continuous variable.

3.5.4 Lifestyle variables

Average number of hours of light physical activity per week was categorized as <1, 1-3, ≥3 or unknown (Paper1). For Papers II and III, light physical activity was categorized as <1, ≥1 or unknown hours per week. Physical activity was defined based on participant response to the following HUNT2 question: "How much of your leisure time have you been physically active during the last year? (Think of a weekly average for the year. Your commute to work counts as leisure time)" Participants qualified their physical activity as light (no sweating/not out of breath) or vigorous (sweating/out of breath). Daily smoking was categorized as never, current, former or unknown (Papers I and II). For Paper III, daily smoking was categorized as never or ever (current/former). Frequency of alcohol consumption was based on participant response to the following question, "How many times a month do you normally drink alcohol?", and was categorized in Paper I as abstain/once, 1-4, ≥5 or unknown.

3.5.5 Allergy status

In Paper II, allergic rhinitis was used as a proxy measure for allergy status (yes, no or unknown) based on participant response to the following HUNT3 question, "Do you have or have you had allergic rhinitis or hay fever?"

3.5.6 Medication and supplement use

Daily intake of cod liver oil (5mL/400 IUs of vitamin D per day by recommendation) for at least one month was categorized as yes, no or unknown (Paper I). For Paper 2, ever use of asthma medication was included as yes, no or unknown. Also for Paper 2, regular use of inhaled corticosteroids (ICS) in the last 6 months was dichotomized as yes or no, and daily dosage of ICS was included as a continuous variable in a sensitivity analysis.

3.6 Statistical methods

STATA versions 12.1 and 13.1 were used for all statistical analyses (StataCorp LP, College Station, Texas).

3.6.1 Paper I

In this study, we estimated the prevalence of vitamin D deficiency (serum 25(OH)D <50nmol/L) in a general Norwegian population (n=2505) and examined factors associated with serum 25(OH)D level and vitamin D deficiency using a random sample and cross-sectional study design. We calculated the prevalence of vitamin D deficiency overall and by season of blood sample collection, and demonstrated the frequency distribution of serum 25(OH)D levels by histogram. We used Poisson regression[156] to estimate the crude and adjusted prevalence ratio (PR) and 95% confidence intervals (CIis) of factors associated with vitamin D deficiency. Analysis of variance was used to calculate the mean difference in serum 25(OH) D level by socio-demographics, season, BMI and lifestyle variables. We used Fischer's exact test for small sample sizes to test differences in baseline characteristics between those with serum 25(OH)D level <25nmol/L compared to the ≥25nmol/L group. The multivariable

models included age, sex, education, receipt of social benefits, economic difficulties, season of blood sample collection, BMI, cod liver oil intake, physical activity, smoking and alcohol consumption. Missing data for education, social benefits, economic difficulties, BMI, cod liver oil intake, physical activity, smoking, and alcohol consumption were categorized as "unknown" and included in the analysis. Missing data were assumed missing at random (MAR). In addition, we used the multivariate imputation by chained equations (MICE) method to impute fifty datasets of missing data on the above covariates and repeated the analyses.

3.6.2 Paper II

In this cross-sectional observational study, we assessed the association between serum 25(OH)D level and lung function in Norwegian adults with asthma (n=760). We also examined possible interactions by sex and allergy status. Based on our hypothesis, we used the likelihood ratio test to evaluate the modifying effect of sex and allergy status on lung function . Based on a significant three-way interaction term (categorical serum 25(OH)D*sex*allergic rhinitis) on lung function parameters (p=0.03) we performed the analysis separately in women (n=446) and men (n=314), and further stratified the data by allergic rhinitis. We used Pearson's chi-squared to test differences between baseline characteristics in women compared to men in the analysis group. Further, we used Pearson's chi-squared to test differences between baseline characteristics in the analysis group compared to participants excluded due to missing information on either exposure or outcome (n=138). We used linear regression analysis to estimate the association between serum 25 (OH)D levels and lung function measures (FEV_1 % pred., FVC % pred., and FEV_1/FVC ratio). Serum 25(OH)D level was

analyzed as either a categorical (<50 nmol/L, 50-74.9 nmol/L or ≥75nmol/L) or continuous independent variable. Crude and adjusted regression coefficients (β) and 95% CIis are given. Multivariable linear regression models included BMI, education, receipt of social benefits, economic difficulties in the last year, season of blood sample collection, physical activity, smoking status, ever use of asthma medication and regular use of ICS in the last 6 months as important covariates. Missing data on education, social benefits, economic difficulties, physical activity, smoking status and ever use of asthma medication were categorized as "unknown" and included in the analysis. Missing data were assumed MAR. We used the MICE method to impute fifty datasets of missing data on the above covariates and repeated the analyses. To minimize possible misclassification of reported asthma, we excluded those who reported having COPD, chronic bronchitis or emphysema (n=83) and repeated the analysis.

3.6.3 Paper III

In this observational study, we assessed the cross-sectional (n=1220) and prospective (n=869) interrelationship between serum 25(OH)D level, smoking and lung function or lung function changes in a random sample of Norwegian adults. The analyses were performed overall and separately in never and ever smokers, based on priori information from the literature, and our hypothesis. We used multivariate linear and logistic regression analyses to estimate the crude and adjusted differences in LF or LF decline, crude and adjusted odds ratios (ORs) for LF impairment or development of LF impairment, and 95% CIs. We conducted the analyses using serum 25(OH)D level as a categorical (<50nmol/L or ≥50nmol/L), or continuous independent variable. Outcome measures for linear models included continuous values of FEV_1 % pred., FVC

% pred., and FEV_1/FVC ratio. Outcome measures for logistic models included FEV_1/FVC ratio <70% and FEV_1/FVC ratio <LLN. T FEV_1/FVC ratio models based on actual measurements rather than predicted equations were further adjusted for age, sex and height. Multivariable linear and logistic regression models included BMI, education, socio-economic status, season of blood sample collection and physical activity as important covariates. Missing data on socio-economic status, and physical activity were categorized as "unknown" and included in the analyses. After participants with complete data on serum 25(OH)D, smoking, and lung function were included in the studies, missing data on the above covariates was less than 2%, therefore, multiple imputations of missing data were not performed. To minimize possible residual confounding, we further controlled for pack-years of smoking as a continuous variable amongst ever smokers. To test the robustness of our findings, we excluded subjects who reported ever asthma (n=110), ever COPD (n=10), or both (n=12), and repeated the analyses.

3.7 Ethics

The HUNT Study including all adult HUNT surveys, non-response surveys and sub-studies (Lung Health Study), received ethics approval from the Regional Committee for Ethics in Medical Research (REK), and the Norwegian Data Inspectorate. All participants gave informed written consent.

This doctoral research received ethics approval from the REK and utilized data and blood samples that had already been collected by the HUNT Research Center. All names and personal identification were removed from the data files received from the HUNT Research Center. Only anonymous case numbers were used to link questionnaire

data and biological samples. Thus, the anonymity of all study participants was protected. No persons were directly contacted and no new information was gathered as part of this doctoral research. The values of serum 25(OH)D were not provided to study participants.

4 Results

4.1 Factors associated with vitamin D deficiency in a Norwegian population

Using data from the HUNT Study, we estimated the overall prevalence of vitamin D deficiency to be 40%, ranging from 20% in the summer to 64% in the winter ($p<0.001$). Winter season (adjusted prevalence ratio (PR): 3.16, 95% CI: 1.45-2.10) and obesity (BMI $\geq$30.0 kg/m^2) (PR: 1.74, 95% CI: 1.45-2.10) were strongly associated with prevalent vitamin D deficiency (serum 25(OH)D level<50nmol/L). Our results further indicate that potentially modifiable lifestyle factors were also independently associated with vitamin D status.

In addition to winter season and high BMI, current smoking also showed an increased PR (1.41, 95% CI: 1.21-1.65). Whereas daily intake of cod liver oil (PR: 0.60, 95% CI: 0.41-0.77), increased physical activity (PR: 0.80, 95% CI: 0.68-0.95) and more frequent alcohol consumption (PR: 0.76, 95% CI: 0.60-0.95) were associated with a reduced PR. Similar results were found when serum 25(OH)D level <75nmol/L was used as the cut-point (data not presented).

Approximately 4% of study participants (n=107) had very low serum 25(OH)D levels (<25nmol/L) ranging from 10 to 25 nmol/L (figure 2). The median serum 25(OH)D level in this sub-group was 21 nmol/L. A comparison between participants with very low serum 25(OH)D level (<25 nmol/L)and those with serum 25(OH)D levels $\geq$25 nmol/L showed that the group with very low serum 25(OH)D levels were younger, had lower socio-economic status, higher BMI, were less physically active, were less likely to take cod liver oil, and had a greater proportion of current smokers.

The <25nmol/L group also consumed alcohol fewer times per month, and had a higher proportion of blood samples collected during the winter season (table 1). When serum 25(OH)D level <25nmol/L was used as the cut-point, winter season, high BMI and current smoking showed an increased PR, although the confidence intervals were much wider compared to data obtained with the <50nmol/L cut-point (table 1). Daily intake of cod liver oil, increased physical activity and more frequent alcohol consumption were still associated with reduced PR. Although the socio-economic (SES) variables remained statistically non-significant, increasing age demonstrated a reduced PR in the <25nmol/L group compared to the ≥25nmol/L group.

Figure 2 Unadjusted frequency distribution of study participants with serum 25(OH)D<25nmol/L

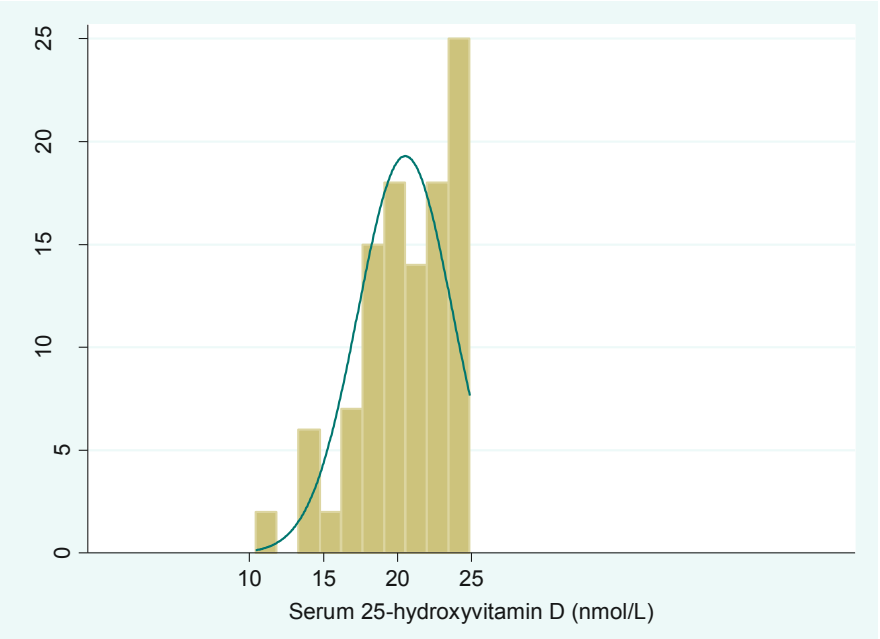


Table 1 Baseline characteristics and adjusted prevalence ratio with 95% confidence intervals for serum 25(OH)D level <25nmol/L, in a random sample of adults, the HUNT Study, 1995-1997

	<25nmol/L n=107	≥25nmol/L n=2,398	p-value[¶]	Adjusted PR	95% CI
Age, years			0.03		
19-29	25 (23)	353 (15)		1.00	Reference
30-39	37 (35)	714 (30)		0.72	0.43, 1.21
40-49	33 (31)	951 (40)		0.49	0.28, 0.85
50-55	12 (11)	380 (16)		0.44	0.21, 0.91
Sex			0.62		
Female	62 (58)	1322 (55)		1.00	Reference
Male	45 (42)	1076 (45)		1.02	0.68, 1.53
Education years			0.05		
<10	21 (20)	468 (20)		1.00	Reference
10-12	67 (63)	1280 (53)		1.07	0.63, 1.81
≥13	17 (16)	630 (26)		0.82	0.42, 1.62
Unknown	2 (1)	20 (1)			
Social benefit recipient			0.01		
No	60 (56)	1548 (65)		1.00	Reference
Yes	32 (30)	434 (18)		1.36	0.86, 2.15
Unknown	15 (14)	416 (17)			
Economic difficulties			0.01		
No	53 (50)	1430 (60)		1.00	Reference
Yes	42 (39)	664 (28)		1.12	0.73, 1.73
Unknown	12 (11)	304 (12)			
Season[#]			<0.001		
Summer	2 (1)	305 (13)		1.00	Reference
Autumn	12 (11)	792 (33)		2.24	0.50, 10.0
Winter	67 (63)	688 (29)		12.67	3.10, 51.9
Spring	26 (24)	613 (26)		6.43	1.52, 27.2
Body mass index $kg \cdot m^{-2}$			0.001		
<25.0	36 (34)	1068 (45)		1.00	Reference
25.0-29.9	45 (42)	1049 (44)		1.44	0.92, 2.26
≥30.0	26 (24)	274 (11)		2.54	1.51, 4.26
Unknown		7 (0.3))			
Cod liver oil intake			0.004		
No	74 (69)	1459 (61)		1.00	Reference
Yes	5 (4)	336 (14)		0.37	0.15, 0.92
Unknown	28 (27)	603 (25)			
Physical activity h·week			0.01		
<1	40 (37)	532 (22)		1.00	Reference
1-2	35 (34)	827 (35)		0.67	0.42, 1.06
≥3	28 (26)	745 (31)		0.72	0.44, 1.19
Unknown	4 (3)	294 (12)			
Smoking status			<0.001		
Never	35 (34)	996 (42)		1.00	Reference
Current	49 (46)	646 (27)		1.96	1.24, 3.11
Former	18 (16)	620 (26)		0.84	0.47, 1.50
Unknown	5 (4)	136 (6)			
Alcohol consumption, times per month			<0.001		
Abstain or ≤1	49 (46)	636 (27)		1.00	Reference
1-4	45 (42)	1342 (56)		0.43	0.29, 0.66
≥5	11 (10)	333 (14)		0.56	0.28, 1.12
Unknown	2 (1)	87 (4)			

Data are presented as n (%), unless otherwise stated. 25(OH)D: 25-hydroxyvitamin D; PR: prevalence ratio; CI: confidence interval. # Season: summer, June-August; autumn, September-November; winter, December-February; spring, March-May. ¶ Fischer's exact test performed to analyze the difference between <25nmol/L group and ≥25nmol/L group (missing excluded).

4.2 Serum 25-hydroxyvitamin D levels and lung function in adults with asthma

Using data from the HUNT Study, we conducted a cross-sectional analysis to assess the association between serum 25(OH)D level and lung function in a sample of Norwegian adults with asthma (n=898). We also examined possible interactions by sex and allergy status.

A comparison between participants in the final analysis group (n=760) and those excluded due to missing information on either exposure or outcome (n=138) showed that the analysis group had higher serum 25(OH)D levels, a higher proportion of never smokers, were less likely to report regular use of ICS, and had better LF.

The overall prevalence of vitamin D deficiency (serum 25(OH) D level <50nmol/L) in this adult asthma cohort was 44%, which was slightly higher than the prevalence of vitamin D deficiency (40%) in the general HUNT population[157].

The associations between serum 25(OH)D level and LF measures seemed to be modified by sex and allergic rhinitis ($p<0.03$ for 3-way interaction term). In summary, serum 25(OH)D level <50 nmol/L was not associated with lower LF in asthmatic women with or without allergic rhinitis, or in asthmatic men with allergic rhinitis. However, in men with asthma but without allergic rhinitis, serum 25(OH)D level <50 nmol/L was significantly associated with a much lower FEV_1/FVC ratio in comparison with the serum 25(OH)D level ≥50nmol/L group (β= -8.60%; 95% CI: -16.95% to -0.25%).

When participants with self-reported COPD, chronic bronchitis or emphysema were excluded (women: n=40; men: n=43), the association between serum 25(OH)D level and LF measures in women with asthma and with or without allergic rhinitis remained null. The association between serum 25(OH)D level and FEV_1/FVC ratio in men with asthma but without allergic rhinitis, remained (β= -6.98; 95% CI: -12.88 to -1.06).

In a further sensitivity analysis we examined how dosage of ICS would influence our results. Information on dosage of ICS was obtained from the following question, "What is the average daily dosage of ICS that you used last week? (dosage + number of inhalations)." The difference in ICS dosage per day between women and men was not statistically significant (p=0.237). When dosage of ICS per day was included as a covariate in the final adjusted models, we found no change in point estimates or confidence intervals (data not presented). Finally, we also obtained similar analytical results after multiple imputations of missing data on allergic rhinitis and other covariates were performed in adjusted models (data not presented).

4.3 Serum 25-hydroxyvitamin D level, smoking and lung function changes in adults

Using data from the HUNT Study, we studied the cross-sectional (n=1293) and prospective (n=922) interrelationship of serum 25(OH)D, smoking and LF or LF changes in a random sample of Norwegian adults.

At baseline, 40% of adults in this study had serum 25(OH)D level<50nmol/L which was consistent with the prevalence of vitamin D deficiency found in Paper I[158]. Overall, serum 25(OH)D level<50nmol/L showed worse LF, and increased odds for development of LF impairment compared to the ≥50nmol/L group. These associations tended to be stronger amongst ever smokers who showed more decline in FEV_1/FVC ratio (%) (adjusted difference: 1.2; 95% CI: 0.1 to 2.2) and greater odds for development of LF impairment (adjusted OR for FEV_1/FVC<70%: 2.4; 95% CI: 1.2 to 4.9). Amongst never smokers, no associations between serum 25(OH)D levels and LF or LF changes were observed. Cross-sectional and prospective results were generally consistent.

A sensitivity analysis which excluded study participants who self-reported ever asthma (n=110), ever COPD (n=10) or both (n=12), gave further confirmation of more decline in FEV_1/FVC ratio (%) amongst ever smokers with serum 25(OH)D level <50nmol/L (adjusted difference: 1.3; 95% CI: 0.1 to 2.5). Associations amongst never smokers remained null. In another sensitivity analysis, we adjusted for pack-years of smoking amongst ever smokers and obtained similar analytical results in both cross-sectional and follow-up studies (data not presented). When LLN was used as a cut-point to define impaired LF or development of impaired LF, results were weaker but

showed a similar trend in comparison to results obtained when FEV_1/FVC ratio <70% was used as a cut-point (data not presented).

Table 2 shows a supplementary analysis looking at the difference in baseline characteristics between high (≥ 50nmol/L) versus low (<50nmol/L) serum 25(OH)D level amongst never smokers. Never smokers with serum 25(OH)D level ≥ 50nmol/L were more physically active, had lower BMI, and had a greater proportion of blood samples collected during summer months compared to the <50nmol/L group. These factors were adjusted in multivariable analysis when the relation of serum 25(OH)D levels and LF was assessed in never smokers as well as in ever smokers, and the association remained null in never smokers.

Table 2 Baseline characteristics of never smokers in a random sample of Norwegian adults, the HUNT Study 1995-1997

	Serum 25(OH)D ≥50nmol/L (n=340)	Serum 25(OH) <50nmol/L (n=185)	p-value[#]
Age, years	39±9.2	38±8.8	0.07
Sex			0.15
Female	195 (57)	94 (51)	
Male	145 (43)	91 (54)	
25(OH)D (nmol/L)	73±18	38±9	<0.001
Body mass index $kg \cdot m^{-2}$	25±4	27±4	<0.001
Socio-economic status			0.99
High	200 (57)	105 (57)	
Low	109 (31)	57 (31)	
Unknown	31 (12)	23 (12)	
Season			<0.001
December-May	129 (38)	141 (76)	
June-November	211 (62)	44 (24)	
Physical activity h·week			0.003
<1	61 (18)	52 (28)	
≥1	235 (69)	105 (57)	
Unknown	44 (13)	28 (15)	
Ever asthma			0.30
Yes	304 (89)	171 (92)	
No	35 (35)	14 (8)	
Unknown	1 (0.3)	0	
FEV_1 % pred.	99±13.5	99±11.7	0.50
FVC % pred.	100±12.1	100±11.2	0.42
FEV_1/FVC ratio	0.82±0.06	0.82±0.06	0.83

Data are presented as mean±sd or n(%), unless otherwise stated. HUNT: Nord-Trøndelag Health Study; 25(OH)D: 25-hydroxyvitamin D; FEV_1 % pred.: forced expiratory volume in 1 second percent predicted; FVC: forced vital capacity. #: A t-test was performed to analyze the difference between never smokers with serum 25(OH)D level ≥50nmol/L and <50nmol/L for continuous variables and a Chi-squared test was applied for categorical variables (missing data were excluded).

5 Discussion

What follows is a presentation of key results from Papers I-III, a discussion of strengths and limitations of each study, and a comparison to other papers.

5.1 Key results

Our principle findings are hereby summarized:

- Vitamin D deficiency (serum 25(OH)D level<50nmol/L) is relatively high in a general Norwegian population (40%), and slightly higher in a Norwegian asthma cohort (44%);
- Winter season and high BMI were strongly associated with vitamin D deficiency (serum 25(OH)D level<50nmol/L) in a general Norwegian population.
- Several potentially modifiable lifestyle factors including smoking status, physical activity, intake of cod liver oil, and alcohol consumption were also independently associated with vitamin D status in a general Norwegian population;
- Low serum 25(OH)D level was not associated with airway obstruction in most of the adults with asthma, with the exception of men with asthma but without allergic rhinitis; and
- Amongst never smokers, we found no significant associations between serum 25(OH)D levels and lung function or lung function changes in a general population of Norwegian adults, however, ever smokers with low serum 25(OH)D level showed more lung function decline, and increased odds for development of lung function impairment.

5.2 Methodological considerations

Methodological considerations related to strengths and weaknesses of the epidemiologic studies included in this dissertation (Papers I-III) will now be discussed in turn.

5.2.1 Random error (imprecision of estimate)

Random error refers to the likelihood of chance findings and potential variability around the data that can be reduced by increasing the sample size[159]. Conversely, precision may be defined as lack of random error. Epidemiologic studies provide point estimates which, of themselves, do not express variability in the data. For this purpose, confidence intervals (CIs) are used. In Papers I-III we estimated precision with 95% CIs, which is to say, if we replicated data collection and analysis *n* times, the true value of the measure would be within the reported CIs, 95% of the time[160]. Increased sample size may reduce variance and improve the precision of the estimates. As such, studies with large samples have increased precision and narrow CIs, whereas studies with small samples have reduced precision and wide CIs.

In Papers I-III, we had ample sample size to estimate the prevalence of vitamin D deficiency (n=2505), the association between serum 25(OH)D level and LF in an adult asthma cohort (n=760), as well as the cross-sectional (n=1220) and prospective (n=869) associations between serum 25(OH)D level and LF or LF changes in a general population. However, some sub-group analyses in Paper II had fewer within stratum observations and wider CIs to indicate reduced precision. These estimates should be interpreted with care.

5.2.2 Systematic error (lack of internal validity)

Systematic error is an umbrella term for different types of bias that may be present in an epidemiologic study. Unlike random error which is reduced by increasing sample size, systematic error (or bias) is an inherent, non-random deviation of results that cannot be minimized by increasing sample size[159]. The three categories of systematic error are selection bias, information bias and confounding.

5.2.2.1 Selection bias

Selection bias is introduced into the study when the study population is erroneously selected[160]. This may include selecting the wrong study participants such that there is a discernable difference between those who participated in the study and those who did not. Other examples of selection bias include loss to follow-up due to migration, death, illness or disinterest.

According to HUNT2[144] and HUNT3[146] non-responder surveys, the main reasons for non-participation across age groups reflected in our studies (19-55 years), were lack of time, moved away, or inconvenient session. Given the age range of our study participants, non-attendants were likely in school, working, or caring for family. We may surmise that non-response due to illness is less likely in this age group. As an example, no significant difference in the prevalence of asthma was found between responders and non-responders in either HUNT2 or HUNT3, thus minimizing selection bias in our asthma cohort. However, there were significantly more smokers in the non-responder group (36%) compared to the responder group (30%) in HUNT2. Following this, there were significantly less never smokers in the non-responder group (43%) compared to the responder group (46%) in HUNT3. This may have led to an

underestimation of current or former smokers, and an overestimation of never smokers in our analysis cohorts. Also, men with COPD in HUNT3 had a lower participation rate than men without COPD, and women with or without COPD. In Papers II-III, we conducted sensitivity analyses that excluded ever COPD patients, so male COPD non-responders to HUNT3 would not influence our results as they would be otherwise excluded from our analyses. Also, we selected a random sample for serum 25(OH)D measurement from the overall cohort, which may compensate for some potential selection bias. Some loss to follow up is common in population based studies, particularly if persons who are loss to follow up due to migration, illness, death, etc., differ in comparison to those who remain in the study. In our Paper III we were able to follow-up 70% of study participants from HUNT2 to HUNT3. Strong follow-up in conjunction with non-responder data gives us some confidence that we were able to minimize selection bias.

Missing data from survey non-response is another type of selection bias. For Papers I and II, we used the multivariate imputation by chained equations (MICE) method to impute fifty datasets of missing data on the covariates with missing information and we observed similar results (data not presented) (Section 3.8).

5.2.2.2 Information bias

Information bias occurs during data collection and refers to measurement error that results in an incorrect estimate of the association between exposure and outcome[160]. Inaccurate measurement of exposure, covariate or outcome variables can occur. Information bias may further be divided into misclassification bias, detection bias, recall and observer bias, and reporting bias.

There are two types of misclassification to be aware of in epidemiologic studies, namely, non-differential and differential misclassification[159]. If all groups of subjects or categories of a variable have equal probability of being misclassified and therefore have the same error rate, the misclassification is said to be non-differential. In contrast, if the probability of being misclassified differs across groups of study subjects resulting in a different error rate in each group to be compared, the misclassification is said to be differential. Erroneous information, whether non-differentially or differentially misclassified, can give a biased estimate either toward or away from the null.

Detection bias, particularly systematic differences in measurement of outcome variables, is important to consider in cohort studies[160]. Valid and reliable outcome measures will greatly reduce detection bias. Recall and observer bias are similar in that the participant or interviewer may lack impartiality and may be guided by some form of prior knowledge that may lead to a biased response. Finally, reporting bias often happens when the participant reports incorrect information, if for example the inaccurate response is deemed more socially desirable than the accurate response. Furthermore, responses given by a proxy may prove to be a source of inaccurate information. Many forms of information bias can be minimized by participant or interviewer blinding, or by implementing a standardized protocol[160].

By nature of the HUNT study design, some information bias within questionnaire data might be expected. For example, questionnaire 1 in both HUNT2 and HUNT3 surveys were mailed to each potential participant who then completed the questionnaire by self or by proxy which may contribute to reporting bias. However, participants then attended a clinical examination where trained health professionals

received the questionnaire and provided additional support if needed. This follow-up may limit potential erroneous or missing information. Regarding our asthma cohort in Paper II, participants with asthma may forget a childhood diagnosis of asthma, or may have been given a diagnosis like recurrent bronchitis instead of asthma[150]. Participant results from clinical exam were shared with the family physician[142]. Participant awareness around the possibility of validated responses by family physician may have reduced reporting bias.

The serum 25(OH)D levels and lung function measures used in our studies were obtained by trained professionals using standardized protocols and quality controlled methods (Section 3.5-3.6). However, spirometry is dependent on technique and standardization may be difficult to adhere to in population based studies. Important failures may include too low initial flow or too short expiration time, however FEV_1 is a robust parameter that seems to have high validity[150]. In addition, some covariates may have been misclassified due to self-report. For example, a skin prick test or measurement of allergen-specific IgE would give more accurate information on allergy status[63]. Also, assessment of cotinine levels in biological fluid may provided a more accurate categorization of smoking status[161]. In future, studies using HUNT biobank data could conduct validation studies in sub-samples of the survey population to determine self-report accuracy.

5.2.2.3 Confounding

Confounding is a critical concept in epidemiology, particularly in observational studies when it is rarely possible for individuals to be randomly assigned[162]. Study participants who share a particular risk, vitamin D deficiency for example, also have

other characteristics in common that influence their risk of disease. In experimental studies, randomization ensures that each treatment group has a similar risk of the outcome at baseline. In this context, the difference between treatment groups is said to be causal[162]. However, observational studies are limited in that one cannot be sure if subjects in different exposure groups are similar with respect to all other factors. Confounding can be defined as a mixture of the effect of an exposure with effects of other factors[159]. Confounding may lead to an underestimation, overestimation, or a change in the direction of the estimate[159].

In order for a variable to be a confounder it must be independently associated with both the exposure and the outcome, must not be along the causal pathway between the exposure and outcome, and must not be a consequence of the outcome[159, 162]. There is no statistical test for confounding[162]. One's ability to accurately identify a confounder is based on expert and/or priori knowledge from the literature.

The HUNT studies constitute a comprehensive database of clinical, lifestyle and environmental information collected over 25 years. Using data from HUNT2 and HUNT3, we were able to control for a wide range of possible confounding factors in our studies (Sections 3.8 and 3.9). Literature on serum 25(OH)D levels and lung function in adults is growing, but still sparse. Information on potential confounders of this association is also lacking.. We can therefore not discount the possibility of residual or unmeasured confounding in our studies. For example, in Paper I we lacked full information on alcohol consumption including quantity, frequency and type of alcohol consumed. Therefore, the association between increased alcohol consumption and lower prevalence ratio for vitamin D deficiency may be subject to residual

confounding. For Paper II we had information on cod liver oil intake, however, other dietary intakes and nutrition may be a common cause of both vitamin D deficiency and asthma onset or severity[163]. Regarding adverse lung function changes in Paper III, we did not control for possible workplace exposure which may or may not be associated with vitamin D status. However, for unknown confounding to substantially impact our findings, the variable must not be associated with any other variable we controlled for in the analyses, and must be strongly associated with both exposure and outcome. Future studies on vitamin D status and lung function in adult asthma or adult general populations will provide further information concerning confounding.

5.3 Generalizability (external validity)

Regarding the generalization of epidemiologic results, this section is not concerned with statistical representativeness based on sampling from a source population as previously discussed under selection bias (Section 5.1.2.1). Rather, in this section we consider generalization of epidemiologic results within the context of biological representativeness of scientific findings[159]. For example, our study results from a general adult population (aged 19-55) in Norway cannot be considered biologically comparable to infants, children, the elderly or diseased populations. Likewise, our findings within the adult asthma cohort cannot be considered biologically comparable to general or healthy populations, or COPD patients. Furthermore, the HUNT study population is mostly Caucasian (97%)[141] which may reduce the generalizability of our research findings to more ethnically diverse populations. However, since the HUNT study represents a population based cohort, the prevalence and incidence of diseases and determinants of disease are comparable to those of other

northern European countries[33]. Finally, our analysis cohorts can be considered representative of ethnic Norwegian populations which provides a platform for biomarker-based research that may be undertaken in the future[33].

5.4 Comparison with other studies

5.4.1 Prevalence of vitamin D deficiency

In our study, the prevalence of vitamin D deficiency (serum 25(OH)D level <50 nmol/L) was high in a general population of Norwegian adults (40%) and higher in an asthma cohort of Norwegian adults (44%). Comparisons on the prevalence of vitamin D deficiency are difficult due to the lack of general consensus regarding cut-points, and varying use of terminology to define these cut-points[24, 27]. For example, a serum 25(OH)D level between 30-50nmol/L has been defined as "inadequate"[24], and levels below 50nmol/L have been defined as "deficient"[27], or "insufficient"[29]. The widely recommended cut-points to define vitamin D deficiency and insufficiency are serum 25(OH)D levels <50nmol/L, and <75nmo/L, respectively[17]. In this paper, we considered serum 25(OH)D level <50nmol/L as deficient.

The mean level of serum 25(OH)D in Paper I (59 nmol/L) was comparable to that from another population based study of adults aged 25-84 years conducted in Northern Norway (55 nmol/L)[164]. However, the prevalence of vitamin D deficiency in our study tended to be higher than was found in some other studies. For example, the prevalence of vitamin D deficiency between May and January was 14% in a cross-sectional study of healthy Norwegian adults living in Oslo[42], compared to 34% of participants in our study whose blood samples were collected during the same time. The Oslo study selected a random sample of participants aged 45, 60, and 75. Older

participants may be more likely to supplement with vitamin D[51]. A recent Canadian study reported a 20% overall prevalence of vitamin D deficiency in adults aged over 35, but a larger proportion of the Canadian study participants reported regular intake of vitamin D through supplementation or fortified food intake[165]. In contrast, an earlier study from our research group estimated that only 18% of HUNT participants took cod liver oil regularly[51]. The blood sample collection in our study (1995-97) was 10 years previous to the Canadian study (2005-07). However, results from a recent prospective study in the United States suggested high intra-individual reproducibility[166]. This may indicate that the two studies can be compared despite serum 25(OH)D levels being measured at different time points. On the other side, this intra-individual reproducibility may be lacking in high latitude populations undergoing a cultural shift resulting in a change away from traditional practices, including traditional diet. A new study of Arctic Inuit in Greenland showed a marked decline in serum 25(OH)D levels over 13 years, with a decrease in serum 25(OH)D concentrations ranging from 33% to 58%, depending on age and sex[43]. The authors suggest that a change away from traditional diet may be the main reason for this dramatic shift in vitamin D status. Despite documented belief that higher vitamin D status in Norway is due to a traditional diet[14], 82% of HUNT participants do not take cod liver oil regularly[51]. Like the Greenlandic population, departure from a traditional Norwegian diet may have influenced the overall vitamin D status in our study population.

5.4.2 Factors associated with vitamin D deficiency

We found that winter season and high BMI were the two strongest factors associated with vitamin D deficiency in our large random sample of a general Norwegian population. Our results also indicate that potentially modifiable lifestyle factors including intake of cod liver oil, physical activity, smoking and alcohol consumption were independently associated with vitamin D status. These associations persisted when we used serum 25(OH)D level <75nmol/L and <25nmol/L as additional cut-points.

Winter season was the strongest factor associated with vitamin D deficiency in our study, as found in many other studies[15, 42, 43, 62, 164]. This finding is not at all surprising considering the high latitude of Nord-Trøndelag county (64° North) and the lack of UVB radiation available at this latitude during winter season[1, 38]. High BMI was the second strongest factor associated with low vitamin D status in our study. Due to the cross-sectional design of our study we cannot infer causality, but the literature suggests a potentially harmful cycle between high BMI and low 25(OH)D levels. Vitamin D may be stored in adipose tissue due to its fat soluble character. In this way, high BMI leads to low vitamin D status[52, 167]. However, low vitamin D may lead to obesity due to the promotion of lipogenesis in adipocyte tissue[61, 147]. A recent study that used genetic markers as an instrumental variable to explore the causality and direction of the relationship between circulating 25(OH)D levels and BMI, suggested that higher BMI led to lower 25(OH)D levels, but the reverse was not true[54]. Further research is warranted to clarify the causal relationship between vitamin D status and BMI.

As expected, regular intake of cod liver oil was associated with higher serum 25(OH)D levels as shown in other studies[41, 45, 46]. Increased awareness around the benefits of taking cod liver oil, especially during winter months, could be an important public health message in Norway considering the long tradition of cod liver oil intake in the country, and the lower than expected proportion of HUNT participants who reported taking cod liver oil regularly.

Increased hours of light physical activity was associated with higher serum 25(OH)D levels, but physical activity may be a proxy measure for sun exposure due to increased leisure time spent outdoors, as found in other studies[165, 168, 169]. Still, some evidence suggests that increased physical activity may be a factor associated with vitamin D status independent of the effect of sun exposure[53]. Thus, the role of physical activity in modulating circulating 25(OH)D levels, independent of sun exposure, is a potential area for future research.

In our study, current smoking was associated with an increased prevalence ratio (PR) for vitamin D deficiency. Previous European studies have shown a positive association between smoking and vitamin D deficiency [58, 59, 170, 171]. One study in northern Norway found higher serum 25(OH)D levels in smokers, which the authors believed to be most likely due to measurement error and smokers were therefore excluded from further analysis[164]. Other studies found no association between smoking and vitamin D status [53, 60]. Cigarette smoke causes inflammation [121], and vitamin D may modulate an immune response triggered by smoking-induced inflammation[122]. This process may reduce the bioavailable concentrations

of serum 25(OH)D in smokers, resulting in lower levels of serum 25(OH)D available for measurement.

Interestingly, we found that more regular alcohol consumption was associated with higher levels of serum 25(OH)D. Since the publication of Paper I, at least two additional studies report a similar association in support of our finding[43, 172]. Although the mechanism for how alcohol consumption might affect serum 25(OH)D level is unclear, alcohol induced impairment of parathyroid hormone (PTH) secretion may be one reason[61]. PTH secretion increases the enzyme responsible for converting serum 25(OH)D to the biologically active form[61]. If this process is impaired, unconverted serum 25(OH)D may lead to higher serum 25(OH)D levels in the circulation when measured. However, this mechanistic theory is highly speculative and the association between serum 25(OH)D levels and alcohol consumption should be further evaluated using well defined variables including quantity, frequency and type of alcohol consumption.

None of the socio-demographic markers were substantially associated with vitamin D deficiency in this Norwegian population which seems plausible given the socio-political structure of Norway as a social democratic welfare state[173, 174]. Our results were consistent with findings from two other Norwegian studies in which no difference between women and men were found[42, 164]. Still, marginalized socio-demographic status has been identified as a risk factor for low vitamin D status in other populations[11, 171, 172]. Findings by socio-demographics in other studies may be related to more pronounced strata by social class in countries with socio-political structures that differ from Norway. Reduced health literacy, particularly in younger

age groups, may also explain some risk for vitamin D deficiency in these populations. Moreover, reduced health literacy in the youngest ages of our HUNT population, may partly explain why a significant proportion of younger people in our study had serum 25(OH)D level<25nmol/L, and why increasing age was associated with a reduced prevalence ratio in this <25nmol/L group, but not in our overall cohort. The potential association between socio-demographics and vitamin D deficiency in our study may also be mediated by lifestyle factors.

5.4.3 Serum 25(OH) D level and airway obstruction in adult asthma

In our study, low serum 25(OH)D level was not associated with airway obstruction in most adults with asthma, with the exception of a sub-group of men. In men with asthma but without allergic rhinitis, low serum 25(OH)D level was associated with a considerably reduced FEV_1/FVC ratio. Multiple imputation of missing data and a sensitivity analysis that excluded persons who reported ever COPD status gave similar results.

Although several studies have observed an association between serum 25(OH)D level and lung function in a general population of adults[104-106, 108, 137, 175], or in children with asthma[100, 176], prior studies in adult asthma cohorts are scarce. A small clinical trial of 54 US adults with persistent asthma reported an association between reduced continuous serum 25(OH)D with impaired FEV_1 and increased AHR[110]. A Chinese study of 435 adults with asthma found a significant association between vitamin D deficiency (<50 nmol/L) and low values for FEV_1/FVC ratio and FEV_1[111]. However, this study did not report sex-specific results.

Regarding a sex difference, a most recent report in children with asthma provided consistent results of an association between low plasma 25(OH)D levels with low FEV_1/FVC ratio and FEV_1 in boys[177]. Two studies in general adult populations provide additional support to our sex specific finding in adults with asthma[106, 108]. A study of 3359 Canadian adults observed an association between vitamin D deficiency (<50 nmol/L) and worse lung function (FEV_1 and FVC, but not FEV_1/FVC ratio) in men [108]. In the Longitudinal Aging Study Amsterdam, a strong association between serum 25(OH)D and peak expiratory flow rate (PEFR) was observed in older men but not in older women[106]. Our observation in asthmatic men but not women does not seem to be explained by type 2 error in women (false negative finding) due to a comparable number of women (n=446) and men (n=314) in our analyses. Asthmatic men in our study had lower lung function compared with asthmatic women (FEV_1/FVC ratio (%): men, 75±0.5; women, 78±0.4). Women with asthma in our study were more likely than men with asthma to report use of asthma medication, which may indicate greater compliance with recommended treatment for asthma and thus better lung function. However, a previous Canadian study indicated that sex may modify the association between asthma and lung function, i.e. the association of asthma with lower lung function was stronger in men than in women[178]. Even though a sex-specific finding seems plausible, further investigation and confirmation regarding the association between serum 25(OH)D and lung function in adult asthma is needed.

Our finding of an association between low serum 25(OH)D level and reduced FEV_1/FVC ratio in men with asthma but without allergic rhinitis is consistent with an

earlier study from our research group in which an association between low serum 25(OH)D and incident asthma was demonstrated only among men with no allergy status[91]. Furthermore, an association between low serum 25(OH)D levels and ever asthma in non-atopic subjects has been reported [90]. A recent genome-wide association study reported an association between T_H1 non-allergic pathway genes and lung function in asthmatic subjects[179], while lower serum 25(OH)D_3 levels have also been associated with thicker airway smooth muscle (ASM) mass in children with severe asthma [99]. The modulating effect of serum 25(OH)D on the contraction, inflammation and remodeling of ASM function[79] is one possible mechanism for airway obstruction in asthma subjects. In light of this evidence, we suggest that low serum 25(OH)D levels may be associated with airway obstruction and may influence the development of asthma, as well as asthma severity and control via the non-allergic pathway.

5.4.4 Serum 25(OH)D level, smoking and lung function changes in adults

In this general population of Norwegian adults, serum 25(OH)D levels <50nmol/L were associated with worse LF and increased odds for development of LF impairment compared to the ≥50 nmol/L group. These associations seemed stronger in the ever smoker group, amongst whom more LF decline in FEV_1/FVC ratio was also observed. Associations in never smokers were null. Cross-sectional and prospective results were consistent.

Several cross-sectional studies have reported an association between low vitamin D status and lower lung function in general adult populations[104, 108, 175]. To date, three prospective studies on vitamin D status and lung function changes in general

adult populations have been published[105, 114, 115], but findings from these studies lack consistency. Our findings were somewhat supported by Afzal et. al[115] who found a prospective association between smokers with lowest plasma 25(OH)D quintile and more decline in LF in comparison to smokers with highest plasma 25(OH)D quintile (difference in decline in FEV_1 % pred./year: 0.47, 95% CI: 0.38-0.56). Afzal et al. also report an association between low serum 25(OH)D levels and increased risk for development of COPD[115].These results were replicated in two independent samples. However, these findings have recently been criticized by Young and Hopkins[138]. First, Young and Hopkins suggest a threshold effect for the association between vitamin D status and lung function. Second, they cite concern with the overall estimates for an association between vitamin D status and FEV_1 % pred. which were consistently mirrored by a corresponding association between vitamin D status and FVC % pred., while no effect was observed for FEV_1/FVC ratio[138, 139]. In response to this critique, Afzal et. al state that the observed significant interaction suggests a stronger association between vitamin D status and FEV_1% pred. in individuals with airway obstruction[139]. Young and Hopkins theorize further that the reduction in lung function found by Afzal et. al, could be explained by the confounding or mediating effects of frailty in older persons or systemic inflammation, both of which correlate with low vitamin D status (serum 25(OH)D level <50 nmol/L)[138] . These critiques also have bearing on our results. Although it is true that increased systemic inflammation has been linked to greater LF decline[129], it is also true that cigarette smoke can directly induce systemic inflammation[128]. Our analysis was conducted overall, and separately in ever and never smokers. To some extent, we thereby controlled for systemic inflammation

caused by smoking. Furthermore, our study population consisted of relatively young participants aged 19-55 years, thus reducing the likelihood of confounding due to frailty. Finally, we observed a change in FEV_1 % pred. and FEV_1/FVC ratio <70%, which is the recommended cut-point to determine possible COPD[102], but we found no significant change in FVC % pred. However, due to the observational nature of our study and due to the fact that our study lacked post bronchodilator spirometry for COPD diagnosis, causal statements regarding the interrelationship between serum 25(OH)D level, smoking and COPD are not possible.

Our results are also consistent with those of a longitudinal cohort of elderly men in the US[105] that suggested a serum 25(OH)D level ≥50nmol/L may protect against lower LF and more rapid LF decline in smokers[105]. However, this US study included male only participants aged 21 to 80 years, some of whom had chronic conditions. Thus, a generalization of these findings to our general adult population may not be possible.

In contrast to our findings and to those of Afzal et. al[115], another prospective Danish study by Thuesen et. al[114] found higher levels of serum 25(OH)D to be significantly associated with adverse LF changes. Given that both Danish studies had large sample sizes derived from a general adult population in Copenhagen, the prospective results in opposite directions are not easily explained. Method-related differences based on different assays used for measurement of serum 25(OH)D levels may be one explanation. Like our study, Afzal et. al[115] used DiaSorin radioimmunoassay for serum 25(OH)D measurement, whilst Thuesen et. al[114] used liquid chromatography for serum 25(OH)D measurement. It has been suggested that

results given by different assay methods lack comparability, and that results from liquid chromatography are relatively high compared to those of DiaSorin radioimmunoassay[116].

Smoking is the main risk factor for COPD[119], and COPD is rarely reversible[180]. By using a standard cut-point to define airflow limitation (FEV_1/FVC ratio <70%)[102], our finding amongst ever smokers suggests that lower serum 25(OH)D levels may indicate a potential risk for future development of COPD. As mentioned above, at least one prospective study reported an association between lowest plasma 25(OH)D quintile and risk of COPD[115], although the accuracy of this finding is being debated[138, 139]. A small randomized trial of participants with moderate to very severe COPD and a history of recent exacerbations (n=182), reported a reduction in COPD exacerbations when high doses of vitamin D supplementation (100,000 IU every 4 weeks for 1 year) were given to trial participants with baseline serum 25(OH)D levels <25nmol/L[135]. To be noted this post hoc finding was only true for the <25nmol/L group, where no reduction in COPD exacerbations was found in the overall study population[135]. Moreover, a longitudinal study of COPD patients found no prospective association between baseline vitamin D status and rate of LF decline in slow versus rapid decliners[136]. Further evidence to fully elucidate the association between serum 25(OH)D levels, smoking and COPD, is needed. It is also important to gain an understanding on the relevance and efficacy of vitamin D supplementation as an intervention strategy to mitigate the risk of disease onset amongst smokers, or to reduce exacerbations amongst COPD patients.

6 Conclusions and future perspectives

Findings from this dissertation are derived from three original epidemiologic studies based on vitamin D status and serum 25(OH)D level as the exposure of interest, and lung function or lung function changes as outcomes of interest. Asthma, allergic rhinitis, smoking status, and COPD were additional key study elements.

Data from Paper I suggests a high prevalence of vitamin D deficiency (serum 25(OH)D level <50 nmol/L) in a Norwegian adult population. Besides season and BMI, several potentially modifiable lifestyle factors were independently association with vitamin D status. Regular intake of cod liver oil is a potential area for increased public health messaging in Norway.

In Paper II, we found no association between serum 25(OH)D level and lung function in most adults with asthma, with the exception of men with asthma but without allergic rhinitis. The observed interactions by sex and allergy status warrant further investigation and replication. A well-designed prospective study on serum 25(OH)D and lung function changes in an asthma cohort are called for.

In Paper III, data amongst never smokers showed no clear associations between serum 25(OH)D levels and lung function (cross-sectionally) or lung function changes (prospectively). However, lower serum 25(OH)D levels seemed to be associated with more lung function decline and increased odds for development of lung function impairment amongst ever smokers. These findings suggest that higher 25(OH)D concentrations may be partially protective against future development of COPD in smokers.

If confirmed by other studies, these results suggest that vitamin D supplementation may be a potentially cost effective, safe and straightforward means of asthma control and COPD prevention. Continued research on serum 25(OH)D levels and lung function changes in well designed prospective and intervention studies are called for. By measuring serum 25(OH)D levels in a large sample of Norwegian participants from the HUNT2 Study, our research group has built up serum 25(OH)D samples which can benefit future vitamin D research, including biomarker studies.

7 References

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Paper I

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