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Modulation of airway epithelial cell function by vitamin D in COPD

Schrumpf, J.A.

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Author: Schrumpf, J.A.

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CHAPTER

7

Impact of the local inflammatory environment on mucosal vitamin D metabolism and signaling in chronic inflammatory lung diseases

Jasmijn A. Schrumpf, Anne M. van der Does, Pieter S. Hiemstra

Department of Pulmonology, Leiden University Medical Center, Leiden,
The Netherlands

Abstract

Vitamin D plays an active role in the modulation of innate and adaptive immune responses as well as in the protection against respiratory pathogens. Evidence for this immunomodulatory and protective role is derived from observational studies showing an association between vitamin D deficiency, chronic airway diseases and respiratory infections, and is supported by a range of experimental studies using cell culture and animal models. Furthermore, recent intervention studies have now shown that vitamin D supplementation reduces exacerbation rates in vitamin D-deficient patients with chronic obstructive pulmonary disease (COPD) or asthma and decreases the incidence of acute respiratory tract infections. Vitamin D is known to contribute to the integrity of the mucosal barrier, promote killing of pathogens (via the induction of antimicrobial peptides) and to modulate inflammation and immune responses. These mechanisms may partly explain its protective role against infections and exacerbations in COPD and asthma patients. The respiratory mucosa is an important site of local vitamin D metabolism and signaling, a process that can be affected by exposure to inflammatory mediators. As a consequence, mucosal inflammation and other disease-associated factors, as observed in e.g. COPD and asthma, may modulate the protective actions of vitamin D. Here, we discuss the potential consequences of various disease-associated processes such as inflammation and exposure to pathogens and inhaled toxicants on vitamin D metabolism and local responses to vitamin D in both immune- and epithelial cells. We furthermore discuss potential consequences of disturbed vitamin D bioavailability for chronic lung diseases. Additional insight into the relationship between disease-associated mechanisms and local effects of vitamin D is expected to contribute to the design of future strategies aimed at improving local vitamin D bioavailability in chronic inflammatory lung diseases.

Introduction

Vitamin D is a pleiotropic hormone that is well known for its role in the regulation of calcium and phosphate homeostasis and bone mineralization. The receptor for vitamin D (VDR) is however expressed in nearly all tissues and cells and regulates a large number of genes (approximately 0.8-5% of the total genome) (1, 2). As a result, vitamin D affects many additional processes including cell proliferation and differentiation, apoptosis, DNA repair, ion transport, metabolism, cell adhesion, and oxidative stress responses (1, 3). Vitamin D deficiency (serum 25(OH)D < 50 nmol/L; 25(OH)D is the main circulating form of vitamin D and its levels are used to assess vitamin D status in the clinic (4, 5)) affects more than 30% of the children and adults worldwide and is a major cause of bone diseases such as rickets and osteoporosis (6). Increasing evidence has indicated that vitamin D deficiency is also associated with various other diseases such as cancer, cardiovascular disease, Alzheimer's disease and muscle myopathy, as well as several immune-related diseases such as type 1 diabetes, multiple sclerosis, inflammatory bowel disease (IBD), psoriasis and chronic inflammatory lung diseases including asthma, cystic fibrosis (CF) and chronic obstructive pulmonary disease (COPD) (6-9).

Several studies have now shown that vitamin D deficiency is prevalent in COPD patients and inversely correlated with lung function and severity of the disease (8, 10-12). It is currently unknown whether vitamin D deficiency is a cause or consequence of COPD, since many COPD patients have low physical activity levels and spend most time indoors (13). There are however studies suggesting that low vitamin D levels are associated with development of COPD, based on observed associations between polymorphisms in the vitamin D binding protein (VDBP), vitamin D serum levels and COPD severity (8, 10, 11, 14). In addition, one study in mice showed that maternal vitamin D deficiency can impair lung -development, -structure and -function in the offspring and suggests that even before birth, maternal 25(OH)D serum levels are important for a healthy lung development (15). This might be relevant, since associations have been found between lower childhood lung function and development of COPD later in life (16). The link between maternal 25(OH)D status and asthma development is however much clearer, since two recent randomized controlled trials (RCTs) have shown that maternal vitamin D supplementation reduces the risk of childhood asthma/recurrent wheeze (17). This might be explained by the fact that multiple

vitamin D-regulated genes are transcriptionally active during alveolar maturation and a number of these genes are differentially expressed in asthma (18). Additionally, this protective effect was linked to the GG-genotype of the 17q21 functional SNP rs12936231, which is associated with lower expression of *ORMDL3* and increased sphingolipid metabolism (19). Moreover, maternal circulating 25(OH)D levels affect the gut microbiota and can therefore indirectly modulate immune responses in the lung via the gut-lung-axis (20). Also later in life, optimal 25(OH)D levels remain crucial for keeping the lungs healthy. For example, Heulens *et al.* showed that subacute and chronic cigarette smoke (CS) exposure decreased lung function and promoted early signs of emphysema and airway inflammation in vitamin D-deficient mice compared to vitamin D-sufficient animals (21). Similarly in an elastase-induced COPD mouse model, topical administration of vitamin D in the lungs counteracted alveolar damage and improved lung function (22). Yet in humans, it is still unclear whether vitamin D affects COPD development and disease progression. Taken together, these observations suggest an important role for vitamin D during fetal and childhood lung maturation, and indicate that sufficient vitamin D levels might contribute to protection against development of childhood asthma and possibly COPD at older age.

Systemic levels of biologically active vitamin D are tightly regulated to preserve sufficient levels of calcium (Ca^{2+}) and phosphate (PO_4^{2-}) for optimal bone mineralization, whereas in mucosal tissues locally produced (autocrine) active vitamin D levels and signaling can be elevated or decreased upon exposure to inflammatory mediators, pathogens or inhaled toxicants (6). This could be important, since the inflamed airway mucosa of patients suffering from chronic inflammatory lung diseases is constantly exposed to these disease-associated factors (8, 23, 24). Impaired vitamin D bioavailability and signaling might have consequences for disease pathogenesis and progression. Dysregulated host defenses as found in patients with chronic inflammatory airway diseases include aberrant immune responses, altered microbiome composition, impaired epithelial barrier function and aberrant secretion of host defense molecules (25-27). Adequate vitamin D levels may provide protection against these dysregulated processes by maintaining the integrity of the mucosal barrier and promotion of killing of pathogens (e.g. via the induction of the antimicrobial peptide [AMP] hCAP18/LL-37) and via the modulation of both innate and adaptive immune responses (7, 28, 29).

In this review, we first discuss the effects of these disease-associated factors on local bioavailability of vitamin D and vitamin D-induced responses in the lung mucosa. In the second part of the review we will describe the mechanistic links between vitamin D deficiency and the pathogenesis of chronic inflammatory lung diseases such as asthma, CF and COPD, and discuss recent evidence related to the protective effects of vitamin D on COPD and on COPD exacerbations.

Regulation of mucosal vitamin D metabolism in health

Vitamin D enters the circulation either via food intake or as a result of its synthesis in the skin by UVB radiation. It subsequently binds to the VDBP (30, 31), after which this complex is transported to the liver where it is converted by vitamin D-25-hydroxylases (CYP2R1 and CYP27A1) into 25-hydroxy-vitamin D [25(OH)D]. However, recent studies showed that also other cell types such as airway epithelial cells, keratinocytes, intestinal epithelial cells, and monocytes/macrophages express CYP2R1 and CYP27A1, and thus are able to (locally) convert vitamin D₃ into 25(OH)D₃ (32, 33). This inactive 25(OH)D needs to be converted into the active 1,25 dihydroxy-vitamin D (1,25(OH)₂D) by 25-hydroxyvitamin D-1 α -hydroxylase (CYP27B1) in the kidney and in other cells, including several immune- and epithelial cells (34-40). 1,25(OH)₂D regulates expression of several genes by binding the nuclear VDR, which heterodimerizes with the retinoic acid receptor (RXR) to interact with vitamin D response elements (VDREs) that are present on the promoter region of these genes (1, 2). VDR is most abundantly expressed in intestinal enterocytes, pancreatic islets, renal distal tubules and osteoblasts, but is also present at lower levels in most other tissues and several other epithelial- and immune cells (41-45). Expression of VDR is classically regulated by 1,25(OH)₂D, growth factors and hormones such as FGF-23 and PTH respectively, circulating calcium-levels, bile acids, transcriptional co-activators/repressors, and genetic- and epigenetic modifications, which is tissue specific (46-49). 1,25(OH)₂D regulates its own negative feedback by several mechanisms, including induction of expression of the catabolic enzymes 25-hydroxyvitamin D-24-hydroxylase (CYP24A1) and CYP3A4 (50, 51). CYP24A1 is expressed in most tissues and converts both 25(OH)D and 1,25(OH)₂D into biologically inactive 24,25(OH)₂D and 1,24,25(OH)₂D

respectively (50), whereas CYP3A4, mainly expressed in the liver and small intestines, contributes to the metabolic clearance of 25(OH)D and 1,25(OH)₂D by converting 25(OH)D into 4β,25(OH)₂D, and 1,25(OH)₂D into 1,23R,25(OH)₂D or 1,24S,25(OH)₂D (51). Expression of both CYP27B1 and CYP24A1 in the kidneys is tightly regulated to maintain optimal Ca²⁺- and PO₄²⁻ levels in the circulation, which are important for bone mineralization(52). The complex mechanisms that explain how vitamin D and its metabolic enzymes maintain sufficient Ca²⁺ and PO₄²⁻ levels in the circulation are explained by Quarles *et al.* (52). In summary, it has become increasingly evident that the effects of vitamin D are not limited to homeostasis of Ca²⁺ and PO₄²⁻ and bone mineralization, because several extra-renal cells such as airway epithelial cells and immune cells express the VDR and are capable of converting circulating 25(OH)D into the active 1,25(OH)₂D metabolite (**Figure 1**).

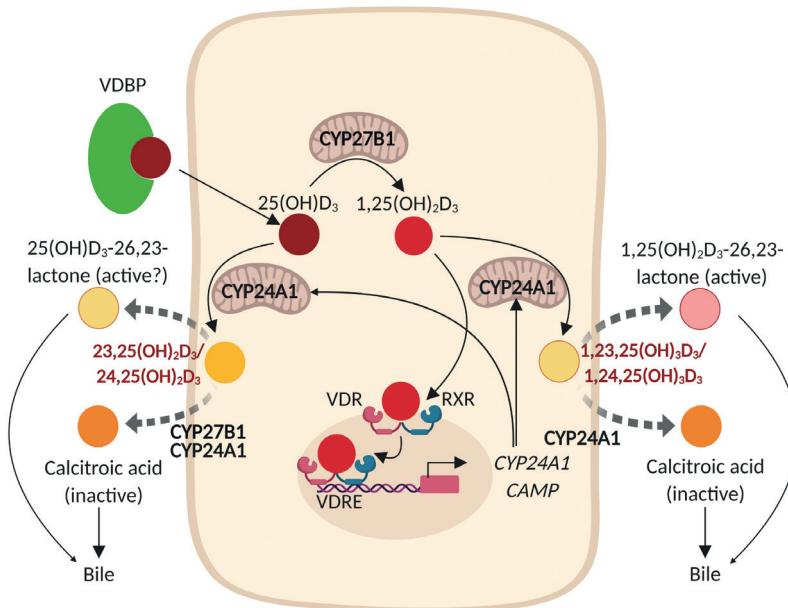


Figure 1. Vitamin D metabolism and expression of hCAP18/LL-37 (CAMP) in epithelial cells. The vitamin D binding protein (VDBP)-25(OH)D complex enters the epithelial cell from the circulation and 25(OH)D is subsequently released from the complex. In the cytoplasm, 25(OH)D is hydroxylated by 25-hydroxyvitamin D-1α-hydroxylase (CYP27B1; localized to the inner mitochondrial membrane) into the active metabolite 1,25(OH)₂D. 1,25(OH)₂D subsequently binds to the nuclear vitamin D receptor (VDR) which heterodimerizes with the retinoic acid receptor (RXR) to interact with vitamin D response elements (VDREs) that are present on the promoter region of numerous genes, including *CAMP* (hCAP18/LL-37) and *CYP24A1* (25-hydroxyvitamin D-24-hydroxylase). 1,25(OH)₂D thereby regulates its own negative feedback via direct induction of *CYP24A1* that converts both 25(OH)D and 1,25(OH)₂D to biologically inactive 24,25(OH)₂D and 1,24,25(OH)₂D respectively (53).

Altered regulation of mucosal vitamin D metabolism and vitamin D responsiveness in chronic inflammatory airway diseases

Local bioavailability and activity of 1,25(OH)₂D are in part determined by expression of VDR and the equilibrium between the vitamin D metabolic enzymes CYP27B1 and CYP24A1. It is important to realize that mucosal expression of CYP24A1, CYP27B1 and also VDR can be affected by several disease-associated inflammatory mediators, toxicants and pathogens, summarized in **Table I**. As a consequence of this, the local bioavailability of vitamin D in tissues such as the inflamed airways of patients that suffer from chronic inflammatory airway diseases might be reduced.

Cell/tissue type	Stimulus	Effect	References
Primary airway epithelial cells	Poly(I:C); RSV; IL-13; IL-4; PM	CYP27B1 ↑	(38, 54-56)
	TNF-α; IL-1β; IL-17A; TGF-β1; NTHi	CYP24A1 ↑	(57, 58)
	CSE	CYP27B1 ↓	(59, 60)
	<i>A. fumigatus</i> ; HRV; RSV	VDR ↓	(56, 61)
BEAS-2B (bronchial epithelial cell line)	HRV; RSV	VDR ↓	(56)
16HBE (bronchial epithelial cell line)	PM	VDR ↑	(55)
	<i>A. fumigatus</i>	VDR ↑	(62)
A549 (lung carcinoma cell line)	TGF-β1	CYP27B1 ↑	(54)
	<i>A. fumigatus</i>	CYP27B1 ↑	(62)
HCT116 (colon cancer epithelial cell line)	CSE	VDR translocation ↓	(63)
	LPS; TNF-α	CYP27B1 ↑	(64)
	LPS; TNF-α	VDR ↓	(64, 65)
COGA-1A (colon cancer epithelial cell line)	LPS	CYP24A1 ↓	(64)
	TNF-α ± IL-6	CYP27B1 ↓	(66)

Trophoblasts	TNF- α ; IL-1 β ; IL-6	CYP24A1 \uparrow	(67)
	IFN- γ	CYP27B1 \uparrow	(67)
Macrophages	ss-RNA	CYP27B1 \uparrow	(68)
Macrophages (derived from THP-1)	CSE	VDR \uparrow	(69)
		VDR \uparrow	
Macrophages (derived from THP-1)	BaP	CYP24A1 \uparrow	(70)
Monocytes	TLR2/1L \pm IFN- γ ; LPS; IL-15	CYP27B1 \uparrow	(39, 71-73)
		VDR \uparrow	
	IL-4 \pm TLR2/1L	CYP24A1 \uparrow	(39)
Neutrophils	IFN- γ	CYP27B1 \uparrow	(74)
	<i>S. pneumoniae</i> T4R	VDR \uparrow	
T cells	T cell activators (anti-CD3/anti- CD28; PHA; PMA/ionomycin)	CYP27B1 \uparrow	(75)
		VDR \uparrow	
B cells	B cell activators (anti-IgM/anti- CD40/IL-21)	CYP27B1 \uparrow	(76)
		VDR \uparrow	

Table 1: Effects of inflammatory mediators on the expression of VDR, CYP24A1 and CYP27B1 in immune cells and epithelial cells.

Abbreviations: Polyinosinic:polycytidylic acid (Poly[I:C]); Particulate matter (PM); nontypeable *Haemophilus influenzae* (NTHi); *Aspergillus fumigatus* (*A. fumigatus*); Cigarette smoke extract (CSE); Human rhinovirus (HRV); Respiratory syncytial virus (RSV); Single stranded RNA (ssRNA); Benzo[a]pyrene (BaP); Toll like receptor 2/1 Ligand (TLR2/1L); Phytohemagglutinin (PHA); Phorbol 12-myristate 13-acetate (PMA).

Epithelial cells

Chronic lung diseases are characterized by airway inflammation and impaired respiratory host defense, which is illustrated by the increased susceptibility for respiratory infections and exacerbations (77-79). Furthermore, exposure to inhaled toxicants such as cigarette smoke and air pollutants are associated with disease pathogenesis and exacerbations in COPD, CF and in asthma patients (80-82). It would therefore be of great interest to investigate these effects on vitamin D bioavailability and vitamin D-mediated respiratory host defense in the airway mucosa. Studies in airway epithelial cells have shown that exposure to UV-inactivated nontypeable *Haemophilus influenzae* (NTHi) increased expression of the vitamin D-degrading enzyme CYP241, whereas exposure to viral double stranded-RNA analogue polyinosinic:polycytidylic acid (Poly[I:C]) increased expression of CYP27B1 and conversion of 25(OH)D into the active metabolite (38,

57). On the other hand in the bronchial cell line BEAS-2B, expression of VDR was decreased after infection with respiratory viruses such as human rhinovirus (HRV) and respiratory syncytial virus (RSV) (56). Collectively, these studies have shown in airway epithelial cells that respiratory viral- and bacterial infections can either promote or impair vitamin D activation and responses.

A local airway inflammatory milieu can also exert differential effects on vitamin D bioavailability and signaling, dependent on the type of inflammatory mediators that are predominantly present. We have shown in differentiated primary airway epithelial cells that Th2 cytokines such as IL-4 and IL-13, enhance expression of CYP27B1 and vitamin D-mediated expression of hCAP18/LL-37, which suggests that a Th2-inflammatory environment, as found in allergic airway inflammation increases vitamin D bioavailability (81, 83). The observation that levels of both 1,25(OH)₂D and hCAP18/LL-37 were increased in bronchoalveolar lavage (BAL) after allergen challenge is in line with this proposed mechanism (84). This effect of Th2 cytokines was in contrast to the effects (chronic) exposures to the proinflammatory cytokines IL-1 β , TNF- α and IL-17A that strongly increased the expression of the vitamin D-degrading CYP24A1, even in absence of vitamin D (57). Furthermore, short-term exposures to TGF- β 1, a pleiotropic growth factor which is elevated in the lungs of COPD, CF and asthma patients, also increases the expression of CYP24A1 (85). As a consequence, 1,25(OH)₂D-mediated expression of the AMP hCAP18/LL-37 was impaired, which was likely the result of the enhanced degradation of both 25(OH)D and 1,25(OH)₂D by this enzyme (57, 58). In addition to pathogens and cytokines, exposure to inhaled toxicants such as cigarette smoke (CS) and particulate matter (PM) may also alter expression or activity of VDR and CYP27B1. Studies have demonstrated that cigarette smoking or exposure to CS extract (CSE) decreases expression of CYP27B1 and inhibited membrane bound (m)VDR translocation to the cell membrane in airway epithelial cells and A549 cells (an alveolar tumor cell line) respectively (59, 60, 63) This inhibition reduces the conversion of 25(OH)D to 1,25(OH)₂D and 1,25(OH)₂D-mediated gene expression as well as non-genomic actions of 1,25(OH)₂D-membrane associated, rapid response steroid-binding (MARRS)- signalling (59, 60, 63). This adverse effect of cigarette smoking on vitamin D bioavailability and effects in airway epithelial cells was recently confirmed *in vivo* by Vargas Buonfiglio *et al.*, who demonstrated that vitamin D supplementation increased antimicrobial activity in apical surface liquid (ASL) in the airway of healthy non-smokers, but not in smokers (59). On the other hand, exposure to PM increases the expression of both CYP27B1 and VDR in airway

epithelial cells, thereby possibly promoting vitamin D bioavailability (55). It is however important to consider that several retrospective and observational studies have demonstrated that air pollution is an independent risk factor for developing vitamin D deficiency (86). In conclusion, exposure to CS, TGF- β 1 and presence of a proinflammatory milieu appeared to most strongly decrease vitamin D bioavailability and signaling in airway epithelial cells.

Immune cells

Whereas various studies show that exposure to proinflammatory stimuli affects vitamin D metabolism and reduces the effects of vitamin D in (airway) epithelial cells, the opposite appears to be the case for immune cells. In monocytes, macrophages and neutrophils, effects on vitamin D bioavailability and vitamin D-mediated antimicrobial responses were generally enhanced by these proinflammatory stimuli as illustrated by increased expression of both VDR and CYP27B1 (39, 68, 71-74). It is therefore tempting to speculate that this apparent increase in vitamin D-mediated antimicrobial responses in immune cells in an inflammatory environment may serve as a second line of defense and compensate for the enhanced epithelial degradation of vitamin D during inflammation. Inhaled toxicants may also affect vitamin D responsiveness of immune cells. This is illustrated by two recent studies studying the effects of cigarette smoke on the human monocyte/macrophage-like cell line THP-1. One study showed that treatment with cigarette smoke extract (CSE) increased the expression of VDR without enhancing vitamin D responses (69), while the other study -that focused on the effects of Benzo[a]pyrene (BaP) (a component produced by cigarette combustion)- demonstrated that vitamin D-mediated CYP24A1 expression was induced, which was found to further enhance degradation of 1,25(OH)₂D (70). In summary, proinflammatory stimuli generally increased vitamin D responses and bioavailability in immune cells, whereas more studies are needed to fully determine the impact of exposure to cigarette smoke and other inhaled toxicants.

Lung mucosa

Whereas these studies provide evidence that inflammation and inhaled toxicants may affect vitamin D metabolism and responsiveness in epithelial cells and immune cells, it is not clear whether this has an impact on these events in lung tissue of patients with chronic lung diseases. Although evidence is limited, we can speculate that vitamin D bioavailability and responses are also affected by disease-associated factors in mesenchymal cells that are present in the lung mucosa. One study that

showed in a bleomycin fibrosis model and in primary lung mouse fibroblasts that TGF- β 1 reduced expression of the VDR might support this assumption (87). It is currently insufficiently studied whether exposures to disease-associated factors promote or impair vitamin D bioavailability and responses in immune-, mesenchymal and epithelial cells combined to give a better reflection of the *in vivo* situation. Interestingly, one study did already show that nasal CYP27B1- and 1,25(OH) $_2$ D-levels are both reduced in chronic rhinosinusitis (CRS) patients with nasal polyps as compared to CRS-patients without nasal polyps, whereas no difference was found in circulating 1,25(OH) $_2$ D-levels (88). Since most other studies were performed *in vitro* using monocultures of epithelial cells or immune cells, more complex models are needed to delineate this. Therefore, animal models or preferably more complex animal-free cell culture models using co-cultures or organ-on-a-chip models of primary fully differentiated epithelial cells, airway-derived fibroblasts or smooth muscle cells and immune cells could be considered in future studies.

7

Protective effects of vitamin D on mucosal homeostasis

After discussing altered vitamin D metabolism and responsiveness in the inflamed airway mucosa, it is important to consider the possible consequences of these inflammation-induced changes in the airway mucosa keeping in mind the pleiotropic effects of vitamin D that were introduced earlier. In several cells, tissues and organs, vitamin D regulates multiple cellular processes that affect normal and malignant cell growth and differentiation (89, 90). Vitamin D displays furthermore protective effects on mucosal host defense by maintaining the integrity of the epithelial barrier, inhibition of epithelial-to-mesenchymal transition (EMT), stimulating production of AMPs and modulating both innate- and adaptive immune functions (29, 91, 92). In addition, vitamin D maintains both energetic and survival homeostasis in the mucosal epithelium through the modulation of stress and damage responses, including clearance of disturbing and stressful agents (3, 93) (**Figure 2**).

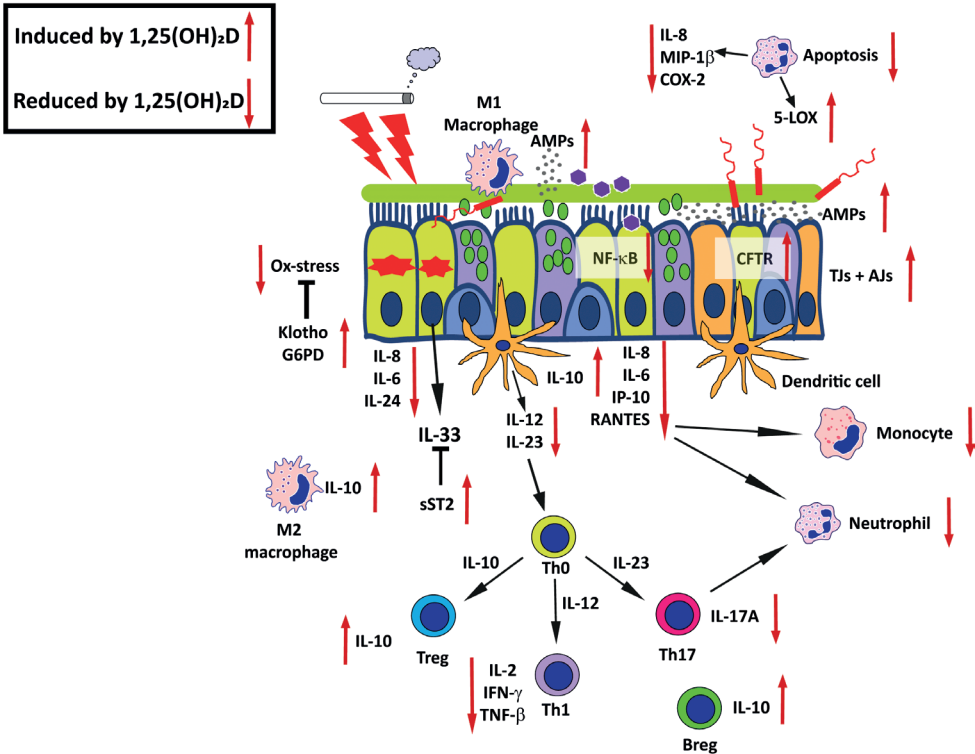


Figure 2. Effect of vitamin D on airway epithelial host defense-mechanisms in chronic airway disease. The promoting or inhibitory effects of vitamin D are indicated by the red arrows.

Abbreviations: Antimicrobial peptides (AMPs); Cystic fibrosis transmembrane conductance regulator (CFTR); Oxidative stress (Ox-stress); Suppressor of cytokine signaling proteins (SOCS); Tight junctions (TJs); Adherens junctions (AJs); Glucose-6-phosphate dehydrogenase (G6PD); Soluble suppression of tumorigenicity 2 (sST2); Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB); Naive T cell (Th0); Regulatory T cell (Treg); T helper type 1 cell (Th1); T helper type 2 cell (Th2); T helper type 17 cell (Th17); Regulatory B cell (Breg); See text for details and references.

Epithelial barrier function

In chronic inflammatory lung diseases, epithelial barrier function is impaired, and as a consequence the susceptibility towards respiratory infections is increased (94). There is increasing evidence that vitamin D promotes epithelial barrier integrity or protects against epithelial barrier destruction. In cells of the bronchial epithelial cell line 16HBE, vitamin D inhibited CSE-mediated reduction of the epithelial barrier and expression of E-cadherin and β -catenin (95). Recently, two murine studies were published that investigated the effects of vitamin D on pulmonary epithelial barrier function. Shi *et al.* showed that vitamin D-supplementation alleviated lung injury in LPS-treated mice through maintenance of the pulmonary barrier by inducing expression of Zonula occludens (ZO)-1 and occludin in whole lung homogenates (96), whereas Gorman *et al.* showed in healthy mice, fed with a vitamin D-poor diet, that vitamin D supplementation had little effect on epithelial integrity (97). Only the first study that used a more severe mouse model with higher levels of inflammation and edema found an effect on vitamin D on epithelial barrier function. Since inflammation is detrimental for epithelial barrier integrity (98), it cannot be excluded that the main protective effects of vitamin D on the epithelial barrier in the first study by Shi *et al.* were in fact exerted through inhibition of inflammation rather than via direct induction of cell junction proteins. Vitamin D might also promote epithelial barrier function through its ability to increase expression of cystic fibrosis transmembrane conductance regulator (CFTR) in airway epithelial cells (99). CFTR maintains optimal ASL- and mucus hydration, volume and pH that support mucociliary clearance and activity of AMPs (100). Moreover, CFTR is also affected in the airways of smokers and COPD patients (101). In summary, these studies indicate that vitamin D promotes both the integrity and function of the epithelial barrier and might additionally protect against epithelial damage by dampening inflammatory responses.

Anti-fibrotic effects of vitamin D

The loss of epithelial barrier function with a decrease in epithelial polarization and cell-junction proteins and a gain of expression of mesenchymal markers is a hallmark of EMT (94). EMT is primarily involved in development, wound healing and stem cell differentiation, and TGF- β signaling plays a major role in this process (102). Elevated TGF- β 1 levels are found in the lungs of patients with chronic inflammatory lung diseases and this was associated with cigarette smoking, inflammation and fibrosis (77, 103). There are indications that vitamin D counteracts various pathways leading to EMT. In mouse models and in airway

epithelial cell lines, vitamin D has been shown to inhibit EMT and fibrosis, in particular when this process is induced by TGF- β 1 (87, 104-107).

Effects of vitamin D on epithelial antimicrobial responses

In addition to maintenance of the epithelial barrier and inhibition of fibrosis as discussed in the previous paragraphs, vitamin D is also actively involved in respiratory host defense by a variety of mechanisms (3, 29). Vitamin D is an important inducer of AMPs, which are mostly cationic peptides that have a broad-spectrum antimicrobial activity, the ability to modulate immune responses and to promote epithelial wound repair and angiogenesis (108). hCAP18/LL-37 is likely to be the most prominent AMP that is induced by vitamin D and is expressed in several types of mucosal epithelial cells and immune cells such as monocytes and neutrophils (38, 74, 109). In macrophages and intestinal epithelial cells, vitamin D also increases expression of human β -defensin-2 (hBD-2), whereas in keratinocytes expression of both hBD-2 and human β -defensin-3 (hBD-3) is increased by vitamin D (110-113). Collectively these data show that AMPs are modulated by vitamin D in mucosal tissues, which could have impact on susceptibility to both bacterial and viral infections and on the composition of the microbiota, which will be discussed in the next section .

Effects of vitamin D on innate and adaptive immune responses

Diseases such as COPD and asthma are characterized by chronic inflammation, a low-grade and prolonged inflammation that may result in destruction and aberrant repair of surrounding tissue by growth factors, proteases and cytokines that are released at the site of inflammation (114-116). Cumulative data suggest that vitamin D exerts anti-inflammatory effects via its actions on both innate and adaptive immune responses. Upon viral infection or exposure of pro-inflammatory stimuli such as Poly(I:C) or PM, vitamin D attenuates induced expression of cytokines and chemokines e.g. via inhibition of nuclear factor (NF)- κ B or oxidative stress respectively in (airway) epithelial cells (38, 55, 117). Furthermore, vitamin D increases expression of the soluble decoy receptor for IL-33 (sST2) by airway epithelial cells, which in turn inhibits the actions of the type 2 alarmin IL-33 (118). Further effects of vitamin D on local innate and adaptive immune responses in the epithelial mucosa are mediated through its actions on immune and structural cells and have been reviewed by Heulens *et al.*, Vanherwegen *et al.* and Pfeffer *et al.* (29, 119, 120).

Taken together, these findings suggest that on the one hand vitamin D protects against infections by enhancing epithelial barrier function and production of AMPs, and on the other hand vitamin D induces tolerance and dampens proinflammatory responses in various cell types of the airway mucosa. Thereby, vitamin D may prevent exaggerated inflammatory responses and further damage to the mucosal tissue, qualities that are very relevant in the context of chronic inflammatory (lung) diseases (**Figure 2**).

Effects of vitamin D on epithelial oxidative stress and aging

COPD is considered to be a disease of accelerated ageing lungs, underscored by markers of aging being increased in these patients partly as a result of oxidative stress (121). Evidence that vitamin D may protect epithelial cells from oxidative stress was provided by Pfeffer *et al.*, who demonstrated that vitamin D increased expression of the antioxidant gene *G6PD* in airway epithelial cells. Furthermore, vitamin D increased the ratio of reduced to oxidized glutathione and decreased the formation of 8-isoprostane after exposure to PM (55). The induction of *klotho* by vitamin D might be another vitamin D-mediated anti-ageing mechanism (122). *Klotho* is an anti-ageing protein that is mainly expressed in the kidney, brain and in the lung by airway epithelial cells and exerts its protective effects through the inhibition of inflammation, insulin/IGF-1 signaling and activation of forkhead transcription factor (FoxO) signaling, which enables removal of reactive oxygen species (ROS) (123-125). Expression of *klotho* is impaired in the airways of smokers and further decreased in the airways of COPD patients and in cultures of the bronchial epithelial cell line 16HBE after CSE exposure (125). These studies suggest that vitamin D may protect against ageing via inhibition of oxidative stress and possibly via its ability to restore *klotho* expression (**Figure 2**). However, direct evidence showing that vitamin D indeed increases expression of *klotho* in airway epithelial cells is currently lacking.

Effects of vitamin D on epithelial autophagy and apoptosis

In addition to providing protection against oxidative stress and ageing, data from studies using intestinal epithelial cells suggest that vitamin D may also promote cellular survival via the induction of autophagy and reduction of apoptosis (126, 127). In chronic inflammatory lung diseases, aberrant activation of autophagy plays a role in disease pathogenesis (128). A recent study showed that club cells and autophagy-related proteins were both decreased in COPD patients and that these proteins were important for club cell structure and function in airways (129).

However, the effects of vitamin D on autophagy in the airway mucosa of chronic inflammatory lung diseases are still unclear and need to be further evaluated (128).

Role of vitamin D in the treatment of chronic airway diseases

Clearly vitamin D has pivotal actions in host defense that are relevant in the context of chronic inflammatory lung diseases, in which vitamin D deficiency may be prevalent. Strategies to promote local bioavailability of vitamin D or use it as a treatment itself could be therefore of interest. Here, we will discuss the latest clinical evidence accompanied with functional *in vitro*- and animal studies that may explain the effects of vitamin D treatment on typical hallmarks of chronic airway diseases.

Effect of vitamin D on inhaled corticosteroid responsiveness in chronic airway diseases

Currently, inhaled corticosteroid (ICS)-use with or without long acting bronchodilators is the most frequently used treatment for COPD and asthma patients (130). However, the response to corticosteroids is not always effective in many COPD patients and in patients with steroid resistant (SR)-asthma (131). There are several complex mechanisms that underlie the resistance to corticosteroids in both COPD and SR-asthma that include but are not limited to genetic background, impaired glucocorticoid receptor binding, T helper type 17 cell (Th17)-inflammation and oxidative stress (e.g. from air pollution or smoking) and decreased numbers of IL-10 secreting regulator T cells (Tregs), which normally prevent skewing towards Th17-inflammation (131). Direct evidence of the ability of vitamin D to reverse SR was provided by a study showing that *ex-vivo* stimulation with 25(OH)D promoted generation of IL-10-secreting Tregs which restored sensitivity towards corticosteroids in CD4+ T cells that were derived from SR-asthma patients (132). A further potential treatment role of vitamin D was elegantly illustrated by studies that showed that vitamin D deficiency is associated with decreased steroid responsiveness in asthmatics and by the fact that several potential underlying mechanisms of SR such as oxidative stress and Th17-mediated inflammatory responses could be reversed by vitamin D treatment (55, 133-138). Interestingly,

the corticosteroid dexamethasone was shown to increase expression of the vitamin D degrading enzyme CYP24A1 in renal cells and osteoblasts (139), which suggests a bidirectional interaction between corticosteroids and vitamin D and could further limit its bioavailability for patients. Additional research is needed to determine if vitamin D may also improve corticosteroid responsiveness in COPD.

Vitamin D and exacerbations in COPD

Exacerbations are a major burden for COPD patients, they accelerate decline in lung function and frequently result into hospital admissions (140, 141). Exacerbations are often triggered by pollutants or by bacterial- and/or viral infections (80, 142, 143). COPD patients generally have lower serum 25(OH)D-levels than age- and smoking-matched controls, which is associated with more and more severe exacerbations (8, 10). Several *in vivo* and *in vitro* studies have provided evidence that explain the protective effects of vitamin D on exacerbations in COPD patients and this will be discussed accordingly.

Air pollution

First of all, Pfeiffer and colleagues showed that 25(OH)D and 1,25(OH)₂D reduce the production of proinflammatory cytokines in part via the ability to enhance antioxidant responses in airway epithelial cells that were exposed to PM (55). This was also demonstrated in human DCs that were matured in presence of PM, where treatment with 1,25(OH)₂D counteracted the expansion of proinflammatory IL-17A⁺ and IFN- γ ⁺ Th17.1 cells (136). In line with this, Bolcas *et al.*, showed that vitamin D supplementation counteracted the development of airway hyperresponsiveness and accumulation of Th2/Th17 cells in mice that had been repeatedly exposed to both diesel exhaust and house dust mite allergens (144). Vitamin D could therefore exert a protective role in air pollution-triggered exacerbations.

Respiratory viral infections

In addition to its protective effects against pollutants, there is also increasing evidence that vitamin D may enhance clearance of respiratory viral infections that account for 30-50% as underlying cause of exacerbations in COPD patients (145). Infections with respiratory viruses such as HRV, coronaviruses and to a lesser extend respiratory syncytial virus (RSV) and (para)influenza virus are present during exacerbations and may predispose the host towards secondary bacterial infections that can eventually lead to uncontrolled bacterial outgrowth, more severe exacerbations and neutrophilic inflammation (145, 146). Two recent *in vitro* studies

showed that acute exposure to relatively high doses (100 - 1000 nM) of 1,25(OH)₂D reduced HRV-infection in undifferentiated cultures of airway epithelial cells (56, 147). In those models, vitamin D most likely interfered with viral replication by increasing expression of interferon-stimulated genes and expression of hCAP18/LL-37, which has been shown to have direct antiviral activity (56, 147, 148). In fully differentiated airway epithelial cells, treatment with lower concentrations of 1,25(OH)₂D (10 nM) during epithelial differentiation had no effect on acute HRV infection (149). As for other viruses than HRV, both Hansdottir *et al.* and Telcian *et al.* showed that vitamin D did not decrease RSV infection in airway epithelial cells, but did reduce virus-induced inflammatory responses (56, 117). In addition, two other studies reported in influenza (H9N2 and H1N1)-infected A549 cells comparable findings (150, 151). Moreover, inhibitory effects of vitamin D on poly(I:C)-induced inflammatory responses were furthermore confirmed in primary airway epithelial cells Hansdottir *et al.* and by our group (38, 83). Up to now, the afore mentioned studies suggest that higher doses of vitamin D might be protective against HRV-infections in undifferentiated airway epithelial cells only, whereas for other respiratory viral infections vitamin D mainly reduces inflammatory responses without affecting viral clearance. However, more studies are needed, especially in differentiated airway epithelial cells using multiple HRV-serotypes that use different receptors for infection to verify if vitamin D indeed is capable of promoting HRV-clearance. There is more consensus about vitamin D reducing virus-induced inflammatory responses and this may certainly help to alleviate the burden of exacerbations in COPD (38, 83).

Bacterial infections

In addition to viral infections, also bacterial infections are associated with COPD exacerbations and account for approximately 50% of all exacerbations (152). Due to improved study design and sampling techniques from the lower airways using bronchoscopy in recent decades, the causative role of bacteria in COPD-related exacerbations has become clear (152). This was additionally supported by Sethi *et al.*, who found that acquisition of a new strain of pathogenic bacterial species into the airways was linked to COPD exacerbations (153). Recent developments in assessing the airway microbiota using 16S rRNA sequencing techniques further demonstrated that during exacerbations, the relative abundance of Haemophilus, Pseudomonas, and Moraxella was increased and the microbial composition was shifted towards the Proteobacteria phylum (143). The ability of vitamin D to promote antibacterial activity was recently demonstrated in cultures of airway

epithelial cells. In differentiated airway epithelial cells, we have shown that vitamin D treatment enhances epithelial expression of hCAP18/LL-37 and antibacterial activity against NTHi, a Gram-negative bacterium, which is associated with COPD exacerbations (57, 154). In addition, Yim *et al.* demonstrated that vitamin D treatment increased expression of the AMP hCAP18/LL-37 and killing of *Pseudomonas aeruginosa* and *Bordetella bronchiseptica*, which are both Gram-negative bacteria (155). These observed antibacterial effects of vitamin D on airway epithelium *in vitro* were recently confirmed *in vivo* by Vargas Buonfiglio *et al.*. The authors demonstrated that vitamin D supplementation increased antimicrobial activity against the Gram-positive *Staphylococcus aureus* in ASL in healthy non-smokers and was dependent on presence of hCAP18/LL-37 (59).

In murine airways, studies showed no effects of vitamin D on the expression of *Defb4* or *mCramp* (the murine homologue for *CAMP*) (156). This can be explained by the fact that both the promoters of *mCramp* and *Defb4* lack VDREs, suggesting that mice might not be suitable for studying the role of vitamin D in AMP-mediated host defense in infection (157). Indeed, Niederstrasser and colleagues showed no effects of vitamin D deficiency on the susceptibility of mice to pulmonary infection with *Streptococcus pneumoniae* or *Pseudomonas aeruginosa* (158). However, in a recently developed mouse model by Lowry *et al.*, who transfected *mCramp* knockout mice with the human *CAMP* gene, topical vitamin D treatment increased expression of *CAMP* and promoted antibacterial effects on the mucosa of the skin (159). There are also multiple other murine studies that demonstrate protective effects of vitamin D on bacterial infections in the gut, indicating that vitamin D-mediated antibacterial effects are additionally modulated by other mechanisms such as via enhancement of epithelial barrier integrity (64, 160). In conclusion, these observations show that vitamin D promotes protection against pollutants and enhances clearance of viral- and bacterial infections (both Gram-positive and negative bacteria) in combination with a dampening effect on exaggerated immune responses and these features might explain why vitamin D (deficiency) is linked to COPD exacerbations.

Modulation of microbiota by vitamin D

There are strong indications that modulation of immune responses and antibacterial activities by vitamin D and/or vitamin D-regulated AMPs as well as autophagy have implications for the composition of the microbiota at the epithelial mucosa of the airways and the gut (161). Evidence for a role of AMPs in regulating

the composition of the microbiota in the gut came from a variety of studies, including those showing that Paneth cell-derived defensins may modulate the composition of the microbiome (162). This notion is further supported by observations showing that many commensal gut bacteria are protected from killing by AMPs such as the vitamin D-inducible hCAP18/LL-37 and hBD-2, whereas pathogens are in general more sensitive (163). Alterations in the gut microbiota have been linked to many diseases of the gut such as IBD but also with diseases affecting the lungs such as COPD and asthma, implicating an important role for the so-called gut–lung axis (164, 165). The mechanisms that explain how gut microbiota affect lung health and disease are complex and include the production of short chain fatty acids (SCFAs). SCFA have a wide range of effects on both immune and structural cells, and the effect of SCFA produced in the intestine on lung immunity may in part be explained by modulation of myeloid cells in the bone marrow, which subsequently migrate to the airways and modulate local immune responses (165). Microbiota that are diverse, rich and contain a higher abundance of SCFA-producing species within these populations are considered to be associated with health (166). In the gut there is strong evidence that both vitamin D deficiency and/or supplementation affect composition of the adult and infant microbiota (166, 167), specifically in relation to disease (168). However, due to the limited number of RCTs and small sample sizes, the precise effects on the microbiota and the mechanisms involved in this are still unclear (166). Alterations in the lung microbiota are also observed in COPD and asthma patients and are likely the result of environmental exposures, airway remodeling, infections and treatments such as the use of antibiotics. This may contribute to disease pathogenesis through altered epithelial innate and adaptive immune responses that damages the airway epithelial barrier and provokes further changes in the lung microbiome that accumulates with increasing disease severity (169, 170). To date only 2 studies describe a possible influence of vitamin D on composition of the microbiota in the airways (171, 172). Toivonen *et al.* showed an association between low serum 25(OH)D-levels and reduced richness of the nasopharyngeal microbiota and bronchiolitis severity in patients with low 25(OH)D-levels (171), whereas in another study vitamin D supplementation decreased the abundance of *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Corynebacterium* species in sputum samples in vitamin D-deficient CF patients compared to sufficient CF patients (172). In summary, there is evidence that alterations in the airway or gut microbiota can affect chronic airway disease and that these changes could be related to both

vitamin D deficiency and/or supplementation. However, due to the limited number of RCTs and small sample sizes more RCTs are needed in larger patient populations.

Effect of vitamin D supplementation on chronic airway diseases

COPD

The above described protective and therapeutic possibilities of vitamin D, together with observations that many COPD patients are vitamin D deficient, suggest that COPD patients might benefit from vitamin D supplementation. As discussed elsewhere in this review, the link between circulating 25(OH)D-levels and the number of exacerbations has been extensively studied (8). So far however, only 4 RCTs have investigated the effect of vitamin D supplementation in the context of COPD: only 2 out of 4 RCTs showed that vitamin D supplementation reduces the number of exacerbations (173-176). However, in a post-hoc analysis, selecting those patients that were vitamin D deficient, exacerbations were indeed reduced after vitamin D supplementation. Jolliffe *et al.* summarized these 4 RCTs and performed a recent individual participant data meta-analysis and concluded that vitamin D supplementation is only protective against exacerbations in COPD patients with baseline serum 25(OH)D levels < 25 nmol/L (177). These important findings suggest that exacerbations in this specific subset of COPD patients are connected to vitamin D deficiency and this part can be resolved with supplementation. In summary, the protective effects of vitamin D in patients suffering from COPD are most prominent in those with vitamin D deficiency and this would indicate that serum levels 25(OH)D in these patients should always be determined before considering using vitamin D supplementation. Since only 4 RCTs with relatively small patient populations have been conducted in both vitamin D-sufficient and -deficient COPD patients, more RCTs are needed, especially in vitamin D-deficient patients. Currently, a multicenter RCT is being conducted by Rafiq and colleagues in a group of vitamin-deficient COPD patients (25(OH)D < 50 nmol/L), which may reveal whether vitamin D is indeed protective against exacerbations in this group (178).

Vitamin D supplementation in asthma, cystic fibrosis and acute respiratory tract infections

In addition to the effects of vitamin D supplementation in COPD patients, the effects of vitamin D supplementation has also been extensively investigated in other lung diseases (which have associations with vitamin D deficiency) such as asthma, cystic fibrosis, upper respiratory tract infections. Most RCTs that investigated the effects of vitamin D supplementation were performed in acute respiratory tract infections (ARTIs) and asthma. A recent meta-analysis that assessed the effects of vitamin D supplementation in 25 RCTs (11 321 participants) showed that indeed vitamin D supplementation was protective against ARTIs and this effect was again more profound in patients with vitamin D deficiency $25(\text{OH})\text{D} < 25 \text{ nmol/L}$ at baseline (179). A recent meta-analysis in asthma that included a total of 14 randomized controlled trials (1421 participants), indicated that vitamin D supplementation reduced the rate of asthma exacerbations and increased lung function, especially in patients with vitamin D insufficiency ($25(\text{OH})\text{D} < 75 \text{ nmol/L}$) (180). Interestingly, in asthma patients that were supplemented with vitamin D, the frequency of respiratory infections was reduced, and this effect was related to the increase of hCAP18/LL-37 (181). CF patients with vitamin D deficiency had a higher rate of exacerbations as compared to patients with sufficient vitamin D levels (182). However only one recent multicenter RCT was conducted and indicated that vitamin D supplementation did not affect the number of exacerbations in CF patients with serum $25(\text{OH})\text{D}$ concentrations between 25 and 137.5 nmol/L (183). In summary, the protective effects of vitamin D supplementation in patients suffering from COPD, asthma or ARTIs are most prominent in those with vitamin D deficiency and this would indicate the importance of establishing serum levels $25(\text{OH})\text{D}$ in these patients as supplementation could reduce unnecessary aggravated disease pathology as a result of this deficiency.

Conclusion and Perspectives

Many drivers of COPD pathogenesis such as chronic exposure to noxious particles and gases, which are present in CS and air pollution, proteolytic enzymes, cytokines and chemokines that are released by infiltrating inflammatory cells, are known to harm the epithelial barrier and cause aberrant remodeling of the airway epithelium with important functional consequences for e.g. host defense. A dysfunctional epithelial barrier increases the susceptibility towards bacterial and viral infections, which are important triggers of COPD exacerbations and these exacerbations contribute importantly to disease progression. Sufficient levels of vitamin D may provide partial protection against these effects by reducing the effects of oxidative stress induced by exposure to inhaled oxidants or those derived from recruited inflammatory cells. Vitamin D furthermore protects against impairment of epithelial barrier function by promoting the integrity of the epithelial barrier, and by modulating both innate and adaptive immune responses. Protection against the detrimental effects of both bacterial and viral infections is provided by the ability of vitamin D to promote of antiviral responses, induce expression of AMPs and modulate of inflammatory responses. Taken together, these activities suggest that vitamin D may provide protection against development and progression of COPD, and against disease exacerbations.

In addition, the local inflammatory milieu as well as the chronic exposure to noxious particles and gases, which are present in CS and air pollution, may negatively affect vitamin D bioavailability and signaling. Here we discussed the recent *in vitro* studies that demonstrated that disease-associated factors such as inflammation and exposure to CS and air pollution could interfere with vitamin D signaling and its degradation and activation by affecting expression of VDR, CYP24A1 and CYP27B1 respectively. These findings indicate that vitamin D bioavailability and the protective effects of vitamin D on the airway mucosa might be impaired especially in patients with COPD with elevated exposures to cigarette smoke and cytokines such as TNF- α , IL-1 β , IL-17A and TGF- β 1. This suggests that even in patients with sufficient vitamin D serum levels the local activity of vitamin D in the lungs can be improved. We have to start generating more information on both systemic and local bioavailability of vitamin D and gene expression signatures related to vitamin D metabolism or vitamin D responses in COPD (and other chronic inflammatory diseases that are related to vitamin D deficiency), both at baseline and after vitamin

D supplementation. This information could lead to improved treatment strategies that enhance local efficacy of vitamin D, using e.g. specific CYP24A1-inhibitors such as VID400 (184). Alternatively, degradation by CYP24A1 could be prevented by using 1,25(OH)₂D analogs that are insensitive to CYP24A1-mediated degradation, such as sulfone and sulfoximine derivatives, that also act as a VDR agonist (185). A third option is to entail the use of combination treatment with vitamin D and anti-inflammatory or certain anti-fibrotic drugs that target cytokines/proteins that are known to potentially decrease bioavailability of vitamin D by inducing expression of CYP24A1 (48, 186, 187). When considering such strategies, it should be noted that these may enhance the calcemic side effects and lead to unwanted inhibition of the immune system. We therefore need to carefully analyze the preclinical *in vivo* and *in vitro* studies and balance the pros and cons of the different strategies. In conclusion, future studies in COPD and but also in other chronic inflammatory diseases that are related to vitamin D deficiency, should be designed with more focus on assessing and improving local bioavailability of vitamin D. These new insights may lead to the development of new treatment strategies, such as those targeting CYP24A1 to enhance local bioavailability of vitamin D resulting in improved homeostasis and protection of the airway mucosa in patients with chronic inflammatory lung diseases.

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References

1. Bouillon R, Carmeliet G, Verlinden L, van Etten E, Verstuyf A, Luderer HF, Lieben L, Mathieu C, Demay M. Vitamin D and human health: lessons from vitamin D receptor null mice. *Endocrine reviews* 2008; 29: 726-776.
2. Wang T-T, Tavera-Mendoza LE, Laperriere D, Libby E, Burton MacLeod N, Nagai Y, Bourdeau V, Konstorum A, Lallemand B, Zhang R, Mader S, White JH. Large-Scale in Silico and Microarray-Based Identification of Direct 1,25-Dihydroxyvitamin D3 Target Genes. *Molecular Endocrinology* 2005; 19: 2685-2695.
3. Christakos S, Dhawan P, Verstuyf A, Verlinden L, Carmeliet G. Vitamin D: Metabolism, Molecular Mechanism of Action, and Pleiotropic Effects. *Physiological Reviews* 2016; 96: 365-408.
4. Smith JE, Goodman DS. The turnover and transport of vitamin D and of a polar metabolite with the properties of 25-hydroxycholecalciferol in human plasma. *Journal of Clinical Investigation* 1971; 50: 2159-2167.
5. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Murad MH, Weaver CM. Evaluation, Treatment, and Prevention of Vitamin D Deficiency: an Endocrine Society Clinical Practice Guideline. *The Journal of Clinical Endocrinology & Metabolism* 2011; 96: 1911-1930.
6. Holick MF. The vitamin D deficiency pandemic: Approaches for diagnosis, treatment and prevention. *Reviews in Endocrine and Metabolic Disorders* 2017; 18: 153-165.
7. Herr C, Greulich T, Koczulla RA, Meyer S, Zakharkina T, Branscheidt M, Eschmann R, Bals R. The role of vitamin D in pulmonary disease: COPD, asthma, infection, and cancer. *Respiratory Research* 2011; 12: 31-31.
8. Zhu M, Wang T, Wang C, Ji Y. The association between vitamin D and COPD risk, severity, and exacerbation: an updated systematic review and meta-analysis. *International Journal of Chronic Obstructive Pulmonary Disease* 2016; 11: 2597-2607.
9. Brehm JM. Vitamin D and Asthma—Life After VIDA? *Current Allergy and Asthma Reports* 2014; 14: 461.
10. Janssens W, Bouillon R, Claes B, Carremans C, Lehouck A, Buyschaert I, Coolen J, Mathieu C, Decramer M, Lambrechts D. Vitamin D deficiency is highly prevalent in COPD and correlates with variants in the vitamin D-binding gene. *Thorax* 2010; 65: 215-220.
11. Persson LJ, Aanerud M, Hiemstra PS, Hardie JA, Bakke PS, Eagan TM. Chronic obstructive pulmonary disease is associated with low levels of vitamin D. *PLoS One* 2012; 7: e38934.
12. Burkes RM, Ceppe AS, Doerschuk CM, Couper D, Hoffman EA, Comellas AP, Barr RG, Krishnan JA, Cooper C, Labaki WW, Ortega VE, Wells JM, Criner GJ, Woodruff PG, Bowler RP, Pirozzi CS, Hansel NN, Wise R, Brown TT, Drummond MB. Associations between 25-hydroxy-vitamin D levels, lung function, and exacerbation outcomes in COPD: An analysis of the SPIROMICS cohort. *Chest* 2020.
13. Kokturk N, Baha A, Oh Y-M, Young Ju J, Jones PW. Vitamin D deficiency: What does it mean for chronic obstructive pulmonary disease (COPD)? a comprehensive review for pulmonologists. *The Clinical Respiratory Journal* 2018; 12: 382-397.

14. Persson LJ, Aanerud M, Hiemstra PS, Michelsen AE, Ueland T, Hardie JA, Aukrust P, Bakke PS, Eagan TM. Vitamin D, vitamin D binding protein, and longitudinal outcomes in COPD. *PLoS One* 2015; 10: e0121622.
15. Zosky GR, Berry LJ, Elliot JG, James AL, Gorman S, Hart PH. Vitamin D deficiency causes deficits in lung function and alters lung structure. *Am J Respir Crit Care Med* 2011; 183: 1336-1343.
16. Bui DS, Burgess JA, Lowe AJ, Perret JL, Lodge CJ, Bui M, Morrison S, Thompson BR, Thomas PS, Giles GG, Garcia-Aymerich J, Jarvis D, Abramson MJ, Walters EH, Matheson MC, Dharmage SC. Childhood Lung Function Predicts Adult Chronic Obstructive Pulmonary Disease and Asthma-Chronic Obstructive Pulmonary Disease Overlap Syndrome. *Am J Respir Crit Care Med* 2017; 196: 39-46.
17. Wolsk HM, Chawes BL, Litonjua AA, Hollis BW, Waage J, Stokholm J, Bønnelykke K, Bisgaard H, Weiss ST. Prenatal vitamin D supplementation reduces risk of asthma/recurrent wheeze in early childhood: A combined analysis of two randomized controlled trials. *PLoS one* 2017; 12: e0186657-e0186657.
18. Kho AT, Sharma S, Qiu W, Gaedigk R, Klanderman B, Niu S, Anderson C, Leeder JS, Weiss ST, Tantisira KG. Vitamin D related genes in lung development and asthma pathogenesis. *BMC medical genomics* 2013; 6: 47-47.
19. Kelly RS, Chawes BL, Guo F, Zhang L, Blighe K, Litonjua AA, Raby BA, Levy BD, Rago D, Stokholm J, Bonnelykke K, Bisgaard H, Zhou X, Lasky-Su JA, Weiss ST. The role of the 17q21 genotype in the prevention of early childhood asthma and recurrent wheeze by vitamin D. *Eur Respir J* 2019; 54: 1900761.
20. Sordillo JE, Zhou Y, McGeachie MJ, Ziniti J, Lange N, Laranjo N, Savage JR, Carey V, O'Connor G, Sandel M, Strunk R, Bacharier L, Zeiger R, Weiss ST, Weinstock G, Gold DR, Litonjua AA. Factors influencing the infant gut microbiome at age 3-6 months: Findings from the ethnically diverse Vitamin D Antenatal Asthma Reduction Trial (VDAART). *J Allergy Clin Immunol* 2017; 139: 482-491.e414.
21. Heulens N, Korf H, Cielen N, De Smidt E, Maes K, Gysemans C, Verbeken E, Gayan-Ramirez G, Mathieu C, Janssens W. Vitamin D deficiency exacerbates COPD-like characteristics in the lungs of cigarette smoke-exposed mice. *Respiratory research* 2015; 16: 110-110.
22. Horiguchi M, Hirokawa M, Abe K, Kumagai H, Yamashita C. Pulmonary administration of 1,25-dihydroxyvitamin D3 to the lungs induces alveolar regeneration in a mouse model of chronic obstructive pulmonary disease. *Journal of Controlled Release* 2016; 233: 191-197.
23. Limketkai BN, Mullin GE, Limsui D, Parian AM. Role of Vitamin D in Inflammatory Bowel Disease. *Nutr Clin Pract* 2017; 32: 337-345.
24. Jolliffe DA, Greenberg L, Hooper RL, Griffiths CJ, Camargo CA, Kerley CP, Jensen ME, Mauger D, Stelmach I, Urashima M, Martineau AR. Vitamin D supplementation to prevent asthma exacerbations: a systematic review and meta-analysis of individual participant data. *The Lancet Respiratory Medicine* 2017; 5: 881-890.
25. De Rose V, Molloy K, Gohy S, Pilette C, Greene CM. Airway Epithelium Dysfunction in Cystic Fibrosis and COPD. *Mediators of Inflammation* 2018; 2018: 20.
26. Martini E, Krug SM, Siegmund B, Neurath MF, Becker C. Mend Your Fences: The Epithelial Barrier and its Relationship With Mucosal Immunity in Inflammatory Bowel Disease. *Cellular and molecular gastroenterology and hepatology* 2017; 4: 33-46.
27. Heijink IH, Nawijn MC, Hackett TL. Airway epithelial barrier function regulates the pathogenesis of allergic asthma. *Clin Exp Allergy* 2014; 44: 620-630.

28. Colotta F, Jansson B, Bonelli F. Modulation of inflammatory and immune responses by vitamin D. *Journal of Autoimmunity* 2017; 85: 78-97.
29. Heulens N, Korf H, Janssens W. Innate Immune Modulation in Chronic Obstructive Pulmonary Disease: Moving Closer toward Vitamin D Therapy. *Journal of Pharmacology and Experimental Therapeutics* 2015; 353: 360-368.
30. Reboul E. Intestinal absorption of vitamin D: from the meal to the enterocyte. *Food & Function* 2015; 6: 356-362.
31. Holick MF. The Cutaneous Photosynthesis of Previtamin D3: A Unique Photoendocrine System. *Journal of Investigative Dermatology* 1981; 77: 51-58.
32. DiFranco KM, Mulligan JK, Sumal AS, Diamond G. Induction of CFTR gene expression by 1,25(OH)₂ vitamin D₃, 25OH vitamin D₃, and vitamin D₃ in cultured human airway epithelial cells and in mouse airways. *The Journal of Steroid Biochemistry and Molecular Biology* 2017.
33. Vantieghem K, Overbergh L, Carmeliet G, De Haes P, Bouillon R, Segaert S. UVB-induced 1,25(OH)₂D₃ production and vitamin D activity in intestinal CaCo-2 cells and in THP-1 macrophages pretreated with a sterol Delta7-reductase inhibitor. *J Cell Biochem* 2006; 99: 229-240.
34. Adams JS, Clemens TL, Parrish JA, Holick MF. Vitamin-D Synthesis and Metabolism after Ultraviolet Irradiation of Normal and Vitamin-D-Deficient Subjects. *New England Journal of Medicine* 1982; 306: 722-725.
35. Stoffels K, Overbergh L, Giuliatti A, Verlinden L, Bouillon R, Mathieu C. Immune Regulation of 25-Hydroxyvitamin-D₃-1 α -Hydroxylase in Human Monocytes. *Journal of Bone and Mineral Research* 2006; 21: 37-47.
36. Zehnder D, Bland R, Williams MC, McNinch RW, Howie AJ, Stewart PM, Hewison M. Extrarenal Expression of 25-Hydroxyvitamin D₃-1 α -Hydroxylase1. *The Journal of Clinical Endocrinology & Metabolism* 2001; 86: 888-894.
37. Cross HS, Kállay E, Khorchide M, Lechner D. Regulation of extrarenal synthesis of 1,25-dihydroxyvitamin D₃—relevance for colonic cancer prevention and therapy. *Molecular Aspects of Medicine* 2003; 24: 459-465.
38. Hansdottir S, Monick MM, Hinde SL, Lovan N, Look DC, Hunninghake GW. Respiratory Epithelial Cells Convert Inactive Vitamin D to Its Active Form: Potential Effects on Host Defense. *The Journal of Immunology* 2008; 181: 7090-7099.
39. Edfeldt K, Liu PT, Chun R, Fabri M, Schenk M, Wheelwright M, Keegan C, Krutzik SR, Adams JS, Hewison M, Modlin RL. T-cell cytokines differentially control human monocyte antimicrobial responses by regulating vitamin D metabolism. *Proceedings of the National Academy of Sciences of the United States of America* 2010; 107: 22593-22598.
40. Pillai S, Bikle DD, Elias PM. 1,25-Dihydroxyvitamin D production and receptor binding in human keratinocytes varies with differentiation. *Journal of Biological Chemistry* 1988; 263: 5390-5395.
41. Wang Y, Zhu J, DeLuca HF. Where is the vitamin D receptor? *Archives of Biochemistry and Biophysics* 2012; 523: 123-133.
42. Boland RL. VDR activation of intracellular signaling pathways in skeletal muscle. *Molecular and Cellular Endocrinology* 2011; 347: 11-16.
43. O'Connell TD, Simpson RU. Immunochemical identification of the 1,25-dihydroxyvitamin D₃ receptor protein in human heart. *Cell Biology International* 1996; 20: 621-624.

44. Chen S, Glenn DJ, Ni W, Grigsby CL, Olsen K, Nishimoto M, Law CS, Gardner DG. Expression of the Vitamin D Receptor Is Increased in the Hypertrophic Heart. *Hypertension* 2008; 52: 1106-1112.
45. Gascon-Barré M, Demers C, Mirshahi A, Néron S, Zalzal S, Nanci A. The normal liver harbors the vitamin D nuclear receptor in nonparenchymal and biliary epithelial cells. *Hepatology* 2003; 37: 1034-1042.
46. Saccone D, Asani F, Bornman L. Regulation of the vitamin D receptor gene by environment, genetics and epigenetics. *Gene* 2015; 561: 171-180.
47. Lee SM, Meyer MB, Benkusky NA, O'Brien CA, Pike JW. The impact of VDR expression and regulation in vivo. *The Journal of Steroid Biochemistry and Molecular Biology* 2017.
48. Solomon JD, Heitzer MD, Liu TT, Beumer JH, Parise RA, Normolle DP, Leach DA, Buchanan G, DeFranco DB. VDR Activity is Differentially Affected by Hic-5 in Prostate Cancer and Stromal Cells. *Molecular cancer research : MCR* 2014; 12: 1166-1180.
49. Makishima M, Lu TT, Xie W, Whitfield GK, Domoto H, Evans RM, Haussler MR, Mangelsdorf DJ. Vitamin D Receptor As an Intestinal Bile Acid Sensor. *Science* 2002; 296: 1313-1316.
50. Jones G, Prosser DE, Kaufmann M. 25-Hydroxyvitamin D-24-hydroxylase (CYP24A1): Its important role in the degradation of vitamin D. *Archives of Biochemistry and Biophysics* 2012; 523: 9-18.
51. Wang Z, Schuetz EG, Xu Y, Thummel KE. Interplay between vitamin D and the drug metabolizing enzyme CYP3A4. *The Journal of steroid biochemistry and molecular biology* 2013; 136: 54-58.
52. Quarles LD. Endocrine functions of bone in mineral metabolism regulation. *J Clin Invest* 2008; 118: 3820-3828.
53. *Created with Biorendercom.*
54. Wang J, Liu X, Wang H, Li Y, Lan N, Yuan X, Wu M, Liu Z, Li G. Allergen specific immunotherapy enhanced defense against bacteria via TGF- β 1-induced CYP27B1 in asthma. *Oncotarget* 2017; 8: 68681-68695.
55. Pfeffer PE, Lu H, Mann EH, Chen Y-H, Ho T-R, Cousins DJ, Corrigan C, Kelly FJ, Mudway IS, Hawrylowicz CM. Effects of vitamin D on inflammatory and oxidative stress responses of human bronchial epithelial cells exposed to particulate matter. *PLoS one* 2018; 13: e0200040-e0200040.
56. Telcian AG, Zdrengeha MT, Edwards MR, Laza-Stanca V, Mallia P, Johnston SL, Stanciu LA. Vitamin D increases the antiviral activity of bronchial epithelial cells in vitro. *Antiviral Research* 2017; 137: 93-101.
57. Schrupf JA, Amatngalim GD, Veldkamp JB, Verhoosel RM, Ninaber DK, Ordonez SR, Does AMvd, Haagsman HP, Hiemstra PS. Proinflammatory Cytokines Impair Vitamin D-Induced Host Defense in Cultured Airway Epithelial Cells. *American Journal of Respiratory Cell and Molecular Biology* 2017; 56: 749-761.
58. Schrupf JA, Ninaber DK, van der Does AM, Hiemstra PS. TGF- β 1 Impairs Vitamin D-Induced and Constitutive Airway Epithelial Host Defense Mechanisms. *Journal of Innate Immunity* 2019.
59. Vargas Buonfiglio LG, Cano M, Pezzulo AA, Vanegas Calderon OG, Zabner J, Gerke AK, Comellas AP. Effect of vitamin D(3) on the antimicrobial activity of human airway surface liquid: preliminary results of a randomised placebo-controlled double-blind trial. *BMJ open respiratory research* 2017; 4: e000211-e000211.

60. Mulligan JK, Nagel W, O'Connell BP, Wentzel J, Atkinson C, Schlosser RJ. Cigarette smoke exposure is associated with vitamin D3 deficiencies in patients with chronic rhinosinusitis. *Journal of Allergy and Clinical Immunology* 2014; 134: 342-349.e341.
61. Coughlan CA, Chotirmall SH, Renwick J, Hassan T, Low TB, Bergsson G, Eshwika A, Bennett K, Dunne K, Greene CM, Gunaratnam C, Kavanagh K, Logan PM, Murphy P, Reeves EP, McElvaney NG. The effect of *Aspergillus fumigatus* infection on vitamin D receptor expression in cystic fibrosis. *Am J Respir Crit Care Med* 2012; 186: 999-1007.
62. Li P, Wu T, Su X, Shi Y. Activation of vitamin D regulates response of human bronchial epithelial cells to *Aspergillus fumigatus* in an autocrine fashion. *Mediators of inflammation* 2015; 2015: 208491-208491.
63. Uh S-T, Koo S-M, Kim YK, Kim KU, Park SW, Jang AS, Kim DJ, Kim YH, Park CS. Inhibition of vitamin d receptor translocation by cigarette smoking extracts. *Tuberculosis and respiratory diseases* 2012; 73: 258-265.
64. Du J, Wei X, Ge X, Chen Y, Li YC. Microbiota-Dependent Induction of Colonic Cyp27b1 Is Associated With Colonic Inflammation: Implications of Locally Produced 1,25-Dihydroxyvitamin D3 in Inflammatory Regulation in the Colon. *Endocrinology* 2017; 158: 4064.
65. Bakke D, Sun J. Ancient Nuclear Receptor VDR With New Functions: Microbiome and Inflammation. *Inflammatory Bowel Diseases* 2018; 24: 1149-1154.
66. Hummel DM, Fetahu IS, Gröschel C, Manhardt T, Kállay E. Role of proinflammatory cytokines on expression of vitamin D metabolism and target genes in colon cancer cells. *The Journal of Steroid Biochemistry and Molecular Biology* 2014; 144: 91-95.
67. Noyola-Martínez N, Díaz L, Zaga-Clavellina V, Avila E, Halhali A, Larrea F, Barrera D. Regulation of CYP27B1 and CYP24A1 gene expression by recombinant pro-inflammatory cytokines in cultured human trophoblasts. *The Journal of Steroid Biochemistry and Molecular Biology* 2014; 144: 106-109.
68. Campbell GR, Spector SA. Toll-Like Receptor 8 Ligands Activate a Vitamin D Mediated Autophagic Response that Inhibits Human Immunodeficiency Virus Type 1. *PLOS Pathogens* 2012; 8: e1003017.
69. Heulens N, Korf H, Mathyssen C, Everaerts S, De Smidt E, Dooms C, Yserbyt J, Gysemans C, Gayan-Ramirez G, Mathieu C, Janssens W. 1,25-Dihydroxyvitamin D Modulates Antibacterial and Inflammatory Response in Human Cigarette Smoke-Exposed Macrophages. *PLoS one* 2016; 11: e0160482-e0160482.
70. Matsunawa M, Amano Y, Endo K, Uno S, Sakaki T, Yamada S, Makishima M. The Aryl Hydrocarbon Receptor Activator Benzo[a]pyrene Enhances Vitamin D3 Catabolism in Macrophages. *Toxicological Sciences* 2009; 109: 50-58.
71. Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, Ochoa MT, Schaubert J, Wu K, Meinken C, Kamen DL, Wagner M, Bals R, Steinmeyer A, Zügel U, Gallo RL, Eisenberg D, Hewison M, Hollis BW, Adams JS, Bloom BR, Modlin RL. Toll-Like Receptor Triggering of a Vitamin D-Mediated Human Antimicrobial Response. *Science* 2006; 311: 1770-1773.
72. Adams JS, Ren S, Liu PT, Chun RF, Lagishetty V, Gombart AF, Borregaard N, Modlin RL, Hewison M. Vitamin d-directed rheostatic regulation of monocyte antibacterial responses. *J Immunol* 2009; 182: 4289-4295.

73. Krutzik SR, Hewison M, Liu PT, Robles JA, Stenger S, Adams JS, Modlin RL. IL-15 links TLR2/1-induced macrophage differentiation to the vitamin D-dependent antimicrobial pathway. *J Immunol* 2008; 181: 7115-7120.
74. Subramanian K, Bergman P, Henriques-Normark B. Vitamin D Promotes Pneumococcal Killing and Modulates Inflammatory Responses in Primary Human Neutrophils. *Journal of Innate Immunity* 2017; 9: 375-386.
75. Baeke F, Korf H, Overbergh L, van Etten E, Verstuyf A, Gysemans C, Mathieu C. Human T lymphocytes are direct targets of 1,25-dihydroxyvitamin D3 in the immune system. *The Journal of Steroid Biochemistry and Molecular Biology* 2010; 121: 221-227.
76. Chen S, Sims GP, Chen XX, Gu YY, Chen S, Lipsky PE. Modulatory Effects of 1,25-Dihydroxyvitamin D₃ on Human B Cell Differentiation. 2007; 179: 1634-1647.
77. Thomas BJ, Kan-o K, Loveland KL, Elias JA, Bardin PG. In the Shadow of Fibrosis: Innate Immune Suppression Mediated by Transforming Growth Factor- β . *American Journal of Respiratory Cell and Molecular Biology* 2016; 55: 759-766.
78. Barnes PJ. Targeting cytokines to treat asthma and chronic obstructive pulmonary disease. *Nature Reviews Immunology* 2018.
79. De Rose V, Molloy K, Gohy S, Pilette C, Greene CM. Airway Epithelium Dysfunction in Cystic Fibrosis and COPD. *Mediators of inflammation* 2018; 2018: 1309746-1309746.
80. Wedzicha JA, Seemungal TAR. COPD exacerbations: defining their cause and prevention. *The Lancet* 2007; 370: 786-796.
81. Loxham M, Davies DE. Phenotypic and genetic aspects of epithelial barrier function in asthmatic patients. *J Allergy Clin Immunol* 2017; 139: 1736-1751.
82. Skolnik K, Quon BS. Recent advances in the understanding and management of cystic fibrosis pulmonary exacerbations. *F1000Research* 2018; 7: F1000 Faculty Rev-1575.
83. Schrupf JA, van Sterkenburg MA, Verhoosel RM, Zuyderduyn S, Hiemstra PS. Interleukin 13 exposure enhances vitamin D-mediated expression of the human cathelicidin antimicrobial peptide 18/LL-37 in bronchial epithelial cells. *Infect Immun* 2012; 80: 4485-4494.
84. Liu MC, Xiao HQ, Brown AJ, Ritter CS, Schroeder J. Association of vitamin D and antimicrobial peptide production during late-phase allergic responses in the lung. *Clin Exp Allergy* 2012; 42: 383-391.
85. Schrupf JA, Ninaber DK, van der Does AM, Hiemstra PS. TGF- β 1 Impairs Vitamin D-Induced and Constitutive Airway Epithelial Host Defense Mechanisms. *J Innate Immun* 2020; 12: 74-89.
86. Barrea L, Savastano S, Di Somma C, Savanelli MC, Nappi F, Albanese L, Orio F, Colao A. Low serum vitamin D-status, air pollution and obesity: A dangerous liaison. *Reviews in endocrine & metabolic disorders* 2017; 18: 207-214.
87. Tzilias V, Bouros E, Barbayianni I, Karampitsakos T, Kourtidou S, Ntassiou M, Ninou I, Aidinis V, Bouros D, Tzouveleki A. Vitamin D prevents experimental lung fibrosis and predicts survival in patients with idiopathic pulmonary fibrosis. *Pulmonary Pharmacology & Therapeutics* 2019; 55: 17-24.
88. Schlosser RJ, Carroll WW, Soler ZM, Pasquini WN, Mulligan JK. Reduced sinonasal levels of 1 α -hydroxylase are associated with worse quality of life in chronic rhinosinusitis with nasal polyps. *Int Forum Allergy Rhinol* 2016; 6: 58-65.
89. Souberbielle J-C, Body J-J, Lappe JM, Plebani M, Shoenfeld Y, Wang TJ, Bischoff-Ferrari HA, Cavalier E, Ebeling PR, Fardellone P, Gandini S, Gruson D, Guérin AP, Heckendorff L, Hollis

- BW, Ish-Shalom S, Jean G, von Landenberg P, Largura A, Olsson T, Pierrot-Deseilligny C, Pilz S, Tincani A, Valcour A, Zittermann A. Vitamin D and musculoskeletal health, cardiovascular disease, autoimmunity and cancer: Recommendations for clinical practice. *Autoimmunity Reviews* 2010; 9: 709-715.
90. DeLuca GC, Kimball SM, Kolasinski J, Ramagopalan SV, Ebers GC. Review: The role of vitamin D in nervous system health and disease. *Neuropathology and Applied Neurobiology* 2013; 39: 458-484.
91. Herr C, Greulich T, Koczulla RA, Meyer S, Zakharkina T, Branscheidt M, Eschmann R, Bals R. The role of vitamin D in pulmonary disease: COPD, asthma, infection, and cancer. *Respir Res* 2011; 12: 31.
92. Dankers W, Colin EM, van Hamburg JP, Lubberts E. Vitamin D in Autoimmunity: Molecular Mechanisms and Therapeutic Potential. *Frontiers in immunology* 2017; 7: 697-697.
93. Berridge MJ. Vitamin D cell signalling in health and disease. *Biochemical and Biophysical Research Communications* 2015; 460: 53-71.
94. Aghapour M, Raei P, Moghaddam SJ, Hiemstra PS, Heijink IH. Airway Epithelial Barrier Dysfunction in Chronic Obstructive Pulmonary Disease: Role of Cigarette Smoke Exposure. *American Journal of Respiratory Cell and Molecular Biology* 2018; 58: 157-169.
95. Zhang R, Zhao H, Dong H, Zou F, Cai S. 1 α ,25-Dihydroxyvitamin D3 counteracts the effects of cigarette smoke in airway epithelial cells. *Cellular Immunology* 2015; 295: 137-143.
96. Shi Y-Y, Liu T-J, Fu J-H, Xu W, Wu L-L, Hou AN, Xue X-D. Vitamin D/VDR signaling attenuates lipopolysaccharide-induced acute lung injury by maintaining the integrity of the pulmonary epithelial barrier. *Molecular medicine reports* 2016; 13: 1186-1194.
97. Gorman S, Buckley AG, Ling K-M, Berry LJ, Fear VS, Stick SM, Larcombe AN, Kicic A, Hart PH. Vitamin D supplementation of initially vitamin D-deficient mice diminishes lung inflammation with limited effects on pulmonary epithelial integrity. *Physiological reports* 2017; 5: e13371.
98. Luissint A-C, Parkos CA, Nusrat A. Inflammation and the Intestinal Barrier: Leukocyte-Epithelial Cell Interactions, Cell Junction Remodeling, and Mucosal Repair. *Gastroenterology* 2016; 151: 616-632.
99. DiFranco KM, Mulligan JK, Sumal AS, Diamond G. Induction of CFTR gene expression by 1,25(OH)₂ vitamin D(3), 25OH vitamin D(3), and vitamin D(3) in cultured human airway epithelial cells and in mouse airways. *The Journal of steroid biochemistry and molecular biology* 2017; 173: 323-332.
100. Pezzulo AA, Tang XX, Hoegger MJ, Abou Alaiwa MH, Ramachandran S, Moninger TO, Karp PH, Wohlford-Lenane CL, Haagsman HP, van Eijk M, Bánfi B, Horswill AR, Stoltz DA, McCray PB, Jr., Welsh MJ, Zabner J. Reduced airway surface pH impairs bacterial killing in the porcine cystic fibrosis lung. *Nature* 2012; 487: 109-113.
101. Rab A, Rowe SM, Raju SV, Bebok Z, Matalon S, Collawn JF. Cigarette smoke and CFTR: implications in the pathogenesis of COPD. *Am J Physiol Lung Cell Mol Physiol* 2013; 305: L530-L541.
102. Lamouille S, Xu J, Derynck R. Molecular mechanisms of epithelial-mesenchymal transition. *Nat Rev Mol Cell Biol* 2014; 15: 178-196.
103. Milara J, Peiró T, Serrano A, Cortijo J. Epithelial to mesenchymal transition is increased in patients with COPD and induced by cigarette smoke. 2013; 68: 410-420.

104. Ricca C, Aillon A, Viano M, Bergandi L, Aldieri E, Silvagno F. Vitamin D inhibits the epithelial-mesenchymal transition by a negative feedback regulation of TGF- β activity. *The Journal of Steroid Biochemistry and Molecular Biology* 2018.
105. Jiang F, Yang Y, Xue L, Li B, Zhang Z. $1\alpha,25$ -dihydroxyvitamin D₃ Attenuates TGF- β -Induced Pro-Fibrotic Effects in Human Lung Epithelial Cells through Inhibition of Epithelial-Mesenchymal Transition. *Nutrients* 2017; 9: 980.
106. Tan Z-X, Chen Y-H, Xu S, Qin H-Y, Zhang C, Zhao H, Xu D-X. Calcitriol inhibits bleomycin-induced early pulmonary inflammatory response and epithelial-mesenchymal transition in mice. *Toxicology Letters* 2016; 240: 161-171.
107. Fischer KD, Hall SC, Agrawal DK. Vitamin D Supplementation Reduces Induction of Epithelial-Mesenchymal Transition in Allergen Sensitized and Challenged Mice. *PLoS One* 2016; 11: e0149180.
108. Hancock REW, Haney EF, Gill EE. The immunology of host defence peptides: beyond antimicrobial activity. *Nat Rev Immunol* 2016; 16: 321-334.
109. Wang T-T, Nestel FP, Bourdeau V, Nagai Y, Wang Q, Liao J, Tavera-Mendoza L, Lin R, Hanrahan JW, Mader S, White JH. Cutting Edge: $1,25$ -Dihydroxyvitamin D₃ Is a Direct Inducer of Antimicrobial Peptide Gene Expression. *The Journal of Immunology* 2004; 173: 2909-2912.
110. Huang FC. The differential effects of $1,25$ -dihydroxyvitamin D₃ on Salmonella-induced interleukin-8 and human beta-defensin-2 in intestinal epithelial cells. *Clinical and experimental immunology* 2016; 185: 98-106.
111. Wang T-T, Dabbas B, Laperriere D, Bitton AJ, Soualhia H, Tavera-Mendoza LE, Dionne S, Servant MJ, Bitton A, Seidman EG, Mader S, Behr MA, White JH. Direct and Indirect Induction by $1,25$ -Dihydroxyvitamin D₃ of the NOD2/CARD15-Defensin β 2 Innate Immune Pathway Defective in Crohn Disease. *Journal of Biological Chemistry* 2010; 285: 2227-2231.
112. Gonzalez-Curiel I, Trujillo V, Montoya-Rosales A, Rincon K, Rivas-Calderon B, deHaro-Acosta J, Marin-Luevano P, Lozano-Lopez D, Enciso-Moreno JA, Rivas-Santiago B. $1,25$ -dihydroxyvitamin D₃ induces LL-37 and HBD-2 production in keratinocytes from diabetic foot ulcers promoting wound healing: an in vitro model. *PLoS one* 2014; 9: e111355-e111355.
113. Dai X, Sayama K, Tohyama M, Shirakata Y, Hanakawa Y, Tokumaru S, Yang L, Hirakawa S, Hashimoto K. PPAR γ mediates innate immunity by regulating the $1\alpha,25$ -dihydroxyvitamin D₃ induced hBD-3 and cathelicidin in human keratinocytes. *Journal of Dermatological Science* 2010; 60: 179-186.
114. Hou W, Hu S, Li C, Ma H, Wang Q, Meng G, Guo T, Zhang J. Cigarette Smoke Induced Lung Barrier Dysfunction, EMT, and Tissue Remodeling: A Possible Link between COPD and Lung Cancer. *Biomed Res Int* 2019; 2019: 2025636-2025636.
115. Ito JT, Lourenço JD, Righetti RF, Tibério IFLC, Prado CM, Lopes FDTQS. Extracellular Matrix Component Remodeling in Respiratory Diseases: What Has Been Found in Clinical and Experimental Studies? *Cells* 2019; 8: 342.
116. Shimshoni E, Yablecovitch D, Baram L, Dotan I, Sagi I. ECM remodelling in IBD: innocent bystander or partner in crime? The emerging role of extracellular molecular events in sustaining intestinal inflammation. *Gut* 2015; 64: 367-372.

117. Hansdottir S, Monick MM, Lovan N, Powers L, Gerke A, Hunninghake GW. Vitamin D decreases respiratory syncytial virus induction of NF-kappaB-linked chemokines and cytokines in airway epithelium while maintaining the antiviral state. *J Immunol* 2010; 184: 965-974.
118. Pfeffer PE, Chen Y-H, Woszczek G, Matthews NC, Chevretton E, Gupta A, Saglani S, Bush A, Corrigan C, Cousins DJ, Hawrylowicz CM. Vitamin D enhances production of soluble ST2, inhibiting the action of IL-33. *J Allergy Clin Immunol* 2015; 135: 824-827.e823.
119. Pfeffer PE, Hawrylowicz CM. Vitamin D in Asthma: Mechanisms of Action and Considerations for Clinical Trials. *Chest* 2018; 153: 1229-1239.
120. Vanherwegen A-S, Gysemans C, Mathieu C. Regulation of Immune Function by Vitamin D and Its Use in Diseases of Immunity. *Endocrinology and Metabolism Clinics of North America* 2017; 46: 1061-1094.
121. Mercado N, Ito K, Barnes PJ. Accelerated ageing of the lung in COPD: new concepts. *Thorax* 2015; 70: 482-489.
122. Haussler MR, Whitfield GK, Kaneko I, Haussler CA, Hsieh D, Hsieh J-C, Jurutka PW. Molecular Mechanisms of Vitamin D Action. *Calcified Tissue International* 2013; 92: 77-98.
123. Torres PU, Prié D, Molina-Blétry V, Beck L, Silve C, Friedlander G. Klotho: An antiaging protein involved in mineral and vitamin D metabolism. *Kidney International* 2007; 71: 730-737.
124. Krick S, Grabner A, Baumlin N, Yanucil C, Helton S, Grosche A, Sailland J, Geraghty P, Viera L, Russell DW, Wells JM, Xu X, Gaggar A, Barnes J, King GD, Campos M, Faul C, Salathe M. Fibroblast growth factor 23 and Klotho contribute to airway inflammation. *Eur Respir J* 2018; 52: 1800236.
125. Gao W, Yuan C, Zhang J, Li L, Yu L, Wiegman CH, Barnes PJ, Adcock IM, Huang M, Yao X. Klotho expression is reduced in COPD airway epithelial cells: effects on inflammation and oxidant injury. *Clinical science (London, England : 1979)* 2015; 129: 1011-1023.
126. Li YC, Chen Y, Du J. Critical roles of intestinal epithelial vitamin D receptor signaling in controlling gut mucosal inflammation. *J Steroid Biochem Mol Biol* 2015; 148: 179-183.
127. Lu R, Zhang YG, Xia Y, Sun J. Imbalance of autophagy and apoptosis in intestinal epithelium lacking the vitamin D receptor. *FASEB J* 2019; 33: 11845-11856.
128. Racanelli AC, Kikkers SA, Choi AMK, Cloonan SM. Autophagy and inflammation in chronic respiratory disease. *Autophagy* 2018; 14: 221-232.
129. Malvin NP, Kern JT, Liu TC, Brody SL, Stappenbeck TS. Autophagy proteins are required for club cell structure and function in airways. *Am J Physiol Lung Cell Mol Physiol* 2019; 317: L259-L270.
130. <https://goldcopd.org>. 2019.
131. Barnes PJ. Corticosteroid resistance in patients with asthma and chronic obstructive pulmonary disease. *Journal of Allergy and Clinical Immunology* 2013; 131: 636-645.
132. Xystrakis E, Kusumakar S, Boswell S, Peek E, Urry Z, Richards DF, Adikibi T, Pridgeon C, Dallman M, Loke T-K, Robinson DS, Barrat FJ, O'Garra A, Lavender P, Lee TH, Corrigan C, Hawrylowicz CM. Reversing the defective induction of IL-10-secreting regulatory T cells in glucocorticoid-resistant asthma patients. *J Clin Invest* 2006; 116: 146-155.
133. Sutherland ER, Goleva E, Jackson LP, Stevens AD, Leung DYM. Vitamin D levels, lung function, and steroid response in adult asthma. *American journal of respiratory and critical care medicine* 2010; 181: 699-704.

134. Lan N, Luo G, Yang X, Cheng Y, Zhang Y, Wang X, Wang X, Xie T, Li G, Liu Z, Zhong N. 25-Hydroxyvitamin D3-deficiency enhances oxidative stress and corticosteroid resistance in severe asthma exacerbation. *PLoS One* 2014; 9: e111599.
135. Konya V, Czarnewski P, Forkel M, Rao A, Kokkinou E, Villablanca EJ, Almer S, Lindfors U, Friberg D, Höög C, Bergman P, Mjösberg J. Vitamin D downregulates the IL-23 receptor pathway in human mucosal group 3 innate lymphoid cells. *Journal of Allergy and Clinical Immunology* 2018; 141: 279-292.
136. Mann EH, Ho T-R, Pfeffer PE, Matthews NC, Chevretton E, Mudway I, Kelly FJ, Hawrylowicz CM. Vitamin D Counteracts an IL-23-Dependent IL-17A(+)|IFN- γ (+) Response Driven by Urban Particulate Matter. *American journal of respiratory cell and molecular biology* 2017; 57: 355-366.
137. Fawaz L, Mrad MF, Kazan JM, Sayegh S, Akika R, Khoury SJ. Comparative effect of 25(OH)D3 and 1,25(OH)2D3 on Th17 cell differentiation. *Clinical Immunology* 2016; 166-167: 59-71.
138. Nanzer AM, Chambers ES, Ryanna K, Richards DF, Black C, Timms PM, Martineau AR, Griffiths CJ, Corrigan CJ, Hawrylowicz CM. Enhanced production of IL-17A in patients with severe asthma is inhibited by 1 α ,25-dihydroxyvitamin D3 in a glucocorticoid-independent fashion. *Journal of Allergy and Clinical Immunology* 2013; 132: 297-304.e293.
139. Dhawan P, Christakos S. Novel regulation of 25-hydroxyvitamin D3 24-hydroxylase (24(OH)ase) transcription by glucocorticoids: Cooperative effects of the glucocorticoid receptor, C/EBP β , and the Vitamin D receptor in 24(OH)ase transcription. *Journal of Cellular Biochemistry* 2010; 110: 1314-1323.
140. Donaldson GC, Seemungal TAR, Bhowmik A, Wedzicha JA. Relationship between exacerbation frequency and lung function decline in chronic obstructive pulmonary disease. *Thorax* 2002; 57: 847-852.
141. Decramer M, Janssens W, Miravittles M. Chronic obstructive pulmonary disease. *The Lancet* 2012; 379: 1341-1351.
142. Leung JM, Tiew PY, Mac Aogáin M, Budden KF, Yong VFL, Thomas SS, Pethe K, Hansbro PM, Chotirmall SH. The role of acute and chronic respiratory colonization and infections in the pathogenesis of COPD. *Respirology* 2017; 22: 634-650.
143. Wang Z, Bafadhel M, Haldar K, Spivak A, Mayhew D, Miller BE, Tal-Singer R, Johnston SL, Ramsheh MY, Barer MR, Brightling CE, Brown JR. Lung microbiome dynamics in COPD exacerbations. *European Respiratory Journal* 2016; 47: 1082-1092.
144. Bolcas PE, Brandt EB, Zhang Z, Biagini Myers JM, Ruff BP, Khurana Hershey GK. Vitamin D supplementation attenuates asthma development following traffic-related particulate matter exposure. *Journal of Allergy and Clinical Immunology* 2019; 143: 386-394.e383.
145. Wang H, Anthony D, Selemidis S, Vlahos R, Bozinovski S. Resolving Viral-Induced Secondary Bacterial Infection in COPD: A Concise Review. *Frontiers in immunology* 2018; 9: 2345-2345.
146. Stolz D, Papakonstantinou E, Grize L, Schilter D, Strobel W, Louis R, Schindler C, Hirsch HH, Tamm M. Time-course of upper respiratory tract viral infection and COPD exacerbation. *Eur Respir J* 2019; 54: 1900407.
147. Schogler A, Muster RJ, Kieninger E, Casaulta C, Tapparel C, Jung A, Moeller A, Geiser T, Regamey N, Alves MP. Vitamin D represses rhinovirus replication in cystic fibrosis cells by inducing LL-37. *European Respiratory Journal* 2016; 47: 520-530.

148. Sousa FH, Casanova V, Findlay F, Stevens C, Svoboda P, Pohl J, Proudfoot L, Barlow PG. Cathelicidins display conserved direct antiviral activity towards rhinovirus. *Peptides* 2017; 95: 76-83.
149. Brockman-Schneider RA, Pickles RJ, Gern JE. Effects of vitamin D on airway epithelial cell morphology and rhinovirus replication. *PLoS one* 2014; 9: e86755-e86755.
150. Gui B, Chen Q, Hu C, Zhu C, He G. Effects of calcitriol (1, 25-dihydroxy-vitamin D₃) on the inflammatory response induced by H9N2 influenza virus infection in human lung A549 epithelial cells and in mice. *Virology journal* 2017; 14: 10-10.
151. Khare D, Godbole NM, Pawar SD, Mohan V, Pandey G, Gupta S, Kumar D, Dhole TN, Godbole MM. Calcitriol [1, 25[OH]₂ D₃] pre- and post-treatment suppresses inflammatory response to influenza A (H1N1) infection in human lung A549 epithelial cells. *European Journal of Nutrition* 2013; 52: 1405-1415.
152. Sethi S, Murphy TF. Infection in the pathogenesis and course of chronic obstructive pulmonary disease. *N Engl J Med* 2008; 359: 2355-2365.
153. Sethi S, Evans N, Grant BJB, Murphy TF. New Strains of Bacteria and Exacerbations of Chronic Obstructive Pulmonary Disease. *New England Journal of Medicine* 2002; 347: 465-471.
154. Wilkinson TMA, Aris E, Bourne S, Clarke SC, Peeters M, Pascal TG, Schoonbroodt S, Tuck AC, Kim V, Ostridge K, Staples KJ, Williams N, Williams A, Wootton S, Devaster J-M, Group AS. A prospective, observational cohort study of the seasonal dynamics of airway pathogens in the aetiology of exacerbations in COPD. *Thorax* 2017; 72: 919-927.
155. Yim S, Dhawan P, Ragunath C, Christakos S, Diamond G. Induction of cathelicidin in normal and CF bronchial epithelial cells by 1,25-dihydroxyvitamin D₃. *Journal of cystic fibrosis : official journal of the European Cystic Fibrosis Society* 2007; 6: 403-410.
156. Dimitrov V, White JH. Species-specific regulation of innate immunity by vitamin D signaling. *The Journal of Steroid Biochemistry and Molecular Biology* 2016; 164: 246-253.
157. Gombart AF, Borregaard N, Koeffler HP. Human cathelicidin antimicrobial peptide (CAMP) gene is a direct target of the vitamin D receptor and is strongly up-regulated in myeloid cells by 1,25-dihydroxyvitamin D₃. *FASEB J* 2005; 19: 1067-1077.
158. Niederstrasser J, Herr C, Wolf L, Lehr CM, Beisswenger C, Bals R. Vitamin D Deficiency Does Not Result in a Breach of Host Defense in Murine Models of Pneumonia. *Infection and immunity* 2016; 84: 3097-3104.
159. Lowry MB, Guo C, Zhang Y, Fantacone ML, Logan IE, Campbell Y, Zhang W, Le M, Indra AK, Ganguli-Indra G, Xie J, Gallo RL, Koeffler HP, Gombart AF. A mouse model for vitamin D-induced human cathelicidin antimicrobial peptide gene expression. *The Journal of steroid biochemistry and molecular biology* 2019: 105552-105552.
160. He L, Liu T, Shi Y, Tian F, Hu H, Deb DK, Chen Y, Bissonnette M, Li YC. Gut Epithelial Vitamin D Receptor Regulates Microbiota-Dependent Mucosal Inflammation by Suppressing Intestinal Epithelial Cell Apoptosis. *Endocrinology* 2018; 159: 967-979.
161. Clark A, Mach N. Role of Vitamin D in the Hygiene Hypothesis: The Interplay between Vitamin D, Vitamin D Receptors, Gut Microbiota, and Immune Response. *Front Immunol* 2016; 7: 627.
162. Salzman NH. Paneth cell defensins and the regulation of the microbiome: détente at mucosal surfaces. *Gut Microbes* 2010; 1: 401-406.
163. Cullen TW, Schofield WB, Barry NA, Putnam EE, Rundell EA, Trent MS, Degnan PH, Booth CJ, Yu H, Goodman AL. Gut microbiota. Antimicrobial peptide resistance mediates resilience of

- prominent gut commensals during inflammation. *Science (New York, NY)* 2015; 347: 170-175.
164. Budden KF, Gellatly SL, Wood DLA, Cooper MA, Morrison M, Hugenholtz P, Hansbro PM. Emerging pathogenic links between microbiota and the gut–lung axis. *Nature Reviews Microbiology* 2016; 15: 55.
165. Dang AT, Marsland BJ. Microbes, metabolites, and the gut-lung axis. *Mucosal Immunol* 2019; 12: 843-850.
166. Waterhouse M, Hope B, Krause L, Morrison M, Protani MM, Zakrzewski M, Neale RE. Vitamin D and the gut microbiome: a systematic review of in vivo studies. *Eur J Nutr* 2019; 58: 2895-2910.
167. Talsness CE, Penders J, Jansen EHJM, Damoiseaux J, Thijs C, Mommers M. Influence of vitamin D on key bacterial taxa in infant microbiota in the KOALA Birth Cohort Study. *PLoS one* 2017; 12: e0188011-e0188011.
168. Schaffler H, Herlemann DP, Klinitzke P, Berlin P, Kreikemeyer B, Jaster R, Lamprecht G. Vitamin D administration leads to a shift of the intestinal bacterial composition in Crohn's disease patients, but not in healthy controls. *J Dig Dis* 2018; 19: 225-234.
169. Sullivan A, Hunt E, MacSharry J, Murphy DM. 'The Microbiome and the Pathophysiology of Asthma'. *Respiratory research* 2016; 17: 163-163.
170. Mammen MJ, Sethi S. COPD and the microbiome. *Respirology* 2016; 21: 590-599.
171. Toivonen L, Hasegawa K, Ajami NJ, Celedon JC, Mansbach JM, Petrosino JF, Camargo CA, Jr. Circulating 25-hydroxyvitamin D, nasopharyngeal microbiota, and bronchiolitis severity. *Pediatr Allergy Immunol* 2018; 29: 877-880.
172. Kanhere M, He J, Chassaing B, Ziegler TR, Alvarez JA, Ivie EA, Hao L, Hanfelt J, Gewirtz AT, Tangpricha V. Bolus Weekly Vitamin D3 Supplementation Impacts Gut and Airway Microbiota in Adults With Cystic Fibrosis: A Double-Blind, Randomized, Placebo-Controlled Clinical Trial. *J Clin Endocrinol Metab* 2018; 103: 564-574.
173. Zendedel A, Gholami M, Anbari K, Ghanadi K, Bachari EC, Azargon A. Effects of Vitamin D Intake on FEV1 and COPD Exacerbation: A Randomized Clinical Trial Study. *Global journal of health science* 2015; 7: 243-248.
174. Khan DM, Ullah A, Randhawa FA, Iqtadar S, Butt NF, Waheed K. Role of Vitamin D in reducing number of acute exacerbations in Chronic Obstructive Pulmonary Disease (COPD) patients. *Pakistan journal of medical sciences* 2017; 33: 610-614.
175. Lehouck A, Mathieu C, Carremans C, Baeke F, Verhaegen J, Van Eldere J, Decallonne B, Bouillon R, Decramer M, Janssens W. High doses of vitamin D to reduce exacerbations in chronic obstructive pulmonary disease: a randomized trial. *Ann Intern Med* 2012; 156: 105-114.
176. Martineau AR, James WY, Hooper RL, Barnes NC, Jolliffe DA, Greiller CL, Islam K, McLaughlin D, Bhowmik A, Timms PM, Rajakulasingham RK, Rowe M, Venton TR, Choudhury AB, Simcock DE, Wilks M, Degun A, Sadique Z, Monteiro WR, Corrigan CJ, Hawrylowicz CM, Griffiths CJ. Vitamin D3 supplementation in patients with chronic obstructive pulmonary disease (ViDiCO): a multicentre, double-blind, randomised controlled trial. *Lancet Respir Med* 2015; 3: 120-130.
177. Jolliffe DA, Greenberg L, Hooper RL, Mathysen C, Rafiq R, de Jongh RT, Camargo CA, Griffiths CJ, Janssens W, Martineau AR. Vitamin D to prevent exacerbations of COPD: systematic review and meta-analysis of individual participant data from randomised controlled trials. *Thorax* 2019; 74: 337-345.

178. Rafiq R, Aleva FE, Schruppf JA, Heijdra YF, Taube C, Daniels JMA, Lips P, Bet PM, Hiemstra PS, van der Ven AJAM, den Heijer M, de Jongh RT. Prevention of exacerbations in patients with COPD and vitamin D deficiency through vitamin D supplementation (PRECOVID): a study protocol. *BMC Pulmonary Medicine* 2015; 15: 106.
179. Martineau AR, Jolliffe DA, Hooper RL, Greenberg L, Aloia JF, Bergman P, Dubnov-Raz G, Esposito S, Ganmaa D, Ginde AA, Goodall EC, Grant CC, Griffiths CJ, Janssens W, Laaksi I, Manaseki-Holland S, Mauger D, Murdoch DR, Neale R, Rees JR, Simpson S, Stelmach I, Kumar GT, Urashima M, Camargo CA. Vitamin D supplementation to prevent acute respiratory tract infections: systematic review and meta-analysis of individual participant data. *The BMJ* 2017; 356: i6583.
180. Wang M, Liu M, Wang C, Xiao Y, An T, Zou M, Cheng G. Association between vitamin D status and asthma control: A meta-analysis of randomized trials. *Respiratory Medicine* 2019; 150: 85-94.
181. Ramos-Martínez E, López-Vancell MR, Fernández de Córdova-Aguirre JC, Rojas-Serrano J, Chavarría A, Velasco-Medina A, Velázquez-Sámamo G. Reduction of respiratory infections in asthma patients supplemented with vitamin D is related to increased serum IL-10 and IFN γ levels and cathelicidin expression. *Cytokine* 2018; 108: 239-246.
182. Wani WA, Nazir M, Bhat JI, Malik E-u-h, Ahmad QI, Charoo BA, Ali SW. Vitamin D status correlates with the markers of cystic fibrosis-related pulmonary disease. *Pediatrics & Neonatology* 2019; 60: 210-215.
183. Tangpricha V, Lukemire J, Chen Y, Binongo JNG, Judd SE, Michalski ES, Lee MJ, Walker S, Ziegler TR, Tirouvanziam R, Zughaier SM, Chesdachai S, Hermes WA, Chmiel JF, Grossmann RE, Gaggar A, Joseph PM, Alvarez JA. Vitamin D for the Immune System in Cystic Fibrosis (DISC): a double-blind, multicenter, randomized, placebo-controlled clinical trial. *The American Journal of Clinical Nutrition* 2019; 109: 544-553.
184. Luo W, Hershberger PA, Trump DL, Johnson CS. 24-Hydroxylase in cancer: impact on vitamin D-based anticancer therapeutics. *The Journal of steroid biochemistry and molecular biology* 2013; 136: 252-257.
185. Amatngalim GD, Broekman W, Daniel NM, van der Vlugt LEPM, van Schadewijk A, Taube C, Hiemstra PS. Cigarette Smoke Modulates Repair and Innate Immunity following Injury to Airway Epithelial Cells. *PLoS one* 2016; 11: e0166255-e0166255.
186. Toshiyuki S, Kaori Y, Atsushi K, Keiko Y, Tai CC. CYP24A1 as a Potential Target for Cancer Therapy. *Anti-Cancer Agents in Medicinal Chemistry* 2014; 14: 97-108.
187. Lachapelle P, Li M, Douglass J, Stewart A. Safer approaches to therapeutic modulation of TGF-beta signaling for respiratory disease. *Pharmacol Ther* 2018; 187: 98-113.