

REVIEW

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The immunobiological and clinical role of vitamin D in obstructive lung diseases

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ABSTRACT

Vitamin D is a fat-soluble vitamin, which is obtained by conversion of 7-dehydrocholesterol in the skin by UV ray and by diet. Its role on bone mineralization has been known for over two hundred years, while its non-skeletal effects have been acknowledged only in the last few years. The discovery of important vitamin D properties on the innate and adaptive immune system created a lot of interest in a potential role of vitamin D on diseases characterized by heightened inflammation and oxidative response, and impaired antimicrobial response, such as asthma and chronic obstructive pulmonary disease (COPD). Recent studies have demonstrated that vitamin D and its deficiency have a number of biological effects which are potentially important in altering the pathogenesis and severity of both asthma and COPD. Vitamin D may improve lung function and response to steroids therapy, reduce airway remodeling and disease exacerbations. The aim of this study is to review the role of Vitamin D in asthma and COPD.

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Asthma and chronic obstructive pulmonary disease (COPD) are chronic obstructive airways diseases characterized by heightened airway inflammation, airway hyper-responsiveness with high incidence and prevalence. It was estimated that over 300 million of people suffer from asthma ¹ and, in approximately 50% of cases, the disease is not optimally controlled, even in developed countries. COPD is the fourth leading cause of death worldwide, prevalence and mortality rates are still increasing.² The costs of asthma and COPD are largely due to uncontrolled disease, and are likely to rise as their prevalence and severity are likely to increase.

In the last years, the association between

different nutrients and airway disease has been evaluated. Among those, vitamin D has received particular attention, probably because of its multiple functions.

Vitamin D has been shown in recent studies to play a role in asthma and COPD pathogenesis by acting on innate and adaptive immune system and on inflammation.

The aim of this study is to review the role of vitamin D in asthma and COPD.

Vitamin D metabolism

Vitamin D is a fat-soluble vitamin. Few foods (fatty fish, cod liver oil, eggs, etc.) contain it naturally, so the dietary intake of vita-

min D is not enough to satisfy the metabolic needs. Most of cholecalciferol or vitamin D₃ is produced in the skin from 7-dehydrocholesterol by ultraviolet B exposure. Sensible sun exposure can provide an adequate amount of vitamin D, which is stored in body fat and released during the winter, when it cannot be activated by sun.³

Vitamin D from the diet or dermal synthesis is biologically inactive and requires dihydroxylation to become a metabolic active form: 1-25-dihydroxy vitamin D, also known as calcitriol. The first vitamin D hydroxylation occurs in the liver in order to produce 25-hydroxy vitamin D, also known as calcidiol. Calcidiol is the stored form of vitamin D, and its systemic status is reliably indicated by the serum level of 25-hydroxyvitamin D.⁴ Calcidiol is then converted by CYP27B1 (1 α -hydroxylase) into the biologically-active 1-25-dihydroxy vitamin D; this reaction occurs mainly in the kidneys, but also in extrarenal sites.⁵ In the kidneys, CYP27B1 expression and thus 1,25-dihydroxyvitamin D production are regulated by calcium, phosphorus, and their regulating hormones (including parathyroid hormone), which regulate bone metabolism. Negative feedback is provided by 1,25-dihydroxyvitamin D itself through downregulation of CYP27B1 and upregulation of CYP24A1 (24-hydroxylase), which catalyzes the first step in vitamin D catabolism, thereby preventing the excessive production of 1,25-dihydroxyvitamin D.

This vitamin form performs biologic functions by binding to the vitamin D receptor (VDR). VDR complex binds to specific genomic sequences (vitamin D response elements) in the promoter region of target genes, whereby gene expression is regulated. In this way, 1-25-dihydroxy vitamin D performs endocrine, paracrine and autocrine functions.⁶

Vitamin D deficiency

Although there is no universal consensus on the criteria for optimal serum levels of 25-hydroxyvitamin D, usually a level of 25-hydroxy vitamin D of 21 to 29 ng/mL (or 30 to 50 nmol/L) is considered a relative in-

sufficiency of vitamin D, and a level of 30 ng/ml or greater (or >75 nmol/L) as sufficient vitamin D.⁷ However, higher 25-hydroxy vitamin D levels (e.g. >100 nmol/l) have been suggested to be necessary for optimal immune function and respiratory outcomes.⁸ By these definitions, it has been estimated that 1 billion people worldwide have vitamin D deficiency or insufficiency,⁶ so 25-hydroxy vitamin D deficiency is a health problem worldwide.

Vitamin D deficiency or insufficiency is due to an inadequate sun exposure to maintain adequate vitamin D,⁹ clothes that shield most of the skin from the sun,¹⁰ skin pigmentation, season, aging, sunscreen use, and air pollution,¹¹ obesity,¹² pregnancy, and breastfeeding.¹³

To minimize such deficiency, in many countries — but not in Italy — many kinds of food and drinks are available which are fortified with vitamin D.

The role of vitamin D in the bone metabolism has been known for over two hundred years, while in the last years the association between vitamin D deficiency and a lot of disease has been described, such as: malabsorption due to chronic inflammatory bowel disease, celiac enteropathy, total or subtotal gastrectomy, pathologies of the biliary secretion, intestinal bacterial pollution, kidney failure, cancer, diabetes, autoimmune disorders, hypertension, atherosclerosis, muscle weakness, and neuronal disorders has been reported.⁶

Many studies have been demonstrating the role of vitamin D in airway disease.

Immunological effects of vitamin D on COPD

As recently published, many *in-vitro* and *in-vivo* studies suggest a potential involvement of vitamin D in numerous pathogenic processes in respiratory disease.¹⁴

The biologically active form of vitamin D is produced in the lungs by airway epithelial cells, macrophages, and neutrophils, which express both 1 α -hydroxylase and VDR. Therefore, macrophages express a truncated, catabolically inactive form of 24-hydroxylase, thereby preventing catabolism of 1,25-dihy-

droxyvitamin D; in this way macrophages may maintain an overproduction of 1,25-dihydroxyvitamin D, which could promote innate effector functions.

The active form of vitamin D has anti-inflammatory effects due to inhibition of NF- κ B and mitogen-activated protein kinase (MAPK) activity. NF- κ B and MAPK activity lead to the enhanced secretion of a number of inflammatory cytokines and chemokines, including interleukin-1 β (IL-1 β), IL-6, IL-8, TNF- α , and monocyte chemoattractant protein-1. In response to these inflammatory signals, neutrophils, monocytes, and T lymphocytes rapidly migrate into the lungs and further promote the inflammatory response. Hence, 1,25 vitamin D decrease the inflow of inflammatory cells producing the decreased expression of pro-inflammatory cytokine, chemokines,¹⁴ antiapoptotic factors as well as enzyme involved in the generation of pro-inflammatory mediation such as COX-2.¹⁵

Vitamin D can reduce oxidative stress, NF- κ B and p38 MAPK pathways related, by inhibiting antiprotease activity, impairing antimicrobial defenses, increasing the release of TGF- β ¹⁶ and acting on nuclear factor erythroid 2-related factor 2 (Nrf2, a transcriptional regulator of most antioxidant genes). These effects of vitamin D on Nrf2 might also have implications for the phagocytic capacity of alveolar macrophages.¹⁴

Vitamin D plays a preventive role against infection: 1,25-dihydroxy vitamin D increased the maturation and proliferation of monocytes into macrophages¹⁷ and promotes the production of cathelicidin and defensin- β 2, by up-regulating their transcription.¹⁸ These peptides are produced by several cell types in the lungs such as airway epithelial cells, macrophages and neutrophils. Cathelicidin and defensin- β 2 are very effective in killing both Gram-positive and -negative antibiotic-resistant strains, such as *Pseudomonas* and *Staphylococcus aureus*, and different viruses.¹⁹ They also suppress inflammation,²⁰ and subsequently reduce the severity of infection.

Berg *et al.* demonstrated that low 25-hydroxyvitamin D serum levels were associated

with the severity of computed tomography-defined emphysema.²¹ This might be because vitamin D plays a critical role in tissue repair and remodeling: 1,25-dihydroxy vitamin D has been showed a potential inhibitory effect in expression of several matrix metalloproteinases in monocytes and alveolar macrophages, in proliferation and activation of murine fibroblasts and in reduction of expression of extracellular matrix proteins by these fibroblasts.²² These observations suggest that under TGF- β stimulation, 1,25-dihydroxy vitamin D inhibits the pro-fibrotic phenotype of lung fibroblasts and epithelial cells.

Vitamin D status, lung function and exacerbation in COPD

There are several cross-sectional and longitudinal studies that demonstrated the positive association between vitamin D serum levels and lung function, as assessed by forced expiratory volume in 1 second (FEV₁);^{21, 23-27} while the role of vitamin D on lung function decline has been evaluated in few longitudinal studies. Kunisaki *et al.* followed 196 continuous smokers with COPD during 6 years and found no difference in baseline 25(OH)D levels between patients with rapid and slow decline in FEV₁.²⁸ This is in contrast to the findings of a large population-based study, where lower 25(OH)D levels were associated with faster decline in FEV₁ in 1647 COPD patient,²⁹ and to a community-based sample of 626 elderly men followed for 20 years, the combination of current smoking and vitamin D deficiency was significantly associated with a faster decline in FEV₁.²⁶ Finally, Persson *et al.* showed that severe vitamin D deficiency was associated with the decline in lung function.³⁰

The role of vitamin D on acute exacerbation of the disease is still debated. In a secondary analysis of a prospective cohort study of Kunisaki *et al.*, performed in exacerbation-prone COPD patients,²⁸ no association was found between baseline vitamin D levels and subsequent risk of acute exacerbations; negative results have also been reported in a primary care setting³¹ and in a London COPD cohort.³²

These results were probably due to the fact that some patients in the study were already taking vitamin D supplementation at baseline for osteopenia or osteoporosis. Hence, of course, once supplemented, vitamin D levels no longer reflected the underlying COPD severity. In a second analysis of a study by Kunisaki *et al.*²⁸ excluding those patients taking supplements, Heulens *et al.*³³ demonstrated that patients with vitamin D levels below 10 ng/mL had the shortest time to first exacerbation and experienced the highest number of COPD acute exacerbation. Similar positive results were observed in our study,³⁴ where severe vitamin D deficiency was related to more frequent disease exacerbations and hospital admission for acute exacerbation of the disease independently of patients' characteristics and comorbidities.

Recently, Zhang *et al.* published a meta-analysis of case-control study analyzing the relationship of vitamin D on asthma and COPD. It showed that vitamin D insufficiency at baseline is not associated with increased odds ratio of COPD exacerbation.³⁵ The vitamin D add on has recently demonstrated effects on disease control. To date, two double-blind, randomized placebo-controlled trials were performed to analyze the effect of vitamin D supplementation on the incidence of exacerbations in COPD patients. Lehouck *et al.* randomized 182 patients with moderate to very severe COPD with history of recent exacerbations to receive 100,000 IU of vitamin D supplementation or placebo every 4 weeks for 1 year. They did not show significant differences among two groups in the median time to first exacerbation, exacerbation rates, FEV₁, hospitalization, quality of life, and death. However, a post hoc analysis in 30 participants with severe vitamin D deficiency at baseline (serum vitamin D levels <10 ng/mL) showed a significant reduction in exacerbations in the vitamin D add on group.³⁶ Similar results were found in ViDiCo Trial: 240 COPD patients, enrolled by 60 general practices and four Acute National Health Service Trust clinics in London, were randomly allocated to vitamin D₃ add-on group (receiving a 2-monthly oral dose of

120,000 IU vitamin D₃) or placebo group for 1 year. Vitamin D₃ compared to placebo did not affect time to first moderate or severe exacerbation, but vitamin D₃ add-on protected against moderate or severe exacerbation, in patients with COPD with baseline 25-hydroxyvitamin D levels lower than 50 nmol/L (*i.e.*, 30 ng/mL).³⁷

According to findings mentioned above, our preliminary results on 45 COPD patients with vitamin D deficiency, supplemented with vitamin D 1000 UI daily, suggest a decrease in acute exacerbation and hospital admission for COPD after 1 year of vitamin D replenishment.³⁸

There are two double-blind, randomized placebo-controlled trials which are currently still ongoing. One is the PRECOVID multi-center intervention study that enrolled 240 COPD patients aged 40 and above, after an exacerbation, and randomized them in a 1:1 ratio to receive vitamin D₃ 16800 IU or placebo orally once a week during 1 year.³⁹ The other one is LungVITAL study, taking place in the United States among over 20,000 participants aged 50 years or older, including a subgroup of COPD patients, to evaluate the influences of vitamin D and marine omega-3 fatty acid supplementation, on pneumonia risk, respiratory exacerbation episodes and lung function.⁴⁰

Immunological effects of vitamin D on asthma

As showed in a recent review of the literature,⁴¹ vitamin D may influence the pathogenesis of asthma, in addition to the above mentioned immunological effects.

During life *in utero*, vitamin D regulates lung growth,⁴² and a maternal vitamin D deficiency was associated, in male children, with asthma at the age of 6.⁴³

1,25-dihydroxy vitamin D acts on adaptive immune system by inhibiting the proliferation, differentiation and production of immunoglobulins of B lymphocytes, suppressing the secretion of pro-inflammatory cytokines by CD4+ T cells, inhibiting the development and function of multiple T-helper (Th) cells, such

as Th1, Th9, Th17, upgrading the development of regulatory T cells and promoting tolerogenic phenotype of dendritic cells.⁴⁴

Up today, the role of vitamin D is not established on Th2 cells: there are many studies with contradictory results; research with human cord blood cells has demonstrated inhibition of both Th1 and Th2 differentiation with 1,25-dihydroxy-vitamin D,⁴⁴ whereas in another study⁴⁵ 1,25-dihydroxy-vitamin D has decreased Th1 cytokines and has increased Th2 cytokines in stimulated peripheral blood mononuclear cells in subjects with inflammatory bowel disease.⁴⁶

Another important feature of vitamin D is the correlation with IL-10 expression. Active vitamin D response elements have been identified in the IL-10 gene and 1,25-dihydroxy vitamin D serum level is associated with increased IL-10 gene expression in the T cells of patients with steroids refractory asthma. IL-10 inhibits activation and effector function of T cells, monocytes, and macrophages. In addition to these activities, IL-10 regulates growth and differentiation of B cells, NK cells, mast cells, granulocytes, dendritic cells, keratinocytes, and endothelial cells. IL-10 plays a key role in differentiation and function of Tregs and regulates response both of T-helper 1 (Th1)-type and Th2-type. These data suggest that vitamin D serum levels may be important for asthma control.⁴⁴

Vitamin D has been linked to reduced risk of viral respiratory illness due to direct influence on chemokine synthesis and the alteration of growth and differentiation of airway epithelial cells. This effect takes place because vitamin D supplementation increases the secretion of CXCL8 and CXCL10 that induce thickening of the cell layers and proliferation of cytokera-tin-5-expressing cells.⁴⁴

Vitamin D may also influence airway remodeling: it suppresses bronchial airway muscle cells proliferation and has direct inhibitory effects on passively sensitized human airway smooth muscle cells *in vitro*, including inhibition of cell proliferation and expression of MMP-9 and ADAM33.⁴⁴

Vitamin D is also associated with steroid

response and may attenuate steroid resistance. The administration of vitamin D₃ to healthy individuals and steroid resistance asthmatic patients enhanced subsequent responsiveness to dexamethasone for induction of IL-10 by CD4⁺T cells.⁴⁷ In another research performed on an experimental model of steroid resistance in which dexamethasone alone did not inhibit T cell proliferation, addition of vitamin D resulted in significant dose-manner suppression of cell proliferation, due to the increase of MAPK 1 and IL10 mRNA.⁴⁸

Vitamin D can act in synergy with steroids, decreasing responsiveness to self and external allergens by inducing a tolerogenic dendritic cell phenotype.⁴⁴

Vitamin D status, lung function and exacerbation in asthma

The effect of vitamin D on asthma was analyzed by many more studies than those on COPD. Recently, a review that examined all of these studies was published.⁴⁹ In this review 25 case control studies were analyzed, evaluating 2568 asthmatic cases and 4376 controls, that examined vitamin D intake, status as well as correlations with respiratory and atopic parameters in asthma cases compared to controls; 36 cross sectional studies, involving 386,584 subjects. The results of these studies suggest that low 25-hydroxy vitamin D serum level is associated with poor control, increased exacerbation, reduced lung function and increased medication use in asthmatics.

Lately, Zhang *et al.*³⁵ published a meta-analysis of case-control study analyzing the relationship of vitamin D to asthma and COPD. It showed that vitamin D insufficiency at baseline is associated with increased odds ratio of severe asthma exacerbation, and children with insufficient vitamin D levels have a slightly lower mean FEV1 compared with children with sufficient levels.

These results are interesting, but they cannot demonstrate whether the vitamin D deficiency influences asthma pathogenesis, severity and control or vice versa.

More robust information is provided by in-

terventional studies; up today 17 intervention- al studies were performed, among which 9 are randomized clinical trials.

Most of these trials demonstrated that vita- min D add-on reduces significantly the number of asthma acute exacerbations and improves lung function and symptoms, but the best known was a negative one, the VIDA trial.⁵⁰ In this randomized clinical trial 408 patients were enrolled. Patients received oral vitamin D₃ (100,000 IU once, then 4000 IU/day for 28 weeks) or placebo added to inhaled ciclesonide (320 µg/day); if asthma control was achieved after 12 weeks, ciclesonide was tapered to 160 µg/day for 8 weeks, then to 80 µg/day for 8 weeks if asthma control was maintained. This study assessed the effect of vitamin D supplementation on asthmatics with vitamin D deficiency and did not find an effect of vitamin D supplementation on the primary outcome of time to first asthma exacerbation. How- ever, the trial was underpowered, and when analyses were limited to those that achieved a sufficient level of vitamin D (30 ng/mL or greater), an effect was seen for decreasing the overall number of asthma exacerbations. A small difference in the overall dose of cicle- sonide required to maintain asthma control in the vitamin D₃ group (111.3 µg/day) *versus* the placebo group (126.2 µg/day) was observed.

Our preliminary data demonstrated that vi- tamin D deficiency was related to airway ob- struction, higher inhaled steroids dosage and higher rate of asthma acute exacerbation, and the vitamin D add on was associated with improvement of airway obstruction and de- creased rate of asthma acute exacerbation.⁵¹

Recently, it was published a meta-analysis about the benefits and harms of vitamin D sup- plementation in asthmatic children: Fares *et al.* matched four trials with a total of 149 children and do not confirm or rule out beneficial ef- fects of vitamin D supplementation.⁵²

As mentioned above, vitamin D has a role on airway remodeling, this is demonstrated also in some clinical study. Sutherland ER *et al.* showed that reduced vitamin D levels, in asthma, are associated not only with impaired lung function and reduced glucocorticoid re-

sponse but also with increased airway hyper responsiveness.⁵³

Gupta *et al.*⁵⁴ found that 25-hydroxy vita- min D levels were significantly lower in severe asthma than either mild asthmatics or controls, bronchodilator response and IgE and inverse- ly related with airway smooth muscle mass in children with severe asthma; further low 25-hydroxy vitamin D was related with asthma exacerbation and medication usage.

At the end, there is an increasing interest in association of higher vitamin D serum level and reduction of inhaled steroids dosages, es- pecially in children.^{48, 55-58}

Conclusions

Studies on vitamin D suggest that it has an important role in lung diseases, especially in asthma and COPD, depending on its immuno- modulatory function. Most of clinical studies demonstrated that dietary or pharmacological supplementation should reduce exacerbations and remodeling. Nutritional lack and low sun exposure in large parts of world population seem to underline the need of attention on this yet controversial topic. More studies are need- ed to confirm these findings.

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