CHAPTER 1
General introduction
The role of vitamin D as an important actor in bone and calcium homeostasis has long been established. In the past few years, a large number of studies have examined the extra-skeletal role of vitamin D as well. Research pointing towards a role of vitamin D in the immune system and lungs have led us to study the potential role of vitamin D in inflammation and pulmonary function. In this general introduction vitamin D metabolism, the classical effects of vitamin D and its role in the immune system and lungs will be discussed. Finally, the aims and outline of this thesis will be presented.

**Vitamin D pathway**

**Metabolism & Regulation**

Vitamin D is a prohormone produced in the skin under the influence of ultraviolet radiation (Figure 1). Next to production in the skin, vitamin D is consumed in food, especially fatty fish. In the skin, the precursor 7-dehydrocholesterol is converted to the unstable previtamin D3, and subsequently to vitamin D3. In the circulation, vitamin D is then bound to vitamin D binding protein (VDBP) and transported through the body. As vitamin D is a fat-soluble molecule a large part is sequestrated in adipose tissue. In the liver, vitamin D is hydroxylated into 25-hydroxyvitamin D (25(OH)D) by the enzymes CYP2R1 and CYP27A1. Whereas 25(OH)D is presumed to be metabolically inactive, it is the quantitatively most important circulating form in the body and used to determine vitamin D status. 25(OH)D is then further hydroxylated in the kidneys by CYP27B1 into 1,25-dihydroxyvitamin D (1,25(OH)_2D), the active metabolite. This hydroxylation has also been shown to take place in a large number of other organs, such as the lungs, immune system and intestines. Inactivation of 25(OH)D and 1,25(OH)_2D takes place through degradation by the enzyme CYP24A1 into 24,25-dihydroxyvitamin D and 1,24,25-trihydroxyvitamin D, and sequentially into further inactive metabolites, which are excreted in the bile (1-4).

**Vitamin D Receptor**

Vitamin D exerts its effects through binding of the Vitamin D Receptor (VDR). The VDR is a nucleus receptor and upon binding of 1,25(OH)_2D and subsequent dimerization with the Vitamin A Receptor, activates several target genes. Several studies have shown that the 1,25(OH)_2D-VDR-complex controls almost 3% of the human genome. The discovery of the VDR on nearly all tissues in the human body has led to the hypothesis that vitamin D has a larger role in human physiology next to its classical effects in bone and mineral homeostasis (5).

The important role of the VDR is demonstrated by the VDR knock-out mouse. These mice lacking VDR failed to thrive, demonstrated alopecia, hypocalcemia and a severe impairment
of bone formation and mineralisation (6). They also showed a higher sensitivity to autoimmune diseases such as inflammatory bowel disease. Next to these physical effects, VDR knock-out mice also showed different behaviour, such as a disturbed grooming pattern and an increased anxiety behaviour. These defects were not observed in vitamin D–deficient animals and point towards a critical role for VDR, not only in growth and bone formation, but also in the immune system and neurobehavioural development.

Classical effects

The classical role of vitamin D consists of its effects in calcium and bone metabolism. The effects of vitamin D are tightly regulated by the parathyroid hormone (PTH). PTH is produced in the parathyroid glands in the case of low calcium and/or high phosphate serum levels. PTH leads to mobilization of calcium from bone and stimulates the renal hydroxylation of 25(OH)D. 1,25(OH)_{2}D stimulates absorption of calcium and phosphorus in the gut. In addition, both PTH and 1,25(OH)_{2}D stimulate the reabsorption of calcium in the distal renal tubules, whereas PTH leads to an inhibition of phosphorus reabsorption. These effects lead to a net increase of serum calcium and decrease of serum phosphorus.
Serum PTH levels are in turn regulated by a negative feedback loop through higher serum calcium levels and a direct effect of \(1,25(\text{OH})_2\text{D}\) (7). Finally, \(1,25(\text{OH})_2\text{D}\) also directly influences osteoblast and osteoclast signaling, influencing bone remodeling (7). The effects of \(1,25(\text{OH})_2\text{D}\) are mostly anabolic, leading to a net increase in bone formation.

**Vitamin D deficiency**

Because of its long half-life, serum 25(OH)D concentrations are used to measure vitamin D status. Reference ranges for serum 25(OH)D concentrations have classically been defined based on the cut-off values above which PTH levels normalise. Based on these cut-off values, serum 25(OH)D concentrations above 50 nmol/L are generally considered as adequate levels (8, 9). However, guidelines of different institutes and societies differ in their opinion of the appropriate reference ranges and supplementation dose (Table 1) (10-13). Based on the cut-off value of 50 nmol/L vitamin D deficiency is present in 40-100% of the general population (8, 14). The Dutch Health Council does not mention reference ranges, but advises supplementation doses according to different risk groups. The most important predictor of vitamin D status is sunshine exposure. The availability of ultraviolet light varies with latitude, season and time of the day. Other important determinants of vitamin D status are skin pigmentation, clothing habits, nutrition and use of supplements. Risk groups of vitamin D deficiency are pregnant women, children, the elderly and non-Western immigrants (13, 15).

**Table 1.** Reference ranges of serum 25(OH)D concentrations (nmol/L) according to different guidelines

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<th>Severe deficiency</th>
<th>Deficiency</th>
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<td>-</td>
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<td>Endocrine Society, 2011</td>
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<td>&lt;25</td>
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<td>ECTS, 2019</td>
<td>&lt;30</td>
<td>30-50</td>
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<td>&gt;50</td>
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*IOM: Institute of Medicine; SACN: Scientific Advisory Committee on Nutrition; ECTS: European Calcified Tissue Society*

Vitamin D deficiency can result in a defect in bone mineralisation and formation, which increases the risk of fractures. Severe vitamin D deficiency can lead to rickets in children and osteomalacia in adults. In the past few years, however, vitamin D deficiency has been linked to a large number of other health effects. These studies mainly consist of association studies and a causal link has not yet been proven.
Vitamin D and the immune system

Role of vitamin D in the immune system

The expression of the VDR and CYP27B1 by several immune cells, have led researchers to hypothesize that vitamin D plays a role in the immune system. In the past two decades, a large amount of in vitro and in vivo studies have been published confirming this hypothesis (Fig 2). In the innate immune system, it has been shown that Toll-like receptor activation (TLR) by bacterial antigens leads to the induction of the expression of VDR and CYP27B1 in macrophages and monocytes, leading to upregulation of target genes and catalyzing the production of the active 1,25(OH)₂D (16). 1,25(OH)₂D improves phagocytosis and the production of antimicrobial agents such as LL-37 (16) and β-defensins (17). In the adaptive immune system VDR has been shown to be present in lymphocytes. In CD4+ helper T-cells (Th cells), 1,25(OH)₂D inhibits the expression of cell-mediated Th₁ cytokines that are involved in cell-mediated immunity, while promoting Th₂ cytokines that are involved in humoral immunity (18, 19). This promotion of a less cell-mediated immune response has been proposed as a potential mechanism by which vitamin D might have beneficial effects in autoimmune diseases. In addition, 1,25(OH)₂D promotes the regulation of T-regulatory lymphocytes (T_REG) proliferation, supporting a more anti-inflammatory state of the immune system. Finally, bridging the innate and adaptive immune response, 1,25(OH)₂D suppresses the maturation of myeloid dendritic cells and enhances the production of specific cytokines such as IL-10, thereby promoting a more tolerogenic phenotype (20).

Clinical studies

The results of in vitro studies of vitamin D in the immune system have proposed an important role of vitamin D in antimicrobial defense and autoimmune diseases. Indeed, already in the 19th century cod liver oil and UV-B light were used for the treatment of tuberculosis (21). Lower levels of vitamin D concentrations have been associated with a higher risk of progression to active tuberculosis (22, 23). Two randomized clinical trials showed that additional vitamin D supplementation led to a faster recovery of symptoms and conversion of the sputum smear to culture negativity. These results, however, were not replicated in a later, larger study (24). Several cohort studies have shown that, next to tuberculosis, lower levels of 25(OH)D are related to an increased risk of respiratory tract infections (25, 26). A recent meta-analysis of intervention studies with individual participant data showed that vitamin D supplementation protected against respiratory tract infections in participants with serum 25(OH)D levels below 25 nmol/L (27).
Next to infectious diseases, vitamin D has also been related to several autoimmune diseases. Lower levels of vitamin D concentrations have been linked to a higher risk of type 1 diabetes, multiple sclerosis and Crohn’s disease. Increasing prevalences of these diseases with higher latitude have supported these findings. In addition, several studies have shown associations between VDR polymorphisms and type 1 diabetes (28) and MS (29, 30). Randomized clinical trials on the effects of vitamin D supplementation on clinical outcomes in these diseases, however, are scarce and several large studies are still ongoing.

Figure 2. Immunomodulatory effects of 1,25(OH)₂D₃
From Mathieu et al. IBMS BoneKEy. 2011 April;8(4):178-186
1,25(OH)₂D₃ targets different players of the innate and adaptive immune compartment. 1,25(OH)₂D₃ stimulates innate immune responses by enhancing the chemotactic and phagocytic responses of macrophages as well as the production of antimicrobial proteins such as cathelicidin. On the other hand, 1,25(OH)₂D₃ also modulates adaptive immunity. At the level of the APC (like the DC), 1,25(OH)₂D₃ inhibits the surface expression of MHC-II-complexed antigen and of costimulatory molecules, in addition to production of the cytokines IL-12 and IL-23, thereby indirectly shifting the polarization of T cells from a Th1 and Th17 phenotype towards a Th2 phenotype. In addition, 1,25(OH)₂D₃ directly affects T cell responses, by inhibiting the production of Th1 cytokines (IL-2 and IFN-γ) and Th17 cytokines (IL-17 and IL-21), and by stimulating Th2 cytokine production (IL-4). Moreover, 1,25(OH)₂D₃ favors Treg cell development via modulation of DCs and by directly targeting T cells. Finally, 1,25(OH)₂D₃ blocks plasma cell differentiation, IgG and IgM production and B cell proliferation.
Vitamin D and the lungs

Role of vitamin D in the lungs

Several in vitro studies have shown that lung epithelial cells and alveolar macrophages express CYP27B1, suggesting an active role of 1,25(OH)₂D locally (16, 31). One of the first epidemiologic studies to point towards a potential role of vitamin D in pulmonary function was a large study performed in the third National Health and Nutrition Examination Survey. Black and colleagues showed that lower serum 25(OH)D concentrations were associated with a worse pulmonary function (32). This relationship has since then been studied, but the results have remained inconsistent (33-36).

The underlying mechanism remains unclear, but it has been proposed that vitamin D affects tissue remodeling in the lungs due to its effects on metalloproteinases and inflammatory cytokines (37, 38). Several studies showed an inverse association between systemic inflammatory markers and pulmonary function (39, 40).

COPD

The combination of anti-inflammatory and antimicrobial effects have led researchers to believe vitamin D might be a therapeutic agent in patients with chronic obstructive pulmonary disease (COPD) (41). COPD is a disease characterized by a progressive, non-reversible airflow limitation associated with an abnormal inflammatory reaction of the lungs. In addition, patients with COPD can have acute exacerbations that are characterized by acute worsening of the respiratory symptoms and are often triggered by bacterial or viral infections (42). These exacerbations are an important predictor of quality of life and health status in patients with COPD, and are associated with a faster decline of the pulmonary function (43, 44). Several studies have shown a high prevalence of vitamin D deficiency in COPD (41, 45). One explaining factor is the impaired capacity of the skin for vitamin D synthesis due to aging and toxic smoke effects in patients with COPD. Another factor is reduced sun exposure due to less outdoor activities because of their disability. On the other hand, vitamin D catabolism might be increased due to inflammation and glucocorticoid use (46). Several studies have shown that lower serum 25(OH)D concentrations are associated with worse pulmonary function and a faster decline in patients with COPD. Two previous RCTs showed that, overall, vitamin D supplementation did not affect exacerbation rate in patients with COPD. However, post-hoc analyses in subgroups with severe vitamin D deficient patients (serum 25(OH)D <25 nmol/L) showed that exacerbation rate was lower in patients receiving vitamin D supplementation.
Physical performance

Next to its effects in the lungs, vitamin D plays an important role in muscle function. It is thought that vitamin D affects skeletal function through VDR signaling as well as through more rapid nongenomic effects on cellular Ca\textsuperscript{2+} influx (47). Several observational studies in the general population have shown that higher serum 25(OH)D concentrations are associated with better physical performance scores and muscle strength. In addition, several meta-analyses have shown that vitamin D supplementation has positive effects on muscle strength and balance, but only in participants with vitamin D deficiency (serum 25(OH)D < 50 nmol/L) and of 60 years and older (48, 49). In COPD, impaired physical performance and skeletal muscle dysfunction form a major part of the disease burden and are independent predictors of respiratory failure and death (50, 51). Studies on the relationship between vitamin D status and muscle function in patients with COPD show inconsistent results (52, 53). One study showed that a polymorphism in the VDR gene was associated with quadriceps muscle strength in patients with COPD (54). Two trials on the effects of vitamin D supplementation on physical performance in patients with COPD did not show any effect (55, 56).

Aims and outline of the thesis

The emerging evidence of the role of vitamin D in the immune system and lungs have led us to investigate the role of vitamin D in inflammation, pulmonary function and as a potential therapeutic in patients with COPD. In addition, this thesis studies several outcomes related to inflammation and pulmonary function to help us understand potential underlying mechanisms of these relationships. A schematic form of the hypothesis is presented in Figure 2.

The first part of this thesis investigates the relationship between 25(OH)D concentrations and inflammation, and the role of adiposity therein. As obesity has been associated with both inflammation and vitamin D deficiency, we will study whether the association between vitamin D levels and inflammation is influenced by adiposity. In Chapter 2 the relationship between several body fat deposits and 25(OH)D concentrations is studied. In Chapter 3 the relationship between 25(OH)D concentrations with CRP, leptin and adiponectin is described.

In the second part of this thesis the relationship between 25(OH)D concentrations, pulmonary function and inflammation is studied. In Chapter 4 associations between 25(OH)D concentrations and pulmonary function, airway inflammation and common colds in a cohort of the general population are examined. In Chapter 5 the relationship between 25(OH)D and pulmonary function is studied in an elderly population. In addition,
potential mediation by physical performance and inflammation was analysed.

In the third part of this thesis the relationship between vitamin D and quality of life and the effects of supplementation in patients with COPD are studied. It is known that people with chronic diseases, such as COPD, cardiovascular diseases and arthritis often score lower in assessments of quality of life. As vitamin D status has also been related to quality of life, we will study the relationship between 25(OH)D and quality of life and the role of physical performance and chronic diseases therein in Chapter 6. Finally, as previous studies have pointed towards a beneficial effect of vitamin D supplementation in patients with COPD we have set-up two trials assessing these effects. Chapter 7 presents the results of our pilot trial on the effects of vitamin D supplementation on respiratory muscle strength and physical performance in patients with COPD. Chapter 8 discusses the design of our ongoing RCT on the effects of vitamin D supplementation on exacerbation rate in patients with COPD. In Chapter 9 we included a recent meta-analysis on the effects of vitamin D supplementation in patients with COPD, including our pilot trial.

Cohort studies

In this thesis we have used data from two large prospective cohort studies: the Longitudinal Aging Study Amsterdam (LASA) and the Netherlands Epidemiology of Obesity (NEO) study. Below, a short description of these studies will be given.

LASA

The Longitudinal Aging Study Amsterdam (LASA) is a an ongoing population-based, prospective cohort study and aims to determine predictors and consequences of aging. The
study consists of a nationally representative sample obtained from registries of 11 municipalities across 3 culturally distinct regions in the Netherlands. Data collection started in 1992-1993 among a cohort of respondents aged 55-84 years old (N=3,107, wave B, baseline). Since then, measurement cycles have been conducted about every three years. An additional cohort of respondents aged 55-64 years was included from the same sampling frame and was measured for the first time exactly ten years after the original baseline measurement, in 2002-2003 (cohort 2, N=1,002, wave 2B). Since then, respondents from this second cohort of respondents have been included in regular LASA measurement waves. In 2012-2013, exactly twenty years after the baseline measurement, a third cohort study (cohort 3, wave 3B) was initiated with new respondents aged 55-64 years from the same sampling frame. This sample is also included in regular LASA measurement waves. For this thesis data from the first (1995/1996) and third (2012/2013) cohort were used. A detailed description of the study can be found elsewhere (57).

NEO
The Netherlands Epidemiology of Obesity (NEO) study is an ongoing population-based prospective cohort study in men and women aged 45–65 years at baseline. Individuals with a body mass index (BMI) of 27 kg/m² or higher were oversampled in the study. The study was designed to investigate pathways leading to obesity-related diseases. In total, 6,671 individuals were included between 2008 and 2012. Men and women with self-reported BMI ≥27 kg/m² living in the greater area of Leiden (in the west of the Netherlands) were eligible to participate in the NEO study. In addition, in one municipality (Leiderdorp) all inhabitants aged 45 to 65 years were invited, irrespective of their BMI, allowing for a reference distribution of BMI. Participants were invited for a baseline visit at the NEO study center of the Leiden University Medical Center (LUMC) after an overnight fast. Prior to this study visit, participants completed a general questionnaire at home to report demographic, lifestyle and clinical information. All participants underwent an extensive physical examination, including blood sampling and spirometry. Design and data collection of the study has been described in detail previously (58).
References


