VITAMIN D AND EXTRASKELETAL EFFECTS IN CHILDREN

Studies on infections, allergies and inflammation

Jenni Rosendahl

ACADEMIC DISSERTATION

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Supervisors

Professor Sture Andersson
Children’s Hospital
University of Helsinki and Helsinki University Hospital
Helsinki, Finland

Professor Outi Mäkitie
Children’s Hospital
University of Helsinki and Helsinki University Hospital
Helsinki, Finland

Reviewers

Professor Ville Peltola
Department of Paediatrics and Adolescent Medicine
University of Turku and Turku University Hospital
Turku, Finland

Professor Harri Niinikoski
Department of Physiology and Pediatrics
University of Turku
Turku, Finland

Opponent

Professor Marjo Renko
Department of Pediatrics
University of Eastern Finland
Kuopio, Finland

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To my family
Vitamin D is essential for normal childhood growth and bone development. For almost 90 years, vitamin D supplementation has been recommended for children in Finland. At present, due to the widely adopted use of vitamin D supplementation, rickets has been largely eradicated, but interest towards other potential benefits of vitamin D has increased. Besides its essential effects on bone and mineral metabolism, vitamin D has several extraskeletal actions and is an important modulator of the immune system. Vitamin D status is defined by serum 25-hydroxyvitamin D concentration (25(OH)D), and a 25(OH)D concentration ≥ 50 nmol/L is usually regarded as an indicator of vitamin D sufficiency. However, this may only apply to skeletal health; the optimal vitamin D status for immune defense and overall health remains unestablished.

The main aim of this thesis was to study the extraskeletal effects of vitamin D focusing on infections, allergies and inflammation. The effect of high-dose vitamin D supplementation, as compared with the standard recommended dose, in prevention of childhood infections and allergic diseases was evaluated. The study enabled examination of vitamin D status of Finnish children at various ages and determination of factors influencing vitamin D status.

The Vitamin D Intervention in Infants study (VIDI) comprised 975 healthy term infants randomized to daily vitamin D₃ supplementation of either 10 µg or 30 µg for the first two years of life. Data on infectious diseases were collected prospectively from the parents. Occurrence of allergic diseases was determined by questionnaires and specific IgE antibodies towards common allergens were measured. Serum 25(OH)D was analyzed during pregnancy, at birth from cord blood, and at ages 1 and 2 years. Inflammatory markers were measured from cord blood. In addition, vitamin D status and related dietary and health factors at 10 years of age were assessed from another study sample of 171 fourth graders.

The children in both study populations were mostly vitamin D sufficient, as defined by serum 25(OH)D concentration ≥ 50 nmol/L. Over 95% of the children participating in the vitamin D intervention study were vitamin D sufficient throughout the study, from birth up to age 2 years. Of the 10-year-old children, almost 84% were vitamin D sufficient.

In the vitamin D intervention study, the incidence of parent-reported infections did not differ between the intervention groups at age 2 years. Allergic sensitization and clinical allergies were not prevented by high-dose
vitamin D supplementation during the first year of life. In contrast, higher cord blood 25(OH)D predicted higher concentration of inflammatory markers at birth, and associated with increased risk of allergic sensitization at age 1.

In conclusion, in contrast to previous research, most of the participating Finnish children had adequate vitamin D status. We found no additional benefit of high-dose vitamin D supplementation compared with the standard dose in the prevention of infections or allergic diseases. Daily 10 μg vitamin D supplementation was adequate in maintaining vitamin D sufficiency in children from birth to age 2 years.

Tämän väittöskirjatutkimuksen tavoitteena oli selvittää D-vitamiinin luuston ulkopuolisia vaikutuksia, keskitytyn erityisesti pienentävän lasten infektioihin, allergioihin ja tulehdustilaan. Tutkimuksessa selvitettiin, miten nykyvuotiaiden korkeampi D-vitamiininoso vaikuttaa infektiosairastuvuuteen ja allergoiden kehittymiseen varhaislapsuudessa. Tutkimuksessa tarkasteltiin myös suomalaislasten D-vitamiinitasoa eri ikäkausina sekä tutkittiin siihen vaikuttavia tekijöitä.


CONTENTS

Abstract .......................................................................................................................... 4
Tiivistelmä ..................................................................................................................... 6
Contents ....................................................................................................................... 8
List of original publications ....................................................................................... 11
Abbreviations .............................................................................................................. 12
1 Introduction .............................................................................................................. 13
2 Review of the literature .......................................................................................... 14
   2.1 History of vitamin D ....................................................................................... 14
   2.2 Vitamin D metabolism .................................................................................. 15
   2.3 Physiological actions of vitamin D ................................................................. 17
      2.3.1 Skeletal homeostasis ............................................................................. 17
         2.3.1.1 Rickets ....................................................................................... 17
      2.3.2 Immunological actions ......................................................................... 19
         2.3.2.1 Vitamin D and innate immunity ..................................................... 19
         2.3.2.2 Vitamin D and adaptive immunity ............................................. 20
         2.3.2.3 Vitamin D and inflammation ....................................................... 21
      2.3.3 Other extraskeletal actions .................................................................... 22
   2.4 Vitamin D status ............................................................................................. 22
      2.4.1 Measurement of vitamin D status ......................................................... 22
      2.4.2 Definition of vitamin D status ............................................................... 23
      2.4.3 Vitamin D intake ................................................................................... 24
         2.4.3.1 Recommendations for vitamin D intake ....................................... 24
         2.4.3.2 Vitamin D supplementation guidelines ..................................... 25
         2.4.3.3 Food fortification ......................................................................... 26
2.4.4 Vitamin D status in Finnish children ............................................ 26

2.5 Clinical studies on vitamin D, infections and allergies ...................... 27
2.5.1 Vitamin D and infectious diseases .................................................... 27
2.5.1.1 Observational studies ............................................................... 27
2.5.1.2 Randomized trials ................................................................. 28
2.5.2 Vitamin D and allergic diseases ..................................................... 31
2.5.2.1 Observational studies .............................................................. 31
2.5.2.2 Randomized trials ................................................................ 31

3 Aims of the study ............................................................................. 33

4 Subjects and methods ........................................................................ 34
4.1 Study subjects and design ................................................................. 34
4.1.1 Vitamin D Intervention in Infants study (I, II, III) ....................... 34
4.1.1.1 Randomization and blinding ................................................. 34
4.1.2 ISCOLE cohort (IV) ....................................................................... 34
4.2 Data collection .................................................................................. 35
4.2.1 Family background data (I, II, III, IV) .......................................... 35
4.2.2 Adherence and follow-up (I, II) ..................................................... 35
4.2.3 Nutritional Data (I) ...................................................................... 35
4.2.4 Infection data (I, II) ................................................................... 35
4.2.5 Allergy questionnaire (II, IV) ......................................................... 36
4.3 Laboratory measurements ................................................................. 36
4.3.1 25-hydroxyvitamin D (I, II, III, IV) ............................................. 36
4.3.2 Matrix metalloproteinase 8 (III) ................................................... 37
4.3.3 High-sensitivity C-reactive protein (III) ....................................... 37
4.3.4 Immunoglobulin E (II) ................................................................. 37
4.4 Ethical considerations ........................................................................ 37
4.5 Statistical analyses ........................................................................... 38
5 Results ................................................................................................................................. 39

5.1 Characteristics of study subjects (I-IV) ................................................................. 39

5.1.1 Vitamin D Intervention in Infants study (I, II, III) ........................................ 40

5.2 Vitamin D status .......................................................................................................... 41

5.2.1 Pregnancy (I, II) ................................................................................................. 41

5.2.2 Birth (I, II) ........................................................................................................... 41

5.2.3 Early childhood (I, II) ......................................................................................... 42

5.2.4 School age (IV) ................................................................................................... 43

5.3 Vitamin D supplementation and infectious diseases (I) ................................ 44

5.3.1 Characteristics of infections .............................................................................. 44

5.3.2 Effect of vitamin D supplementation on infections ........................................ 45

5.4 Vitamin D supplementation and allergic diseases (II) .................................. 46

5.5 Cord blood vitamin D and inflammatory and allergy markers ..................... 48

5.5.1 Markers of inflammation (III) ....................................................................... 48

5.5.2 Allergic sensitization (II) .................................................................................. 49

6 Discussion ...................................................................................................................... 50

6.1 Vitamin D status .......................................................................................................... 50

6.2 Vitamin D and infections .......................................................................................... 52

6.3 Vitamin D, allergies and inflammation .................................................................. 53

6.4 Strengths and limitations ......................................................................................... 55

6.5 Future prospects ......................................................................................................... 56

6.6 Conclusions ................................................................................................................ 57

7 Acknowledgements ........................................................................................................ 58

8 References ...................................................................................................................... 61
LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications:


* equal contribution

The publications are referred to by their Roman numerals in the text. The articles have been reprinted with the permission of the copyright holders.
# Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>hs-CRP</td>
<td>High-sensitivity C-reactive protein</td>
</tr>
<tr>
<td>DBP</td>
<td>Vitamin D-binding protein</td>
</tr>
<tr>
<td>DEQAS</td>
<td>Vitamin D Quality Assessment Scheme</td>
</tr>
<tr>
<td>D$_2$</td>
<td>Vitamin D$_2$</td>
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<td>D$_3$</td>
<td>Vitamin D$_3$</td>
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<tr>
<td>25(OH)D</td>
<td>25-hydroxyvitamin D</td>
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<tr>
<td>1,25(OH)$_2$D</td>
<td>1,25-dihydroxyvitamin D</td>
</tr>
<tr>
<td>FGF-23</td>
<td>Fibroblast growth factor 23</td>
</tr>
<tr>
<td>IgE</td>
<td>Immunoglobulin E</td>
</tr>
<tr>
<td>IOM</td>
<td>Institute of Medicine</td>
</tr>
<tr>
<td>NNR</td>
<td>Nordic Nutrition Recommendations</td>
</tr>
<tr>
<td>PTH</td>
<td>Parathormone</td>
</tr>
<tr>
<td>RDA</td>
<td>Recommended dietary allowance</td>
</tr>
<tr>
<td>RI</td>
<td>Recommended intake</td>
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<tr>
<td>Tc cell</td>
<td>Cytotoxic T cell</td>
</tr>
<tr>
<td>Th cell</td>
<td>Helper T cell</td>
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<tr>
<td>Treg cell</td>
<td>Regulatory T cell</td>
</tr>
<tr>
<td>TLR</td>
<td>Toll-like receptor</td>
</tr>
<tr>
<td>UL</td>
<td>Tolerable upper intake level</td>
</tr>
<tr>
<td>UVB</td>
<td>Ultraviolet B</td>
</tr>
<tr>
<td>VDR</td>
<td>Vitamin D receptor</td>
</tr>
<tr>
<td>VDSP</td>
<td>Vitamin D Standardization Program</td>
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1 INTRODUCTION

Vitamin D is essential for life. Especially children need vitamin D for normal growth and bone development, and severe vitamin D deficiency results in rickets and poor health. Classical actions of vitamin D are its effects on bone mineralization and maintenance of calcium-phosphate balance in the body, but various extraskeletal effects of vitamin D have recently been acknowledged. In addition to liver and kidneys, important regulators of vitamin D metabolism, many other tissues and cells express enzymes required for vitamin D hydroxylation and the vitamin D receptor which mediate the actions of vitamin D on gene regulation.

Vitamin D is formed in the skin by sunlight radiation. Dietary sources include fish, eggs, some plants, vitamin D-fortified food products and vitamin D supplements. Vitamin D status is determined by measuring serum 25-hydroxyvitamin D (25(OH)D) concentration. Vitamin D sufficiency is commonly defined as serum 25(OH)D concentration above 50 nmol/L, but there is no precise consensus for the optimal vitamin D status for overall health.

Many immune cells are targets for vitamin D-related regulation, and in observational studies, vitamin D deficiency has been associated with impaired immune function and susceptibility to infectious and allergic diseases. However, the findings are not conclusive and especially the results from randomized vitamin D supplementation trials in disease prevention show mixed findings. Moreover, sufficiently powered randomized trials involving infants and young children are largely lacking.

In Finland, regular daily vitamin D supplementation is recommended for all children under the age of 18 years. Vitamin D supplementation guidelines have mostly been based on studies focusing on prevention of rickets. Considering the expanding knowledge of the various biological actions of vitamin D, the potential extraskeletal effects of vitamin D in children deserve further evaluation.
2 REVIEW OF THE LITERATURE

2.1 HISTORY OF VITAMIN D

Vitamin D is regarded an ancient molecule which has existed in phytoplankton since the early evolution of life. It is believed that it protected early organisms against DNA damage induced by ultraviolet B (UVB) radiation. Plankton, many plants and animals that are exposed to sunlight have the capacity to synthesize vitamin D (Holick 2003).

The human history of vitamin D began with the discovery of rickets, a disease characterized by bone deformities and impaired growth. Already in ancient Greece, the historian Herodotus noticed that Persian warriors, who covered their head with turbans, had much softer skulls than Egyptians who went bareheaded from childhood, exposing their heads to sunlight. In the 1600s, rickets was first described in England by the two doctors Glisson and Whistler, and was originally called the English disease. It affected children living in industrialized cities with limited sunshine exposure and poor diet. Almost two centuries passed until the pathology of rickets was understood to relate to vitamin D (Hernigou et al. 2018, Wolf 2004).

In 1919, Sir Edward Mellanby observed that rickets in dogs could be cured by providing cod liver oil (Mellanby 1919). It was first thought that the vitamin A in cod liver oil was the key ingredient, but Elmer McCollum, an American professor, identified another nutrient factor that cured rickets, called vitamin D (McCollum 1922). The chemical structure of vitamin D was characterized in the 1930s by the chemist Adolf Windaus, who received the Nobel prize in chemistry in 1928 for his research on sterols and vitamins.

Already in the early 1800s, cod liver oil was used for the treatment of tuberculosis. In 1903, Dr. Neils Finsen was awarded the Nobel Prize for curing patients suffering from lupus vulgaris, a cutaneous form of tuberculosis, with phototherapy. Similarly, in the 1940s, sunlight exposure in sanatoria was recommended for tuberculosis patients (McCullough and Lehrer 2018). All these treatments, although not recognized at that time, took advantage of the immunomodulatory functions of vitamin D.
2.2 VITAMIN D METABOLISM

Vitamin D is a secosteroid hormone by chemical structure. There are two forms of vitamin D: vitamin D₃ and vitamin D₂. In human or animal skin, a photochemical reaction induced by solar UVB radiation converts 7-dehydrocholesterol to previtamin D₃, which is further converted to vitamin D₃ by isomerization dependent on skin temperature (Holick et al. 1977). Several factors limit the cutaneous synthesis of vitamin D: e.g. skin pigmentation, the content of 7-dehydrocholesterol in the skin, and the solar zenith angle depending on latitude, season and time of day (Chen TC et al. 2007). Excessive cutaneous vitamin D synthesis is physiologically restricted; thus if sunlight exposure is prolonged, higher UVB exposure doses result in the conversion of previtamin D₃ to inactive isomers lumisterol and tachysterol (Holick et al. 1981). Egg yolk, fatty fish and liver contain vitamin D₃ naturally, while vitamin D₂ is formed from its precursor ergosterol in plants, yeasts and fungi by UVB radiation.

To function biologically, the vitamin D (either vitamin D₃ or vitamin D₂, hereafter referred to as vitamin D) produced in the skin or obtained from diet needs to be activated twice. First, vitamin D is transported to the liver bound to vitamin D-binding protein (DBP) where it is hydroxylated by cytochrome P450 25-hydroxylases into 25(OH)D. Of the 25-hydroxylases, CYP27A1 and CYP2R1 are the main hydroxylating enzymes of vitamin D, but also other cytochrome P450-linked oxidases (i.e. CYP3A4, CYP2J3) have been identified (Prosser and Jones 2004). The DBP-bound 25(OH)D is then transported to the kidneys where the second hydroxylation occurs: 1α-hydroxylase (CYP27B1) catalyzes hydroxylation of 25(OH)D to 1,25-dihydroxyvitamin D (1,25(OH)₂D), the active metabolite of vitamin D.

The active 1,25(OH)₂D exerts its biological actions by binding to vitamin D receptor (VDR) and forming a heterodimer with retinoid X receptor. This complex binds to vitamin D response elements in the target genes and regulates gene transcription. VDR is present in various tissues and cell types of the human body, and vitamin D is thus estimated to participate in the regulation of hundreds of genes (Norman 2008, Wang et al. 2012).

The renal synthesis of 1,25(OH)₂D is tightly regulated by parathormone (PTH), serum calcium and phosphate levels, and fibroblast growth factor 23 (FGF-23). 1,25(OH)₂D also regulates its own level by a negative feedback loop and by activating 25-hydroxyvitamin D-24-hydroxylase (CYP24A1), which catalyzes the catabolism of 25(OH)D and 1,25(OH)₂D into inactive water-soluble metabolites (Jones et al. 2012).
Besides kidneys, many other tissues can contribute to $1,25(\text{OH})_2\text{D}$ synthesis. For example, cells of the placenta, prostate and skin, and immune cells such as macrophages, T lymphocytes and dendritic cells express $1\alpha$-hydroxylase, and are capable of independently synthesizing $1,25(\text{OH})_2\text{D}$ from $25(\text{OH})\text{D}$ (Adams and Hewison 2012, Hewison et al. 2007).

**Figure 1.** Principal steps of vitamin D metabolism. $25(\text{OH})\text{D}$, 25-hydroxyvitamin D. $1,25(\text{OH})_2\text{D}$, 1,25-dihydroxyvitamin D.
2.3 PHYSIOLOGICAL ACTIONS OF VITAMIN D

Classical actions of vitamin D are its effects on bone and mineral metabolism, but the discovery of extrarenal synthesis of 1,25(OH)₂D and existence of VDR in various cell types has extended our knowledge on the functions of vitamin D in the human body.

2.3.1 SKELETAL HOMEOSTASIS

Vitamin D is essential in calcium and phosphate metabolism. Active 1,25(OH)₂D promotes the absorption of calcium and phosphate from the intestine ensuring adequate serum levels of these minerals for bone mineralization and other metabolic functions. Renal reabsorption of calcium is induced by 1,25(OH)₂D, whereas renal phosphate handling also involves FGF-23, PTH and other metabolic factors (Blaine et al. 2015).

Bone remodeling and mineralization is regulated by vitamin D, but the mechanisms are not fully understood. If dietary calcium intake is inadequate, 1,25(OH)₂D induces the maturation of osteoclasts, which in turn dissolve bone to release calcium and phosphate into circulation (Suda et al. 2012). On the other hand, 1,25(OH)₂D induces the differentiation of osteoblasts, enhancing bone mineralization (van Driel and van Leeuwen 2017).

2.3.1.1 Rickets

Rickets is defined as a bone disease affecting growing bones while osteomalacia affects adults or older children with completed growth and closed growth plates. Most commonly, rickets is seen in vitamin D-deficient infants younger than 18 months of age (Holick 2006). The characteristic clinical signs of rickets include bone deformities such as bending of the leg bones (genu varum and genu valgum), growth retardation, delayed fontanelle closure, bone pain, failure to thrive and delayed gross motor development. Radiologically, widening of the growth plates, metaphyseal cupping and fraying, osteopenia and bowing of long bones can be observed. The abnormal metaphyses may also appear as clinically prominent wrists and rachitic rosary (enlarged costochondrial junctions of ribs) (Elder and Bishop 2014).

The most common cause of acquired rickets is severe vitamin D deficiency (Munns et al. 2016). In the absence of vitamin D, absorption of dietary calcium and phosphate from the intestine is impaired. To maintain normal serum calcium levels, the secretion of PTH is increased, resulting in secondary hyperparathyroidism. PTH enhances renal reabsorption of calcium and induces osteoclasts to mobilize calcium from the bone. PTH also decreases renal phosphate reabsorption leading to loss of phosphate in the
Review of the literature

urine and low serum phosphate levels. Hypophosphatemia plays a major role in the development of ricketic skeletal manifestations impairing mineralization of the skeleton, especially in the growth plates of rapidly growing bones. Chondrocyte proliferation is defective, resulting in chondrocyte hypertrophy in the growth plates (Tiosano and Hochberg 2009).

Rickets is associated with susceptibility to respiratory tract infections (Haider et al. 2010, Muhe et al. 1997, Najada et al. 2004). Weakening of the thoracic wall and respiratory muscles causes defective ventilation with respiratory obstruction and atelectasis (Holick 2006, Muhe et al. 1997). In addition, immunological factors related to vitamin D deficiency may predispose ricketic children to infections (Walker and Modlin 2009).

Besides vitamin D deficiency, low calcium intake and genetic defects in vitamin D and mineral metabolism may cause rickets. Genetic diseases include vitamin D pathway defects, hypophosphataemic rickets and hypophosphatasia (Elder and Bishop 2014).

Figure 2. Child with rachitic deformity of the thorax. St Bartholomew’s Hospital Archives & Museum, Wellcome Collection.
2.3.2 IMMUNOLOGICAL ACTIONS

2.3.2.1 Vitamin D and innate immunity

Innate immunity forms the first-line defense of the host against invading pathogens. Anatomical barriers include the skin, mucosa, cilia of the respiratory tract, and normal bacterial flora and peristalsis of the gut. Monocytes, macrophages and neutrophils, also called phagocytes, and dendritic cells are the most important cells of innate immunity. In addition, molecules of the complement system, acute phase proteins and antimicrobial peptides such as cathelicidins and defensins participate in innate immune responses (Parkin and Cohen 2001).

The antimicrobial peptides cathelicidins are able to kill bacteria and fungi, inhibit and destroy bacterial biofilms, and have antiviral and antiparasitic activity. Cathelicidins are expressed in epithelial cells of the skin, intestine and airway, for example, but also in cells of the innate immune system such as neutrophils, monocytes, macrophages and dendritic cells (Vandamme et al. 2012). Several factors influence the expression of cathelicidins but the finding of the cathelicidin gene being a direct target of the vitamin D receptor VDR has suggested that vitamin D may be a major regulator of its production (Gombart et al. 2005, Vandamme et al. 2012).

Monocytes, macrophages and dendritic cells have 1α-hydroxylase activity and VDR (Provvedini et al. 1983). Monocytes and macrophages recognize pathogens by sensing pathogen-associated molecular patterns with their pattern-recognition receptors, such as toll-like receptors (TLR) (Takeda and Akira 2005). As presented in Figure 3, pathogen-recognition with TLR1-TLR2 heterodimer induces 1α-hydroxylase activity and VDR expression in the cell, and 1,25(OH)2D is synthesized from circulating 25(OH)D. The locally produced 1,25(OH)2D binds to VDR and modulates the expression of target genes, including those coding for antimicrobial peptides such as cathelicidin. The produced antimicrobial peptides participate in bacterial killing by formation of autophagosomes (Hewison 2011). This vitamin-D-dependent induction of monocyte antimicrobial activity is best characterized for the pathogen Mycobacterium tuberculosis, but may apply to other pathogens as well, as various microbial components are recognized with TLR2 (Liu et al. 2006, Takeda and Akira 2005).
2.3.2.2 Vitamin D and adaptive immunity

Adaptive immunity consists of antigen-specific immune responses mediated by T and B lymphocytes. T lymphocytes can be divided into helper T cells (Th) and cytotoxic T (Tc) cells. Th cells are essential in the activation and regulation of the overall adaptive immune response, while Tc cells defend the host against pathogens by direct induction of cell death. B cells produce antibodies against foreign antigens to form a humoral immune response.

Dendritic cells are important antigen-presenting cells which introduce bacterial antigens to T cells. In vitro studies demonstrate that vitamin D affects dendritic cells by suppressing their maturation and function (Baeke et al. 2007). The expression of dendritic cell-derived cytokines is modulated by 1,25(OH)2D. For example, 1,25(OH)2D inhibits the production of interleukins 12 and 23, which regulate the differentiation of T helper cells to type 1 T helper cells (Th1) and type 17 T helper cells (Th17) (Penna and Adorini 2000). Inhibition of Th1 and Th17 responses alters the T cell balance towards the type 2 helper cell (Th2) immune response, which is considered important in the pathogenesis of allergy. Vitamin D also induces the development of regulatory T cells (Treg), a subgroup of Th cells that participate in suppressing immune responses and in maintaining immune tolerance (Adorini et al. 2004, Gorman et al. 2007).

The influence of vitamin D on B cells and antibody production is less clear. Like T cells, also B cells express VDR (Provvedini et al. 1983). The active 1,25(OH)2D has been shown to inhibit B cell differentiation and immunoglobulin secretion (Chen S et al. 2007, Hartmann et al. 2011).
2.3.2.3 Vitamin D and inflammation

Inflammation is a consequence of immune responses against invading pathogens or a reaction to repair tissue damage. Cells of the innate immune system release inflammatory mediators on sites of infection or injury to activate acute phase reactions. Macrophages and monocytes produce cytokines, such as interleukin 6, which induce the formation of C-reactive protein (CRP), an acute phase protein produced in the liver. CRP interacts with ligands of bacteria and damaged cells, and participates in the activation of the complement system and enhancement of phagocytosis (Black et al. 2004).

In an acute infection, the plasma level of CRP rises rapidly and thus CRP is widely used as a marker of acute inflammation. However, low concentrations of CRP can be detected in apparently healthy individuals. This mild elevation of CRP, assessed with quantitative high-sensitive assays and thus referred to as high-sensitivity CRP (hs-CRP), reflects chronic systemic inflammation and has been associated with obesity, cardiovascular disease and other chronic inflammatory states (Cook et al. 2000, Haffner 2006, Nappo et al. 2013).

Vitamin D is linked to CRP by findings of experimental studies in which 1,25(OH)₂D inhibits the synthesis of interleukin 6, an activator of CRP (Müller et al. 1991, Zhang et al. 2012). Observational studies demonstrate associations between vitamin D status and CRP levels. In adults, inverse correlations between 25(OH)D and CRP concentration have been documented (de Oliveira et al. 2017, Kruit and Zanen 2016, Liefaad et al. 2015). Similarly, in neonates with vitamin D deficiency, an inverse correlation with cord blood 25(OH)D and CRP was observed (Tao et al. 2015). However, the findings are inconsistent, and absence of associations, positive associations or U-shaped associations have also been reported (Blondon et al. 2016, Mellenthin et al. 2014, Williams et al. 2012). A meta-analysis of vitamin D supplementation trials showed that vitamin D supplementation decreased circulating hs-CRP levels, but there was considerable heterogeneity across the studies and none included children (Chen N et al. 2014).

On sites of infection and inflammation, neutrophils produce proteolytic enzymes, which include matrix metalloproteinase 8 (MMP-8). Like other matrix metalloproteinases, MMP-8 participates in the turnover of the extracellular matrix, but is also involved in inflammatory reactions, for example by cleaving chemokines and cytokines and thereby controlling their activity (Parks et al. 2004, Sorsa et al. 2006). Clinically, MMP-8 has been used as a biomarker of inflammation in periodontitis, sepsis patients, cardiovascular disease, and intra-amniotic infection (Kim et al. 2007, Lauhio et al. 2011, Mäntylä et al. 2003, Maymon et al. 2000, Noack et al. 2017, Tuomainen et al. 2007).
There are no studies on the effects of vitamin D on MMP-8 metabolism, but *in vitro*, 1,25(OH)₂D has been shown to inhibit the production of other matrix metalloproteinases, such as MMP-9 in fetal airway smooth muscle cells, MMP-7 and MMP-10 in *M. tuberculosis*-infected human leucocytes, and MMP-2 and MMP-9 in human uteroid fibroid cells (Britt et al. 2015, Coussens et al. 2009, Halder et al. 2013).

### 2.3.3 OTHER EXTRASKELETAL ACTIONS

Various other cellular actions of vitamin D have been documented as many cells express VDR. For example, VDRs have been found in the muscle tissue, and muscle pain and weakness are typical in severe vitamin D deficiency (Ceglia and Harris 2013). Vitamin D has regulatory effects on the renin-angiotensin-aldosteron system and may have effects on blood pressure control (Li et al. 2004). In addition, vitamin D has antiproliferative actions, and it promotes apoptosis and inhibits angiogenesis in cellular studies (Picotto et al. 2012). Still, there is little evidence of a causal relationship between vitamin D supplementation and cancer prevention (Dimitrakopoulou et al. 2017).

In some epidemiological studies, vitamin D deficiency has been associated with increased risk of autoimmune diseases such as type I diabetes. The findings are, however, inconsistent and have not been confirmed in intervention studies (Pittas and Dawson-Hughes 2010). Vitamin D deficiency is also a suggested environmental risk factor for multiple sclerosis due to its immunomodulatory functions, but also central neurological mechanisms of vitamin D may affect the disease. The detailed mechanisms of action of vitamin D in multiple sclerosis are still unclear (Pierrot-Deseilligny and Souberbielle 2017).

### 2.4 VITAMIN D STATUS

#### 2.4.1 MEASUREMENT OF VITAMIN D STATUS

Serum 25(OH)D, the main circulating form of vitamin D, is considered the best indicator of vitamin D status. The half-life of 25(OH)D is around 15 days, compared with 10-20 hours of 1,25(OH)₂D (Jones 2008). As serum 1,25(OH)₂D concentration is tightly regulated by PTH, calcium and phosphate, it does not reflect vitamin D reserves and therefore it is not used for monitoring vitamin D status.

There are various commercial assays to measure serum 25(OH)D of which immunoassays and liquid chromatography-tandem mass spectrometry are the most common. There has been considerable variability in the measured
25(OH)D concentrations between 25(OH)D assays, making it difficult to compare results of different studies, but the development of the Vitamin D Standardization Program (VDSP) has improved the situation. In collaboration with VDSP, the Vitamin D Quality Assessment Scheme (DEQAS) monitors the accuracy of 25(OH)D assays by comparing the 25(OH)D results against those of a recognized reference method. Participating laboratories are provided reference samples for analysis and the results are sent to DEQAS. The accuracy of the data is calculated and presented as a % bias from the target reference value (Binkley and Carter 2017, Carter et al. 2017).

2.4.2 DEFINITION OF VITAMIN D STATUS

No direct consensus exists on the definition of optimal vitamin D status for health. However, there is increasing agreement that adequate serum 25(OH)D levels should be above 50 nmol/L. The US Institute of Medicine (IOM) suggests 25(OH)D concentration above 50 nmol/L as sufficient based on studies on bone health (Ross et al. 2011). Recently, a global consensus group published a consensus statement on the prevention of rickets, where vitamin D sufficiency was defined as 25(OH)D above 50 nmol/L, insufficiency as 30–50 nmol/L and deficiency as below 30 nmol/L (Munns et al. 2016). In contrast, the Endocrine Society promotes higher thresholds for 25(OH)D and considers vitamin D sufficiency as 25(OH)D above 75 nmol/L, insufficiency as 50–75 nmol/L and deficiency as below 50 nmol/L (Holick et al. 2011).

High concentrations of 25(OH)D can cause hypercalcemia, hypercalciuria, nephrocalcinosis and eventually, renal failure. Symptoms indicative of vitamin D intoxication include poor appetite, weight loss, abdominal pain, vomiting, constipation, polyuria and polydipsia (Barrueto et al. 2005). Reports on vitamin D intoxication in children indicate that the severe adverse effects are seen after exposure to very high doses of vitamin D (i.e. 6,000-100,000 μg) leading to 25(OH)D concentrations exceeding 600 nmol/L (Vogiatzi et al. 2014).

The Pediatric Endocrine Society recommended monitoring for hypercalcemia in children with 25(OH)D concentrations above 375 nmol/L (Vogiatzi et al. 2014). The global rickets consensus defined vitamin D toxicity as serum 25(OH)D above 250 nmol/L with hypercalciuria and suppressed PTH (Munns et al. 2016). However, the IOM has been even more cautious in defining safe upper levels for 25(OH)D. It annotated that there are limited data on the benefits of raising 25(OH)D levels above 75 nmol/L, and that U-shaped associations have been described between vitamin D and various chronic diseases and all-cause mortality, suggesting that both subnormal and very high concentrations may have harmful effects. Thus, the IOM advocated
for maintaining 25(OH)D concentrations below 125 nmol/L until more evidence of the health benefits of vitamin D has been obtained (Ross et al. 2011).

2.4.3 VITAMIN D INTAKE

2.4.3.1 Recommendations for vitamin D intake

For achieving optimal vitamin D status, dietary requirement values for vitamin D have been established. These include the Recommended Dietary Allowance (RDA) or Recommended Intake (RI) that describe the level of intake that meets the requirements of at least 97.5% of the population. The IOM and the Nordic Nutrition Recommendations (NNR) have estimated the requirement values using serum 25(OH)D concentration above 50 nmol/L as a target. According to IOM, adequate intake of vitamin D is 10 μg/d for infants under age 1 year and 15 μg/d for 1- to 18-year-olds (Ross 2011). The NNR's recommended vitamin D intake is 10 μg/d for all children under age 18 (Nordic Nutrition Recommendations 2012).

Similarly, tolerable upper intake levels (UL) for vitamin D have been implemented. UL refers to the highest daily intake of the nutrient that is likely to pose no risk. The recommendations by NNR, IOM and the Endocrine Society for vitamin D intake and upper intake levels in children are presented in Table 1.

Table 1. Serum 25(OH)D concentration defining vitamin D sufficiency, recommended vitamin D intakes and upper intake levels in different-aged children by selected authorities.

<table>
<thead>
<tr>
<th>Age (y)</th>
<th>NNR</th>
<th>IOM</th>
<th>Endocrine Society</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-0.5</td>
<td>10</td>
<td>25</td>
<td>10</td>
</tr>
<tr>
<td>0.5-1</td>
<td>10</td>
<td>25</td>
<td>10</td>
</tr>
<tr>
<td>1-3</td>
<td>10</td>
<td>50</td>
<td>15</td>
</tr>
<tr>
<td>4-8</td>
<td>10</td>
<td>50</td>
<td>15</td>
</tr>
<tr>
<td>9-10</td>
<td>10</td>
<td>50</td>
<td>15</td>
</tr>
<tr>
<td>11-17</td>
<td>10</td>
<td>100</td>
<td>15</td>
</tr>
</tbody>
</table>


*for patients at risk for vitamin D deficiency
2.4.3.2 Vitamin D supplementation guidelines

To improve vitamin D status, vitamin D supplementation guidelines have been implemented by many authorities and scientific organizations. Table 2 summarizes the varying vitamin D supplementation recommendations for children by different authorities and countries.

In Finland, vitamin D supplementation has been recommended for children since the 1930s, first in the form of cod liver oil. Over the years, the recommended daily dose has gradually decreased from an average 100 μg in the 1950s to 10 μg today (Hallman et al. 1964). The current Finnish vitamin D supplementation guidelines recommend 10 μg vitamin D₃ daily for children aged 2 weeks to 2 years and 7.5 μg daily up to age 17, throughout the year. Pregnant and lactating women are recommended a supplemental dose of 10 μg vitamin D₃ daily. Vitamin D supplementation is also recommended for the elderly (≥ 75 years) with a dose of 20 μg daily. For 18- to 74-year-olds, supplementation is recommended in winter months if daily dietary vitamin D intake is less than 10 μg (Elintarviketurvallisuusvirasto 2014).

Table 2. Recommendations for daily vitamin D supplementation and total vitamin D intake in children according to different authorities and countries.

<table>
<thead>
<tr>
<th>Authority or organization</th>
<th>Country</th>
<th>Daily supplemental vitamin D dose</th>
<th>Daily total vitamin D intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>National Nutrition Council</td>
<td>Finland</td>
<td>Age 2 weeks-2 y: 10 μg Age 2-17 y: 7.5 μg</td>
<td>All children 10 μg</td>
</tr>
<tr>
<td>National Food Agency</td>
<td>Sweden</td>
<td>Age 0-2 y: 10 μg Age 2-5 y, if dark skin, no outdoor activity, not consuming fortified food products or fish: dose not defined</td>
<td>All children 10 μg</td>
</tr>
<tr>
<td>Danish Health Authority</td>
<td>Denmark</td>
<td>Age 0-2 y: 10 μg Older children, if dark skin or not exposed to sun: 10 μg</td>
<td>All children 10 μg</td>
</tr>
<tr>
<td>Norwegian Directorate of Health</td>
<td>Norway</td>
<td>Age 4 weeks-1 y: 10 μg</td>
<td>All children 10 μg</td>
</tr>
<tr>
<td>Public Health England</td>
<td>UK</td>
<td>Age 0-1 y, breastfed or formulafed &lt; 500 ml/d: 8.5-10 μg Age 1-4 y: 10 μg</td>
<td>All children 10 μg</td>
</tr>
<tr>
<td>American Academy of Pediatrics</td>
<td>USA</td>
<td>All infants until vitamin D-fortified formula or milk used &gt; 1 l/d: 10 μg Older children, if not obtained from diet: 10 μg</td>
<td>Age 0-1 y: 10 μg Age &gt; 1 y: 15 μg</td>
</tr>
<tr>
<td>Global consensus recommendation for rickets prevention</td>
<td>Global</td>
<td>Age 0-1 y: 10 μg Age &gt; 1 y, if not obtained from diet: dose not defined</td>
<td>Age 0-1 y: 10 μg Age &gt; 1 y: 15 μg</td>
</tr>
</tbody>
</table>

a based on Nordic Nutrition Recommendations (NNR) 2012
c based on American Academy of Pediatrics (AAP) guidelines 2008 (Wagner et al. 2008) and Institute of Medicine (IOM) report 2011 (Ross et al. 2011)
d Munns et al. 2016
2.4.3.3 Food fortification
The main natural dietary sources of vitamin D₃ are oily fish and eggs. Vitamin D₂ can be obtained from plants such as some fungi and yeast. As it is difficult to achieve adequate vitamin D intake from naturally vitamin D-containing food products, many countries have implemented food fortification with vitamin D. As vitamin D₃ is considered to be more biologically effective compared with vitamin D₂, it is mostly the form of vitamin D used in food fortification as well as in vitamin D supplements (Tripkovic et al. 2012).

Food fortification policies vary greatly between countries (Spiro and Buttriss 2014). In Finland, fortification of fluid milk products and fat spreads with vitamin D was started in 2003. In 2010, the fortification was doubled, and currently the recommended fortification is 1 µg vitamin D₃/100 mL for milk products and 20 µg/100 g for fat spreads (National Nutrition Council. Valtion ravitsemusneuvottelukunta.) Although not mandatory, most fluid milk products, including non-dairy alternatives, and fat spreads are fortified according to the recommendation in Finland. In addition, all infant formulas are fortified with vitamin D.

2.4.4 VITAMIN D STATUS IN FINNISH CHILDREN
As mentioned above, fortification of fluid milk products and fat spreads with vitamin D was implemented in Finland in 2003 because of the low vitamin D intake in the population (Männistö et al. 2003). In 2010, the vitamin D fortification was doubled to further improve vitamin D status, as vitamin D intake was still inadequate as indicated by the National FINDIET Survey 2007 (Helldan et al. 2013). In addition, to further increase vitamin D intake, in 2011 the recommendation on daily vitamin D supplementation was extended to year-round including all children under age 18, as well as pregnant and lactating women.

Previously, the prevalence of vitamin D deficiency, defined as 25(OH)D concentration below 50 nmol/L, in Finnish pregnant mothers has been reported to be high (Miettinen et al. 2012). In 2007, 77% of the pregnant mothers and 52% of the newborns (based on cord blood samples) were vitamin D deficient (Viljakainen et al. 2010). Similarly, in a study conducted in 2010–2011, the mean cord blood 25(OH)D concentration of healthy newborns was on average 53 nmol/L (Holmlund-Suila et al. 2012).

In Finnish schoolchildren and adolescents studied in 2006–2008, vitamin D deficiency was observed in 71% (Pekkinen et al. 2012). Another study examining 6- to 8-year-olds in 2007–2009, reported vitamin D deficiency in 20% of the children. Mean dietary vitamin D intake was 5.9 µg/d, fortified
milk being the main dietary source of vitamin D; 41% of the children did not use vitamin D supplements (Soininen et al. 2016).

Findings from the Type I Diabetes Prediction and Prevention (DIPP) study indicate that after the implementation of the food fortification in 2003, the vitamin D status of Finnish children improved: the prevalence of vitamin D deficiency in children aged 0–12 years almost halved from 70% in 1998–2002 to 37% in 2003–2006 (Mäkinen et al. 2014). Similarly, improvement of vitamin D status has been reported in the adult population (Raulio et al. 2017).

2.5 CLINICAL STUDIES ON VITAMIN D, INFECTIONS AND ALLERGIES

2.5.1 VITAMIN D AND INFECTIOUS DISEASES

2.5.1.1 Observational studies
Observational studies propose vitamin D deficiency to be associated with increased risk of infections in both children and adults. Already in 1975 in a study on 200 rachitic hospitalized children in Iran, in nearly 75% of the cases, rickets was found to associate with some infectious disease, most commonly bronchopneumonia or gastroenteritis (Salimpour 1975). Low 25(OH)D concentration has been associated with increased risk of respiratory tract infections, gastrointestinal and ear infections in children (Chowdhury et al. 2017, Science et al. 2013, Thornton et al. 2013). In a case-control study, children with urinary tract infection also had significantly lower 25(OH)D levels compared with controls (Shalaby et al. 2018).

The relationship between prenatal 25(OH)D exposure and childhood respiratory tract infections has been extensively studied, with inconsistent results. In a Spanish study, serum 25(OH)D concentrations were measured from 1,724 mothers at on average 12 weeks of gestation, and when the child was 1 year of age, history of physician-confirmed lower respiratory tract infections or wheezing was inquired. Higher maternal 25(OH)D concentrations were associated with lower risk of lower respiratory tract infections but not wheezing (Morales et al. 2012). A smaller study examining 156 healthy neonates reported an increased risk of respiratory syncytial virus bronchiolitis in the first year of life in infants born with vitamin D deficiency (Belderbos et al. 2011). Camargo et al. reported a similar inverse association between cord blood 25(OH)D concentration and risk of parent-reported respiratory infections at age 3 months (Camargo et al. 2011). On the other hand, a Danish study concluded that cord blood 25(OH)D level did not have
an effect on the occurrence of pneumonia and/or bronchiolitis at age 0–3 years, with diagnoses confirmed by the research pediatricians (Chawes et al. 2014). Furthermore, de Jongh et al. found that compared with those with 25(OH)D > 75 nmol/L, mothers with late pregnancy 25(OH)D < 50 nmol/L reported fewer respiratory symptoms and physician-diagnosed lower respiratory tract infections in their children at 6 months (de Jongh et al. 2014). Authors speculated that the positive association between 25(OH)D and self-reported respiratory symptoms was due to health-conscious women having higher 25(OH)D levels and being more eager to report their child’s symptoms. This finding demonstrates the general weakness of observational studies in proving causality.

### 2.5.1.2 Randomized trials

Table 3 represents a summary of the randomized controlled trials evaluating the effect of vitamin D supplementation on the prevention of infections in children. The trials are heterogeneous and comprise children of different age-groups; only four of them included infants. Two studies were conducted in Afghanistan evaluating the effect of vitamin D supplementation on the incidence of pneumonia in infants aged 1–36 months. High-dose (2,500 μg) vitamin D supplementation, administered by single bolus or every three months for 18 months, did not reduce the duration or incidence of radiologically-confirmed pneumonia (Manaseki-Holland et al. 2010, Manaseki-Holland et al. 2012). However, single bolus supplementation reduced the risk of repeat episodes of pneumonia; in contrast, vitamin D supplementation administered every three months, increased the risk of repeat episodes of pneumonia.

Two studies from India examined the effect of vitamin D supplementation, administered to infants orally or through breast milk by the lactating mother, on the rate of hospital admission, death or severe morbidity (including severe infections or fever), or on the number of days of respiratory or diarrheal infection (Chandy et al. 2016, Kumar et al. 2011). Hospital admissions, death or severe morbidity were not prevented by vitamin D supplementation. However, Chandy et al. reported that the number of days with respiratory or diarrheal infection was higher in the placebo group than in the vitamin D groups. All the aforementioned studies from Afghanistan and India were conducted in countries with a high risk of vitamin D deficiency and malnutrition.

In vitamin D-deficient Mongolian schoolchildren, daily 7.5 μg vitamin D supplementation for seven weeks reduced the risk of parent-reported acute respiratory tract infections at 3 months' follow-up (Camargo et al. 2012). On the other hand, a large trial of healthy Canadian children aged 1 to 5 years comparing the effect of daily 10 μg or 50 μg vitamin D supplementation on
wintertime viral upper respiratory tract infections found no difference in the number of laboratory-confirmed respiratory infections between the groups (Aglipay et al. 2017).

Meta-analyses of randomized vitamin D supplementation trials on infection prevention show inconsistent conclusions. Only one systematic review looked at pediatric randomized trials of vitamin D supplementation and acute respiratory infections, concluding that there is lack of evidence of the use of vitamin D supplementation in the prevention of childhood respiratory infections (Xiao et al. 2015). Other meta-analyses have included studies in both adults and children of all ages, with varying baseline 25(OH)D concentrations, vitamin D administration regimens and outcome measure assessments, thus being considerably heterogeneous. However, a large recent meta-analysis of adult and pediatric studies concluded that vitamin D supplementation provides protection against acute respiratory tract infections (Martineau et al. 2017).
<table>
<thead>
<tr>
<th>Study and country</th>
<th>Participants</th>
<th>Age</th>
<th>Vitamin D dose</th>
<th>Duration</th>
<th>Outcome</th>
<th>Baseline 25(OH)D (nmol/L)</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urashima 2010 Japan</td>
<td>430 schoolchildren</td>
<td>6-15 y</td>
<td>30 μg/d vs. placebo</td>
<td>4 mo</td>
<td>Incidence of influenza A</td>
<td>-</td>
<td>Influenza A incidence reduced in vitamin D group</td>
</tr>
<tr>
<td>Manaseki-Holland 2010</td>
<td>453 children with pneumonia</td>
<td>1-36 mo</td>
<td>2500 μg once vs. placebo</td>
<td>3 mo</td>
<td>Duration of pneumonia and repeat episodes</td>
<td>-</td>
<td>No difference between groups in pneumonia duration, but risk of repeat episodes reduced in vitamin D group</td>
</tr>
<tr>
<td>Majak 2011 Poland</td>
<td>48 children with asthma</td>
<td>5-18 y</td>
<td>12.5 μg/d vs. placebo</td>
<td>6 mo</td>
<td>Asthma exacerbation triggered by acute respiratory infection</td>
<td>90.1 vs. 87.6</td>
<td>Asthma exacerbations lower in vitamin D group</td>
</tr>
<tr>
<td>Trilok-Kumar 2011 India</td>
<td>2079 low birthweight infants</td>
<td>0-6 mo</td>
<td>35 μg/wk vs. placebo</td>
<td>6 mo</td>
<td>Rate of hospital admission or death and any severe morbidity⁴</td>
<td>-</td>
<td>No differences between groups in hospital admission, death or any severe morbidity</td>
</tr>
<tr>
<td>Manaseki-Holland 2012</td>
<td>3046 infants</td>
<td>0-11 mo</td>
<td>2500 μg/3 mo vs. placebo</td>
<td>18 mo</td>
<td>Radiologically confirmed pneumonia and repeat episodes</td>
<td>-</td>
<td>No difference between groups in pneumonia, but more repeat episodes in vitamin D group</td>
</tr>
<tr>
<td>Camargo 2012 Mongolia</td>
<td>247 schoolchildren</td>
<td>7-12 y</td>
<td>7.5 μg/d in milk vs. regular milk</td>
<td>7 wk</td>
<td>Number of parent-reported acute respiratory infections over 3 months</td>
<td>17.5 vs. 17.0</td>
<td>Risk of acute respiratory infection reduced in vitamin D group</td>
</tr>
<tr>
<td>Marchisio 2013 Italy</td>
<td>116 otitis-prone children</td>
<td>1-5 y</td>
<td>25 μg/d vs. placebo</td>
<td>4 mo</td>
<td>Number of doctor-diagnosed acute otitis media episodes</td>
<td>66.1 vs. 64.4</td>
<td>Fewer acute otitis media episodes in vitamin D group</td>
</tr>
<tr>
<td>Aluisio 2013 Afghanistan⁴</td>
<td>3046 infants</td>
<td>1-11 mo</td>
<td>2500 μg/3 mo vs. placebo</td>
<td>18 mo</td>
<td>Incidence and time to first diarrheal illness</td>
<td>-</td>
<td>No differences between groups in diarrheal illness</td>
</tr>
<tr>
<td>Urashima 2014④ Japan</td>
<td>247 schoolchildren</td>
<td>15-18 y</td>
<td>50 μg/d vs. placebo</td>
<td>2 mo</td>
<td>Incidence of influenza A during H1N1 pandemic</td>
<td>-</td>
<td>No difference in influenza A incidence</td>
</tr>
<tr>
<td>Dubnov-Raz 2015⑤ Israel</td>
<td>55 vitamin D-insufficient swimmers</td>
<td>12-18 y</td>
<td>50 μg/d vs. placebo</td>
<td>12 wk</td>
<td>Upper respiratory tract infection</td>
<td>All 60.4</td>
<td>No differences between groups in upper respiratory infections</td>
</tr>
<tr>
<td>Chandy 2016 India</td>
<td>230 mother-infant pairs</td>
<td>0-9 mo</td>
<td>Mother 3000 μg/mo or infant 10 μg/d vs. placebo</td>
<td>9 mo</td>
<td>Number of days of diarrhea or respiratory morbidity⁴</td>
<td>-</td>
<td>Number of days of respiratory or diarrheal infection lower in both vitamin D groups</td>
</tr>
<tr>
<td>Aglipay 2017 Canada</td>
<td>703 children</td>
<td>1-5 y</td>
<td>50 μg/d vs. 10 μg/d</td>
<td>4-8 mo</td>
<td>Number of laboratory-confirmed viral upper respiratory tract infections</td>
<td>89.6 vs. 92.1</td>
<td>No difference between groups in number of laboratory-confirmed infections</td>
</tr>
</tbody>
</table>

⁴ Any severe morbidity defined as pneumonia, persistent diarrhea, dysentery, severe fever, meningitis, ear infections, septicemia, severe protein energy malnutrition; ⁵ Same study as Manaseki-Holland 2012, diarrhea as secondary outcome; ⁶ Only abstract available, no full text; ⁷ Secondary outcome.
2.5.2 VITAMIN D AND ALLERGIC DISEASES

2.5.2.1 Observational studies

The role of vitamin D in development of allergic diseases is unclear. In a large cross-sectional study of 3,136 children and 3,454 adults in the US, vitamin D deficiency associated with higher prevalence of immunoglobulin E (IgE) sensitization to various food and environmental allergens in children and adolescents (Sharief et al. 2011). Different findings were presented in a Finnish study on 7,288 adults, where increases in average total IgE concentrations were seen both in participants with low and high 25(OH)D concentrations (Hyppönen et al. 2009).

Studies evaluating associations between maternal or cord blood 25(OH)D and IgE sensitization also show mixed results. Inverse associations, but also no associations, positive or even U-shaped associations between 25(OH)D levels and prevalence of food or aeroallergen sensitization have been reported (Chiu et al. 2015, Bunyavanich et al. 2016, Weisse et al. 2013, Rothers et al. 2011).

Vitamin D deficiency at birth has been associated with early childhood wheezing or troublesome lung symptoms in several studies (Baiz et al. 2014, Camargo et al. 2011, Chawes et al. 2014, Stelmach et al. 2015). Again, there are opposing findings: in a study of two cohorts of infants at high risk for asthma, cord blood 25(OH)D was not associated with wheezing, food allergies, asthma or IgE sensitization (Visness et al. 2015). Similarly, Morales et al. reported no association between maternal 25(OH)D concentration and wheeze/asthma in offspring, even though higher maternal 25(OH)D concentrations associated with lower risk of lower respiratory tract infections at age 1 year (Morales et al. 2012).

2.5.2.2 Randomized trials

There are no randomized trials of vitamin D supplementation in prevention of allergic diseases conducted primarily in infancy. In New Zealand, a research group randomized pregnant women from 27 weeks of gestation to birth and then their children from birth to 6 months to two different dosages of vitamin D supplementation or placebo. At 18 months (i.e. one year after end of trial), the children were measured for IgE and acute primary care visits for respiratory infections were evaluated. Vitamin D supplementation reduced the proportion of children sensitized to house dust mites (Grant et al. 2016).
Three prenatal vitamin D supplementation trials on prevention of early childhood wheezing have been published. Goldring et al. randomized 180 pregnant women to daily vitamin D of 20 μg, single oral bolus of 5,000 μg or placebo from 27 weeks gestation, but found no difference in wheeze in the offspring at 3 years of age (Goldring et al. 2013). Similarly, two recent large trials studied the effect of maternal vitamin D supplementation (60 μg or 100 μg/d compared with 10 μg/d) on the risk of recurrent wheezing in offspring and found no significant protective effect of higher vitamin D supplementation (Chawes et al. 2016, Litonjua et al. 2016). However, when the two research groups merged their datasets and conducted a combined analysis of the trials, the analyses showed a 25% reduced risk of asthma/recurrent wheeze by 3 years of age in the vitamin D supplementation group (Wolsk et al. 2017).

A few vitamin D supplementation trials on prevention of allergic diseases have been conducted in older children. In Mongolia, vitamin D supplementation for one month improved winter-related atopic dermatitis in schoolchildren, a population with high risk of vitamin D deficiency (Camargo et al. 2014). Similar effects were seen in a smaller pilot study in the US by the same research group (Sidbury et al. 2008). One study including schoolchildren with asthma reported improvement of asthma control with vitamin D supplementation, but another study found no differences in asthma parameters between intervention groups (Kerley et al. 2016, Tachimoto et al. 2016). Vitamin D supplementation as a supplementary treatment of grass pollen allergy in children with allergic rhinitis reduced allergy symptoms and medication score during pollen season; however, only 38 children were included in the study (Jerzynska et al. 2018).
3 AIMS OF THE STUDY

Vitamin D is essential for bone health and normal growth of children but it also has wide extraskeletal effects in the human body. Vitamin D modulates both the innate and adaptive immune responses, and vitamin D deficiency is associated with increased susceptibility to infectious diseases. However, the benefit of vitamin D supplementation in the prevention of childhood infections or allergic diseases is unclear.

The aims of this doctoral thesis were:

1. To evaluate the vitamin D status of Finnish children at different ages (I, II, III, IV)

2. To determine the effect of vitamin D supplementation on infection morbidity in early childhood (I)

3. To study the effect of vitamin D supplementation on allergic diseases in early childhood (II)

4. To examine associations between newborn vitamin D status, inflammation and allergy markers (II, III)
4 SUBJECTS AND METHODS

4.1 STUDY SUBJECTS AND DESIGN

4.1.1 VITAMIN D INTERVENTION IN INFANTS STUDY (I, II, III)
The Vitamin D Intervention in Infants (VIDI) study was a randomized controlled double-blinded trial of daily 10 \( \mu \)g or 30 \( \mu \)g vitamin D supplementation administered to infants from 2 weeks to 2 years of age. We recruited the families at Kätilöopisto Helsinki Maternity hospital 1–2 days after delivery. Eligible infants were born healthy and term to mothers of Northern European ethnicity and with a weight appropriate for gestational age (birth weight SD score between -2.0 and +2.0). We excluded infants requiring intravenous glucose, antibiotics, nasal continuous positive airway pressure treatment for more than 1 day, phototerapy for more than 3 days, or nasogastric tube feeding for more than 1 day, and those with seizures. The recruitment occurred between January 2013 and June 2014, and follow-up was completed in May 2016.

4.1.1.1 Randomization and blinding
Infants were randomized to receive either 10 \( \mu \)g or 30 \( \mu \)g vitamin D supplementation. Randomization, in blocks of 50, was performed by a pharmacist of Helsinki University Hospital. All participants and study personnel were masked to group allocation throughout the study period. The study preparations, identical in appearance, were obtained from Orion Pharmaceuticals and contained vitamin D3 in medium-chain triglyceride oil. Parents were instructed to administer 5 drops of the vitamin D supplementation to their child by mouth each day. Supplementation was commenced at 2 weeks of age and continued up to 2-year follow-up visit. Use of other vitamin D supplements was not allowed during the study.

4.1.2 ISCOLE COHORT (IV)
To evaluate vitamin D status of Finnish school-aged children, we used data collected within the Finnish site of the ISCOLE study (International Study on Childhood Obesity, Lifestyle and the Environment). ISCOLE was a multinational cross-sectional study conducted in 12 countries that aimed to determine the relationships between lifestyle behaviors and childhood obesity (Katzmarzyk et al. 2013). Our study sample consisted of 171 10-year-old fourth grade students from 13 primary schools in the capital region of Finland. The data were collected between January and June 2013.
4.2 DATA COLLECTION

4.2.1 FAMILY BACKGROUND DATA (I, II, III, IV)
We used questionnaires to collect data on family demographics, health, lifestyle and socioeconomic factors and parental educational level. Data on the use of vitamin D supplements during pregnancy (VIDI study) and at school age (study IV) were inquired. Electronic hospital records were used to collect data on gestation, delivery, and infant demographics.

4.2.2 ADHERENCE AND FOLLOW-UP (I, II)
In the VIDI study, the families were provided study diaries to record daily use of the vitamin D supplementation. Completed diaries were collected and reviewed at follow-up visits arranged at 6 months, 1 and 2 years. We calculated adherence from the diaries as percentage, comparing days of supplement use with the total duration of follow-up.

4.2.3 NUTRITIONAL DATA (I)
The dietary intake of vitamin D of the children participating in the VIDI study was determined at age 1 from a 3-day food record administered by the parents or daycare personnel. Nutrient intakes were processed with AivoDiet software (Aivo Oy Finland, Turku, Finland), which utilizes Fineli, the National Food Composition Database maintained by the National Institute for Health and Welfare, Finland. The calculated total vitamin D intake did not include intake from breast milk.

4.2.4 INFECTION DATA (I, II)
We used study diaries to collect infection data. Parents recorded prospectively all their child’s infections in the diaries: time and type of the infection, symptoms or specific diagnosis, duration, medication, physician visit, or hospitalization. We calculated the cumulative number of infection episodes for the 2-year study. Simultaneously reported infections were calculated as belonging to the same infection episode.

Post-hoc, the parent-reported infections were categorized into 7 subtypes: 1. upper respiratory tract infections, defined as presence of rhinitis, cough, sore throat, nasal congestion or sneezing, with or without fever over 38.0°C; 2. acute otitis media, diagnosed by a physician; 3. pneumonia, diagnosed by a physician; 4. conjunctivitis, defined as reddish eye and/or discharge from the eye; 5. gastroenteritis, defined as vomiting and/or diarrhea; 6. non-specified viral infection, defined as fever over 38°C with or without skin
manifestations; and 7. other bacterial infections, including miscellaneous bacterial infections, for example urinary tract or skin infections.

The number of hospitalizations due to bronchiolitis or wheezing was also calculated from the study diaries.

4.2.5 **ALLERGY QUESTIONNAIRE (II, IV)**

To collect data on allergic diseases and allergy symptoms, we used a modified International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire (Nwaru et al. 2011). Parents were asked if the child had ever had physician-diagnosed food allergy, atopic eczema or asthma. Allergy symptoms and parental history of allergies were evaluated. Wheezing or breathing difficulty was defined as a positive answer to the question: Has your child ever had wheezing or difficulty in breathing in the preceding 12 months? Persistent cough was defined as a positive answer to the question: Has your child had persistent coughing for at least 6 weeks in the preceding 12 months? Itchy rash was defined as a positive answer to the question: Has your child had dry, red or itchy skin requiring regular care in the preceding 12 months?

4.3 **LABORATORY MEASUREMENTS**

4.3.1 **25-HYDROXYVITAMIN D (I, II, III, IV)**

Serum 25(OH)D concentrations were analyzed with automated immunoassay with chemiluminescence detection. In the VIDI study, we used the IDS-iSYS immunoassay system (Immunodiagnostic Systems Ltd., Bolton, UK) at the research laboratory of Children’s Hospital. In study IV, 25(OH)D concentrations were measured with Roche Diagnostics immunochemiluminescence assay in the Laboratory of Helsinki University Hospital (HUSLAB).

In the VIDI study, pregnancy serum samples were collected at on average 11 weeks of gestation during normal prenatal follow-up visits. Samples were stored in the Finnish Maternity Cohort serum bank. We collected cord blood plasma samples at birth and serum samples from the children at 1- and 2-year follow-up visits. To enable comparison of cord plasma samples with serum samples, the cord plasma 25(OH)D concentrations were corrected with an equation \((19.13+0.897\times\text{cord plasma }25(\text{OH})D\text{ value})\). This equation was based on comparison of 25(OH)D measurements in 84 study subjects for whom both cord plasma and cord serum samples were available. In addition, the IDS-iSYS system manufacturer conducted changes in the immunoassay system in 2014. Therefore, for studies I and II, the cord serum 25(OH)D
concentrations were further corrected by a linear regression equation (correct value = [(initial value)-8.2]/0.99) provided by the manufacturer. We verified the correction by re-analyzing a subsample of 77 subjects (adjusted correlation coefficient = 0.922, standard error of the estimate 9.2 nmol/L).

Intra-assay variation was < 13% for the cord blood samples and < 5% for the 1- and 2-year samples. Our research laboratory participated in the vitamin D External Quality Assessment Scheme (DEQAS) which showed a < 8% positive bias based on the NIST (National Institute of Standards and Technology) Reference Measurement Procedure during 2014 and 2016 when the samples were analyzed.

4.3.2 MATRIX METALLOPROTEINASE 8 (III)
Cord blood plasma MMP-8 concentration was determined with a time-resolved immunofluorometric assay. Monoclonal MMP-8 specific antibodies 8708 and 8706 were used for catching and tracing (Medix Biochemica, Kauniainen, Finland).

4.3.3 HIGH-SENSITIVITY C-REACTIVE PROTEIN (III)
Cord blood plasma hs-CRP concentration was measured with an enzyme immunoassay (IBL international CRP high-sensitive ELISA).

4.3.4 IMMUNOGLOBULIN E (II)
Specific IgE antibodies to food allergens (cow’s milk, egg white, wheat, cod, peanut, soy) and to aeroallergens (birch, mugwort, timothy, horse, cat, dog, Dermatophagoides pteronyssinus, Cladosporium herbarum) were analyzed with ImmunoCAP (Phadia, Uppsala, Sweden) at the Laboratory of Helsinki University Hospital (HUSLAB). We used IgE concentration of 0.35 kU/L or above as a cut-off for allergic sensitization to food or aeroallergens.

4.4 ETHICAL CONSIDERATIONS
All studies in this thesis were conducted in accordance with the Declaration of Helsinki, a statement of ethical principles for medical research. Ethical approvals were provided by the Research Ethics Committee of the Hospital District of Helsinki and Uusimaa. Informed consent at recruitment was obtained from children’s parents or legal guardians, and concerning study IV, the children also provided their assent to participate in the study.
The VIDI study protocol is registered in ClinicalTrials.gov (NCT01723852). An external steering group monitored the study and possible adverse effects. As a safety protocol, the infants were checked for hypercalcemia at follow-up visits, and in case of severe hypercalcemia, defined as the upper age-specific reference limit of ionized calcium exceeding ≥ 10%, a predefined safety protocol was executed.

4.5 STATISTICAL ANALYSES

Sample size calculations were performed when designing the VIDI study. The primary outcomes of the study were incidence of parent-reported infections and bone strength at 2 years of age. Separate power calculations were performed for both primary outcomes. For infections, we assumed that children below age 2 would have an average of 6 infections per year (Denny et al. 1986, Wald et al. 1991). To detect a decrease from 12 to 9 infections during the 2-year study, a sample size of 220 per group was needed. For bone strength, a sample size of at least 210 per group was estimated. Drop-out rate was approximated as 20%, which amounted to a planned sample of 1,000 subjects, 500 in each group.

Normality of the variables was visually inspected, and in case of non-normal distribution, logarithmic transformation was performed or non-parametric tests were used. We used independent t-test or ANOVA, or Mann-Whitney U-test or Kruskall-Wallis test, for group comparisons of continuous variables. For categorical variables, Pearson chi-square or Fisher’s exact test were used. Correlations were tested with Pearson or Spearman correlation, and multivariate linear regression analysis was used for further analyses with adjustment for potential confounders.

To study the effect of vitamin D supplementation group on infection outcomes, a negative binomial model was applied. Incidence was estimated as proportion of follow-up time in person-months. This allowed the use of all available infection data, also from partially completed study diaries. When evaluating the effect of vitamin D supplementation group on allergy outcomes, we used logistic regression analysis. All analyses were conducted with intention-to-treat principle. We used SPSS software (IBM SPSS Statistics for Windows, version 22), and in case of infection outcome analyses, STATA statistical software, version 14 (StataCorp. 2015, College Station, TX, USA).
5 RESULTS

5.1 CHARACTERISTICS OF STUDY SUBJECTS (I-IV)

Table 1 summarizes the main characteristics of the study subjects of studies I-IV. In the VIDI study (I-III), 975 healthy term infants, born to mothers of Northern European origin, were randomized to daily vitamin D supplementation of 10 μg or 30 μg from age 2 weeks to 2 years. Study IV included 171 fourth graders, 94% of them of Northern European ethnicity. Infants in the VIDI study were born in a large tertiary maternity hospital in Helsinki, while the children in study IV were recruited from primary schools in the capital region of Finland.

Table 4. Main study characteristics of participants in studies I-IV.

<table>
<thead>
<tr>
<th></th>
<th>Studies I-III (VIDI study)</th>
<th>Study IV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All</td>
<td>10 μg vitamin D</td>
</tr>
<tr>
<td>Number</td>
<td>975a</td>
<td>489</td>
</tr>
<tr>
<td>Age, years</td>
<td>0-2</td>
<td>0-2</td>
</tr>
<tr>
<td>Girls, %</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Breastfed, months, mean (SD)</td>
<td>11 (6)b</td>
<td>10 (6)</td>
</tr>
<tr>
<td>Siblings, %</td>
<td>37d</td>
<td>34</td>
</tr>
<tr>
<td>Parental education high, %</td>
<td>81f</td>
<td>79</td>
</tr>
<tr>
<td>Parental smoking, %</td>
<td>16h</td>
<td>16</td>
</tr>
<tr>
<td>Year when data collected</td>
<td>2013-2016</td>
<td>2013-2016</td>
</tr>
</tbody>
</table>

a n=939 in study III, b n=854, c n=151, d n=892, e n=159, f n=883, g n=161, h n=837, j n=161
Breastfed defined as receiving any breast milk.
5.1.1 VITAMIN D INTERVENTION IN INFANTS STUDY (I, II, III)

Enrollment, allocation and follow-up of the VIDI study are presented in Figure 4. The baseline characteristics of the intervention groups did not differ (Table 4). A total of 823 (84%) children completed the 2-year follow-up; 152 (16%) discontinued participation. The mean adherence to vitamin D supplementation was 88% with no difference between the groups (p=0.568).

Figure 4. Enrollment, allocation and follow-up of the Vitamin D Intervention in Infants study demonstrating time-points of data collection of studies I-III.
5.2 VITAMIN D STATUS

Serum 25(OH)D concentrations were measured at the following time-points: during pregnancy, at birth (cord blood), and at age 1 and 2 years, from mothers and children participating in the VIDI study (I-III). In study IV, the vitamin D status of 10-year-old schoolchildren was assessed. Table 5 shows the vitamin D status of the children in studies I-IV.

Table 5. Vitamin D status of the children participating in studies I-IV.

<table>
<thead>
<tr>
<th>VIDI study (I-III)</th>
<th>25-hydroxyvitamin D concentration nmol/L</th>
<th>&lt; 50</th>
<th>50-74.9</th>
<th>75-124.9</th>
<th>≥ 125</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnancy</td>
<td>n</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>809</td>
<td>1.0</td>
<td>22.1</td>
<td>72.2</td>
<td>4.7</td>
</tr>
<tr>
<td>Birth</td>
<td>955</td>
<td>4.3</td>
<td>39.6</td>
<td>51.7</td>
<td>4.4</td>
</tr>
<tr>
<td>Age 1 year</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 μg vitamin D</td>
<td>401</td>
<td>2.2</td>
<td>34.9</td>
<td>61.3</td>
<td>1.5</td>
</tr>
<tr>
<td>30 μg vitamin D</td>
<td>404</td>
<td>0.2</td>
<td>5.2</td>
<td>62.1</td>
<td>32.4</td>
</tr>
<tr>
<td>Age 2 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 μg vitamin D</td>
<td>404</td>
<td>1.2</td>
<td>28.2</td>
<td>67.3</td>
<td>3.2</td>
</tr>
<tr>
<td>30 μg vitamin D</td>
<td>410</td>
<td>0.0</td>
<td>4.6</td>
<td>56.6</td>
<td>38.8</td>
</tr>
</tbody>
</table>

| Study IV          |                                          |     |       |       |      |
| Age 10 years      | 171                                      | 16.4| 44.4  | 38.6  | 0.6  |

5.2.1 PREGNANCY (I, II)

The mean pregnancy 25(OH)D concentration was 89.4 (SD 21.9) nmol/L and 99% of the mothers were vitamin D sufficient (≥ 50 nmol/L). The majority (95%) of the mothers used vitamin D supplementation during pregnancy with an average daily vitamin D intake of 15.5 (SD 16.2) μg from the supplements.

5.2.2 BIRTH (I, II)

At birth, measured from cord blood, 96% of the infants were vitamin D sufficient. The mean cord blood 25(OH)D concentration was 81.5 (SD 25.9) nmol/L. There was no difference in cord blood 25(OH)D concentrations between the 10 μg or 30 μg vitamin D groups (81.7 vs 81.3 nmol/L, p=0.825).
5.2.3 EARLY CHILDHOOD (I, II)

Nearly all children (99%) were vitamin D sufficient at age 1 and 2 years. In the 10 μg vitamin D group, the mean 25(OH)D concentrations were 82.7 (SD 19.8) and 86.6 (SD 19.6) nmol/L at 1 and 2 years, respectively. As presented in Figure 5, a dose-dependent response in 25(OH)D concentration was observed, the mean 25(OH)D in children in the 30 μg vitamin D group being 115.0 (SD 27.7) and 117.7 (SD 26.1) nmol/L, at 1 and 2 years, respectively.

At 2 years, 96% of the children in the 10 μg vitamin D group had 25(OH)D concentration between 50-125 nmol/L and in only 3%, 25(OH)D exceeded 125 nmol/L. In the 30 μg vitamin D group, the respective proportions were 61% and 39% (Table 5). The highest 25(OH)D concentration measured was 207.4 nmol/L in a child belonging to the 30 μg vitamin D group.

There was no difference between the groups in mean ionized calcium concentrations at 6 months, 1 and 2 years (p>0.05 for all). At 2 years, 93% of the children in the 10 μg vitamin D group and 91% in the 30 μg vitamin D group were normocalcemic. No severe hypercalcemia was detected during follow-up.

At age 1, the mean daily vitamin D intake from food was 6.2 (SD 3.7) μg and did not differ between the vitamin D supplementation groups (p=0.322).

![Figure 5. Mean serum 25(OH)D concentrations with 95% confidence intervals in children randomized to 10 μg and 30 μg vitamin D supplementation from age 2 weeks to 2 years.](image-url)
5.2.4 SCHOOL AGE (IV)

The mean serum 25(OH)D concentration of the 10-year-olds was 72.6 (SD 21.8) nmol/L. Vitamin D sufficiency was observed in 84% of the children. Female gender, ethnicity other than Northern European, lack of vitamin D supplementation and a history of cow’s milk allergy were all associated with lower 25(OH)D concentrations (Figure 6). Regular use of vitamin D supplements (daily or almost daily) was reported by 60% of the schoolchildren, while 13% did not take supplementation at all.

![Figure 6](image_url)

**Figure 6.** Mean serum 25(OH)D concentrations with SD of the 171 10-year-olds by gender (p<0.001), ethnicity (p=0.003), use of vitamin D supplementation (2–7 times/week vs. no regular use, p=0.002), and history of cow’s milk allergy (p=0.004). Number of subjects: boys n=84, girls n=87; Northern European n=151, other ethnicity n=10; use of vitamin D supplementation n=119, no regular use n=39; no milk allergy n=139, milk allergy n=21.
5.3  VITAMIN D SUPPLEMENTATION AND INFECTIOUS DISEASES (I)

The effect of vitamin D supplementation on the incidence of infections was assessed from study diaries in which parents kept record of their child’s infections from birth to 2 years. We obtained infection data from 449 children in the 10 μg vitamin D group and from 448 in the 30 μg group. Data were missing from 78 (8%) participants.

5.3.1 CHARACTERISTICS OF INFECTIONS

At 2-year follow-up, the mean number of parent-reported infections was 9.18 (95% CI 8.73–9.63) in the 10 μg vitamin D group and 9.14 (95% CI 8.68–9.60) in the 30 μg group. As presented in Table 6, characteristics and distribution of infections were similar in both groups. The majority (78%) were respiratory infections.

<table>
<thead>
<tr>
<th></th>
<th>10 μg vitamin D⁴</th>
<th>30 μg vitamin D⁵</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infection episodes per child mean (SD)</td>
<td>mean (SD)</td>
<td></td>
</tr>
<tr>
<td>0-1 years</td>
<td>4.04 (2.34)</td>
<td>3.92 (2.48)</td>
</tr>
<tr>
<td>0-2 years</td>
<td>9.18 (4.83)</td>
<td>9.14 (4.92)</td>
</tr>
<tr>
<td>Duration per episode, days</td>
<td>6.18 (3.60)</td>
<td>5.68 (2.74)</td>
</tr>
<tr>
<td>Antibiotic treatments</td>
<td>1.76 (2.07)</td>
<td>2.06 (2.27)</td>
</tr>
<tr>
<td>Physician visits</td>
<td>3.15 (2.95)</td>
<td>3.38 (3.17)</td>
</tr>
<tr>
<td>Hospitalizations</td>
<td>0.07 (0.29)</td>
<td>0.09 (0.3)</td>
</tr>
</tbody>
</table>

a n=449, b n=448

Table 6. Characteristics and distribution of parent-reported infection episodes during 2-year follow-up by vitamin D supplementation group.

![Graph showing infection distribution]
5.3.2 EFFECT OF VITAMIN D SUPPLEMENTATION ON INFECTIONS

We observed no differences in incidence rates of infections between the 10 μg and 30 μg vitamin D groups (IRR 1.00, 95% CI 0.93–1.06) at 2-year follow-up (Table 7). The incidence rate of antibiotic treatments was marginally higher in the 30 μg vitamin D group compared with the 10 μg group (IRR 1.17, 95% CI 1.00–1.36). All results remained unchanged after adjustments for multiple confounding factors (parental education, existence of older siblings, parental smoking, daycare attendance, season of birth, or duration of breastfeeding).

Table 7. Incidence rates of parent-reported infections at 2-year follow-up in children randomized to 10 μg or 30 μg vitamin D supplementation.

<table>
<thead>
<tr>
<th></th>
<th>10 μg vitamin D</th>
<th>30 μg vitamin D</th>
<th>IRR (95% CI)¹²</th>
</tr>
</thead>
<tbody>
<tr>
<td>All infection episodes</td>
<td>449 4244 0.41</td>
<td>448 4214 0.41</td>
<td>1.00 (0.93-1.06)</td>
</tr>
<tr>
<td>Upper respiratory tract infections</td>
<td>449 2733 0.27</td>
<td>448 2619 0.26</td>
<td>0.96 (0.89-1.05)</td>
</tr>
<tr>
<td>Acute otitis media</td>
<td>449 561 0.05</td>
<td>448 656 0.06</td>
<td>1.16 (0.97-1.40)</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>449 129 0.01</td>
<td>448 146 0.01</td>
<td>1.13 (0.85-1.51)</td>
</tr>
<tr>
<td>Conjunctivitis</td>
<td>449 379 0.04</td>
<td>448 349 0.03</td>
<td>0.92 (0.79-1.08)</td>
</tr>
<tr>
<td>Gastroenteritis</td>
<td>449 367 0.04</td>
<td>448 366 0.04</td>
<td>1.00 (0.86-1.17)</td>
</tr>
<tr>
<td>Other viral infections</td>
<td>449 48 0.00</td>
<td>448 50 0.00</td>
<td>1.04 (0.68-1.60)</td>
</tr>
<tr>
<td>Other bacterial infections</td>
<td>449 795 0.08</td>
<td>435 922 0.09</td>
<td>1.17 (1.00-1.36)</td>
</tr>
<tr>
<td>Antibiotic treatments</td>
<td>443 1421 0.14</td>
<td>435 1502 0.15</td>
<td>1.07 (0.94-1.21)</td>
</tr>
<tr>
<td>Physician visits</td>
<td>443 33 0.00</td>
<td>435 38 0.00</td>
<td>1.16 (0.71-1.89)</td>
</tr>
</tbody>
</table>

¹ Incidence rate from number of events divided by person-months.
² IRR, incidence rate ratio with 95% confidence interval (CI), from negative binomial regression.
5.4 VITAMIN D SUPPLEMENTATION AND ALLERGIC DISEASES (II)

We evaluated the effect of vitamin D supplementation on allergic sensitization and clinical allergy outcomes of the children participating in the VIDI study at 1-year follow-up. Serum samples for the measurement of specific IgE concentrations were obtained from 723/975 (74%) of the children and data on the occurrence of physician-diagnosed allergic diseases and allergy symptoms from 770/975 (79%).

Positive IgE sensitization to food allergens was observed in 16% of the children in both vitamin D supplementation groups. There was no difference in food IgE sensitization between the groups (adjusted OR 1.01, 95% CI 0.66–1.55, Table 7). Aeroallergen sensitization was rare, with only 3.5% of the children sensitized during the first year of life; no difference between the vitamin D supplementation groups was observed (adjusted OR 0.79, 95% CI 0.34–1.87, Table 7).

As shown in Table 8, at age 1, the occurrence of physician-diagnosed wheat allergy, atopic eczema or asthma reported by parents did not differ between the vitamin D supplementation groups (p>0.1 for all). Diagnosis of cow’s milk allergy was reported more often in the 30 μg vitamin D group compared with the 10 μg dose (adjusted OR 2.23, 95% CI 1.00–4.95). Wheezing or breathing difficulty was reported by 10% of the parents and the occurrence did not differ between the groups (adjusted OR 0.98, 95% CI 0.61–1.59).
Table 8. Allergic sensitization, physician-diagnosed allergic diseases and allergy symptoms at age 1 in children randomized to 10 μg or 30 μg vitamin D supplementation.

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>10 μg vitamin D</th>
<th>30 μg vitamin D</th>
<th>p-value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>OR (95% CI)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Food IgE sensitization</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk</td>
<td>720</td>
<td>42 (5.8)</td>
<td>21 (5.9)</td>
<td>21 (5.8)</td>
<td>0.974</td>
</tr>
<tr>
<td>Wheat</td>
<td>720</td>
<td>17 (2.4)</td>
<td>10 (2.8)</td>
<td>7 (1.9)</td>
<td>0.448</td>
</tr>
<tr>
<td>Egg white</td>
<td>720</td>
<td>75 (10.4)</td>
<td>40 (11.2)</td>
<td>35 (9.7)</td>
<td>0.509</td>
</tr>
<tr>
<td>Cod</td>
<td>720</td>
<td>3 (0.4)</td>
<td>2 (0.6)</td>
<td>1 (0.3)</td>
<td>0.622</td>
</tr>
<tr>
<td>Soybean</td>
<td>720</td>
<td>8 (1.1)</td>
<td>6 (1.7)</td>
<td>2 (0.6)</td>
<td>0.175</td>
</tr>
<tr>
<td>Peanut</td>
<td>720</td>
<td>23 (3.2)</td>
<td>16 (4.5)</td>
<td>7 (1.5)</td>
<td>0.053</td>
</tr>
<tr>
<td><strong>Aeroallergen IgE sensitization</strong></td>
<td>719</td>
<td>25 (3.5)</td>
<td>14 (3.9)</td>
<td>11 (3.0)</td>
<td>0.509</td>
</tr>
<tr>
<td>Birch</td>
<td>718</td>
<td>10 (1.4)</td>
<td>6 (1.7)</td>
<td>4 (1.1)</td>
<td>0.543</td>
</tr>
<tr>
<td>Mugwort</td>
<td>718</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>n/a</td>
</tr>
<tr>
<td>Timothy</td>
<td>718</td>
<td>3 (0.4)</td>
<td>3 (0.8)</td>
<td>0 (0)</td>
<td>0.121</td>
</tr>
<tr>
<td>Dog</td>
<td>718</td>
<td>13 (1.8)</td>
<td>7 (2.0)</td>
<td>6 (1.7)</td>
<td>0.765</td>
</tr>
<tr>
<td>Cat</td>
<td>718</td>
<td>10 (1.4)</td>
<td>5 (1.4)</td>
<td>4 (1.4)</td>
<td>1.000</td>
</tr>
<tr>
<td>Horse</td>
<td>716</td>
<td>2 (0.3)</td>
<td>2 (0.6)</td>
<td>0 (0)</td>
<td>0.245</td>
</tr>
<tr>
<td>Dermatophagoides pteronyssinus</td>
<td>718</td>
<td>1 (0.1)</td>
<td>1 (0.3)</td>
<td>0 (0)</td>
<td>0.496</td>
</tr>
<tr>
<td>Cladosporium herbarum</td>
<td>718</td>
<td>1 (0.1)</td>
<td>1 (0.3)</td>
<td>0 (0)</td>
<td>0.496</td>
</tr>
<tr>
<td><strong>Physician-diagnosed allergic disease</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cow's milk allergy</td>
<td>764</td>
<td>29 (3.8)</td>
<td>9 (2.4)</td>
<td>20 (5.2)</td>
<td>0.044</td>
</tr>
<tr>
<td>Wheat allergy</td>
<td>762</td>
<td>11 (1.4)</td>
<td>5 (1.3)</td>
<td>6 (1.6)</td>
<td>0.788</td>
</tr>
<tr>
<td>Atopic eczema</td>
<td>769</td>
<td>128 (16.6)</td>
<td>72 (18.9)</td>
<td>56 (14.4)</td>
<td>0.097</td>
</tr>
<tr>
<td>Asthma</td>
<td>765</td>
<td>1 (0.1)</td>
<td>1 (0.3)</td>
<td>0 (0)</td>
<td>0.494</td>
</tr>
<tr>
<td><strong>Allergy symptoms</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wheezing or breathing difficulty</td>
<td>763</td>
<td>76 (10.0)</td>
<td>39 (10.3)</td>
<td>37 (9.7)</td>
<td>0.781</td>
</tr>
<tr>
<td>Hospitalization due to bronchiolitis or wheezing</td>
<td>901</td>
<td>25 (2.8)</td>
<td>14 (3.1)</td>
<td>11 (2.4)</td>
<td>0.547</td>
</tr>
<tr>
<td>Persistent cough</td>
<td>765</td>
<td>46 (6.1)</td>
<td>20 (5.3)</td>
<td>26 (6.9)</td>
<td>0.371</td>
</tr>
<tr>
<td>Itchy rash</td>
<td>765</td>
<td>126 (16.5)</td>
<td>72 (18.9)</td>
<td>54 (16.5)</td>
<td>0.067</td>
</tr>
</tbody>
</table>

Allergic sensitization defined as serum food or aeroallergen IgE ≥ 0.35 kU/L.

<sup>a</sup> P-values from Pearson chi-square or Fisher’s exact when cell count ≤ 5.

<sup>b</sup> Odds ratio (OR) from logistic regression, reference group 10 μg vitamin D. Adjusted for parental history of allergy and attendance in daycare in case of allergic sensitization, and parental history of allergy and duration of breastfeeding in case of allergy diagnosis and symptoms. N/a, not applicable due to small number of subjects.
Results

5.5 CORD BLOOD VITAMIN D AND INFLAMMATORY AND ALLERGY MARKERS

5.5.1 MARKERS OF INFLAMMATION (III)

We measured MMP-8 and hs-CRP concentrations from cord blood plasma of 939 infants participating in the VIDI study. The mean concentrations were 65.4 (95% CI 58–72) ng/mL for MMP-8 and 0.13 (95% CI 0.10–0.16) μg/mL for hs-CRP, respectively. There was a positive correlation between cord blood 25(OH)D and MMP-8 (r=0.315, p<0.001), and between 25(OH)D and hs-CRP (r=0.102, p=0.002). Higher cord blood 25(OH)D concentration predicted higher MMP-8 and hs-CRP concentrations (Figure 7). In a linear model, the findings persisted after adjustment for potential confounding factors, i.e. gestational age, mode of delivery, antenatal antibiotic treatment, parity, and maternal pre-pregnancy BMI for hs-CRP (MMP-8 β coefficient = 1.012, 95% CI 1.01–1.02; hs-CRP β coefficient = 1.003, 95% CI 1.00–1.01).

Figure 7. Mean cord blood MMP-8 and hs-CRP concentrations with 95% confidence intervals by cord blood 25(OH)D status. Number of subjects: 25(OH)D < 75 nmol/L n=409, 75-99.9 nmol/L n=363, ≥ 100 nmol/L n=167.
5.5.2 ALLERGIC SENSITIZATION (II)

We evaluated the association of cord blood vitamin D status and allergic sensitization at age 1. The proportion of children sensitized to food allergens varied according to cord blood vitamin D status: when cord blood 25(OH)D concentration was categorized in subgroups below 75 nmol/L, 75–99.9 nmol/L and above 100 nmol/L, the risk for food IgE sensitization was higher in infants with 25(OH)D above 100 nmol/L compared with concentration below 75 nmol/L (OR 1.91, 95% CI 1.14–3.21, Table 9). We repeated the analyses after adjustment for confounding factors (vitamin D supplementation group, sex, parental allergy history and daycare attendance), with similar results (adjusted OR 1.96, 95% CI 1.13–3.41).

Table 9. Food IgE sensitization at age 1 according to cord blood vitamin D status.

<table>
<thead>
<tr>
<th>Cord blood 25(OH)D nmol/L</th>
<th>N</th>
<th>n (%)</th>
<th>OR (95% CI)²</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 75</td>
<td>295</td>
<td>43 (14.6)</td>
<td>reference</td>
</tr>
<tr>
<td>75-99.9</td>
<td>286</td>
<td>40 (14.0)</td>
<td>0.95 (0.60-1.52)</td>
</tr>
<tr>
<td>≥ 100</td>
<td>126</td>
<td>31 (24.6)</td>
<td>1.91 (1.14-3.21)</td>
</tr>
</tbody>
</table>

² Odds ratio (OR) from logistic regression with 95% confidence interval (CI)
Discussion

6 DISCUSSION

6.1 VITAMIN D STATUS

In this thesis, we observed that the vitamin D status of pregnant women, newborns and older children in Finland has improved markedly over the past decade. This improvement is most likely a consequence of the Finnish public health policy that includes fortification of fluid milk products, infant formulas and fat spreads with vitamin D, and guidelines for regular vitamin D supplementation in risk groups for vitamin D deficiency, such as pregnant women and children.

The majority of the mothers in our study took vitamin D supplements, the average vitamin D intake from supplements being 15 μg/d, slightly higher than recommended. The main determinants of maternal vitamin D status were supplemental vitamin D intake, a dietary pattern including consumption of vitamin D-fortified margarine and dairy products, and physical activity, reflecting that an overall healthy lifestyle promotes vitamin D sufficiency in pregnant women (Hauta-alus et al. 2017). As a result, almost all the studied newborns were also vitamin D sufficient, defined as 25(OH)D concentration above 50 nmol/L. To our knowledge, our findings from the VIDI cohort are the first to demonstrate the positive effects of the Finnish food health policy in pregnant women and newborns. It is supported by findings on adults (Jääskeläinen et al. 2017, Raulio et al. 2017). The Health 2011 survey indicated that 91% of the adults aged ≥ 30 years had 25(OH)D concentrations above 50 nmol/L (Jääskeläinen et al. 2017). As vitamin D deficiency is still prevalent in many countries in Europe and worldwide, Finland serves as an example of how vitamin D status can be improved at population level by food fortification and promoting guidelines for vitamin D supplementation (Cashman et al. 2016, Schleicher et al. 2016).

Our study showed that daily 10 μg vitamin D supplementation maintained vitamin D sufficiency in children up to age 2 years. We observed that 96% of the children in the 10 μg vitamin D group had a 25(OH)D concentration between 50–125 nmol/L at 2-year follow-up. The current recommendations by the Finnish National Nutrition Council and some other major authorities referred to previously (see literature review page 24), recommend a daily vitamin D intake of 10 μg in children. For children participating in the VIDI study, at age 1, the mean dietary vitamin D intake was 6.2 μg/d. More detailed analysis showed that in breastfed infants the mean dietary vitamin D intake was 3.8 μg/d and in non-breastfed infants 7.5 μg/d (Hauta-alus et al. 2017). Thus, these results indicate that vitamin D supplementation is still needed to reach the recommended daily intake of 10 μg vitamin D.
On the other hand, in children with 30 μg daily vitamin D supplementation, the 25(OH)D concentrations rose to comparably high levels: almost 40% had a 25(OH)D concentration above 125 nmol/L at age 2 years. We did not observe any direct toxic effects of vitamin D as none of the children had a 25(OH)D concentration above 250 nmol/L or severe hypercalcemia. However, regarding the IOM statement of possible harmful effects of high 25(OH)D concentrations (> 125 nmol/L) in the long term, increasing the vitamin D intake with 30 μg vitamin D supplementation may not be reasonable in children as the 10 μg dosage will maintain sufficiency in the majority of the children (Ross et al. 2011).

We also observed that the vitamin D status of older children had improved compared to earlier reports. In our study comprising 10-year-old schoolchildren, vitamin D deficiency was observed in 16%, while studies from the previous decade reported vitamin D deficiency in 20–70% of the children (Mäkinen et al. 2014, Pekkinen et al. 2012, Soininen et al. 2016). History of cow’s milk allergy was associated with lower 25(OH)D concentrations, which possibly originates from the children’s dietary habits: children who had once been diagnosed with cow’s milk allergy consumed less dairy products even at school age. Other studies also indicate that milk is the main dietary source of vitamin D in Finnish children and underline the importance of vitamin D fortification in improving vitamin D status at population level (Cashman 2015, Soininen et al. 2016). In addition, lack of vitamin D supplementation associated with lower 25(OH)D concentrations. However, almost 60% used regular vitamin D supplementation, and only 16% of the children did not use vitamin D supplementation at all, which is much less than reported earlier. Previously as many as 40–80% of the 6- to 8 year-olds did not use vitamin D supplementation (Kyttilä et al. 2010, Soininen et al. 2016). This suggests that also families with older children have adapted relatively well to the current vitamin D supplementation recommendations updated in 2014.

In Europe, childhood vitamin D supplementation policies, implementation and adherence vary widely. In a European survey, factors that associated with good adherence to vitamin D supplementation were universal guidelines for all infants and children independent of feeding mode, informing parents of vitamin D supplementation at birth, and monitoring adherence to supplementation at child health surveillance visits (Uday et al. 2017). However, for example in the United Kingdom, the vitamin D supplementation guideline is complex and adherence to supplementation has been reported in only 5–20% of children (Uday and Högl 2018, Uday et al. 2017). Alarmingly, the prevalence of rickets is increasing in the UK (Uday and Högl 2018). In Finland, vitamin D supplementation guidelines are universal and simple, including children of all ages and ethnic groups. In addition, all Finnish children are regularly monitored at child health clinics.
throughout childhood, which most likely contributes to the improved adherence to vitamin D supplementation. Similar easy-to-follow vitamin D supplementation guidelines should be considered in other countries as well, especially in those with limited sunshine and without general vitamin D food fortification, and in countries with a population including diverse ethnic groups. As we also observed in our study, school-agers with dark skin had lower vitamin D status compared to children of Northern European ethnicity, underlining the importance of special guidance and monitoring of adequate vitamin D supplementation in these ethnic risk groups.

6.2 VITAMIN D AND INFECTIONS

Although vitamin D supplementation is important in maintaining sufficient vitamin D status in children, based on our findings from the VIDI study, we did not observe any benefit of higher (30 μg) vitamin D supplementation compared with the standard 10 μg dose in prevention of infectious diseases. The incidence of infections did not differ between the intervention groups at the 2-year follow-up.

Several aspects need to considered regarding our findings. First, our study lacked vitamin D-deficient children. Previous work suggests that vitamin D provides benefit against infections, particularly in severely vitamin D-deficient individuals (Martineau et al. 2017, Vuichard Gysin et al. 2016). This may originate from the effect of vitamin D on the immune response: the antibacterial activity induced by vitamin D depends on the concentration of available 25(OH)D which is locally converted to active 1,25(OH)2D by the immune cells (Hewison 2012). When vitamin D-deficient individuals are supplemented with vitamin D, the expression of e.g. antimicrobial cathelicidin is enhanced (Adams et al. 2009). However, our data suggest that when a vitamin D-sufficient state is reached, corresponding to 25(OH)D concentration above 50 nmol/L, there is no additional benefit of further increasing vitamin D status. This is in line with a Canadian study, where high-dose vitamin D supplementation compared with standard dose failed to prevent upper respiratory tract infections during the winter season in vitamin D-sufficient children (Aglipay et al. 2017).

Second, the dosage of supplemental vitamin D may have been too small or trial length too short. This is unlikely, as during the intervention, 25(OH)D concentrations increased to relatively high levels: 40% of the children supplemented with 30 μg vitamin D had a 25(OH)D concentration above 125 nmol/L at the end of the trial. In many pediatric studies, trial length has been relatively short, varying from one month to a maximum of 18 months, while in our study, the intervention continued for 2 years. It is most likely that
possible preventive effects would have been noticeable during this follow-up period.

Differences in genetic factors may affect the response to vitamin D supplementation and the risk for infectious diseases. For example, polymorphism in the gene encoding for DBP generates proteins with different binding affinity, thus affecting circulating 25(OH)D concentrations and bioavailability of 25(OH)D to cells (Chun et al. 2010, Wang et al. 2010). Genetic variation in VDR is associated with susceptibility to infectious diseases and other extraskeletal outcomes (Jolliffe et al. 2016, McNally et al. 2013, Roth et al. 2008). For example, high-dose vitamin D supplementation in adults treated for pulmonary tuberculosis did not improve treatment response in the whole study population while those with a certain VDR genotype benefited from vitamin D supplementation (Ganmaa et al. 2017, Martineau et al. 2011).

Lastly, we observed that children supplemented with 30 μg vitamin D were treated with antibiotics more often than those supplemented with 10 μg. The difference between the groups was still marginal and may be due to chance. However, disadvantageous effects of high-dose vitamin D have also been reported by others. In the trial by Manaseki-Holland, large-dose bolus-administered vitamin D compared with placebo in fact increased the risk of repeat episodes of pneumonia in infants (Manaseki-Holland et al. 2012). A case-control study in Greenland indicated that adults with both low (< 75 nmol/L) and high (> 140 nmol/L) 25(OH)D concentrations had increased risk for tuberculosis infection (Nielsen et al. 2010).

6.3 VITAMIN D, ALLERGIES AND INFLAMMATION

We evaluated the effect of vitamin D supplementation on allergic sensitization, clinical allergies and wheezing at 1 year of age. The higher dose vitamin D supplementation did not prevent these outcomes. Prior to ours, no other study has evaluated the effect of postnatal vitamin D supplementation in allergy prevention. Similarly to the results on infection incidence, our data show that in vitamin D-sufficient children, there is no benefit of increasing vitamin D status for prevention of allergies.

A marginal difference between groups was seen in the occurrence of cow’s milk allergy, which was in fact more common in children supplemented with 30 μg vitamin D compared with the lower dose. Further interpretation is limited by the small number of children with cow’s milk allergy in the study. However, Hyppönen et al. reported previously that the prevalence of atopy and allergic rhinitis in adulthood was higher in participants who had received high-dose (50 μg) vitamin D supplementation during the first year of life.
Discussion

compared with those with irregular or no supplementation (Hyppönen et al. 2004). Furthermore, in a Swedish birth cohort, higher vitamin D intake in infancy associated with increased risk for atopic dermatitis in later childhood (Back et al. 2009).

Two recent prenatal vitamin D supplementation trials showed no reduction in the risk of asthma or recurrent wheeze in the offspring at 3 years of age when comparing high-dose (60 μg or 100 μg) vitamin D supplementation with standard dose (10 μg) in pregnancy (Chawes et al. 2016, Litonjua et al. 2016). Both research groups speculated that the timing of supplementation may have affected the null results, and that possibly also postnatal vitamin D supplementation of the children would have been needed to prevent wheezing illnesses. Our findings do not support these speculations as we found no benefit of postnatal vitamin D supplementation on wheezing or other allergy outcomes during the first year of life. However, longer follow-up of our cohort as well as of the children in the referred prenatal studies is needed as the manifestation of allergic diseases increases by age.

When combining the datasets of the aforementioned trials by Chawes and Litonjua, the researchers did find a 25% reduced risk of asthma/recurrent wheeze in the children by 3 years of age (Wolsk et al. 2017). In fact, the effect was strongest among mothers with 25(OH)D concentration above 75 nmol/L at randomization. The authors concluded that strategies targeted at raising vitamin D levels in pregnant women should be considered, and possibly concentrations as high as 100–150 nmol/L might be needed for optimal immune and lung development in the fetus. Our results, however, indicate that high 25(OH)D levels at birth may not be solely favorable for the offspring as we observed that higher cord blood 25(OH)D concentration (> 100 nmol/L) compared with 25(OH)D in the lowest range (< 75 nmol/L) was associated with increased food allergen sensitization. This is in line with earlier studies where also high 25(OH)D concentrations have been associated with IgE sensitization (Rothers et al. 2011, Weisse et al. 2013).

A similar phenomenon was seen for inflammatory markers in our study: high cord blood 25(OH)D associated with increased inflammation at birth. Previously, low cord blood 25(OH)D levels were found to inversely associate with CRP in healthy vitamin D-deficient neonates; however, no association was seen in those with 25(OH)D above 50 nmol/L (Tao et al. 2015). In a large cross-sectional study including 4,274 9-year-olds, 25(OH)D correlated positively with CRP and interleukin 6 (Williams et al. 2012). U-shaped associations between 25(OH)D and CRP have been reported in the adult population as well, suggesting that higher 25(OH)D levels may have a proinflammatory effect (Mellenthin et al. 2014). There is no final explanation for these results, but also high 25(OH)D levels may modulate the immune
defense some way, for example by altering the T cell differentiation, shifting the Th1-Th2 balance and promoting synthesis of proinflammatory cytokines.

### 6.4 STRENGTHS AND LIMITATIONS

The VIDI study comprised a large and unique cohort of healthy mothers and children followed from birth to 2 years of age. The study design of a randomized controlled trial allowed us to obtain unbiased data on the effects of vitamin D supplementation. Participants adhered well to the study protocol and 84% of the children completed follow-up. The study had sufficient power to detect possible differences in the incidence of infections between intervention groups. However, power was not calculated for the allergy outcomes and therefore these results should be interpreted with caution. In addition, allergy outcomes were assessed at 1 year of age, whereas the effect of vitamin D supplementation on the primary outcome, infections, was evaluated at trial end at age 2 years.

In both groups, children had approximately 9 infections during the 2-year follow-up. This is less than we presumed. From earlier literature we estimated that the children would have an average 6 infections per year (Denny et al. 1986, Wald et al. 1991). Therefore, it can be speculated that the children may have benefited from both dosages of vitamin D supplementation as vitamin D deficiency was completely prevented in both groups, resulting in lower infection burden. A limitation is that we did not have a placebo group. However, use of placebo would have been unethical as vitamin D supplementation is recommended for all children in Finland.

The infection and clinical allergy data were based on parental report. A follow-up visit in case of acute infection would have provided more exact data, especially if the infections had been laboratory-confirmed. Due to the large number of participants, this was not possible to arrange. Follow-up phone calls, for example, might also have lead to more accuracy to recall infections. However, as the study was double-blinded and randomized, the possible misreporting was most likely equal in both groups.

The VIDI cohort was recruited from a single maternity hospital in Helsinki. The mothers were well-educated, mostly primiparous, and all were of Northern European ethnicity. Due to the homogeneous cohort, our results cannot be directly integrated into the general Finnish population. Still, the large study sample has allowed, and will allow in the future, examination of various health-related questions in pregnancy and childhood.

Regarding laboratory assessments, a limitation is that we had to correct the cord blood 25(OH)D results twice with a correction equation: first to be able
to compare cord blood plasma with serum samples, and second due to the manufacturer’s changes in the IDS-iSYS assay. In addition, the cord blood plasma samples were sometimes hemolyzed, which may have challenged some of the immunological methods used in the study. On the other hand, our laboratory participated in the DEQAS which gives validity to all 25(OH)D analyses.

6.5 FUTURE PROSPECTS

In the VIDI study, we collected detailed information from parents on their children’s infections which provides important and up-to-date data on the normal distribution and characteristics of infections in early childhood. Information on medication and antibiotic treatments was also collected. In the future, it would be interesting to further examine the use of antibiotics, their indications and selected treatments. For example, the relationship of antibiotic exposure and childhood obesity could be studied as these have previously been shown to be related (Saari et al. 2015). As infection and clinical allergy data were based on parental report, data from national registers could be used for further validation. This would give more objective data on antibiotic use and diagnosis of infections and allergic diseases.

In this thesis, we studied the association of vitamin D with inflammatory markers hs-CRP and MMP-8 in cord blood. These analyses are planned to be repeated at other time points (pregnancy and early childhood) to evaluate whether the observed association exists already in pregnancy and whether it persists later. It is important to evaluate the impact of vitamin D supplementation on these inflammatory markers and to examine whether low-grade inflammation in the prenatal and postnatal period associates with future health. In the future, it would also be interesting to measure the level of the antimicrobial peptide cathelicidin whose production is specifically regulated by vitamin D metabolites. The relationship between vitamin D and vaccine responses in children also calls for further inspection, as it has been speculated that vitamin D may play a role in the immune response to vaccines (Sadarangani et al. 2015).

For evaluating long-term effects of the vitamin D intervention, a longitudinal follow-up of the VIDI cohort is essential. It will be of importance to examine whether vitamin D supplementation will affect the risk of allergic diseases, autoimmune diseases, growth, bone strength or other health outcomes in later childhood.
6.6 CONCLUSIONS

This thesis shows that the vitamin D status of Finnish children has substantially improved during the past decade. In the large VIDI study, the majority of the pregnant mothers and their newborns had 25(OH)D concentration above 50 nmol/L, indicating vitamin D sufficiency. The prevalence of vitamin D deficiency in 10-year-old schoolchildren was lower compared with previous research. We observed that daily supplementation with 10 μg vitamin D was enough to maintain vitamin D sufficiency in children throughout the first two years of life. No additional benefit in terms of infection morbidity or allergic diseases was achieved by higher vitamin D supplementation compared with the standard recommended dose. In fact, higher cord blood vitamin D status associated with increased inflammation at birth and allergic sensitization during the first year of life.

Our results support earlier conclusions that "the more the better" concept cannot be directly applied to vitamin D. Similarly as previously annotated by the IOM, we observed that higher 25(OH)D concentrations were not consistently associated with greater benefit (Ross et al. 2011). Furthermore, our findings demonstrate the complex immunomodulatory effects of vitamin D. More studies, both in vitro and in vivo, are needed to clarify the actions of vitamin D on the immune system and to define the optimal vitamin D status for overall health.
7 ACKNOWLEDGEMENTS

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Jenni Rosendahl
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