Doctoral Programme in Clinical Research
Pediatric Graduate School and Pediatric Research Center
Children's Hospital

University of Helsinki
Finland

VITAMIN D AND EXTRASKELETAL EFFECTS IN CHILDREN

Studies on infections, allergies and inflammation

Jenni Rosendahl

ACADEMIC DISSERTATION

To be presented, with the permission of the Faculty of Medicine of
the University of Helsinki, for public examination in the Hattivatti lecture hall,
New Children's Hospital, on the 16th of November 2018, at 12 noon.

Helsinki 2018

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

ISBN 978-951-51-4539-0 (paperback)
ISBN 978-951-51-4540-6 (PDF)
http://ethesis.helsinki.fi

Unigrafia
Helsinki 2018

To my family

ABSTRACT

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 D_3 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.

TIIVISTELMÄ

Lapset tarvitsevat D-vitamiinia normaalin kasvun ja luuston kehityksen turvaamiseksi. Suomalaislapsille on suositeltu päivittäistä D-vitamiinilisää jo lähes 90 vuoden ajan, minkä ansiosta riisitauti on maassamme nykyisin harvinainen. D-vitamiini on tärkeä luusto- ja mineraaliaineenvaihdunnan säätelijä, mutta sillä on lisäksi useita luuston ulkopuolisia vaikutuskohteita ja se osallistuu esimerkiksi immuunijärjestelmän toimintaan. Elimistön D-vitamiinitasoa kuvaa parhaiten seerumin 25-hydroksi-D-vitamiinipitoisuus (25(OH)D). Usein riittäväksi D-vitamiinitasoksi määritellään seerumin 25(OH)D-pitoisuus ≥ 50 nmol/l. Terveyden ja puolustuskyvyn kannalta ihanteellisinta D-vitamiinitasoa ei kuitenkaan tarkkaan tiedetä.

Tämän väitöskirjatutkimuksen tavoitteena oli selvittää D-vitamiinin luuston ulkopuolisia vaikutuksia, keskittyen erityisesti pienten lasten infektioihin, allergioihin ja tulehdustilaan. Tutkimuksessa selvitettiin, miten nykysuosituksia korkeampi D-vitamiiniannos vaikuttaa infektiosairastavuuteen ja allergioiden kehittymiseen varhaislapsuudessa. Tutkimuksessa tarkasteltiin myös suomalaislasten D-vitamiinitasoa eri ikäkausina sekä tutkittiin siihen vaikuttavia tekijöitä.

D-vitamiini-interventiotutkimukseen osallistui 975 lasta, jotka satunnaistettiin saamaan päivittäistä D_3-vitamiinilisää joko 10 µg tai 30 µg kahden ensimmäisen elinvuoden ajan. Vanhemmat kirjasivat lapsen sairastamat infektiot ja vastasivat allergiakyselyyn. Lasten allergista herkistymistä tutkittiin mittaamalla seerumin IgE-vasta-aineita tavanomaisille allergeeneille. Seerumin 25(OH)D-pitoisuus mitattiin raskausaikana, napaverestä syntymähetkellä ja lapsista yhden ja kahden vuoden iässä. Napaverestä tutkittiin myös tulehdustekijöiden pitoisuuksia. Lisäksi toisessa tutkimuksessa 171:ltä kymmenvuotiaalta koululaiselta mitattiin 25(OH)D-pitoisuus ja kartoitettiin terveyteen ja ravitsemukseen liittyviä tekijöitä.

Valtaosalla tutkituista lapsista elimistön D-vitamiinitaso oli riittävä (25(OH)D ≥ 50 nmol/l). Interventiotutkimukseen osallistuneista lapsista yli 95 %:lla D-vitamiinitaso oli riittävä syntymästä kaksivuotiaaksi asti. Myös 84 % :lla koululaisista D-vitamiinitaso oli riittävä. D-vitamiini-interventiotutkimuksessa suurempi D-vitamiiniannos ei vähentänyt lasten sairastamien infektioiden lukumäärää kahden ikävuoden aikana. Yhden vuoden iässä allergioissa ei ollut eroa ryhmien välillä. Sen sijaan havaitsimme yhteyden korkean napaveren 25(OH)D-pitoisuuden ja tulehdustekijöiden välillä. Korkea napaveren 25(OH)D-pitoisuus lisäsi myös allergisen herkistymisen riskiä yhden vuoden iässä.

Tutkimustulokset osoittavat, että suomalaislasten D-vitamiinitaso oli aiempiin tutkimuksiin verrattuna kohentunut. Nykysuositusten mukaiseen annokseen verrattuna suurempi D-vitamiiniannos ei kuitenkaan suojannut infektioilta tai allergioilta varhaislapsuudessa. Alle kaksivuotiailla lapsilla 10 µg päivittäinen D-vitamiinilisä oli riittävä hyvän D-vitamiinitason ylläpitämiseksi.

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:

I Rosendahl J*, Valkama S*, Holmlund-Suila E, Enlund-Cerullo M, Hauta-alus H, Helve O, Hytinantti T, Levälahti E, Kajantie E, Viljakainen H, Mäkitie O*, Andersson S*. Effect of higher vs standard dosage of vitamin D_3 supplementation on bone strength and infection in healthy infants: a randomized clinical trial. JAMA Pediatr. 2018; 172(7):646–654.

II Rosendahl J, Pelkonen AS, Helve O, Hauta-alus H, Holmlund-Suila E, Valkama S, Enlund-Cerullo M, Viljakainen H, Hytinantti T, Mäkitie O, Andersson S, Mäkelä MJ. High-dose vitamin D supplementation does not prevent allergic sensitization in infants. Submitted.

III Rosendahl J, Holmlund-Suila E, Helve O, Viljakainen H, Hauta-alus H, Valkama S, Enlund-Cerullo M, Hytinantti T, Tervahartiala T, Sorsa T, Mäkitie O, Andersson S. 25-hydroxyvitamin D correlates with inflammatory markers in cord blood of healthy newborns. Pediatr Res. 2017; 81(5):731-735.

IV Rosendahl J, Fogelholm M, Pelkonen A, Mäkelä MJ, Mäkitie O, Erkkola M. A history of cow's milk allergy is associated with lower vitamin D status in schoolchildren. Horm Res Paediatr. 2017; 88(3-4):244-250.

* 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

CRP	C-reactive protein
hs-CRP	High-sensitivity C-reactive protein
DBP	Vitamin D-binding protein
DEQAS	Vitamin D Quality Assessment Scheme
D_2	Vitamin D_2
D_3	Vitamin D_3
25(OH)D	25-hydroxyvitamin D
$1,25(OH)_2D$	1,25-dihydroxyvitamin D
FGF-23	Fibroblast growth factor 23
IgE	Immunoglobulin E
IOM	Institute of Medicine
MMP-8	Matrix metalloproteinase 8
NNR	Nordic Nutrition Recommendations
PTH	Parathormone
RDA	Recommended dietary allowance
RI	Recommended intake
Tc cell	Cytotoxic T cell
Th cell	Helper T cell
Treg cell	Regulatory T cell
TLR	Toll-like receptor
UL	Tolerable upper intake level
UVB	Ultraviolet B
VDR	Vitamin D receptor
VDSP	Vitamin D Standardization Program

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 D_3 and vitamin D_2. In human or animal skin, a photochemical reaction induced by solar UVB radiation converts 7-dehydrocholesterol to previtamin D_3, which is further converted to vitamin D_3 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 D_3 to inactive isomers lumisterol and tachysterol (Holick et al. 1981). Egg yolk, fatty fish and liver contain vitamin D_3 naturally, while vitamin D_2 is formed from its precursor ergosterol in plants, yeasts and fungi by UVB radiation.

To function biologically, the vitamin D (either vitamin D_3 or vitamin D_2, 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 (CYP27BI) 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 $1,25(OH)_2D$ synthesis. For example, cells of the placenta, prostate and skin, and immune cells such as macrophages, T lymphocytes and dendritic cells express 1α-hydroxylase, and are capable of independently synthesizing $1,25(OH)_2D$ from 25(OH)D (Adams and Hewison 2012, Hewison et al. 2007).

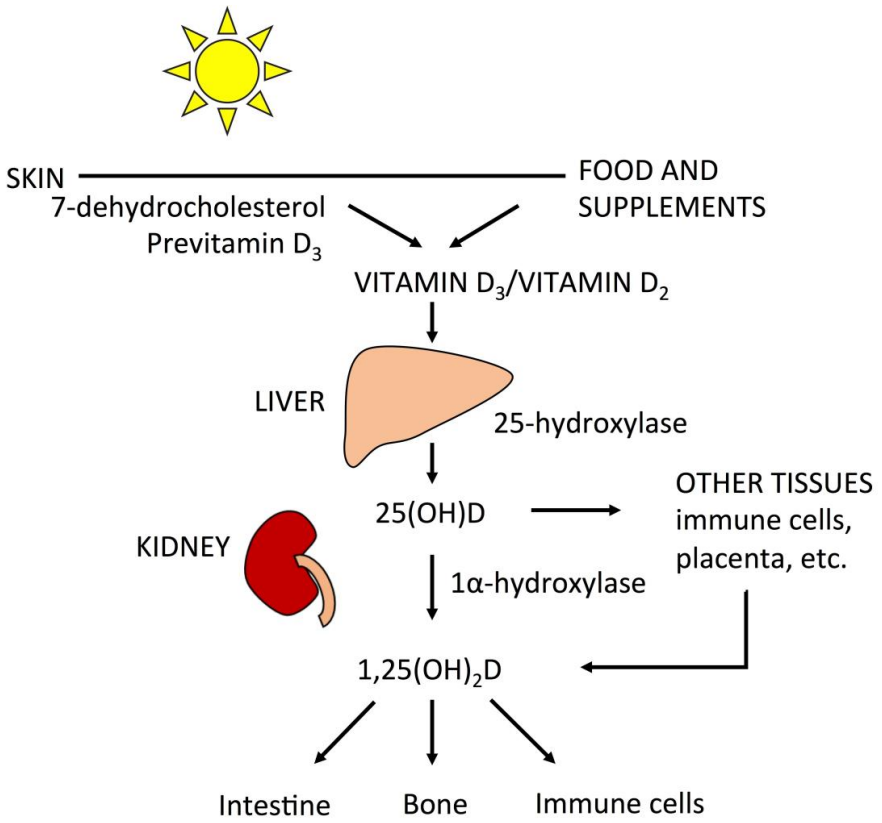


Figure 1. Principal steps of vitamin D metabolism. 25(OH)D, 25-hydroxyvitamin D. $1,25(OH)_2D$, 1,25-dihydroxyvitamin D.

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

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).

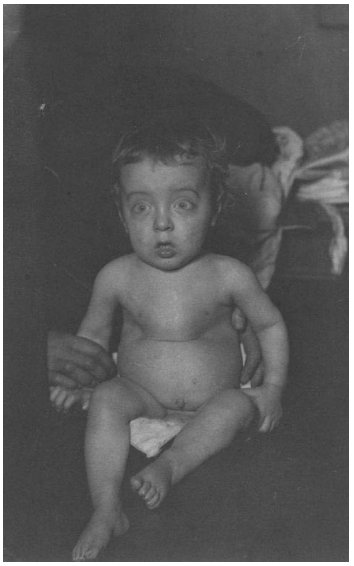


Figure 2. Child with rachitic deformity of the thorax. St Bartholomew's Hospital Archives & Museum, Wellcome Collection.

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).

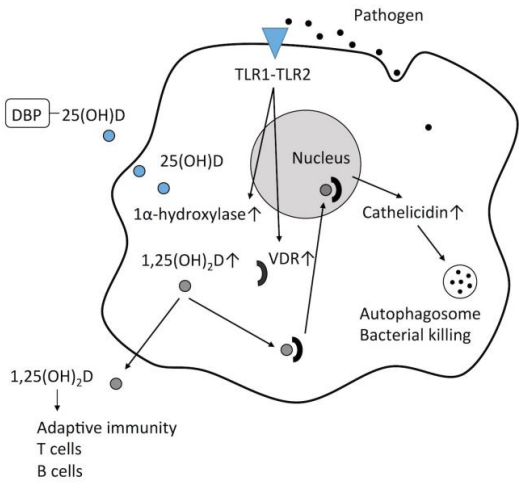


Figure 3. Simplified illustration of vitamin D-induced expression of antimicrobial peptide cathelicidin and bacterial killing in monocyte. Adapted from Hewison et al. 2011.

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, Liefaard 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$(OH)_2$D 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$(OH)_2$D (Jones 2008). As serum 1,25$(OH)_2$D 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.*

	NNR		**IOM**		**Endocrine Society**		
Target 25(OH)D	≥ 50 nmol/L		≥ 50 nmol/L		≥ 75 nmol/L		
Vitamin D	Intake	UL	Intake	UL	Intake	UL	UL risk*
Age (y)	µg/d	µg/d	µg/d	µg/d	µg/d	µg/d	µg/d
0-0.5	10	25	10	25	10	25	50
0.5-1	10	25	10	37.5	10	37.5	50
1-3	10	50	15	62.5	15	62.5	100
4-8	10	50	15	75	15	75	100
9-10	10	50	15	100	15	100	100
11-17	10	100	15	100	15	100	100

NNR, Nordic Nutrition Recommendations 2012. IOM, Institute of Medicine, Ross et al. 2011. Endocrine Society, Holick et al. 2011. UL, tolerable upper intake level.
*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 D_3 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 D_3 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.*

Authority or organization	Country	Daily supplemental vitamin D dose	Daily total vitamin D intake
National Nutrition Council (Valtion ravitsemusneuvottelukunta)[a]	Finland	Age 2 weeks-2 y: 10 µg Age 2-17 y: 7.5 µg	All children 10 µg
National Food Agency (Livsmedelsverket)[a]	Sweden	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	All children 10 µg
Danish Health Authority (Sundhedsstyrelsen)[a]	Denmark	Age 0-2 y: 10 µg Older children, if dark skin or not exposed to sun: 10 µg	All children 10 µg
Norwegian Directorate of Health (Helsedirektoratet)[a]	Norway	Age 4 weeks-1 y: 10 µg	All children 10 µg
Public Health England[b]	UK	Age 0-1 y, breastfed or formulafed < 500 ml/d: 8.5-10 µg Age 1-4 y: 10 µg	All children 10 µg
American Academy of Pediatrics[c]	USA	All infants until vitamin D-fortified formula or milk used > 1 l/d: 10 µg Older children, if not obtained from diet: 10 µg	Age 0-1 y: 10 µg Age > 1 y: 15 µg
Global consensus recommendation for rickets prevention[d]	Global	Age 0-1 y: 10 µg Age > 1 y, if not obtained from diet: dose not defined	Age 0-1 y: 10 µg Age > 1 y: 15 µg

[a] based on Nordic Nutrition Recommendations (NNR) 2012
[b] https://www.gov.uk/government/news/phe-publishes-new-advice-on-vitamin-d, based on Scientific Advisory Committee on Nutrition (SACN) report 2016
[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 D_3 are oily fish and eggs. Vitamin D_2 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 D_3 is considered to be more biologically effective compared with vitamin D_2, 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 D_3/100 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 3. *Randomized controlled trials on vitamin D supplementation in prevention of infections in children.*

Study and country	Participants	Age	Vitamin D dose	Duration	Outcome	Baseline 25(OH)D (nmol/L)	Result
Urashima 2010 Japan	430 schoolchildren	6-15 y	30 µg/d vs. placebo	4 mo	Incidence of influenza A	-	Influenza A incidence reduced in vitamin D group
Manaseki-Holland 2010 Afghanistan	453 children with pneumonia	1-36 mo	2500 µg once vs. placebo	3 mo	Duration of pneumonia and repeat episodes	-	No difference between groups in pneumonia duration, but risk of repeat episodes reduced in vitamin D group
Majak 2011 Poland	48 children with asthma	5-18 y	12.5 µg/d vs. placebo	6 mo	Asthma exacerbation triggered by acute respiratory infection	90.1 vs. 87.6	Asthma exacerbations lower in vitamin D group
Trilok-Kumar 2011 India	2079 low birthweight infants	0-6 mo	35 µg/wk vs. placebo	6 mo	Rate of hospital admission or death and any severe morbidity[a]	-	No differences between groups in hospital admission, death or any severe morbidity
Manaseki-Holland 2012 Afghanistan	3046 infants	0-11 mo	2500 µg/3 mo vs. placebo	18 mo	Radiologically confirmed pneumonia and repeat episodes	-	No difference between groups in pneumonia, but more repeat episodes in vitamin D group
Camargo 2012 Mongolia	247 schoolchildren	7-12 y	7.5 µg/d in milk vs. regular milk	7 wk	Number of parent-reported acute respiratory infections over 3 months	17.5 vs.17.0	Risk of acute respiratory infection reduced in vitamin D group
Marchisio 2013 Italy	116 otitis-prone children	1-5 y	25 µg/d vs. placebo	4 mo	Number of doctor-diagnosed acute otitis media episodes	66.1 vs. 64.4	Fewer acute otitis media episodes in vitamin D group
Aluisio 2013 Afghanistan[b]	3046 infants	1-11 mo	2500 µg/3 mo vs. placebo	18 mo	Incidence and time to first diarrheal illness	-	No differences between groups in diarrhea illness
Urashima 2014[c] Japan	247 schoolchildren	15-18 y	50 µg/d vs. placebo	2 mo	Incidence of influenza A during H1N1 pandemic	-	No difference in influenza A incidence
Dubnov-Raz 2015[c] Israel	55 vitamin D-insufficient swimmers	12-18 y	50 µg/d vs. placebo	12 wk	Upper respiratory tract infection	All 60.4	No differences between groups in upper respiratory infections
Chandy 2016 India	230 mother-infant pairs	0-9 mo	Mother 3000 µg/mo or infant 10 µg/d vs. placebo	9 mo	Number of days of diarrhea or respiratory morbidity[d]	-	Number of days of respiratory or diarrheal infection lower in both vitamin D groups
Aglipay 2017 Canada	703 children	1-5 y	50 µg/d vs. 10 µg/d	4-8 mo	Number of laboratory-confirmed viral upper respiratory tract infections	89.6 vs. 92.1	No difference between groups in number of laboratory-confirmed infections

[a] Any severe morbidity defined as pneumonia, persistent diarrhea, dysentery, severe fever, meningitis, ear infections, septicemia, severe protein energy malnutrition; [b] Same study as Manaseki-Holland 2012, diarrhea as secondary outcome; [c] Only abstract available, no full text; [d] 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 (Baïz 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 μg or 30 μg 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 μg or 30 μg 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 D_3 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 multi-national 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*cord plasma 25(OH)D 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.*

	Studies I-III (VIDI study)			**Study IV**
	All	**10 µg vitamin D**	**30 µg vitamin D**	
Number	975[a]	489	486	171
Age, years	0-2	0-2	0-2	10
Girls, %	50	50	50	51
Breastfed, months, mean (SD)	11 (6)[b]	10 (6)	11 (6)	9 (6)[c]
Siblings, %	37[d]	34	40	87[e]
Parental education high, %	81[f]	79	83	49[g]
Parental smoking, %	16[h]	16	17	20[i]
Year when data collected	2013-2016	2013-2016	2013-2016	2013

[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, [i] 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).

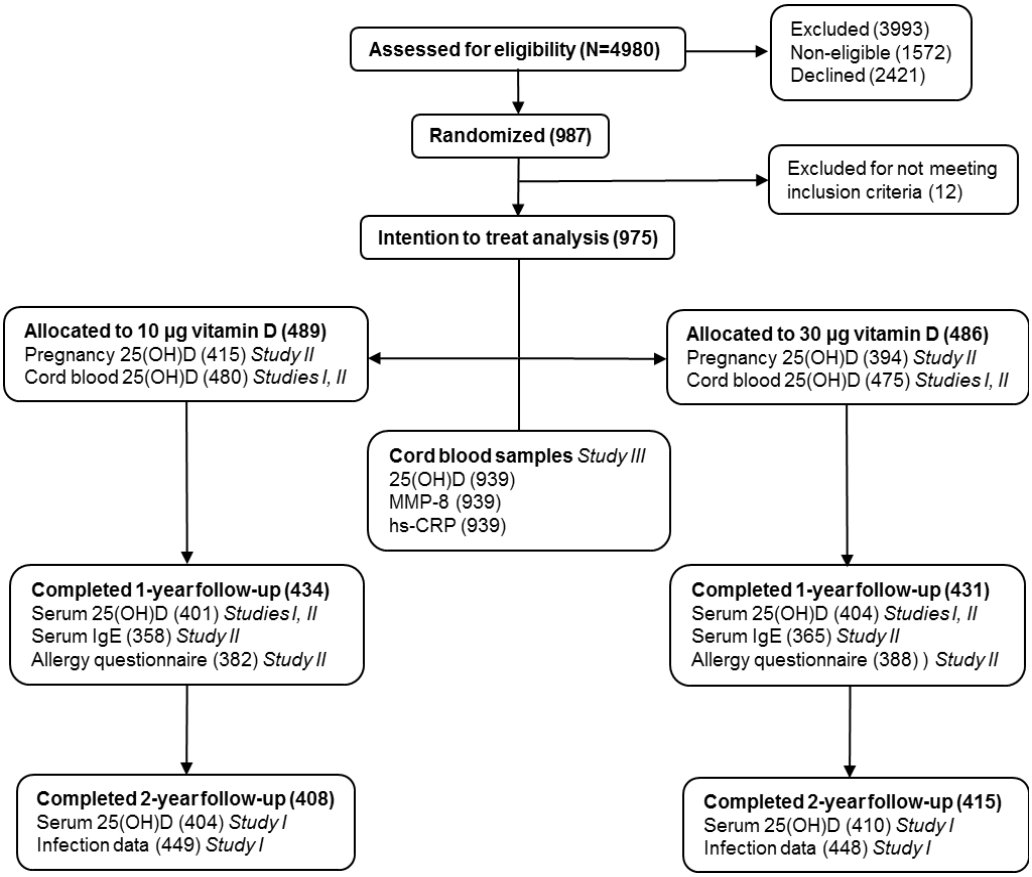


Figure 4. Enrollment, allocation and follow-up of the Vitamin D Intervention in Infants study demonstrating time-points of data collection of studies I-III.

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.*

		25-hydroxyvitamin D concentration nmol/L			
		< 50	**50-74.9**	**75-124.9**	**≥ 125**
VIDI study (I-III)	*n*	*%*	*%*	*%*	*%*
Pregnancy	809	1.0	22.1	72.2	4.7
Birth	955	4.3	39.6	51.7	4.4
Age 1 year					
10 µg vitamin D	401	2.2	34.9	61.3	1.5
30 µg vitamin D	404	0.2	5.2	62.1	32.4
Age 2 years					
10 µg vitamin D	404	1.2	28.2	67.3	3.2
30 µg vitamin D	410	0.0	4.6	56.6	38.8
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 ($p=0.322$).

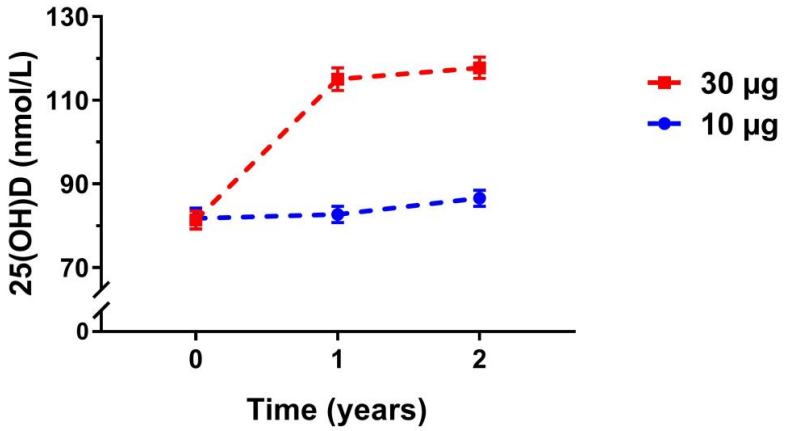


Figure 5. Mean serum 25(OH)D concentrations with 95% confidence intervals in children randomized to 10 µg and 30 µg vitamin D supplementation from age 2 weeks to 2 years.

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.

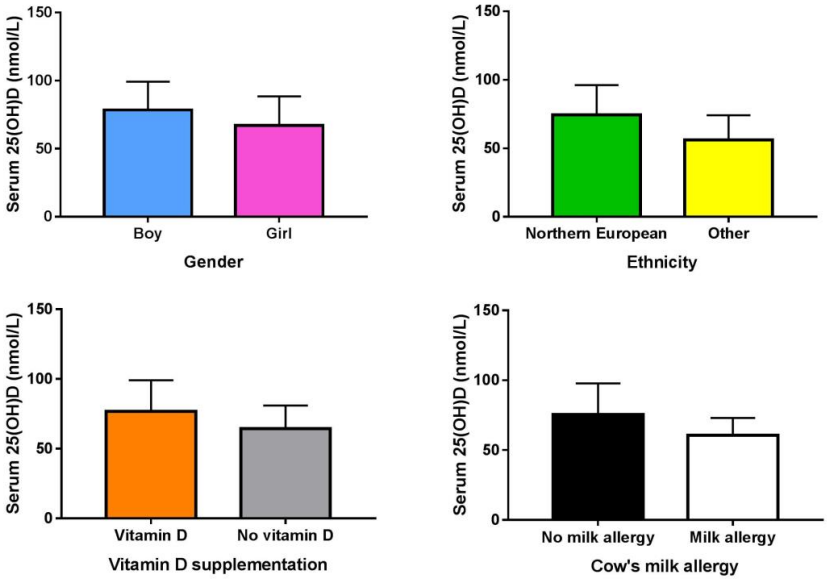


Figure 6. Mean serum 25(OH)D concentrations with SD of the 171 10-year-olds by gender ($p<0.001$), ethnicity ($p=0.003$), use of vitamin D supplementation (2–7 times/week vs. no regular use, $p=0.002$), and history of cow's milk allergy ($p=0.004$). Number of subjects: boys n=84, girls n=87; Northern 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.

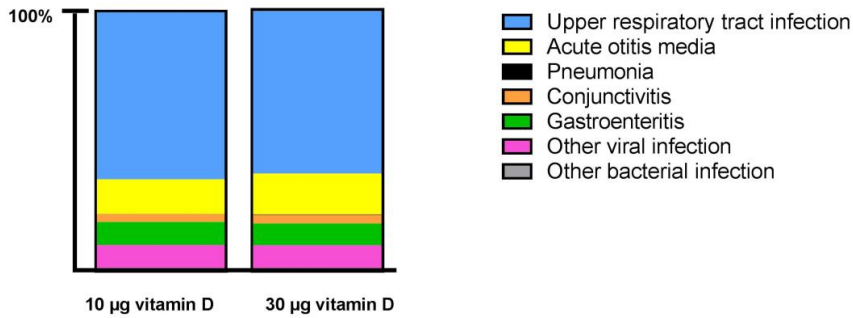
5.3.1 CHARACTERISTICS OF INFECTIONS

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

	10 µg vitamin D[a]	**30 µg vitamin D[b]**
Infection episodes per child	*mean (SD)*	*mean (SD)*
0-1 years	4.04 (2.34)	3.92 (2.48)
0-2 years	9.18 (4.83)	9.14 (4.92)
Duration per episode, days	6.18 (3.60)	5.68 (2.74)
Antibiotic treatments	1.76 (2.07)	2.06 (2.27)
Physician visits	3.15 (2.95)	3.38 (3.17)
Hospitalizations	0.07 (0.29)	0.09 (0.3)

[a] n=449, [b] n=448



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.*

	10 µg vitamin D			30 µg vitamin D			
	n	*Events*	*Incidence rate*[a]	*n*	*Events*	*Incidence rate*[a]	*IRR (95% CI)*[b]
All infection episodes	449	4244	0.41	448	4214	0.41	1.00 (0.93-1.06)
Upper respiratory tract infections	449	2733	0.27	448	2619	0.26	0.96 (0.89-1.05)
Acute otitis media	449	561	0.05	448	656	0.06	1.16 (0.97-1.40)
Pneumonia	449	5	0.00	448	8	0.00	1.60 (0.49-5.26)
Conjunctivitis	449	129	0.01	448	146	0.01	1.13 (0.85-1.51)
Gastroenteritis	449	379	0.04	448	349	0.03	0.92 (0.79-1.08)
Other viral infections	449	367	0.04	448	366	0.04	1.00 (0.86-1.17)
Other bacterial infections	449	48	0.00	448	50	0.00	1.04 (0.68-1.60)
Antibiotic treatments	441	795	0.08	435	922	0.09	1.17 (1.00-1.36)
Physician visits	443	1421	0.14	435	1502	0.15	1.07 (0.94-1.21)
Hospitalizations	443	33	0.00	435	38	0.00	1.16 (0.71-1.89)

[a] Incidence rate from number of events divided by person-months.
[b] 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.*

		All	**10 µg vitamin D**	**30 µg vitamin D**		
	N	*n (%)*	*n (%)*	*n (%)*	*p-value*[a]	*OR (95% CI)*[b]
Food IgE sensitization	723	114 (15.8)	57 (15.9)	57 (15.6)	0.910	1.01 (0.66-1.55)
Milk	720	42 (5.8)	21 (5.9)	21 (5.8)	0.974	n/a
Wheat	720	17 (2.4)	10 (2.8)	7 (1.9)	0.448	n/a
Egg white	720	75 (10.4)	40 (11.2)	35 (9.7)	0.509	n/a
Cod	720	3 (0.4)	2 (0.6)	1 (0.3)	0.622	n/a
Soybean	720	8 (1.1)	6 (1.7)	2 (0.6)	0.175	n/a
Peanut	720	23 (3.2)	16 (4.5)	7 (1.5)	0.053	n/a
Aeroallergen IgE sensitization	719	25 (3.5)	14 (3.9)	11 (3.0)	0.509	0.79 (0.34-1.87)
Birch	718	10 (1.4)	6 (1.7)	4 (1.1)	0.543	n/a
Mugwort	718	0	0	0	n/a	n/a
Timothy	718	3 (0.4)	3 (0.8)	0 (0)	0.121	n/a
Dog	718	13 (1.8)	7 (2.0)	6 (1.7)	0.765	n/a
Cat	718	10 (1.4)	5 (1.4)	4 (1.4)	1.000	n/a
Horse	716	2 (0.3)	2 (0.6)	0 (0)	0.245	n/a
Dermatophagoides pteronyssinus	718	1 (0.1)	1 (0.3)	0 (0)	0.496	n/a
Cladosporium herbarum	718	1 (0.1)	1 (0.3)	0 (0)	0.496	n/a
Physician-diagnosed allergic disease						
Cow's milk allergy	764	29 (3.8)	9 (2.4)	20 (5.2)	0.044	2.23 (1.00-4.96)
Wheat allergy	762	11 (1.4)	5 (1.3)	6 (1.6)	0.788	1.20 (0.36-3.98)
Atopic eczema	769	128 (16.6)	72 (18.9)	56 (14.4)	0.097	0.72 (0.49-2.07)
Asthma	765	1 (0.1)	1 (0.3)	0 (0)	0.494	n/a
Allergy symptoms						
Wheezing or breathing difficulty	763	76 (10.0)	39 (10.3)	37 (9.7)	0.781	0.98 (0.61-1.59)
Hospitalization due to bronchiolitis or wheezing	901	25 (2.8)	14 (3.1)	11 (2.4)	0.547	1.23 (0.50-3.00)
Persistent cough	765	46 (6.1)	20 (5.3)	26 (6.9)	0.371	1.44 (0.77-2.66)
Itchy rash	765	126 (16.5)	72 (18.9)	54 (16.5)	0.067	0.68 (0.46-1.01)

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

[a] P-values from Pearson chi-square or Fisher's exact when cell count ≤ 5.

[b] 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.

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 prepregnancy BMI for hs-CRP (MMP-8 ß coefficient = 1.012, 95% CI 1.01–1.02; hs-CRP ß coefficient = 1.003, 95% CI 1.00–1.01).

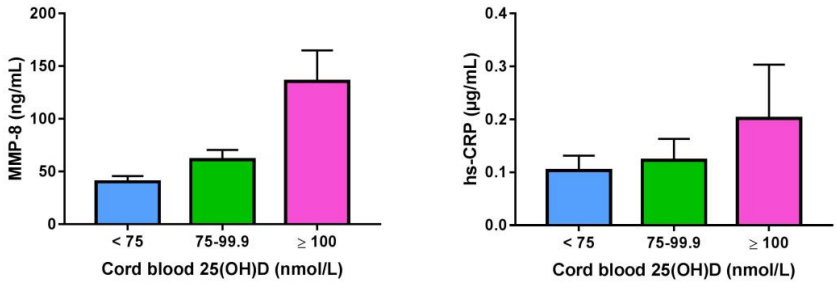


Figure 7. Mean cord blood MMP-8 and hs-CRP concentrations with 95% confidence intervals by cord blood 25(OH)D status. Number of subjects: 25(OH)D < 75 nmol/L n=409, 75-99.9 nmol/L n=363, ≥ 100 nmol/L n=167.

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.*

	Food IgE sensitization		
Cord blood 25(OH)D nmol/L	*N*	*n (%)*	*OR (95% CI)*[a]
< 75	295	43 (14.6)	reference
75-99.9	286	40 (14.0)	0.95 (0.60-1.52)
≥ 100	126	31 (24.6)	1.91 (1.14-3.21)

25(OH)D, 25-hydroxyvitamin D
[a] Odds ratio (OR) from logistic regression with 95% confidence interval (CI)

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 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 (Kyttälä 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 Högler 2018, Uday et al. 2017). Alarmingly, the prevalence of rickets is increasing in the UK (Uday and Högler 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

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

This study was carried out between 2014 and 2018 at the Children's Hospital, Pediatric Research Center, University of Helsinki and Helsinki University Hospital. I wish to thank Jari Petäjä, Director of Children's Hospital, Anne Wikström, Head of Pediatrics, and Eero Jokinen, Head of Tertiary Pediatrics, and Professor Markku Heikinheimo, Professor Mikael Knip and Professor Harri Saxén of the Children's Hospital, University of Helsinki and Helsinki University Hospital for providing excellent research facilities for scientific training. I also warmly thank the former and present heads of the Pediatric Graduate School, Docent Jussi Merenmies and Professor Kim Vettenranta, and Professor Taneli Raivio, head of the Pediatric Research Center, for arranging scientific education, social events and for financial support.

I owe my greatest gratitude to all the children and their families who participated in the studies.

The study was financially supported by the Finnish Medical Foundation, Finska Läkaresällskapet, the Foundation for Pediatric Research, the Orion Research Foundation, the Päivikki and Sakari Sohlberg Foundation and the University of Helsinki. Orion Pharmaceuticals is acknowledged for providing the vitamin D supplements for the intervention study.

I am most grateful to my thesis supervisors Professor Outi Mäkitie and Professor Sture Andersson for their warm and encouraging guidance throughout this study. Outi, your enthusiasm towards science and your international expericence is inspirational. Thank you for the motivation and encouragement. Sture, I am grateful for your wise and fatherly guidance. You have been always available and easy to contact, and your broad knowledge on science, medicine and life in general helped me to see the big picture during this project.

I thank my thesis committee members Professor Harri Saxén and Docent Mikael Kuitunen for advice and encouragement during this work. Harri, I appreciate that you have provided me the opportunity to work at the pediatric infectious diseases unit of the Children's Hospital.

I thank my official external reviewers Professor Ville Peltola and Professor Harri Niinikoski for excellent comments and suggestions to my thesis. I highly appreciate that you took the time to review the work despite your busy schedules and summer vacation.

I express my gratitude to all the co-authors who collaborated in the studies. I thank Professor Mikael Fogelholm and Docent Maijaliisa Erkkola from the Department of Food and Environmental Sciences, University of Helsinki, for allowing me to use the ISCOLE cohort in my work. Maikki, I appreciate your most kind and pedagogical guidance with the article. I thank professor Timo Sorsa and Taina Tervahartiala from the Department of Oral and Maxillofacial Diseases, University of Helsinki, for collaboration with the MMP-8 study. I warmly thank Docent Eero Kajantie and Esko Levälahti from the National Institute for Health and Welfare for their invaluable contribution to the statistical analyses of the intervention study. I also wish to thank Professor Mika Mäkelä and Docent Anna Pelkonen from the Department of Allergy, Skin and Allergy Hospital, for their constructive and encouraging collaboration.

It has been a pleasure working with such a fantastic research group, the VIDI team. A tremendous thanks goes to the world's best research nurses Sirpa, Rhea, Päivi, Nea and Sari; the study would not have completed without your help. Especially Sirpa, your devotion towards the project and the families was exceptional. I lovingly thank my wonderful fellow VIDI researchers, the Täydelliset tutkijanaiset, Helena, Saara, Elisa and Maria. We have shared the ups and downs of the project together. Thank you for your friendship, support, good conversations and fun moments both home and abroad. I also thank Otto, Heli and Timo for advice and encouragement during the study.

I warmly thank all my co-workers at the 6th floor of Biomedicum. Thank you for the relaxing lunch and coffee breaks, conversations and peer support, not to mention the joyful toasting with sparkling wine at times of a successful submission. Thank you especially Laura, Mari, Maija, Maarit, Sonja, Silja, Satu and Anne, among others.

Together with this research work I have been working periodically as a pediatrician at the Children's Hospital, and hospitals of Jorvi and Peijas. I am grateful to Anne Wikström, Head of Pediatrics, for being understanding and flexible in organising the work. I also express my gratitude to all my lovely collegues in all three hospitals.

I am grateful for all my wonderful friends. The Möllit, most important since the teenage years, thank you for always being by my side. Especially Outi and Tuomas, Lotta and Mauri, Katri and Kynde, Karoliina and Perttu, Anu and Janne, and your beautiful children, thank you for your friendship and love towards our family. I thank my dear friends from the medical school, especially the brilliant ladies from the Book club, for the laughter and inspiring conversations, with a special thanks to Hanna for the practical advice when finalizing this thesis. Our neighbours Selina and Vesa, thank you

for the peer support with our family life; sports together and the Sunday sauna helped to keep my head in balance during the hectic final stage of this project.

I owe my deepest gratitude to my family. I thank my parents Maiju and Risto, my sister Outi and her family for the love and faith in me at all times. I thank my father for showing me with his example the significance of scientific work and my mother for lovingly taking care of our children and household whenever needed. My wonderful in-laws Kikka and Hessu, Anna and her family, and other relatives, thank you for always being there for us.

Lastly, I thank my husband Jussi for everything, you are the best companion one could ever have. Without your unconditional love, support and taking care of our everyday family life, I would not have gotten through this work. Our precious children, Kerttu and Eero, I love you more than anything.

Helsinki, August 2018

Jenni Rosendahl

8 REFERENCES

Adams JS, Hewison M (2012) Extrarenal expression of the 25-hydroxyvitamin D-1-hydroxylase. Arch Biochem Biophys 523:95-102.

Adams JS, Ren S, Liu PT, Chun RF, Lagishetty V, Gombart AF, Borregaard N, Modlin RL, Hewison M (2009) Vitamin D-directed rheostatic regulation of monocyte antibacterial responses. J Immunol 182:4289-4295.

Adorini L, Penna G, Giarratana N, Roncari A, Amuchastegui S, Daniel KC, Uskokovic M (2004) Dendritic cells as key targets for immunomodulation by Vitamin D receptor ligands. J Steroid Biochem Mol Biol 89-90:437-441.

Aglipay M, Birken CS, Parkin PC, Loeb MB, Thorpe K, Chen Y, Laupacis A, Mamdani M, Macarthur C, Hoch JS, Mazzulli T, Maguire JL, TARGet Kids! Collaboration (2017) Effect of High-Dose vs Standard-Dose Wintertime Vitamin D Supplementation on Viral Upper Respiratory Tract Infections in Young Healthy Children. JAMA 318:245-254.

Aluisio AR, Maroof Z, Chandramohan D, Bruce J, Mughal MZ, Bhutta Z, Walraven G, Masher MI, Ensink JH, Manaseki-Holland S (2013) Vitamin D3 supplementation and childhood diarrhea: a randomized controlled trial. Pediatrics 132:e832-40.

Back O, Blomquist HK, Hernell O, Stenberg B (2009) Does vitamin D intake during infancy promote the development of atopic allergy? Acta Derm Venereol 89:28-32.

Baeke F, Etten EV, Overbergh L, Mathieu C (2007) Vitamin D3 and the immune system: maintaining the balance in health and disease. Nutr Res Rev 20:106-118.

Baïz N, Dargent-Molina P, Wark JD, Souberbielle J, Annesi-Maesano I (2014) Cord serum 25-hydroxyvitamin D and risk of early childhood transient wheezing and atopic dermatitis. J Allergy Clin Immunol 133:147-153.

Barrueto F Jr, Wang-Flores HH, Howland MA, Hoffman RS, Nelson LS (2005) Acute vitamin D intoxication in a child. Pediatrics 116:e453-6.

Belderbos ME, Houben ML, Wilbrink B, Lentjes E, Bloemen EM, Kimpen JLL, Rovers M, Bont L (2011) Cord Blood Vitamin D Deficiency Is Associated With Respiratory Syncytial Virus Bronchiolitis. Pediatrics 127:e1513-e1520.

Binkley N, Carter GD (2017) Toward Clarity in Clinical Vitamin D Status Assessment: 25(OH)D Assay Standardization. Endocrinol Metab Clin North Am 46:885-899.

Black S, Kushner I, Samols D (2004) C-reactive protein. J Biol Chem 279:48487-48490.

Blaine J, Chonchol M, Levi M (2015) Renal control of calcium, phosphate, and magnesium homeostasis. Clin J Am Soc Nephrol 10:1257-1272.

Blondon M, Cushman M, Jenny N, Michos ED, Smith NL, Kestenbaum B, de Boer IH (2016) Associations of Serum 25-Hydroxyvitamin D With Hemostatic and Inflammatory Biomarkers in the Multi-Ethnic Study of Atherosclerosis. J Clin Endocrinol Metab 101:2348-2357.

Britt RD Jr, Faksh A, Vogel ER, Thompson MA, Chu V, Pandya HC, Amrani Y, Martin RJ, Pabelick CM, Prakash YS (2015) Vitamin D attenuates cytokine-induced remodeling in human fetal airway smooth muscle cells. J Cell Physiol 230:1189-1198.

Bunyavanich S, Rifas-Shiman SL, Platts-Mills TA, Workman L, Sordillo JE, Camargo CA Jr, Gillman MW, Gold DR, Litonjua AA (2016) Prenatal, perinatal, and childhood vitamin D exposure and their association with childhood allergic rhinitis and allergic sensitization. J Allergy Clin Immunol 137:1063-1070.e2.

Camargo CA Jr, Ganmaa D, Sidbury R, Erdenedelger K, Radnaakhand N, Khandsuren B (2014) Randomized trial of vitamin D supplementation for winter-related atopic dermatitis in children. J Allergy Clin Immunol 134:831-835.e1.

Camargo CA Jr, Ganmaa D, Frazier AL, Kirchberg FF, Stuart JJ, Kleinman K, Sumberzul N, Rich-Edwards JW (2012) Randomized trial of vitamin D supplementation and risk of acute respiratory infection in Mongolia. Pediatrics 130:e561-7.

Camargo CA, Ingham T, Wickens K, Thadhani R, Silvers KM, Epton MJ, Town GI, Pattemore PK, Espinola JA, Crane J, the New Zealand Asthma and Allergy Cohort Study Group (2011) Cord-Blood 25-Hydroxyvitamin D Levels

and Risk of Respiratory Infection, Wheezing, and Asthma. Pediatrics 127:e180-e187.

Carter GD, Berry J, Durazo-Arvizu R, Gunter E, Jones G, Jones J, Makin HLJ, Pattni P, Phinney KW, Sempos CT, Williams EL (2017) Quality assessment of vitamin D metabolite assays used by clinical and research laboratories. J Steroid Biochem Mol Biol 173:100-104.

Cashman KD, Dowling KG, Skrabakova Z, Gonzalez-Gross M, Valtuena J, De Henauw S, Moreno L, Damsgaard CT, Michaelsen KF, Molgaard C, Jorde R, Grimnes G, Moschonis G, Mavrogianni C, Manios Y, Thamm M, Mensink GB, Rabenberg M, Busch MA, Cox L, Meadows S, Goldberg G, Prentice A, Dekker JM, Nijpels G, Pilz S, Swart KM, van Schoor NM, Lips P, Eiriksdottir G, Gudnason V, Cotch MF, Koskinen S, Lamberg-Allardt C, Durazo-Arvizu RA, Sempos CT, Kiely M (2016) Vitamin D deficiency in Europe: pandemic? Am J Clin Nutr 103:1033-1044.

Cashman KD (2015) Vitamin D: dietary requirements and food fortification as a means of helping achieve adequate vitamin D status. J Steroid Biochem Mol Biol 148:19-26.

Ceglia L, Harris SS (2013) Vitamin D and its role in skeletal muscle. Calcif Tissue Int 92:151-162.

Chandy DD, Kare J, Singh SN, Agarwal A, Das V, Singh U, Ramesh V, Bhatia V (2016) Effect of vitamin D supplementation, directly or via breast milk for term infants, on serum 25 hydroxyvitamin D and related biochemistry, and propensity to infection: a randomised placebo-controlled trial. Br J Nutr 116:52-58.

Chawes BL, Bonnelykke K, Stokholm J, Vissing NH, Bjarnadottir E, Schoos AM, Wolsk HM, Pedersen TM, Vinding RK, Thorsteinsdottir S, Arianto L, Hallas HW, Heickendorff L, Brix S, Rasmussen MA, Bisgaard H (2016) Effect of Vitamin D3 Supplementation During Pregnancy on Risk of Persistent Wheeze in the Offspring: A Randomized Clinical Trial. JAMA 315:353-361.

Chawes BL, Bonnelykke K, Jensen PF, Schoos AM, Heickendorff L, Bisgaard H (2014) Cord blood 25(OH)-vitamin D deficiency and childhood asthma, allergy and eczema: the COPSAC2000 birth cohort study. PLoS One 9:e99856.

Chen N, Wan Z, Han SF, Li BY, Zhang ZL, Qin LQ (2014) Effect of vitamin D supplementation on the level of circulating high-sensitivity C-reactive

protein: a meta-analysis of randomized controlled trials. Nutrients 6:2206-2216.

Chen S, Sims GP, Chen XX, Gu YY, Chen S, Lipsky PE (2007) Modulatory effects of 1,25-dihydroxyvitamin D3 on human B cell differentiation. J Immunol 179:1634-1647.

Chen TC, Chimeh F, Lu Z, Mathieu J, Person KS, Zhang A, Kohn N, Martinello S, Berkowitz R, Holick MF (2007) Factors that influence the cutaneous synthesis and dietary sources of vitamin D. Arch Biochem Biophys 460:213-217.

Chiu CY, Huang SY, Peng YC, Tsai MH, Hua MC, Yao TC, Yeh KW, Huang JL (2015) Maternal vitamin D levels are inversely related to allergic sensitization and atopic diseases in early childhood. Pediatr Allergy Immunol 26:337-343.

Chowdhury R, Taneja S, Bhandari N, Sinha B, Upadhyay RP, Bhan MK, Strand TA (2017) Vitamin-D deficiency predicts infections in young north Indian children: A secondary data analysis. PLoS One 12:e0170509.

Chun RF, Lauridsen AL, Suon L, Zella LA, Pike JW, Modlin RL, Martineau AR, Wilkinson RJ, Adams J, Hewison M (2010) Vitamin D-binding protein directs monocyte responses to 25-hydroxy- and 1,25-dihydroxyvitamin D. J Clin Endocrinol Metab 95:3368-3376.

Cook DG, Mendall MA, Whincup PH, Carey IM, Ballam L, Morris JE, Miller GJ, Strachan DP (2000) C-reactive protein concentration in children: relationship to adiposity and other cardiovascular risk factors. Atherosclerosis 149:139-150.

Coussens A, Timms PM, Boucher BJ, Venton TR, Ashcroft AT, Skolimowska KH, Newton SM, Wilkinson KA, Davidson RN, Griffiths CJ, Wilkinson RJ, Martineau AR (2009) 1alpha,25-dihydroxyvitamin D3 inhibits matrix metalloproteinases induced by Mycobacterium tuberculosis infection. Immunology 127:539-548.

de Jongh RT, Crozier SR, D'Angelo S, Pike KC, Roberts G, Lucas JS, Inskip H, Godfrey KM, Cooper C, Harvey NC, Southampton Women's Survey Study Group (2014) Maternal 25-hydroxyvitamin D levels in relation to offspring respiratory symptoms and infections. Eur Respir J 43:1181-1183.

de Oliveira C, Biddulph JP, Hirani V, Schneider IJC (2017) Vitamin D and inflammatory markers: cross-sectional analyses using data from the English

Longitudinal Study of Ageing (ELSA). J Nutr Sci 6:e1. doi:10.1017/jns.2016.37.

Denny FW, Collier AM, Henderson FW (1986) Acute respiratory infections in day care. Rev Infect Dis 8:527-532.

Dimitrakopoulou VI, Tsilidis KK, Haycock PC, Dimou NL, Al-Dabhani K, Martin RM, Lewis SJ, Gunter MJ, Mondul A, Shui IM, Theodoratou E, Nimptsch K, Lindstrom S, Albanes D, Kuhn T, Key TJ, Travis RC, Vimaleswaran KS, GECCO Consortium, PRACTICAL Consortium, GAME-ON Network (CORECT, DRIVE, ELLIPSE, FOCI-OCAC, TRICL-ILCCO), Kraft P, Pierce BL, Schildkraut JM (2017) Circulating vitamin D concentration and risk of seven cancers: Mendelian randomisation study. BMJ 359:j4761.

Dubnov-Raz G, Livne N, Raz R, Cohen AH, Constantini NW (2015) Vitamin D Supplementation and Physical Performance in Adolescent Swimmers. Int J Sport Nutr Exerc Metab 25:317-325.

Elder CJ, Bishop NJ (2014) Rickets. Lancet 383:1665-1676.

Elintarviketurvallisuusvirasto (2014) Erityisohjeet ja rajoitukset: D-vitamiinivalmisteet. https://www.evira.fi/elintarvikkeet/terveytta-edistava-ruokavalio/ravitsemussuositukset/erityisohjeet-ja-rajoitukset/

Ganmaa D, Munkhzul B, Fawzi W, Spiegelman D, Willett WC, Bayasgalan P, Baasansuren E, Buyankhishig B, Oyun-Erdene S, Jolliffe DA, Xenakis T, Bromage S, Bloom BR, Martineau AR (2017) High-Dose Vitamin D3 during Tuberculosis Treatment in Mongolia. A Randomized Controlled Trial. Am J Respir Crit Care Med 196:628-637.

Goldring ST, Griffiths CJ, Martineau AR, Robinson S, Yu C, Poulton S, Kirkby JC, Stocks J, Hooper R, Shaheen SO, Warner JO, Boyle RJ (2013) Prenatal vitamin D supplementation and child respiratory health: a randomised controlled trial. PLoS One 8:e66627.

Gombart AF, Borregaard N, Koeffler HP (2005) Human cathelicidin antimicrobial peptide (CAMP) gene is a direct target of the vitamin D receptor and is strongly up-regulated in myeloid cells by 1,25-dihydroxyvitamin D3. FASEB J 19:1067-1077.

Gorman S, Kuritzky LA, Judge MA, Dixon KM, McGlade JP, Mason RS, Finlay-Jones JJ, Hart PH (2007) Topically applied 1,25-dihydroxyvitamin D3 enhances the suppressive activity of CD4+CD25+ cells in the draining lymph nodes. J Immunol 179:6273-6283.

Grant CC, Crane J, Mitchell EA, Sinclair J, Stewart A, Milne T, Knight J, Gilchrist C, Camargo CA Jr (2016) Vitamin D supplementation during pregnancy and infancy reduces aeroallergen sensitization: a randomized controlled trial. Allergy 71:1325-1334.

Haffner SM (2006) The metabolic syndrome: inflammation, diabetes mellitus, and cardiovascular disease. Am J Cardiol 97:3A-11A.

Haider N, Nagi AG, Khan KM (2010) Frequency of nutritional rickets in children admitted with severe pneumonia. J Pak Med Assoc 60:729-732.

Halder SK, Osteen KG, Al-Hendy A (2013) Vitamin D3 inhibits expression and activities of matrix metalloproteinase-2 and -9 in human uterine fibroid cells. Hum Reprod 28:2407-2416.

Hallman N, Hultin H, Visakorpi JK (1964) Riisitaudin ennakkotorjunnasta. Duodecim:185-189.

Hartmann B, Heine G, Babina M, Steinmeyer A, Zugel U, Radbruch A, Worm M (2011) Targeting the vitamin D receptor inhibits the B cell-dependent allergic immune response. Allergy 66:540-548.

Hauta-alus HH, Holmlund-Suila EM, Rita HJ, Enlund-Cerullo M, Rosendahl J, Valkama SM, Helve OM, Hytinantti TK, Surcel HM, Mäkitie OM, Andersson S, Viljakainen HT (2018) Season, dietary factors, and physical activity modify 25-hydroxyvitamin D concentration during pregnancy. Eur J Nutr. 57:1369. https://doi: 10.1007/s00394-017-1417-z.

Hauta-alus HH, Korkalo L, Holmlund-Suila EM, Rosendahl J, Valkama SM, Enlund-Cerullo M, Helve OM, Hytinantti TK, Mäkitie OM, Andersson S, Viljakainen HT (2017) Food and Nutrient Intake and Nutrient Sources in 1-Year-Old Infants in Finland: A Cross-Sectional Analysis. Nutrients 9:12:1309.

Helldan A, Raulio S, Kosola M, Tapanainen H, Ovaskainen ML, Virtanen S (2013) Finravinto 2012 -tutkimus. The National FINDIET 2012 Survey. http://urn.fi/URN:ISBN:978-952-245-951-0. THL Raportti.

Hernigou P, Auregan JC, Dubory A (2018) Vitamin D: part I; from plankton and calcified skeletons (500 million years ago) to rickets. Int Orthop Mar 5. doi:10.1007/s00264-018-3857-3.

Hewison M (2012) An update on vitamin D and human immunity. Clin Endocrinol (Oxf) 76:315-325.

Hewison M (2011) Antibacterial effects of vitamin D. Nat Rev Endocrinol 7:337-345.

Hewison M, Burke F, Evans KN, Lammas DA, Sansom DM, Liu P, Modlin RL, Adams JS (2007) Extra-renal 25-hydroxyvitamin D3-1alpha-hydroxylase in human health and disease. J Steroid Biochem Mol Biol 103:316-321.

Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Murad MH, Weaver CM, Endocrine Society (2011) Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 96:1911-1930.

Holick MF (2006) Resurrection of vitamin D deficiency and rickets. J Clin Invest 116:2062-2072.

Holick MF (2003) Vitamin D: A millenium perspective. J Cell Biochem 88:296-307.

Holick MF, MacLaughlin JA, Doppelt SH (1981) Regulation of cutaneous previtamin D3 photosynthesis in man: skin pigment is not an essential regulator. Science 211:590-593.

Holick MF, Frommer JE, McNeill SC, Richtand NM, Henley JW, Potts JT (1977) Photometabolism of 7-dehydrocholesterol to previtamin D3 in skin. Biochem Biophys Res Commun 76:107-114.

Holmlund-Suila E, Viljakainen H, Hytinantti T, Lamberg-Allardt C, Andersson S, Mäkitie O (2012) High-dose vitamin d intervention in infants--effects on vitamin D status, calcium homeostasis, and bone strength. J Clin Endocrinol Metab 97:4139-4147.

Hyppönen E, Berry DJ, Wjst M, Power C (2009) Serum 25-hydroxyvitamin D and IgE - a significant but nonlinear relationship. Allergy 64:613-620.

Hyppönen E, Sovio U, Wjst M, Patel S, Pekkanen J, Hartikainen AL, Jarvelinb MR (2004) Infant vitamin D supplementation and allergic conditions in adulthood: northern Finland birth cohort 1966. Ann N Y Acad Sci 1037:84-95.

Jerzynska J, Stelmach W, Rychlik B, Majak P, Podlecka D, Woicka-Kolejwa K, Stelmach I (2018) Clinical and immunological effects of vitamin D supplementation during the pollen season in children with allergic rhinitis. Arch Med Sci 14:122-131.

Jolliffe DA, Walton RT, Griffiths CJ, Martineau AR (2016) Single nucleotide polymorphisms in the vitamin D pathway associating with circulating concentrations of vitamin D metabolites and non-skeletal health outcomes: Review of genetic association studies. J Steroid Biochem Mol Biol 164:18-29.

Jones G, Prosser DE, Kaufmann M (2012) 25-Hydroxyvitamin D-24-hydroxylase (CYP24A1): its important role in the degradation of vitamin D. Arch Biochem Biophys 523:9-18.

Jones G (2008) Pharmacokinetics of vitamin D toxicity. Am J Clin Nutr 88:582S-586S.

Jääskeläinen T, Itkonen ST, Lundqvist A, Erkkola M, Koskela T, Lakkala K, Dowling KG, Hull GL, Kröger H, Karppinen J, Kyllönen E, Härkänen T, Cashman KD, Männisto S, Lamberg-Allardt C (2017) The positive impact of general vitamin D food fortification policy on vitamin D status in a representative adult Finnish population: evidence from an 11-y follow-up based on standardized 25-hydroxyvitamin D data. Am J Clin Nutr 105:1512-1520.

Katzmarzyk PT, Barreira TV, Broyles ST, Champagne CM, Chaput JP, Fogelholm M, Hu G, Johnson WD, Kuriyan R, Kurpad A, Lambert EV, Maher C, Maia J, Matsudo V, Olds T, Onywera V, Sarmiento OL, Standage M, Tremblay MS, Tudor-Locke C, Zhao P, Church TS (2013) The International Study of Childhood Obesity, Lifestyle and the Environment (ISCOLE): design and methods. BMC Public Health 13:900-2458-13-900.

Kerley CP, Hutchinson K, Cormican L, Faul J, Greally P, Coghlan D, Elnazir B (2016) Vitamin D3 for uncontrolled childhood asthma: A pilot study. Pediatr Allergy Immunol 27:404-412.

Kim KW, Romero R, Park HS, Park CW, Shim SS, Jun JK, Yoon BH (2007) A rapid matrix metalloproteinase-8 bedside test for the detection of intraamniotic inflammation in women with preterm premature rupture of membranes. Am J Obstet Gynecol 197:292.e1-292.e5.

Kruit A, Zanen P (2016) The association between vitamin D and C-reactive protein levels in patients with inflammatory and non-inflammatory diseases. Clin Biochem 49:534-537.

Kumar GT, Sachdev HS, Chellani H, Rehman AM, Singh V, Arora H, Filteau S (2011) Effect of weekly vitamin D supplements on mortality, morbidity, and growth of low birthweight term infants in India up to age 6 months: randomised controlled trial. BMJ 342:d2975.

Kyttälä P, Erkkola M, Kronberg-Kippila C, Tapanainen H, Veijola R, Simell O, Knip M, Virtanen SM (2010) Food consumption and nutrient intake in Finnish 1-6-year-old children. Public Health Nutr 13:947-956.

Lauhio A, Hästbacka J, Pettila V, Tervahartiala T, Karlsson S, Varpula T, Varpula M, Ruokonen E, Sorsa T, Kolho E (2011) Serum MMP-8, -9 and TIMP-1 in sepsis: high serum levels of MMP-8 and TIMP-1 are associated with fatal outcome in a multicentre, prospective cohort study. Hypothetical impact of tetracyclines. Pharmacol Res 64:590-594.

Li YC, Qiao G, Uskokovic M, Xiang W, Zheng W, Kong J (2004) Vitamin D: a negative endocrine regulator of the renin-angiotensin system and blood pressure. J Steroid Biochem Mol Biol 89-90:387-392.

Liefaard MC, Ligthart S, Vitezova A, Hofman A, Uitterlinden AG, Kiefte-de Jong JC, Franco OH, Zillikens MC, Dehghan A (2015) Vitamin D and C-Reactive Protein: A Mendelian Randomization Study. PLoS One 10:e0131740.

Litonjua AA, Carey VJ, Laranjo N, Harshfield BJ, McElrath TF, O'Connor GT, Sandel M, Iverson RE Jr, Lee-Paritz A, Strunk RC, Bacharier LB, Macones GA, Zeiger RS, Schatz M, Hollis BW, Hornsby E, Hawrylowicz C, Wu AC, Weiss ST (2016) Effect of Prenatal Supplementation With Vitamin D on Asthma or Recurrent Wheezing in Offspring by Age 3 Years: The VDAART Randomized Clinical Trial. JAMA 315:362-370.

Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, Ochoa MT, Schauber J, Wu K, Meinken C, Kamen DL, Wagner M, Bals R, Steinmeyer A, Zugel U, Gallo RL, Eisenberg D, Hewison M, Hollis BW, Adams JS, Bloom BR, Modlin RL (2006) Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science 311:1770-1773.

Majak P, Olszowiec-Chlebna M, Smejda K, Stelmach I (2011) Vitamin D supplementation in children may prevent asthma exacerbation triggered by acute respiratory infection. J Allergy Clin Immunol 127:1294-1296.

Manaseki-Holland S, Qader G, Isaq Masher M, Bruce J, Zulf Mughal M, Chandramohan D, Walraven G (2010) Effects of vitamin D supplementation to children diagnosed with pneumonia in Kabul: a randomised controlled trial. Trop Med Int Health 15:1148-1155.

Manaseki-Holland S, Maroof Z, Bruce J, Mughal MZ, Masher MI, Bhutta ZA, Walraven G, Chandramohan D (2012) Effect on the incidence of pneumonia

of vitamin D supplementation by quarterly bolus dose to infants in Kabul: a randomised controlled superiority trial. The Lancet 379:1419-1427.

Marchisio P, Consonni D, Baggi E, Zampiero A, Bianchini S, Terranova L, Tirelli S, Esposito S, Principi N (2013) Vitamin D supplementation reduces the risk of acute otitis media in otitis-prone children. Pediatr Infect Dis J 32:1055-1060.

Martineau AR, Jolliffe DA, Hooper RL, Greenberg L, Aloia JF, Bergman P, Dubnov-Raz G, Esposito S, Ganmaa D, Ginde AA, Goodall EC, Grant CC, Griffiths CJ, Janssens W, Laaksi I, Manaseki-Holland S, Mauger D, Murdoch DR, Neale R, Rees JR, Simpson S,Jr, Stelmach I, Kumar GT, Urashima M, Camargo CA Jr (2017) Vitamin D supplementation to prevent acute respiratory tract infections: systematic review and meta-analysis of individual participant data. BMJ 356:i6583.

Martineau AR, Timms PM, Bothamley GH, Hanifa Y, Islam K, Claxton AP, Packe GE, Moore-Gillon JC, Darmalingam M, Davidson RN, Milburn HJ, Baker LV, Barker RD, Woodward NJ, Venton TR, Barnes KE, Mullett CJ, Coussens AK, Rutterford CM, Mein CA, Davies GR, Wilkinson RJ, Nikolayevskyy V, Drobniewski FA, Eldridge SM, Griffiths CJ (2011) High-dose vitamin D(3) during intensive-phase antimicrobial treatment of pulmonary tuberculosis: a double-blind randomised controlled trial. Lancet 377:242-250.

Maymon E, Romero R, Pacora P, Gomez R, Athayde N, Edwin S, Yoon BH (2000) Human neutrophil collagenase (matrix metalloproteinase 8) in parturition, premature rupture of the membranes, and intrauterine infection. Am J Obstet Gynecol 183:94-99.

McCollum E (1922) Studies of experimental rickets. XXI. An experimental demonstration of the existence of a vitamin which promotes calcium depositor. Nutr Rev 53:293-312.

McCullough PJ, Lehrer DS (2018) Vitamin D, cod liver oil, sunshine, and phototherapy: Safe, effective and forgotten tools for treating and curing tuberculosis infections - A comprehensive review. J Steroid Biochem Mol Biol 177:21-29.

McNally JD, Sampson M, Matheson LA, Hutton B, Little J (2013) Vitamin D receptor (VDR) polymorphisms and severe RSV bronchiolitis: A systematic review and meta-analysis. Pediatr Pulmonol 49:790-9.

Mellanby E (1919) An experimental investigation on rickets. Lancet 196:407-412.

Mellenthin L, Wallaschofski H, Grotevendt A, Volzke H, Nauck M, Hannemann A (2014) Association between serum vitamin D concentrations and inflammatory markers in the general adult population. Metabolism 63:1056-1062.

Miettinen ME, Reinert L, Kinnunen L, Harjutsalo V, Koskela P, Surcel HM, Lamberg-Allardt C, Tuomilehto J (2012) Serum 25-hydroxyvitamin D level during early pregnancy and type 1 diabetes risk in the offspring. Diabetologia 55:1291-1294.

Morales E, Romieu I, Guerra S, Ballester F, Rebagliato M, Vioque J, Tardon A, Rodriguez Delhi C, Arranz L, Torrent M, Espada M, Basterrechea M, Sunyer J, INMA Project (2012) Maternal vitamin D status in pregnancy and risk of lower respiratory tract infections, wheezing, and asthma in offspring. Epidemiology 23:64-71.

Muhe L, Lulseged S, Mason KE, Simoes EA (1997) Case-control study of the role of nutritional rickets in the risk of developing pneumonia in Ethiopian children. Lancet 349:1801-1804.

Munns CF, Shaw N, Kiely M, Specker BL, Thacher TD, Ozono K, Michigami T, Tiosano D, Mughal MZ, Makitie O, Ramos-Abad L, Ward L, DiMeglio LA, Atapattu N, Cassinelli H, Braegger C, Pettifor JM, Seth A, Idris HW, Bhatia V, Fu J, Goldberg G, Savendahl L, Khadgawat R, Pludowski P, Maddock J, Hyppönen E, Oduwole A, Frew E, Aguiar M, Tulchinsky T, Butler G, Hogler W (2016) Global Consensus Recommendations on Prevention and Management of Nutritional Rickets. J Clin Endocrinol Metab 101:394-415.

Müller K, Diamant M, Bendtzen K (1991) Inhibition of production and function of interleukin-6 by 1,25-dihydroxyvitamin D3. Immunology Letters 28:115-120.

Mäkinen M, Simell V, Mykkänen J, Ilonen J, Veijola R, Hyöty H, Knip M, Simell O, Toppari J, Hermann R (2014) An increase in serum 25-hydroxyvitamin D concentrations preceded a plateau in type 1 diabetes incidence in Finnish children. J Clin Endocrinol Metab 99:E2353-6.

Männistö S, Ovaskainen ML, Valsta L (2003) Finravinto 2002 -tutkimus. The National Findiet 2002 study. Kansanterveyslaitoksen julkaisuja B3. http://urn.fi/URN:NBN:fi-fe2014030615904.

Mäntylä P, Stenman M, Kinane DF, Tikanoja S, Luoto H, Salo T, Sorsa T (2003) Gingival crevicular fluid collagenase-2 (MMP-8) test stick for chair-side monitoring of periodontitis. J Periodontal Res 38:436-439.

Najada AS, Habashneh MS, Khader M (2004) The frequency of nutritional rickets among hospitalized infants and its relation to respiratory diseases. J Trop Pediatr 50:364-368.

Nappo A, Iacoviello L, Fraterman A, Gonzalez-Gil EM, Hadjigeorgiou C, Marild S, Molnar D, Moreno LA, Peplies J, Sioen I, Veidebaum T, Siani A, Russo P (2013) High-sensitivity C-reactive protein is a predictive factor of adiposity in children: results of the identification and prevention of dietary-and lifestyle-induced health effects in children and infants (IDEFICS) study. J Am Heart Assoc 2:e000101.

National Nutrition Council. Valtion ravitsemusneuvottelukunta (2010) D-vitamiiniraportti 2010. https://www.evira.fi/globalassets/vrn/pdf/d-vitamiiniraportti2010.pdf.

Nielsen NO, Skifte T, Andersson M, Wohlfahrt J, Søborg B, Koch A, Melbye M, Ladefoged K (2010) Both high and low serum vitamin D concentrations are associated with tuberculosis: a case–control study in Greenland. Br J Nutr 104: 1487-91.

Noack B, Kipping T, Tervahartiala T, Sorsa T, Hoffmann T, Lorenz K (2017) Association between serum and oral matrix metalloproteinase-8 levels and periodontal health status. J Periodontal Res 52:824-831.

Nordic Nutrition Recommendations 2012 (2012) http://www.norden.org/en/theme/former-themes/themes-2016/nordic-nutrition-recommendation/nordic-nutrition-recommendations-2012

Norman AW (2008) From vitamin D to hormone D: fundamentals of the vitamin D endocrine system essential for good health. Am J Clin Nutr 88:491S-499S.

Nwaru BI, Lumia M, Kaila M, Luukkainen P, Tapanainen H, Erkkola M, Ahonen S, Pekkanen J, Klaukka T, Veijola R, Simell O, Knip M, Virtanen SM (2011) Validation of the Finnish ISAAC questionnaire on asthma against anti-asthmatic medication reimbursement database in 5-year-old children. Clin Respir J 5:211-218.

Parkin J, Cohen B (2001) An overview of the immune system. The Lancet 357:1777-89.

Parks WC, Wilson CL, Lopez-Boado YS (2004) Matrix metalloproteinases as modulators of inflammation and innate immunity. Nat Rev Immunol 4:617-629.

Pekkinen M, Viljakainen H, Saarnio E, Lamberg-Allardt C, Mäkitie O (2012) Vitamin D is a major determinant of bone mineral density at school age. PLoS One 7:e40090.

Penna G, Adorini L (2000) 1 Alpha,25-dihydroxyvitamin D3 inhibits differentiation, maturation, activation, and survival of dendritic cells leading to impaired alloreactive T cell activation. J Immunol 164:2405-2411.

Picotto G, Liaudat AC, Bohl L, Tolosa de Talamoni N (2012) Molecular aspects of vitamin D anticancer activity. Cancer Invest 30:604-614.

Pierrot-Deselligny C, Souberbielle JC (2017) Vitamin D and multiple sclerosis: an update. Mult Scler Relat Disord 14:35-45.

Pittas AG, Dawson-Hughes B (2010) Vitamin D and diabetes. J Steroid Biochem Mol Biol 121: 425-429.

Prosser DE, Jones G (2004) Enzymes involved in the activation and inactivation of vitamin D. Trends Biochem Sci 29:664-673.

Provvedini DM, Tsoukas CD, Deftos LJ, Manolagas SC (1983) 1,25-dihydroxyvitamin D3 receptors in human leukocytes. Science 221:1181-1183.

Raulio S, Erlund I, Männisto S, Sarlio-Lahteenkorva S, Sundvall J, Tapanainen H, Vartiainen E, Virtanen SM (2017) Successful nutrition policy: improvement of vitamin D intake and status in Finnish adults over the last decade. Eur J Public Health 27:268-273.

Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, Durazo-Arvizu RA, Gallagher JC, Gallo RL, Jones G, Kovacs CS, Mayne ST, Rosen CJ, Shapses SA (2011) The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. J Clin Endocrinol Metab 96:53-58.

Roth DE, Jones AB, Prosser C, Robinson JL, Vohra S (2008) Vitamin D receptor polymorphisms and the risk of acute lower respiratory tract infection in early childhood. J Infect Dis 197:676-680.

Rothers J, Wright AL, Stern DA, Halonen M, Camargo Jr. CA (2011) Cord blood 25-hydroxyvitamin D levels are associated with aeroallergen

sensitization in children from Tucson, Arizona. J Allergy Clin Immunol 128:1093-1099.e5.

Saari A, Virta LJ, Sankilampi U, Dunkel L, Saxen H (2015) Antibiotic exposure in infancy and risk of being overweight in the first 24 months of life. Pediatrics 135:617-626.

Sadarangani SP, Whitaker JA, Poland GA (2015) "Let there be light": the role of vitamin D in the immune response to vaccines. Expert Rev Vaccines 14:1427-1440.

Salimpour R (1975) Rickets in Tehran. Study of 200 cases. Arch Dis Child 50:63-66.

Schleicher RL, Sternberg MR, Lacher DA, Sempos CT, Looker AC, Durazo-Arvizu RA, Yetley EA, Chaudhary-Webb M, Maw KL, Pfeiffer CM, Johnson CL (2016) The vitamin D status of the US population from 1988 to 2010 using standardized serum concentrations of 25-hydroxyvitamin D shows recent modest increases. Am J Clin Nutr 104:454-461.

Science M, Maguire JL, Russell ML, Smieja M, Walter SD, Loeb M (2013) Low serum 25-hydroxyvitamin D level and risk of upper respiratory tract infection in children and adolescents. Clin Infect Dis 57:392-397.

Shalaby SA, Handoka NM, Amin RE (2018) Vitamin D deficiency is associated with urinary tract infection in children. Arch Med Sci 14:115-121.

Sharief S, Jariwala S, Kumar J, Muntner P, Melamed ML (2011) Vitamin D levels and food and environmental allergies in the United States: results from the National Health and Nutrition Examination Survey 2005-2006. J Allergy Clin Immunol 127:1195-1202.

Sidbury R, Sullivan AF, Thadhani RI, Camargo CA,Jr (2008) Randomized controlled trial of vitamin D supplementation for winter-related atopic dermatitis in Boston: a pilot study. Br J Dermatol 159:245-247.

Soininen S, Eloranta AM, Lindi V, Venäläinen T, Zaproudina N, Mahonen A, Lakka TA (2016) Determinants of serum 25-hydroxyvitamin D concentration in Finnish children: the Physical Activity and Nutrition in Children (PANIC) study. Br J Nutr 115:1080-1091.

Sorsa T, Tjaderhane L, Konttinen YT, Lauhio A, Salo T, Lee HM, Golub LM, Brown DL, Mäntylä P (2006) Matrix metalloproteinases: contribution to

pathogenesis, diagnosis and treatment of periodontal inflammation. Ann Med 38:306-321.

Spiro A, Buttriss JL (2014) Vitamin D: An overview of vitamin D status and intake in Europe. Nutr Bull 39:322-350.

Stelmach I, Majak P, Jerzynska J, Podlecka D, Stelmach W, Polanska K, Gromadzinska J, Wasowicz W, Hanke W (2015) Cord serum 25-hydroxyvitamin D correlates with early childhood viral-induced wheezing. Respir Med 109:38-43.

Suda T, Takahashi F, Takahashi N (2012) Bone effects of vitamin D - Discrepancies between in vivo and in vitro studies. Arch Biochem Biophys 523:22-29.

Tachimoto H, Mezawa H, Segawa T, Akiyama N, Ida H, Urashima M (2016) Improved control of childhood asthma with low-dose, short-term vitamin D supplementation: a randomized, double-blind, placebo-controlled trial. Allergy 71:1001-1009.

Takeda K, Akira S (2005) Toll-like receptors in innate immunity. Int Immunol 17:1-14.

Tao RX, Zhou QF, Xu ZW, Hao JH, Huang K, Mou Z, Jiang XM, Tao FB, Zhu P (2015) Inverse Correlation between Vitamin D and C-Reactive Protein in Newborns. Nutrients 7:9218-9228.

Thornton KA, Marin C, Mora-Plazas M, Villamor E (2013) Vitamin D deficiency associated with increased incidence of gastrointestinal and ear infections in school-age children. Pediatr Infect Dis J 32:585-593.

Tiosano D, Hochberg Z (2009) Hypophosphatemia: the common denominator of all rickets. J Bone Miner Metab 27:392-401.

Tripkovic L, Lambert H, Hart K, Smith CP, Bucca G, Penson S, Chope G, Hyppönen E, Berry J, Vieth R, Lanham-New S (2012) Comparison of vitamin D2 and vitamin D3 supplementation in raising serum 25-hydroxyvitamin D status: a systematic review and meta-analysis. Am J Clin Nutr 95:1357-1364.

Tuomainen AM, Nyyssönen K, Laukkanen JA, Tervahartiala T, Tuomainen TP, Salonen JT, Sorsa T, Pussinen PJ (2007) Serum matrix metalloproteinase-8 concentrations are associated with cardiovascular outcome in men. Arterioscler Thromb Vasc Biol 27:2722-2728.

Uday S, Högler W (2018) Prevention of rickets and osteomalacia in the UK: political action overdue. Arch Dis Child 0;1-6.

Uday S, Kongjonaj A, Aguiar M, Tulchinsky T, Högler W (2017) Variations in infant and childhood vitamin D supplementation programmes across Europe and factors influencing adherence. Endocr Connect 6:667-675.

Urashima M, Mezawa H, Noya M, Camargo CA Jr (2014) Effects of vitamin D supplements on influenza A illness during the 2009 H1N1 pandemic: a randomized controlled trial. Food Funct 5:2365-2370.

Urashima M, Segawa T, Okazaki M, Kurihara M, Wada Y, Ida H (2010) Randomized trial of vitamin D supplementation to prevent seasonal influenza A in schoolchildren. Am J Clin Nutr 91:1255-1260.

van Driel M, van Leeuwen JPTM (2017) Vitamin D endocrinology of bone mineralization. Molecular and Cellular Endocrinology 453:46-51.

Vandamme D, Landuyt B, Luyten W, Schoofs L (2012) A comprehensive summary of LL-37, the factotum human cathelicidin peptide. Cell Immunol 280:22-35.

Viljakainen HT, Saarnio E, Hytinantti T, Miettinen M, Surcel H, Mäkitie O, Andersson S, Laitinen K, Lamberg-Allardt C (2010) Maternal vitamin D status determines bone variables in the newborn. J Clin Endocrinol Metab 95:1749-57.

Visness CM, Sandel MT, O'Connor G, Gern JE, Jaffee KF, Wood RA, Kattan M, Bloomberg GR, Dresen A, Gergen PJ, Gold DR, Lemanske RF Jr, Rajamanickam V, Camargo CA Jr, Jackson DJ (2015) Cord blood vitamin D concentrations are unrelated to atopy and wheeze in 2 diverse birth cohort studies. J Allergy Clin Immunol 136:1108-10.e2.

Vogiatzi MG, Jacobson-Dickman E, Deboer MD, for the Drugs, and Therapeutics Committee of The Pediatric Endocrine Society (2014) Vitamin D Supplementation and Risk of Toxicity in Pediatrics: A Review of Current Literature. J Clin Endocrinol Metab 99:1132-41.

Vuichard Gysin D, Dao D, Gysin CM, Lytvyn L, Loeb M (2016) Effect of Vitamin D3 Supplementation on Respiratory Tract Infections in Healthy Individuals: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. PLoS One 11:e0162996.

Wagner CL, Greer FR, American Academy of Pediatrics Section on Breastfeeding, American Academy of Pediatrics Committee on Nutrition (2008) Prevention of rickets and vitamin D deficiency in infants, children, and adolescents. Pediatrics 122:1142-1152.

Wald ER, Guerra N, Byers C (1991) Frequency and severity of infections in day care: three-year follow-up. J Pediatr 118:509-514.

Walker VP, Modlin RL (2009) The vitamin D connection to pediatric infections and immune function. Pediatr Res 65:106R-113R.

Wang TJ, Zhang F, Richards JB, Kestenbaum B, van Meurs JB, Berry D, Kiel DP, Streeten EA, Ohlsson C, Koller DL, Peltonen L, Cooper JD, O'Reilly PF, Houston DK, Glazer NL, Vandenput L, Peacock M, Shi J, Rivadeneira F, McCarthy MI, Anneli P, de Boer IH, Mangino M, Kato B, Smyth DJ, Booth SL, Jacques PF, Burke GL, Goodarzi M, Cheung CL, Wolf M, Rice K, Goltzman D, Hidiroglou N, Ladouceur M, Wareham NJ, Hocking LJ, Hart D, Arden NK, Cooper C, Malik S, Fraser WD, Hartikainen AL, Zhai G, Macdonald HM, Forouhi NG, Loos RJ, Reid DM, Hakim A, Dennison E, Liu Y, Power C, Stevens HE, Jaana L, Vasan RS, Soranzo N, Bojunga J, Psaty BM, Lorentzon M, Foroud T, Harris TB, Hofman A, Jansson JO, Cauley JA, Uitterlinden AG, Gibson Q, Jarvelin MR, Karasik D, Siscovick DS, Econs MJ, Kritchevsky SB, Florez JC, Todd JA, Dupuis J, Hyppönen E, Spector TD (2010) Common genetic determinants of vitamin D insufficiency: a genome-wide association study. Lancet 376:180-188.

Wang Y, Zhu J, DeLuca HF (2012) Where is the vitamin D receptor? Archives Biochem Biophys 523:123-133.

Weisse K, Winkler S, Hirche F, Herberth G, Hinz D, Bauer M, Roder S, Rolle-Kampczyk U, von Bergen M, Olek S, Sack U, Richter T, Diez U, Borte M, Stangl GI, Lehmann I (2013) Maternal and newborn vitamin D status and its impact on food allergy development in the German LINA cohort study. Allergy 68:220-228.

Williams DM, Fraser A, Sayers A, Fraser WD, Hingorani A, Deanfield J, Davey Smith G, Sattar N, Lawlor DA (2012) Associations of 25-hydroxyvitamin D2 and D3 with cardiovascular risk factors in childhood: cross-sectional findings from the Avon Longitudinal Study of Parents and Children. J Clin Endocrinol Metab 97:1563-1571.

Wolf G (2004) The discovery of vitamin D: the contribution of Adolf Windaus. J Nutr 134:1299-1302.

Wolsk HM, Chawes BL, Litonjua AA, Hollis BW, Waage J, Stokholm J, Bonnelykke K, Bisgaard H, Weiss ST (2017) Prenatal vitamin D supplementation reduces risk of asthma/recurrent wheeze in early childhood: A combined analysis of two randomized controlled trials. PLoS One 12:e0186657.

Xiao L, Xing C, Yang Z, Xu S, Wang M, Du H, Liu K, Huang Z (2015) Vitamin D supplementation for the prevention of childhood acute respiratory infections: a systematic review of randomised controlled trials. Br J Nutr 114:1026-1034.

Zhang Y, Leung DY, Richers BN, Liu Y, Remigio LK, Riches DW, Goleva E (2012) Vitamin D inhibits monocyte/macrophage proinflammatory cytokine production by targeting MAPK phosphatase-1. J Immunol 188:2127-2135.