Vitamin D deficiency in children with chronic illnesses: Predisposing and protecting factors

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Tiivistelmä – Referat – Abstract

This thesis assesses clinical differences in patients' with low and high vitamin D levels. The factors analyzed included the underlying disease, body size, age, ethnic background, use of vitamin D supplements and the season when the blood sample was taken. Fifty patients with the lowest and 50 patients with the highest vitamin D concentrations were selected from a cohort of 1351 chronically ill children and adolescents who had had their vitamin D status assessed at Children's Hospital. Protective factors appeared to be the usage of vitamin D supplements and young age, especially age <2 years. Predisposing factors included non-Finnish ethnic background and older age, especially age 12-18 years. High vitamin D values were more prevalent in the summer and autumn and low values in the winter and spring. Patients with non-Finnish background were overrepresented in the low value group. No differences regarding the underlying diseases could be detected. Conclusions: In the Northern latitudes UVB-radiation is insufficient for vitamin D synthesis. Vitamin D recommendations appear to be inadequate to fulfill the needs of chronically ill patients whose requirements for vitamin D are elevated compared to the general population. New guidelines for vitamin D supplementation are needed particularly for those at risk of developing vitamin D deficiency.

Avainsanat – Nyckelord – Keywords
Vitamin D, Deficiency, chronic illness , children

Säilytyspaikka – Förvaringställe – Where deposited

Muita tietoja – Övriga uppgifter – Additional information

Contents

1 INTRODUCTION

Concerns regarding low vitamin D levels in patients with long-term underlying diseases have led to this thesis. In the northern latitudes, where Finland is situated, the sun shines adequately for vitamin D synthesis only a couple of months a year. The current guidelines for vitamin D supplementation have been questioned on logic, on science and on effective public health guidance. This thesis aims to clarify the factors affecting vitamin D deficiency and sufficiency in children with chronic illnesses.

For this thesis we carried out a study involving fifty patients with the lowest and fifty patients with the highest vitamin D concentrations, who were selected from a cohort of 1351 chronically ill children and adolescents who had had their vitamin D status assessed at Children's Hospital. The patient groups with low and high vitamin D levels were assessed for clinical differences. The factors analyzed included the underlying disease, body size, age, ethnic background, use of vitamin D supplements and the season when the blood sample was taken.

The literature on vitamin D was carefully reviewed. The functions and metabolism of vitamin D in general are presented. Vitamin D deficiency and toxicity are described and the factors that affect vitamin D concentration in plasma are discussed. Finally the current guidelines in Finland and in the United States are introduced. The third chapter concerns the aims of the thesis more specifically and is followed by an introduction to the study subjects and methods that have been used in the thesis. The fifth chapter presents the results of the thesis. The significance of the findings is further discussed in Discussion and the study findings are correlated with the current state of vitamin D policy. The major conclusion for the thesis is that new recommendations for children with long-term underlying diseases are required for sufficient concentrations of vitamin D throughout the year.

2 REVIEW OF THE LITERATURE

2.1 Vitamin D metabolism and functions

2.1.1 Sources of vitamin D

Vitamin D is a prohormone (7-dehydrocholesterol) that is synthesized in the skin. It is present in the lipid bilayer of plasma membrane in epidermal keratinocytes and dermal fibroblasts. It is most abundant in the stratum spinosum and stratum basale. Exposure to UVB radiation initiates vitamin D synthesis. It causes double bonds to rearrange and 7-hydrocholesterol is converted to previtamin D_3, as shown in Figure 1.

Previtamin D3 then isomerizes to vitamin D and is transferred to dermal capillaries. Vitamin D binds with vitamin D-binding protein (DBP) in the circulation, this drives the conversion equilibrium of previtamin D_3 to vitamin D towards formation of vitamin D. Vitamin D can also be acquired from nutrition. The main sources are fish rich in fat, egg yolk and mushrooms that have grown in sunny places (Table 1). Plant-derived vitamin D is in the form of D_2 and animal-derived is in D_3. Vitamin D from nutrition is absorbed from the gut and is packed into chylomicrons. Chylomicrons circulate first in the lymphatic circulation and then enter venous blood, where vitamin D slowly diffuses to DBP. Chylomicrons are processed by lipoprotein lipase (LPL) in the peripheral tissues such as adipose and muscle tissue. However the liver takes most of the vitamin D when it processes the chylomicron remnant.

Product	**Vitamin D content (µg / 100 mg)**
Eel	25.6
River lamprey	25.6
Pike perch	24.6
Whitefish, pollan, lavaret	22.0
Baltic herring	18.0
Chantarelle	12.8
Margarine	9.2
Salmon	8.9
Tuna	7.2

Egg Yolk	6.5
Lorchel	5.7
Mushroom milk caps	5.3
Boletus edible	2.9
Egg, boiled	2.2
Coalfish	1.5
Milk	1.0
Liver	0.8
Meat	0.2-0.5
Champignon	0.2

Table 1. Vitamin D contents in different dietary sources. Modified from (1)

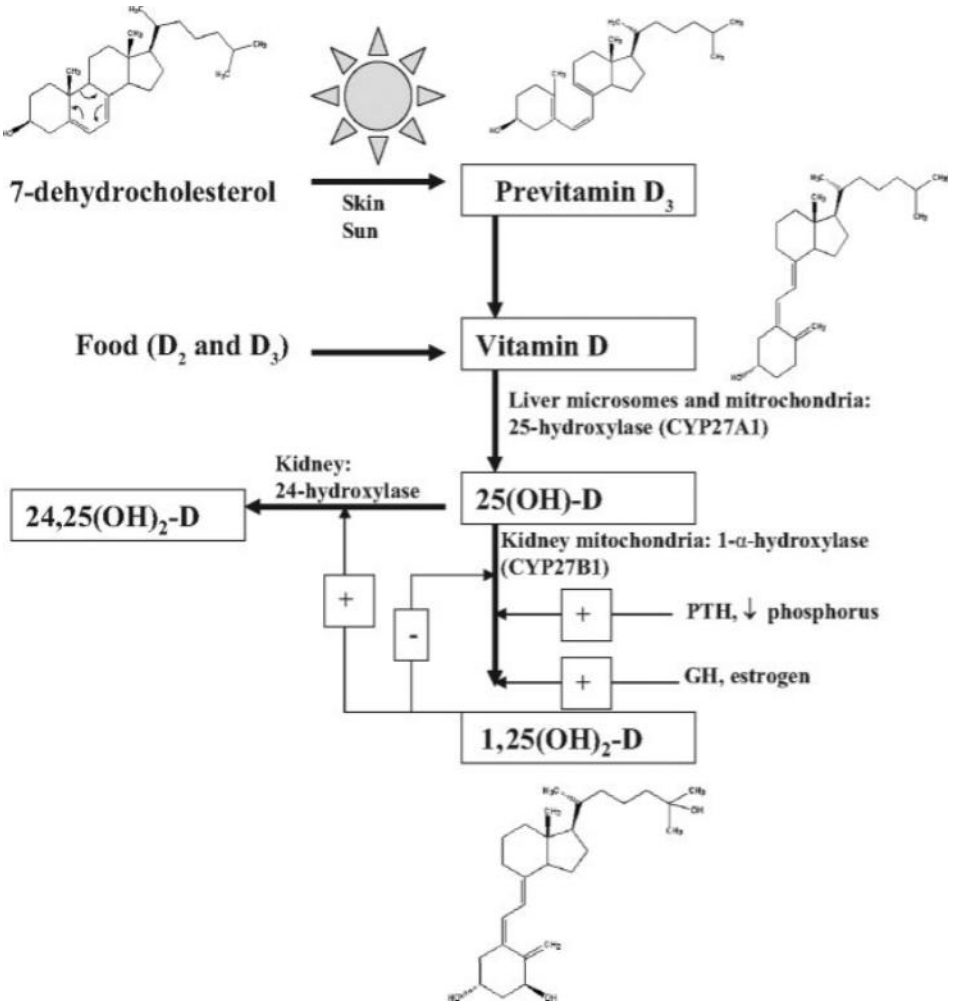

Figure 1. Vitamin D is obtained either from food or synthesized in the skin**.** Its further processing occurs in the liver and then in the kidney. The active form ($1,25(OH)_2D$) is under tight regulation by its own negative feedback mechanism, parathyroid hormone (PTH), plasma concentration of phosphorus, growth hormone (GH) and estrogen. (2)

2.1.2 Metabolism of vitamin D

Vitamin D, whether obtained from nutrition or synthesized in the skin, is processed further in the same way in the liver and then in the kidney. First, vitamin D is 25-hydroxylated in the liver by CYP enzymes and this 25(OH)D is then quickly released to the plasma where it constitutes the main vitamin D pool of the body. Second, 25(OH)D is carried by DBP to the kidney where it is further hydroxylated through 1-α-hydroxylation to $1,25(OH)_2D$ by CYP27B1. This $1,25(OH)_2D$ i.e. calcitriol is the active form of vitamin D. The vitamin D half-life in plasma is ≈4-6 h but in the whole body it is ≈ 2 months (3).

All forms of vitamin D bind to DBP but the affinity differs. There are two forms of 25(OH)D, $25(OH)D_2$ and $25(OH)D_3$ depending whether it is derived from (D_2) ergocalciferol or (D_3) cholecalciferol. $25(OH)D_2$ has stronger affinity to DBP than $25(OH)D_3$ (15 d) and has thus longer half-life. $25(OH)D_3$ is considered to be more efficient than $25(OH)D_2$ due to its ability to up-regulate vitamin D receptor (VDR) and because much more of vitamin D_2 is metabolized to $24(OH)D_2$ than vitamin D_3 to $24(OH)D_3$ (4). Normally in physiologic state only 2-5% of 25(OH)D is bound to DBP. Other metabolites including inactive forms have equal or stronger affinity to DBP than 25(OH)D, and the active form $1,25(OH)_2D$ has the lowest affinity. The affinity to DBP determines the half-life of the metabolites: the stronger the affinity, the longer the half-life. The significance of this is that the inactive metabolites, bound to the DBP, are kept in the plasma pool away from the VDR and nuclear transcriptional machinery while the active form is available for VDR to which its affinity is greatest. Thus the active form has the shortest half life (10-20 h). Under physiologic conditions $1,25(OH)_2D$ is the only active metabolite that binds to VDR. It seems that other metabolites bind to VDR only in vitamin D toxicity. (5)

While sunlight sets the vitamin D synthesis in motion it also controls that concentrations do not rise too high. Sunlight inactivates previtamin-D_3 to inactive $lumisterol_3$ or $tachysterol_3$. It can also inactivate vitamin D_3 to suprasterol I, suprasterol II or 5,6,-transvitamin. This is probably the explanation why there are no reports on vitamin D intoxication due to excessive exposure to sunlight. (6)

It has been shown that 1-α-hydroxylation can also occur in other sites than the kidney, such as alveolar macrophages, lymph nodes, placenta, colon, breasts, osteoblasts, activated macrophages and keratinocytes. This illustrates a paracrine role for $1,25(OH)_2D$. In macrophages stimulation of VDR induces toll-like receptor pathway. This results in up-regulation of mRNA production of cathelicidin, which is antimicrobial protein that kills Myobacterium tuberculosis (7). Also other mechanisms for antimicrobial effects have been demonstrated. (8)

2.1.3 Functions of vitamin D

One of the main functions of Vitamin D is to maintain proper calcium and phosphorous levels. Figure 2 illustrates the plasma calcium homeostasis and the effect of $1,25(OH)_2D$ and PTH. The active vitamin D ($1,25(OH)_2D$) increases calcium absorption in the gut and bone resorption in the skeleton, thereby increasing the plasma calcium pool. However, the overall effect of vitamin D in the skeleton is to increase mineralization: by increasing renal distal tubular reabsorption of phosphorous, it raises the plasma ratio of calcium and phosphate which induces bone mineralization.

In the intestinal epithelium activation of VDR increases transcription of proteins that participate in calcium absorption. Activation of this receptor results in genomic effects that increase synthesis of epithelial calcium channels and binding proteins (e.g. Calbindin) in the epithelial cells. Calbindin is a protein with high affinity for calcium in the cytoplasm of intestinal epithelial cells. It reduces the amount of free ionized calcium and thus hastens absorption of calcium by lowering the potential difference of calcium between gut lumen and epithelial cell cytoplasm. Vitamin D thus stimulates the active transcellular part of calcium absorption but has no effect on the passive paracellular pathway. (9) Only 10-15% of dietary calcium is absorbed without vitamin D (10). Vitamin D also has a negative-feedback mechanism on secretion of parathyroid hormone (PTH) which in turn further decreases bone demineralization. (11,12)

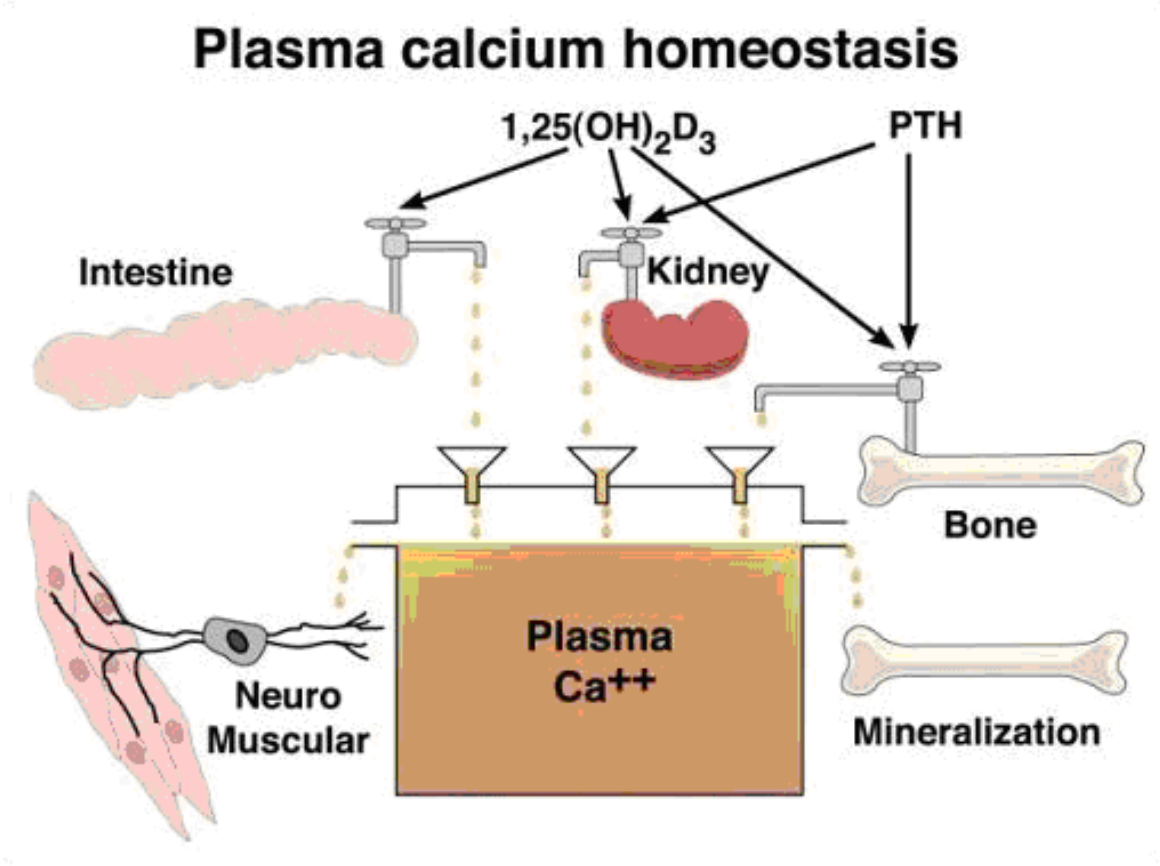

Figure 2. **Plasma calcium homeostasis**. Plasma calcium pool is obtained from nutrition by intestine, from primary urine by kidney and by demineralization of bone. Parathyroid hormone (PTH) and the active form of vitamin D ($1,25(OH)_2D$) controls this system. Plasma calcium pool is essential for normal function of neuromuscular transmission and mineralization of bone.

In addition to a significant role in calcium homeostasis, vitamin D has been shown to function in several other organ systems. Tables 2 and 3 condense these suggested effects.

Category	**Disease**	**Hypothetical mechanism**
Glucose metabolism	• Type 2 Diabetes Mellitus	• Active form of vitamin D directly stimulates insulin receptor
Autoimmune disease	• Multiple sclerosis (MS) • Rheumatoid arthritis • Crohn's Disease • Type 1 diabetes	• Decrease in formation of macrophages from monocytes and thus reduction in antigen representing to T lymphocytes • Decrease in synthesis of immunoglobulins by B lymphocytes • Maturation of dendritic cells is suppressed

Table 2. **Multiple effects of vitamin D** (13-15)

Category	**Disease**	**Hypothetical mechanism**
Cancer	• Colon, breast and prostate cancer	• Antiproliferative effect on cells • promotes ordered differentiation • prevents spreading of tumors by inhibiting apoptosis, telomerase activity and angiogenesis
Cardiovascular disease	• Hypertension • Left ventricular hypertrophy • Congestive heart failure	• Deficiency is associated with malfunction of calcium channels and activation on renin-angiotensin system • Deficiency is associated with formation of foam cells and increased uptake of low-density lipoproteins in macrophages
Glucose metabolism	• Type 2 Diabetes Mellitus	• Correction in vitamin D level improves glucose-stimulated insulin

		release • Uptake of glucose is improved by myocytes and adipocytes when low vitamin D level is corrected • Lowers insulin resistance by suppressing renin-angiotensin-aldosterone system • Improvement in insulin resistance in peripheral tissues by activation of PPAR delta receptor

Table 3. **Multiple effects of vitamin D deficiency** (13-15)

2.2 Vitamin D deficiency and intoxication

2.2.1 Definitions of Vitamin D deficiency

Vitamin D deficiency is associated with rickets in children and osteomalacia in adults. Biochemical manifestations of vitamin D deficiency comprise raised levels of alkaline phosphatase (ALP) and parathyroid hormone (PTH). Plasma concentrations of calcium and phosphate decrease, but in early phase of deficiency they may be normal.(2)(16) Table 4 illustrates the definitions for normal and abnormal vitamin D concentrations.

Definition	**Concentration in plasma**
Severe Vitamin D deficiency	≤ 12,5 nmol/L
Vitamin D insufficiency	≤ 37,5 nmol/L
Vitamin D sufficiency	50-250 nmol/L (80 nmol/L)
Excess	> 250 nmol/L
Intoxication	> 375 nmol/L

Table 4. Definitions for vitamin D concentrations (16)

2.2.2 Mechanisms for vitamin D deficiency

Vitamin D deficiency can be due to defects in formation, supply, absorption or metabolism. Most commonly deficiency results from lack of sunlight exposure due to sunscreen, clothes or geographic location. Deficiency related to supply is mainly a problem at latitudes where sunlight is sufficient only few months a year (latitudes above 37°N (17)) and is due to lack of eating oily fish. Problems with absorption are mainly due to bowel disorders e.g. fat malabsorption, and problems with metabolism are due to renal disease e.g. inherited or acquired kidney disease.

2.2.3 Effects of vitamin D deficiency

Vitamin D deficiency impairs absorption of dietary calcium and phosphorus which results in increased PTH secretion. Only 10 to 15% of dietary calcium and 60% of phosphorus is absorbed without vitamin D (18). Raised PTH levels in secondary hyperparathyroidism increase bone resorption (and thereby decrease bone mineral density) in order to increase plasma calcium levels. In addition, PTH has an effect on renal tubular function. It increases calcium reabsorption and phosphate secretion in renal tubules. This prevents precipitation of calcium and phosphate in tissues, which would otherwise be inevitable when bone is demineralized. In the early phase of vitamin D deficiency calcium and phosphorous levels can be within normal range due to compensatory mechanisms.

Secondary hyperparathyroidism results in inadequate calcium-phosphate supply for bone mineralization. This leads to a decrease in bone mineral density and causes osteopenia and osteoporosis. In growing children this leads to bone deformities. In adults epiphyseal plates are closed and skeletal bones have sufficient calcium and phosphorus reserves which prevent bone deformities, but still BMD is reduced and osteomalacia occurs. This manifests as an increased risk for fractures and isolated or generalized pains in bone and muscle tissue. Vitamin D deficiency causes also muscle weakness especially in the elderly and in children. Affected children have difficulties in standing and walking whereas the elderly have impaired balance and increased risk for falling and fracture. (16)

2.2.4 Vitamin D intoxication

Vitamin D intoxication causes hypercalciuria, hypercalcemia, and soft-tissue calcification. The clinical manifestations of intoxication include kidney disorders, renal insufficiency, gastrointestinal symptoms and constipation, and arterial hypertension.

Three theories about the mechanisms of vitamin D intoxication have been presented:
1. Vitamin D intake raises plasma $1\alpha25(OH)_2D$ concentrations, which increases cellular $1\alpha25(OH)_2D$ concentrations.

2. Vitamin D intake raises plasma 25(OH)D to µmol/L concentrations that exceed the DBP binding capacity and then “free 25(OH)D” enters the cell, where it has direct effects on gene expression.

3. Vitamin D intake increases the concentration of many vitamin D metabolites, especially vitamin D itself and 25(OH)D. These concentrations exceed the DBP binding capacity and cause release of free $1\alpha25(OH)_2D$, which affects target cells.

The actual mechanism for intoxication is more likely to be a combination of all the three mechanisms rather than one of them alone. (5)

All known poisonings in adults with vitamin D reflect misuse on an industrial scale (19). Based on literature review, all reports of vitamin D toxicity showing convincing evidence of hypercalcemia involve serum 25(OH)D concentrations well above 200 nmol/L, which requires a daily intake of ≥ 1000 µg (40 000 IU) (20). These findings are summarized in Figure 3.

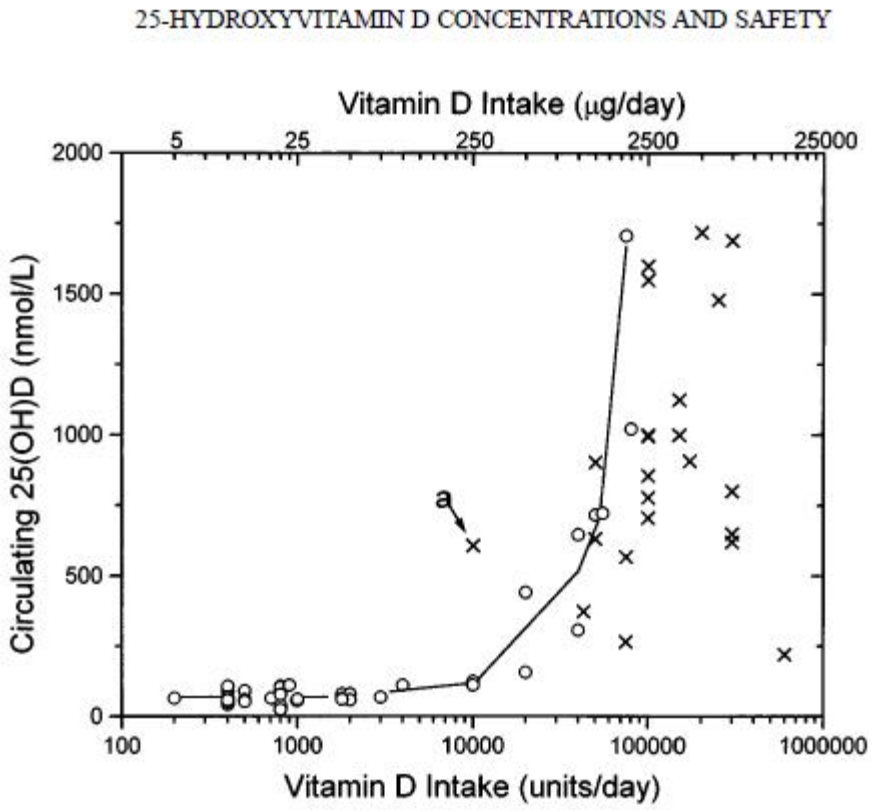

Figure 3. **Dose response of vitamin D to circulating 25(OH)D concentrations**. "X" represents intoxication and the arrow marks the lowest dose that has caused hypercalcemia, it is an outlier because the patient was given a single dose of 7500 µg instead of 250 µg/d. (20)

There are few patient subgroups who can develop vitamin D intoxication because of increased endogenous formation of $1,25(OH)_2D$. Patients with chronic granuloma-forming disorders (e.g. sarcoidosis), some lymphomas and primary hyperparathyroidism have increased metabolism of 25(OH)D to $1,25(OH)_2D$. Patients with these conditions are at risk of developing vitamin D intoxication and yet have low plasma concentrations of 25(OH)D. Because of the chronic inflammation in sarcoidosis there is a notable increase in macrophages at the inflammation site. Macrophages have the CYP27B1 enzyme that converts 25(OH)D to $1,25(OH)_2D$. This results in hypercalciuria and vitamin D deficiency at the same time because $1,25(OH)_2D$ levels are elevated and 25(OH)D levels diminished (21). Also subcutaneous fat necrosis may cause hypercalciuria, which is probably caused by 1α-hydroxylase activity within the inflammatory infiltrate (22).

2.3 Factors affecting plasma concentration of Vitamin D

2.3.1 Primary factors

Factor	Mechanism
Age	Increment in adipose tissue and efficiency of vitamin D photosynthesis decreases with increasing age (17)
Sex	Men have 10-15% less fat with same BMI
BMI	Increment in fat tissue increases volume of sequestration. Release of vitamin D from adipose tissue is extremely slow and proportional to the concentration. Stronger correlation with serum adiposity. (23,24)
UV-exposure	Affects on synthesis of previtamin-D in the base membrane of the skin
Pigment	Affects directly the amount of UV- radiation that reach the base membrane of the skin
Physical activity	Affects lean body mass, time spent outdoors
Nutrition	Dietary intake of vitamin D
Season and geographic location	Affects the amount of UV-radiation

Table 5. Factors affecting vitamin D concentration

2.3.2 Medication and illnesses

Medication and illnesses may have a great influence on vitamin D levels. Mechanisms for drugs to cause hypovitaminosis are mainly due to induction of enzymes that catabolize vitamin D. Diseases that cause hypovitaminosis are usually associated with problems in absorption or increased secretion of vitamin D.

Medication for epilepsy e.g. Carbamazepine therapy decreases levels of vitamin D (25). Carbamazepine induces CYP 450 enzymes that catalyze formation of inactive metabolites of vitamin D in liver and thus reduces levels of vitamin D. Also anticonvulsants, and drugs to treat HIV/AIDS increase catabolism of 25(OH)D and $1,25(OH)_2D$. (2,16)

Disorders affecting vitamin D concentration, categorized by mechanism, include:

1. Intake problems

Eating disorders e.g. anorexia. CP or other neurological problems, food allergies, severe skin diseases in which skins synthesis is impaired can cause insufficient intake of vitamin D.

2. Absorption problems

Patients with malabsorption syndromes and bariatric patients have often vitamin D deficiency because of malabsorption of the fat-soluble vitamin. Hypo function of pancreas, chronic bowel disease, and celiac disease are causes for vitamin D insufficiency due to malabsorption.

3. Metabolism problems

Severe liver disease which prevents 25-hydroxylation in liver and renal diseases where 1-hydoxylation is impaired are causes for hypovitaminosis due to inadequate formation of $1,25(OH)_2D$.

Nephrotic syndrome may cause losing of 25(OH)D bound to DBP in urine and thus cause hypovitaminosis.

Inherited vitamin D dependent rickets 1A (VDDR1A) is an autosomal recessive disorder that manifests itself as rickets. It is caused by a mutation in genes that code the 1-alpha-hydroxylase in the kidney. Thus the active form of vitamin D is not formed.

4. Vitamin D resistance

The defective function of VDR that causes total defect in vitamin D actions even though vitamin D is present. This can be compensated with high doses of $1,25(OH)_2D$.

2.4 Dietary guidelines

2.4.1 Current guidelines in Finland

Group	RDA	UL	Notions
Children under age of 2[1]	10µg (400 IU)	25 µg (1000IU)	Starting at 2 weeks after birth all year round whether or not child has other vitamin D source
Age 2-18[1]	7,5µg (300 IU)	Before age 10 yrs 25µg (1000 IU) After age 10 yrs 50µg (2000 IU)	Regular and remarkable usage of richly fortified product e.g. milk can cause excess intake of vitamin D
Pregnant and lactating women[1]	10µg (400 IU)	50µg (2000 IU)	All year round
Age 18-60	7,5µg (300 IU)	50µg (2000 IU)	Vitamin D supplements should be used from October to end of March if dietary intake of vitamin D is not regular
Over age of 60[2]	20µg (800 IU)	50µg (2000 IU)	All year round, lower dose can be suggested if dietary intake is highly abundant

Table 6.
Sources:
[1] Terveyden ja hyvinvoinnin laitos, Valtion ravitsemusneuvottelukunta ja Suomen Lastenlääkäriyhdistys, 2011

[2] Ikääntyneiden ravitsemussuositukset, 2010

2.4.2 Current recommendations in the United States

Current guidelines for vitamin D intake in the United States are shown in table 7. Those are meant for general population and in addition The Endocrine Society has published their own recommendations for people who are at risk to develop vitamin D deficiency.

Endocrine Society guidelines:

- Agree with the recommendations of Institute of Medicine for general population
- Have different guidelines for patients at risk for vitamin D deficiency
- Screening of 25(OH)D concentration is recommended for patients who are at risk for vitamin D deficiency.
- Do not recommend screening of $1,25(OH)_2D$; it is necessary only in certain conditions, such as in inherited disorders of vitamin D and phosphate metabolism.

AI = Adequate intake
EAR = Estimated average requirement
RDA = Recommended daily allowance
UL = tolerable upper intake limit
NOAEL = No observed adverse effect level
LOAEL = Lowest observed adverse effect level

Life stage group	IOM recommendations				Committee recommendations for patients at risk for vitamin D deficiency	
	AI	EAR	RDA	UL	Daily requirement	UL
Infants						
0 to 6 months	400 IU (10 μg)			1,000 IU (25 μg)	400–1,000 IU	2,000 IU
6 to 12 months	400 IU (10 μg)			1,500 IU (38 μg)	400–1,000 IU	2,000 IU
Children						
1–3 yr		400 IU (10 μg)	600 IU (15 μg)	2,500 IU (63 μg)	600–1,000 IU	4,000 IU
4–8 yr		400 IU (10 μg)	600 IU (15 μg)	3,000 IU (75 μg)	600–1,000 IU	4,000 IU
Males						
9–13 yr		400 IU (10 μg)	600 IU (15 μg)	4,000 IU (100 μg)	600–1,000 IU	4,000 IU
14–18 yr		400 IU (10 μg)	600 IU (15 μg)	4,000 IU (100 μg)	600–1,000 IU	4,000 IU
19–30 yr		400 IU (10 μg)	600 IU (15 μg)	4,000 IU (100 μg)	1,500–2,000 IU	10,000 IU
31–50 yr		400 IU (10 μg)	600 IU (15 μg)	4,000 IU (100 μg)	1,500–2,000 IU	10,000 IU
51–70 yr		400 IU (10 μg)	600 IU (15 μg)	4,000 IU (100 μg)	1,500–2,000 IU	10,000 IU
>70 yr		400 IU (10 μg)	800 IU (20 μg)	4,000 IU (100 μg)	1,500–2,000 IU	10,000 IU
Females						
9–13 yr		400 IU (10 μg)	600 IU (15 μg)	4,000 IU (100 μg)	600–1,000 IU	4,000 IU
14–18 yr		400 IU (10 μg)	600 IU (15 μg)	4,000 IU (100 μg)	600–1,000 IU	4,000 IU
19–30 yr		400 IU (10 μg)	600 IU (15 μg)	4,000 IU (100 μg)	1,500–2,000 IU	10,000 IU
31–50 yr		400 IU (10 μg)	600 IU (15 μg)	4,000 IU (100 μg)	1,500–2,000 IU	10,000 IU
51–70 yr		400 IU (10 μg)	600 IU (15 μg)	4,000 IU (100 μg)	1,500–2,000 IU	10,000 IU
>70 yr		400 IU (10 μg)	800 IU (20 μg)	4,000 IU (100 μg)	1,500–2,000 IU	10,000 IU
Pregnancy						
14–18 yr		400 IU (10 μg)	600 IU (15 μg)	4,000 IU (100 μg)	600–1,000 IU	4,000 IU
19–30 yr		400 IU (10 μg)	600 IU (15 μg)	4,000 IU (100 μg)	1,500–2,000 IU	10,000 IU
31–50 yr		400 IU (10 μg)	600 IU (15 μg)	4,000 IU (100 μg)	1,500–2,000 IU	10,000 IU
Lactation[a]						
14–18 yr		400 IU (10 μg)	600 IU (15 μg)	4,000 IU (100 μg)	600–1,000 IU	4,000 IU
19–30 yr		400 IU (10 μg)	600 IU (15 μg)	4,000 IU (100 μg)	1,500–2,000 IU	10,000 IU
31–50 yr		400 IU (10 μg)	600 IU (15 μg)	4,000 IU (100 μg)	1,500–2,000 IU	10,000 IU

AI, Adequate intake; EAR, estimated average requirement; UL, tolerable upper intake level.

[a] Mother's requirement, 4,000–6,000 IU/d (mother's intake for infant's requirement if infant is not receiving 400 IU/d).

Table 7. Vitamin D intakes recommended by the IOM and the Endocrine Practice Guidelines Committee (18)

3 AIMS OF THE THESIS

Some recent studies have shown that vitamin D deficiency is prevalent in Finnish children. More than 50% of Finnish school children were found to be vitamin D deficient. (26,27) Children with chronic illness may have even greater risk for vitamin D deficiency. The risk factors may be related to the underlying chronic illness or its treatment. This study was carried out to further define factors that predispose to or protect from vitamin D deficiency in children with a chronic illness.

The specific aims were to:

- Define the clinical background of children with high and low levels of vitamin D among pediatric patients visiting the tertiary pediatric out-patient clinics in Children's Hospital, Helsinki University Central Hospital, and assess the impact of the underlying disease on vitamin D status
- Evaluate the effect of patients' age, body size, and ethnic background on vitamin D concentration
- Determine the significance of seasonal variation for vitamin D status in these children.

4 SUBJECTS AND METHODS

The study cohort included 100 subjects who were chosen from a register-based cross-sectional study on 1351 children, who visited the pediatric outpatient clinics at Children´s Hospital, Helsinki University Central Hospital, during 2007-2010. Vitamin D samples had been obtained as part of routine clinical assessment, at the same time with other clinical laboratory tests. The age range of patients was 0-18 years. Subjects included in this study had one or several chronic diseases for which they required follow-up at a tertiary center; hospital inpatients were not included. Results of their S-25-OHD measurements were collected from the database of the Hospital´s Central Laboratory (HUSLAB, Hospital District of Helsinki and Uusimaa), where all the samples had been analyzed.

S-25-OHD measurements were taken based on the judgment of individual clinician, as clinically indicated. Several patients had repeated measurements during the follow-up period but only the first measurement obtained during the study period 2007-2010 was included in the analyses. The selection of the 100 subjects for the present study was made on the basis of vitamin D level. Subjects with highest and lowest vitamin D levels were selected, 50 patients for each group. Patient records of these patients were examined and information was collected for the following variables: height, weight, season for the time of sampling, diagnosis of the underlying disease, medication, use of vitamin D supplements, ethnic background. The study protocol was approved by the Research Ethics Committee of Hospital District of Helsinki and Uusimaa.

Statistical analyses were performed with SPSS version 19.0 for Windows. Chi Square test was used to assess the significance between nominal variables in the two groups. Independent T test was used to assess the statistical significance of differences between various continuous variables in the two groups. All the graphs were done with the same SPSS program. Results were considered statistically significant when $p<0.05$.

5 RESULTS

The study cohort included 50 children with low and 50 children with high S-25-OHD concentration. The mean concentration in the high vitamin D group was 119.3 nmol/L and in the low vitamin D group 15.3 nmol/L. There were no patients with levels suggesting intoxication and the maximum S-25-OHD level was 168 nmol/L. Some patients had such a low vitamin D level that it was below detection limit. For the data analysis such results were registered as 5 nmol/L. There were no statistically significant differences in distribution between sexes in the two groups ($p>0.69$).

Age distribution in the two groups differed drastically. In the high value group younger patients were overrepresented, especially the group of 0-2 year-old patients. In contrast, older patients were overrepresented in the low value group, particularly 12-18 year-old patients, as shown in Figure 4. The mean age in the low value group was 5.9 years and in the high value group 13.7 years with standard deviations of 5.5 and 3.5 years, respectively.

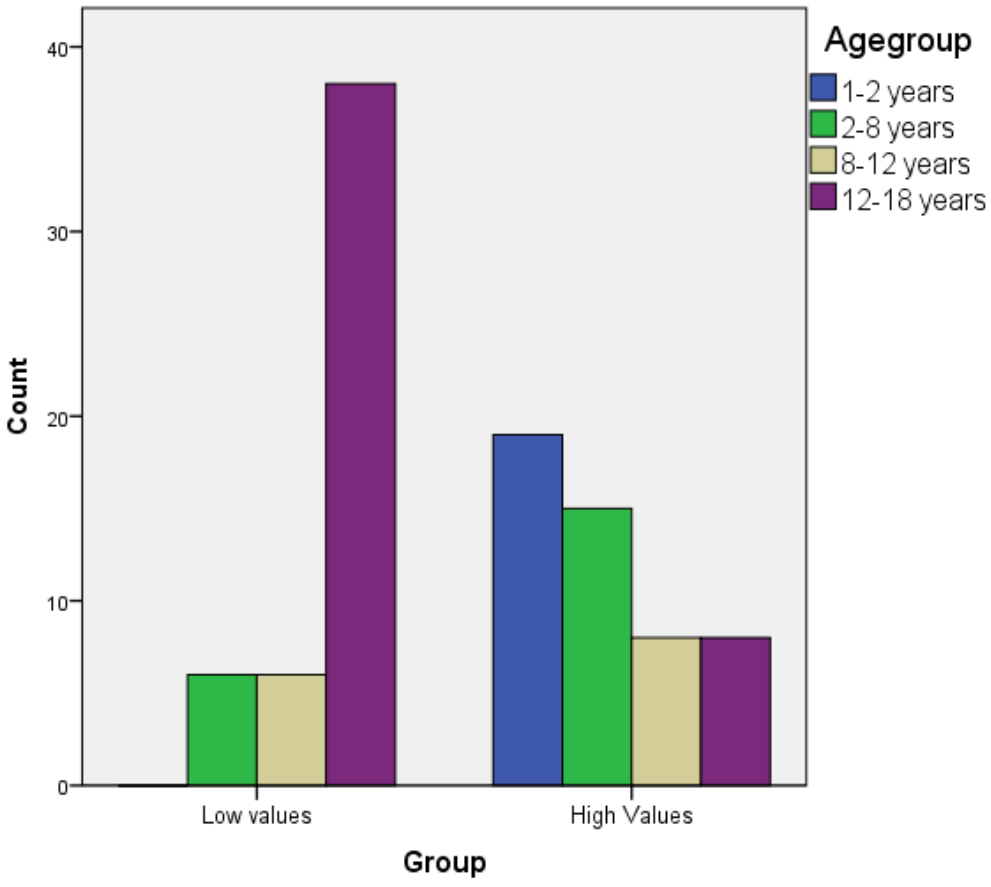

Figure 4. Age distribution in low and high value groups.

There were no differences in the height Z-score or degree of over-weight between the subjects with low and high vitamin D concentrations. The effect of season is shown in Figure 5. The high level samples were taken more often in the summer and autumn and the count progressively decreased toward spring, while low values were taken more often in winter and spring than summer and autumn ($p<0.05$ for the difference between the groups).

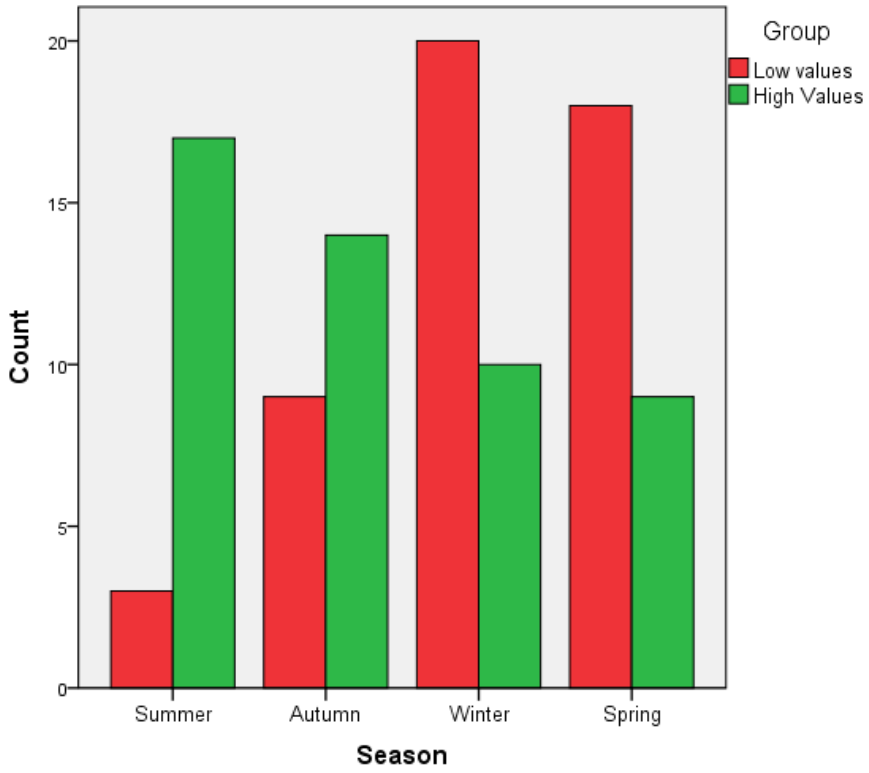

Figure 5. Number of vitamin D samples obtained in different seasons.

The use of vitamin D supplements was significantly more common among those with high than low values ($p<0.05$). Within the group of high values 88% used vitamin D supplements whereas only 23 % in the low vitamin D group used vitamin D supplements. Use of supplements in the different age groups is shown in Table 8.

			Age group			
			1-2 years	2-8 years	8-12 years	12-18 years
Use of vitamin D supplement	Do not use vitamin D supplement	Count	2	4	8	29
		% within Age group	11%	22%	62%	63%
	Uses vitamin D supplement	Count	17	14	5	17
		% within Age group	89%	78%	38%	37%

Table 8. Use of vitamin D supplements in different age groups.

Ethnicity seemed to be a risk factor for low vitamin D levels. In this material 20 out of 100 children had non-Finnish background. Only 2 of them were in the group of high vitamin D values and 18 were in the group of low vitamin D values. ($p < 0.05$) (Figure 6).

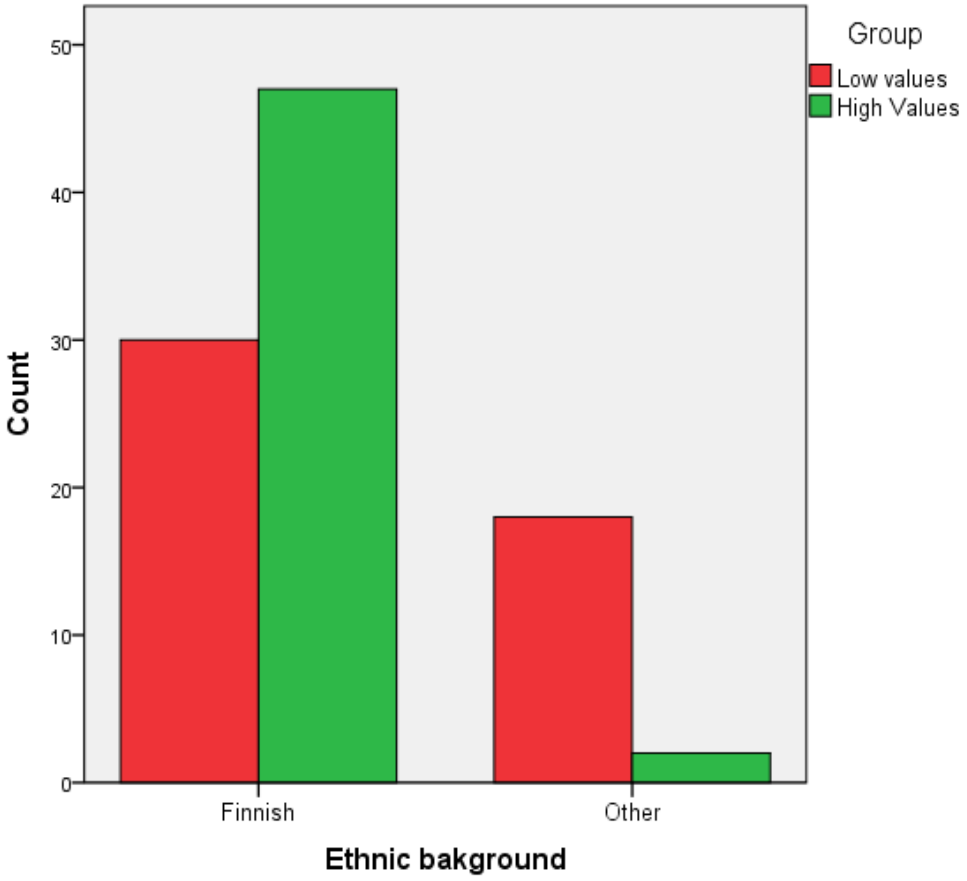

Figure 6. Patients with non-Finnish ethnic background in the low and high vitamin D groups.

There was no correlation between a specific underlying condition and the group of high or low vitamin D levels (p= 0.106). Patients with a nephrologic disease were more prevalent in the high value group. However, almost all of those (6 out of 7) with increased creatinine levels were in the group of high vitamin D levels. High creatinine levels suggest that high S-25-OHD values may partly reflect impaired renal vitamin D hydroxylation and in such case suggest poor rather than good vitamin D status despite high S-25-OHD.

6 DISCUSSION

Vitamin D deficiency is prevalent among Finnish children. Several studies have shown that also children with chronic illness often have low vitamin D levels. This study was carried out to identify the factors that predispose or protect from vitamin D deficiency. The results suggest that the main predisposing factors for vitamin D deficiency in children with chronic illness are older age, lacking use of vitamin D supplements and non-Finnish ethnic background.

The results can be largely explained with the former vitamin D recommendations. The former recommendations commended vitamin D supplementation only up to 2 years of age and older children and adolescents have fallen through the net. The patients in our cohort have been treated according to the old recommendations. The use of supplements was clearly more common in young age groups, especially in the group of 0-2 year-old patients (90%) than in the group of 12-18 year-old patients (37%). Within the group of high vitamin D values 88% used supplements whereas in the group of low values the corresponding proportion was 23%. The recommendations for vitamin D supplementation have recently been changed and the new recommendations cover all age-groups up to 18 years of age, but the dosage for 0-2 year-olds is higher than for 2-18 year-olds, as shown in Table 6. This study does not show the effect of the new recommendations, but it has been already questioned whether the new recommendations are sufficient to satisfy the demands (27,28).

IOM recommendations have also been criticized for being too cautious and illogical (29), even though the recommendations are higher than the new Finnish ones. The Finnish nutritional advisory board states in their report that the recommended dose cannot further be elevated or “it would be even harder or even impossible to fulfill it without usage of food supplements in the whole population”. By saying “then it is not primarily a question of a recommendation of a nutrient” they indicate that, if necessary, the decision should be done by some other instance.

The influence of seasons and the importance of using supplements can be explained by the geographic location. Helsinki is located at the latitude 60°N (comparable to South Alaska). As Holick states (17), sunlight is sufficient only for a few months a year at the latitudes above 37°N (e.g. Southern Italy and Spain). Thus only in the summer one can acquire sufficient

amounts of vitamin D synthesized by irradiation from the sun, but vitamin D stores last only a few months. Inevitably vitamin D concentration lowers in winter and spring without vitamin D supplementation, which is consistent with the results of this thesis.

More people with a non-Finnish background were found in the low level group. People with a non-Finnish background usually have darker pigment and therefore more melanin which functions as the body's natural sun block (6). It seems that vitamin D supplementation is vital for these people in the Northern latitudes. This has already been demonstrated in other studies (30).

Various diagnoses were evenly distributed in the high and low vitamin D level groups. Vitamin D deficiency was a problem regardless of the disease. However, the data showed a trend ($p=0.106$) that patients with a nephrotic disease were abundant in the group of high values. Six out of 13 of these patients had a high creatinine level which may indicate decreased 1-α-hydroxylation in the kidney and accumulation of the measured form (25(OH)D) of vitamin D. Thus it is important to follow the concentration of $1,25(OH)_2D$ in these patients. Another study (31) reveals that also organ transplant recipients and patients with juvenile rheumatological conditions are at risk to develop vitamin D insufficiency. The study indicated that only 3% and 25% of these patients, respectively, had the targeted vitamin D level (≥80nmol/L).

Insufficient vitamin D levels seem to be the common trend in the whole study cohort of 1351 patients (Holmlund-Suila et al., manuscript). Supplementation, being a significant indicator for vitamin D deficiency, should be given more thought when treating children with long-term underlying diseases. This subgroup of patients has many risk factors for developing osteoporosis. Since the most significant prognostic factor for osteoporosis is the peak value of bone density that is achieved in early life, it is important to pay attention to the treatment of the skeletal system. Vitamin D being one of the main environmental factors affecting bone mass, and being easy to administer, it is justified to screen for vitamin D concentration in these patients.

The results of the present study indicate that special recommendations are needed for those who are at risk to develop vitamin D deficiency. It would also be in line with the report of the Finnish Nutrition Advisory Board that states: "certain special groups can be advised and

special recommendations can be made e.g. for little children and the elderly". This has been done in the United States by the Endocrine Society of Clinical Practice as can be viewed in the right side of Table 7. They have also made a list of indications for screening vitamin D levels, shown in Table 9. Patients with long-term underlying diseases need more vitamin D than healthy children. While low levels of vitamin D are measured also in the summer the recommendations should be round-the-year.

TABLE 2. Indications for 25(OH)D measurement (candidates for screening)

- Rickets
- Osteomalacia
- Osteoporosis
- Chronic kidney disease
- Hepatic failure
- Malabsorption syndromes
  - Cystic fibrosis
  - Inflammatory bowel disease
  - Crohn's disease
  - Bariatric surgery
  - Radiation enteritis
- Hyperparathyroidism
- Medications
  - Antiseizure medications
  - Glucocorticoids
  - AIDS medications
  - Antifungals, *e.g.* ketoconazole
  - Cholestyramine
- African-American and Hispanic children and adults
- Pregnant and lactating women
- Older adults with history of falls
- Older adults with history of nontraumatic fractures
- Obese children and adults (BMI > 30 kg/m^2)
- Granuloma-forming disorders
  - Sarcoidosis
  - Tuberculosis
  - Histoplasmosis
  - Coccidiomycosis
  - Berylliosis
- Some lymphomas

Table 9. Candidates for screening vitamin D concentration. (18)

This thesis was a retrospective cross-sectional analysis and has thus limitations in many aspects. Because the sample was not randomly selected we were not able to evaluate correlation between various variables. The strength of this thesis lies in the comparison of the

two chosen groups and in pointing out their clinical differences for further investigation. The thesis shows common factors in patients who have low or high vitamin D levels.

To make the thesis more reliable we could have taken bigger groups under comparison. That could have made it possible to find out predisposing underlying diseases for vitamin D deficiency. The information for the thesis was collected from the patient records and incomplete patient records created the greatest source of error. Because the thesis was made in retrospective manner it precludes the possibility to effect on collection of information.

In conclusion, use of vitamin D supplements is fundamental in achieving sufficient vitamin D concentrations in plasma throughout the year in children with chronic illnesses. The former recommendations failed to attain this state. New recommendations should include special recommendations for this subgroup of patients. The observed severe vitamin D deficiency in several patients is a clear indication for screening 25(OH)D during follow-up in children with chronic illnesses.

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